Symposium on 'Metabolic flexibility in animal and human nutrition' Session I: Early nutrition programming, life performance and cognitive function

Early nutrition programming of long-term health

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Increasing evidence from the EU Project EARNEST and many other investigators demonstrates that early nutrition and lifestyle have long-term effects on later health and the risk of common non-communicable diseases (known as 'developmental programming'). Because of the increasing public health importance and the transgenerational nature of the problem, obesity and associated disorders are the focus of the new EU funded project 'EarlyNutrition'. Currently, three key hypotheses have been defined: the fuel mediated 'in utero' hypothesis suggests that intrauterine exposure to an excess of fuels, most notably glucose, causes permanent changes of the fetus that lead to obesity in postnatal life; the accelerated postnatal weight gain hypothesis proposes an association between rapid weight gain in infancy and an increased risk of later obesity and adverse outcomes; and the mismatch hypothesis suggests that experiencing a developmental 'mismatch' between a sub-optimal perinatal and an obesogenic childhood environment is related to a particular predisposition to obesity and corresponding co-morbidities. Using existing cohort studies, ongoing and novel intervention studies and a basic science programme to investigate those key hypotheses, project EarlyNutrition will provide the scientific foundations for evidence-based recommendations for optimal nutrition considering long-term health outcomes, with a focus on obesity and related disorders. Scientific and technical expertise in placental biology, epigenetics and metabolomics will provide understanding at the cellular and molecular level of the relationships between early life nutritional status and the risk of later adiposity. This will help refine strategies for intervention in early life to prevent obesity.

Early nutrition: Developmental programming: Obesity and related disorders: Key hypothesis

There is a convincing body of research evidence which demonstrates that early nutrition and lifestyle factors have long-lasting programming effects on the risk of later obesity and associated non-communicable diseases, including type 2 diabetes, hypertension and CVD[1–5]. Lifetime experimental studies in animals, historical and prospective cohort studies in human subjects, and experimental, hypothesis-testing interventions in human subjects with long-term follow-up lend support to this conclusion.

Obesity and associated disorders offer some of the best evidence for early nutrition programming[6]. Moreover,

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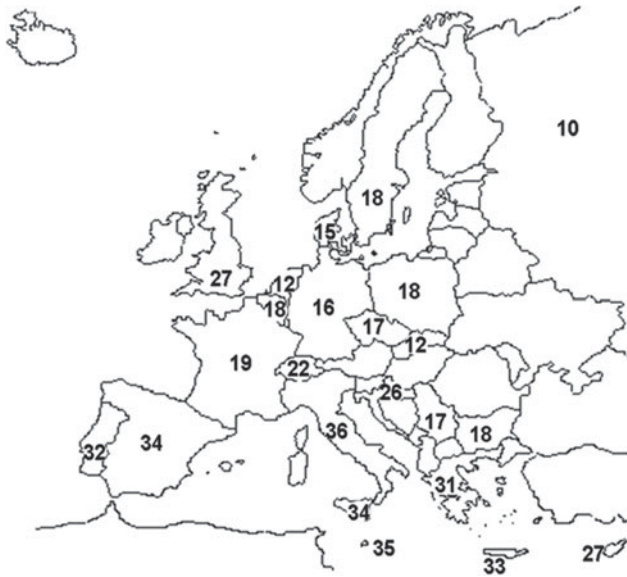
Fig. 1. Prevalence (%) of overweight among children aged 7–11 years across Europe (data redrawn from the International Obesity Taskforce (IOTF)).

focused research on obesity and associated disorders has become increasingly urgent for the following reasons:

obesity in children and adults has increased exponentially and is now of immense public health importance;
the extensive co-morbidities of obesity such as diabetes and CVD mean that research in this area is relevant to many health outcomes;
the trans-generational amplification of obesity programming adds to the public health importance.

Of the 77 million children in the EU in 2004, 11 million were overweight and 3 million were obese, and each year an estimated additional 85 000 children become obese (based on data from the International Obesity Taskforce, www.iotf.org). Fig. 1 shows the prevalence of overweight among children aged 7–11 years in different EU countries, based on a BMI exceeding the equivalent of a BMI >25 kg/m^2 at age 18 years. Rates of obesity and especially of childhood obesity have rapidly increased all over the world during the past two decades, and there is an urgent need to find ways of reversing this alarming trend[7]. Childhood obesity is a problem in its own right because it is not only a prelude to many other childhood diseases but also to adult obesity and early death[8,9]. Recent data show that children with a BMI in the top quartile at age 11 years, compared with those with a BMI in the lowest quartile, are more than twice as likely to die before the age of 55 years[10]. Thus, prevention of overweight childhood and obesity is a high public health priority.

