Regulation of Early Human Growth: Impact on Long-Term Health

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Abstract
Growth and development are central characteristics of childhood. Deviations from normal growth can indicate serious health challenges. The adverse impact of early growth faltering and malnutrition on later health has long been known. In contrast, the impact of rapid early weight and body fat gain on programming of later disease risk have only recently received increased attention. Numerous observational studies related diet in early childhood and rapid early growth to the risk of later obesity and associated disorders. Causality was confirmed in a large, double-blind randomised trial testing the 'Early Protein Hypothesis'. In this trial we found that attenuation of protein supply in infancy normalized early growth and markedly reduced obesity prevalence in early school age. These results indicate the need to describe and analyse growth patterns and their regulation through diet in more detail and to characterize the underlying metabolic and epigenetic mechanisms, given the potential major relevance for public health and policy. Better understanding of growth patterns and their regulation could have major benefits for the promotion of public health, consumer-orientated nutrition recommendations, and the development of improved food products for specific target populations.

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Introduction

Growth and development are central characteristics of childhood. Growth can be characterised quantitatively as a gain in length or height, head circumference, body mass or other measures. The importance of regular monitoring of children's growth patterns as part of standard paediatric health care and of research studies, e.g. by plotting repeated growth measures on percentile references, is widely acknowledged [1, 2]. Up- and downward deviations of growth from normal patterns, based on growth standards or percentile curves, may reflect grave disorders, such as genetic, syndromic or endocrine conditions, infectious or inflammatory diseases, malnutrition, psychosocial deprivation or abuse, or a broad spectrum of other disorders. Based on the results of the Multicentre Growth Reference Study (MGRS), which collected data points on about 8,500 children in six countries around the globe (Brazil, Ghana, India, Norway, Oman and the USA), the World Health Organisation takes the view that a single set of growth curves adequately describes normal growth of all economically advantaged breastfed infants and children up to an age of 5 years [3, 4]. However, a recent systematic review on growth data from studies performed in 55 countries or ethnic groups