The growing prevalence of overweight and obesity is propelling an upsurge in diabetes, hypertension and CVD and the risk of other non-communicable diseases. By 2030 rates of type 2 diabetes are predicted to rise by nearly 40 % in Europe and by nearly 60 % in Asia from those reported in the year 2000[11].

Not only is obesity a prelude to many other diseases, but focusing on childhood obesity as an endpoint has the practical advantage that it occurs over a shorter timeframe and allows a step-by-step understanding of the development of the disease. Questions still to be resolved include:

how is susceptibility to obesity 'programmed' by environmental influences in early life?
what are the relevant environmental stimuli?
what are the sensitive periods of susceptibility during development?
what are the specific effects of developmental exposures on adiposity and co-morbidities in the offspring?

EarlyNutrition researchers appreciate the need to accurately assess body composition, particularly fat mass. The focus on adiposity (i.e. body fat content) is important because it is a better predictor of health outcomes than childhood BMI[12,13].

Currently three key hypotheses are proposed to explain why early nutrition programmes obesity and its co-morbidities. These are not mutually exclusive and could have a greater or lesser impact in different circumstances: (i) the fuel mediated '*in utero*' hypothesis; (ii) the accelerated postnatal weight gain hypothesis and (iii) the mismatch hypothesis.

The early nutrition pathways to programming of obesity are likely to be multifactorial, but Fig. 2 illustrates how these hypotheses could contribute to the developmental programming of non-communicable disease risk (adapted from[14]).

Fuel-mediated *in utero* hypothesis

The hypothesis of fuel-mediated teratogenesis proposes that intrauterine exposure to an excess of fuels, most notably glucose, causes permanent fetal changes that lead to obesity in postnatal life. Recently, the hypothesis that the fetus is susceptible to the humoral influences of maternal obesity has been strengthened by numerous observational studies suggesting that maternal obesity and excessive pregnancy weight gain independently increase the risk of obesity in the child, leading to the 'fuel-mediated' *in utero* hypothesis[15]. Obese women are more than twice as likely to have a large-for-gestational age baby as normal weight women[16]. Children of obese women and of those with an excessive weight gain during pregnancy are at increased risk of becoming overweight and obese themselves[17–21]. While this could be the result of shared genetic predisposition and a similar lifestyle, there is now increasing evidence suggesting that an obesogenic uterine environment is of major importance in modulating long-term risk of adiposity and related outcomes. This evidence includes several reports that suggest that children of obese mothers have a higher risk of obesity than children of obese fathers[22–24]. Also, children born to formerly obese women who had undergone bariatric surgery leading to weight loss, had only half the risk of becoming obese as compared with their older brothers and sisters who had been born prior to bariatric surgery and hence under a less favourable prenatal metabolic and endocrine environment[25]. In addition, some evidence from the diabetes literature could
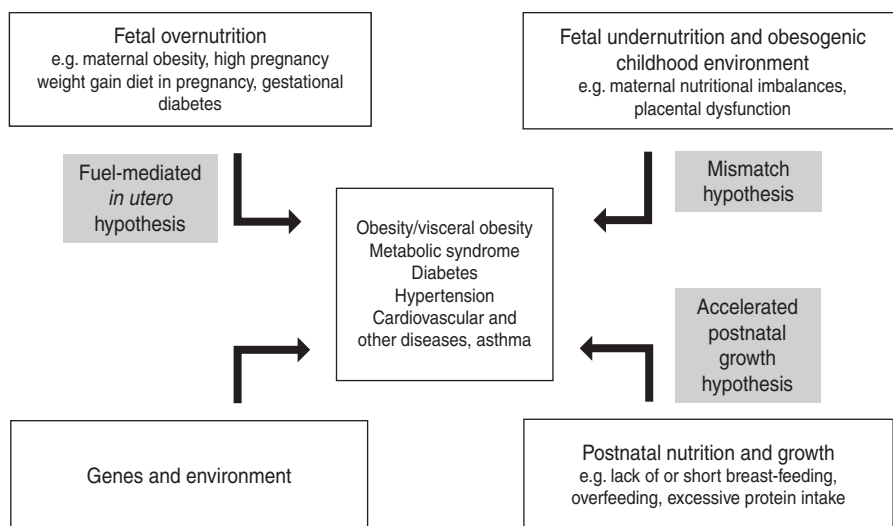
Fig. 2. Integration of hypotheses for programming of obesity and related disorders (modified from[14]).

indirectly support the hypothesis that obesity is associated with an increased risk of gestational diabetes, and two studies have shown that improved glycaemic control in women with gestational diabetes is associated with a reduction of macrosomia or a composite end point including macrosomia[26,27]. Programming the susceptibility to obesity while *in utero* through modified tissue responses and subsequent metabolic changes could potentially lead to acceleration in the risk from generation to generation (see Fig. 3).

The rise in obesity in women of child-bearing age and in the numbers of obese pregnancies is consistent with the recent rise in rates of childhood obesity. Accelerated prenatal fetal growth can result from increased fuel supply to the fetus which occurs when maternal blood glucose and lipid levels are increased due to insulin resistance or gestational diabetes mellitus, both of which are prevalent among obese pregnant women. Indeed, maternal blood glucose and TAG concentrations are directly related to neonatal adiposity in obese women[28]. Better control of blood glucose levels, or reduction of insulin resistance during pregnancy through diet and exercise, offer ways of modifying fetal growth and potentially reducing the child's future risk of obesity[29].

Accelerated postnatal growth hypothesis

Many observational studies have reported that rapid weight gain in infancy is associated with an increased risk of later obesity and other adverse outcomes such as the risk of CVD[30–35]. Accelerated postnatal weight gain can result from high intake of growth-enhancing nutrients such as protein in the infant diet. Available evidence suggests that higher protein intakes increase plasma and tissue levels of insulin-releasing amino acids and of insulin and insulin-like growth factor 1, and thereby increase weight gain and adipogenic activity[36,37].

A meta-analysis of nine studies showed that breast-feeding, which supplies less protein than conventional infant formulae, is associated with an approximately 20%
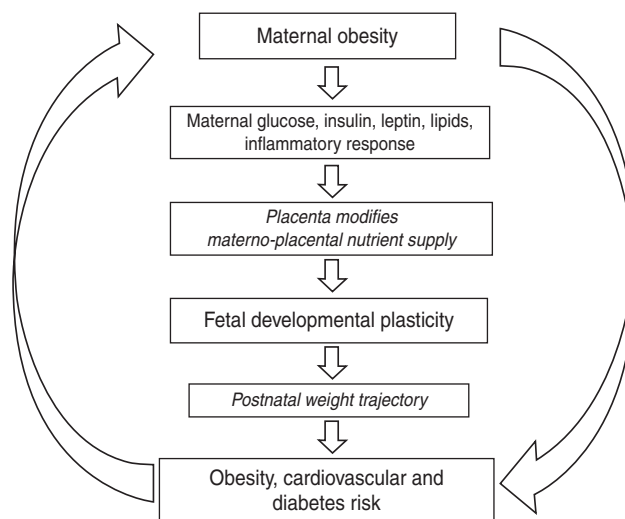

Fig. 3. The potential pathways which may lead to trans-generational acceleration of obesity.

lower risk of obesity at later ages[38,39]. A trial in which 1138 healthy, formula-fed infants in five European countries were randomly assigned to receive infant and follow-on formulae with lower or higher protein contents for the first year found that, at 24 months, the average weight-for-length *z*-score in the lower protein formula group was lower than in the higher protein group and was similar to that of the breast-fed reference group[40]. This difference in weight for length at 2 years is of major benefit as it predicts a 13% lower obesity risk at age 14–16 years with lower protein infant formula[14]. Long-term follow-up of a subset of children assigned in other trials to nutrient-dense or less nutrient-dense infant formulae found that those infants who received the growth promoting formulae had increased body fat mass 5–8 years later[41]. Moreover, in several non-randomised analyses, faster weight gain in infancy was associated with greater fat mass in childhood[30,32,33,40].
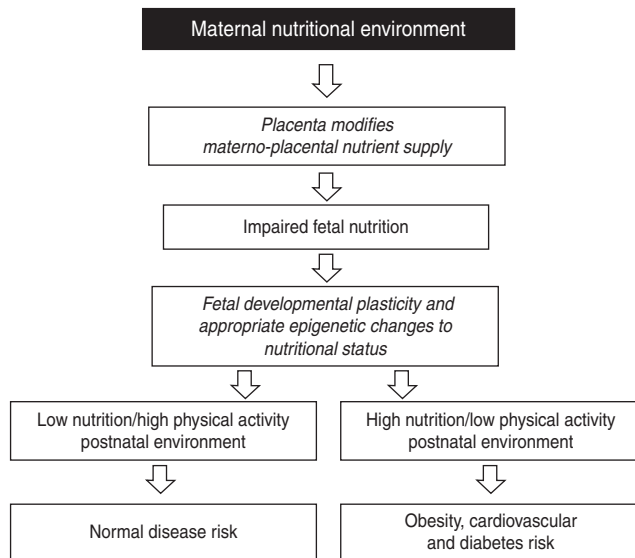
Fig. 4. A developmental 'mismatch' between a sub-optimal pre-natal/infant environment and an obesogenic childhood environment may predispose to obesity and related disorders.

Mismatch hypothesis

The mismatch hypothesis suggests that people who experience developmental 'mismatch' between a sub-optimal pre-natal/infant environment and an obesogenic childhood environment have a particular predisposition to obesity and related co-morbidities[3,42]. During limited time periods of developmental plasticity (see Fig. 4), the fetus responds to cues from the early environment, to produce a phenotype best suited to survival in that environment, leading to irreversible changes in metabolism and endocrine regulation.

After the early phase of developmental plasticity, the established changes become less subject to environmental modulation. Individuals are likely to remain healthy when their resulting phenotype is matched to their environment, as they can mount appropriate responses to everyday challenges. But, when not well matched, their risk of disease increases. The degree of any mismatch hence determines the risk of later disease. People who were small at birth and had poor growth in infancy have an increased risk of CHD, particularly if their impaired early growth is followed by increased childhood weight gain[43,44]. Greater mismatch can arise from altered mother's body composition, unbalanced or low-energy maternal diet or impaired placental nutrient transfer, or through an influence of an obesogenic lifestyle in the later environment. Such changes are important in both affluent settings and in developing societies going through rapid socio-economic transitions.

Potential mechanisms of early nutrition programming effects

The precise mechanisms underlying how early nutrition can cause programming of obesity are unknown, but are thought to be associated with altered development of organ structure or persistent alteration at the cellular level. Some proposed mechanisms[45] include:

> epigenetic memory: transcriptional modification (e.g. DNA-binding proteins, histone acetylation, CpG methylation to 5-methyl-cytosine and altered miRNA expression);
> induction of altered organ structure (vascularisation, innervation and juxtaposition), e.g. altered hepatic architecture during organogenesis which may permanently modify metabolism; reduced nephron number which may influence risk of hypertension;
> alteration of cell number (hyperplasia and hypertrophy);
> clonal selection (disproportionate growth of cells that proliferate rapidly under specific metabolic conditions). Metabolic differentiation (e.g. hepatocellular changes associated with enhanced metabolic activity).

The molecular mechanisms proposed include acute or persistently altered gene expression through a variety of epigenetic pathways. During *in utero* or early postnatal development, short-term changes through environmental influences could permanently change organ development at a time of extreme vulnerability or 'plasticity'. For example, experimental studies and human observations have shown that a reduction in nutrient and oxygen supply differentially affect the growth and development of organs and tissues. Organs affected include the lungs, kidney, gut and liver. Additionally, clinical and experimental studies provide evidence for developmental changes in the homoeostatic set points for many hormones and for alterations in tissue sensitivity to these hormones. Alterations of the fetal hypothalamic–pituitary–adrenal axis, central mechanisms controlling energy balance and sympatho-adrenal responses are likely to be an important mechanism by which developmental exposures affect the offspring's subsequent responses to challenges. One of the keys to understanding how these changes are brought about is to establish whether the placenta plays a facilitatory or protective role in the face of nutritional challenge. As a mandatory preparatory step, we need to establish which maternal exposures are modified by the placenta and how, and to determine what the vulnerable fetus actually experiences. Only then we can begin to unravel the pathways to *in utero* programming which will lead to successful interventions in the mother[46,47]. While conceptually, epigenetic modification provides a framework for understanding how differences in the early environment can lead to permanent changes in metabolism and therefore long-term health risks, much work is still to be done to unravel the specific post epigenetic modifications involved in different disease processes.

The role of the placenta

As the 'gateway' to the fetus, the placenta is located between the maternal and fetal circulation and thus exposed to metabolic, endocrinal and inflammatory changes in the fetal and maternal blood. These factors, which depend on maternal nutrition and lifestyle, have been shown to affect placental transfer of nutrients, through modulation

of placental gene expression[48] or via mammalian target of rapamycin signalling[49]. Maternal macronutrients in particular, have been ascribed a strong influence on fetal development. Maternal overnutrition promotes glucose and lipid supply to the fetus leading to hyperinsulinaemia and enhanced fetal fat accretion. As discussed earlier, this state of nutrient excess may be associated with a greater long-term risk for adult disease[50]. Maternal obesity and increased plasma lipid concentrations are related to fetal obesity, highlighting the importance of lipid status and potentially of placental fatty acid transfer[28,51,52].

While the maternal-to-fetal transfer of fatty acids, especially of PUFA, has been studied in normal pregnancies[53], little is known whether and how this may be changed with maternal overnutrition/obesity or gestational diabetes. Although many of the basic processes of placental lipid transfer and metabolism are established[54], neither the timing nor the magnitude of their modifications with maternal obesity or diet have been defined yet.

Studies in diabetic pregnancies have previously examined the close relationship between increased fetal lipid and total fat accretion, but not in maternal obesity or how they are modified by diet and physical activity[55]. Placental enzymes, receptors, binding proteins and transport proteins all play roles in lipid transfer to the fetus[56,57]. Thus, they strongly contribute to fetal fat accretion and may serve as predictors of fetal and neonatal adiposity. The quantitative disposition of fatty acids and molecular signatures of maternal weight, diet and physical activity in the placenta have to be identified, but will contribute to our understanding of the mechanisms underlying developmental programming and to identification of biomarkers of the long-term effects of factors acting during pregnancy on offspring health.

Epigenetics and metabolomics

Further to the identification of epigenetic modifications leading to modified gene expression and thus function in the placenta, studying the epigenetic changes through environmental factors such as maternal nutrition and lifestyle in other tissues and cell types has become of increased interest in the research of obesity and related disorders. Epigenetics comprises, among other more technically challenging methods, the investigation of DNA methylation profiles as the primary mark of environmentally mediated changes to gene expression. While the exact role of epigenetic mechanisms in fetal programming of metabolic diseases and body weight regulation remains to be further investigated, a number of animal models have demonstrated a causal relationship between early nutrition and later metabolic phenotype with gene dysregulation mediating such adverse metabolic outcomes[58–62]. A recently published article reviews the scientific evidence base of the association between the role of epigenetics in the fetal programming of adverse metabolic outcomes concluding that despite a vast amount of epigenomic data generated, the challenge still is to decipher the biological and clinical relevance of these epigenetic changes[63]. Although studies assessing the role of DNA methylation in

mediating the effect of maternal metabolic factors on offspring obesity have been performed, identification of specific genes as biomarkers have been confined to only a few reports[64,65]. Genome-scale DNA methylation analysis has detected highly variable regions of differential methylation in human subjects, some of which consistently co-vary with BMI over time[66]. Identification of perinatal epigenetic markers holds the potential to prognose individual susceptibility to later obesity and to be applied in monitoring programmes aiming at optimising maternal nutrition and lifestyle. The challenge now is to develop specific studies aimed at investigating how environmental exposures interact with underlying genetic determinants to dysregulate gene expression and lead to metabolic disorders[65].

In parallel to the expanding field of epigenomics, the application of metabolomics has recently gained considerable interest in the area of obesity, metabolic syndrome and diabetes research[67–70]. Exploration of molecular mechanisms of metabolic programming and early growth by metabolomics approaches is an ongoing and still unresolved challenge with considerable relevance, since understanding of the sequence of underlying events and of key metabolic processes may allow for the development of even more targeted and effective intervention. In the field of perinatal development and metabolic programming by early nutrition, metabolomics has been rarely used so far, but a promising example demonstrated the possibility to identify women from metabolite profiles determined in first trimester plasma samples, who later in pregnancy developed preeclampsia[71]. Furthermore, using ultrahigh pressure chromatography coupled to an orbitrap mass spectrometer, biomarkers for small for gestational age babies were identified in plasma samples collected during the 15th week of pregnancy[72]. The already available epigenetic and metabolomic data in the area of metabolic programming and accumulated experience in other fields indicate that both tools are mandatory for the elucidation of the mechanisms linking early nutrition to long-term health.

The EarlyNutrition project

EarlyNutrition (www.project-earlynutrition.eu) is a large-scale collaborative project running from 2012 to 2017 under the umbrella of the EU 7th Framework Programme (FP7) which brings together a multi-disciplinary team of highly successful international scientists and leaders in key areas of the developmental programming field located in twelve European countries, the USA and Australia. Leaders of relevant intervention trials in pregnancy and infancy, some of the best characterised cohorts of pre-pregnant and pregnant women and their children in the world, as well as those involved in the forefront of mechanistic animal studies and in placental biology will work together with partners from industry and small and medium size companies. With a total budget of 11·12 million Euro, the project is supported with 8·96 million from the European Commission.

Project EarlyNutrition presents an integrated work programme designed to fill gaps in the knowledge base, as

addressed by three key hypotheses, as described earlier, linking early nutrition to long-term health and performance. Prevention of adiposity, i.e. increased body fat content that is closely related to disease risk, and of associated disorders are the principal targets. The project and its likelihood of success benefits from concentration on four population target groups, namely women prior to pregnancy, pregnant women, infants (including breast-feeding) and young children. Combining research from animal, observational and human intervention studies, the relative roles of placental function, early growth patterns, pre-pregnancy weight status, pregnancy weight gain, overweight and obesity, gestational diabetes, breast-feeding, genetic variation, environment, gender, lifestyle, physical activity, ethnicity and geographic background as determinants of the risk of obesity and associated disorders in the offspring will result in an increased evidence base to help formulate recommendations.

It is of outmost importance that early nutrition programming research now considers the entire life course and addresses socio-economic priority issues, fosters innovation and ensures rapid translational application, e.g. by leading to health-promoting policies and evidence-based dietary recommendations for the four population target groups. Through the EarlyNutrition project, the field will benefit markedly from close partnership of academia with some of Europe's most successful companies in the food industry as well as small and medium enterprises, with considerable potential for knowledge transfer to the commercial sector and development, for example of novel biomarkers of disease risk and of foods for the health sector. International collaboration across and beyond Europe, including leading investigators in the USA and Australia, will lend to global generalisability of the results and further enhance opportunities for progress and innovation.

Conclusions

Convincing evidence now shows that nutrition during both pre- and early post-natal life can programme long-term health, well-being and performance into adulthood and old age. These advances are widely recognised to offer an important, different and exciting perspective for strategies in the prevention of disease. Among the prerequisites for effective translation to public health recommendations is the need to strengthen the scientific evidence, e.g. on effect sizes of early life programming in contemporary populations, on specific nutritional exposures, on sensitive time periods in early life, on underlying mechanisms, and on potential effect differences in subgroups characterised for example by ethnicity, genetic predisposition or gender. Evidence that diet and lifestyle modifications during pregnancy can reduce the increased risk of obesity in offspring of obese pregnant women will help break an intergenerational cycle of obesity which might otherwise lead to spiralling rates of obesity and associated disorders throughout the world.

A wealth of recommendations for optimised nutrition before and during pregnancy and for infancy and early childhood already exists nationally and internationally.

However, these have usually been based on immediate physiological requirements, and have not often been related to the longer-term health consequences of nutritional status in these critical periods of life. An analysis of twenty-six breast-feeding policy documents from five European countries for the EARNEST project found that only about one-third mentioned long-term outcomes such as a reduction in diabetes risk as a benefit of breast-feeding[73].

A better evidence-base on the effects and mechanistic pathways of early nutrition will allow dietary recommendations for optimised nutrition to be formulated, which will aim at reducing the future risk of obesity and co-morbidities in the following relevant target groups, according to the critical periods for programming and where recommendations are appropriate: pre-pregnant women, pregnant women, infants (including breast-feeding) and young children.

Thus, evidence that excessive weight gain in pregnancy and/or rapid early infant weight gain leads to later obesity will help to formulate policies to reverse the increasing rates of childhood obesity and related disorders. Moreover, further research on dietary modifications in infancy, in particular relating to breast-feeding and complementary feeding practices, as well as novel compositional approaches to infant formula, can reduce the risk of obesity and related disorders in offspring. Research in project EarlyNutrition moves from studies of historical cohorts to a focus on selected key hypotheses that drive research targeted on public health priorities in contemporaneous populations, utilising novel biotechnology methodologies, exploring uniquely characterised prospective cohorts and undertaking interventions that increase the evidence base for practical recommendations and applications.

Acknowledgements

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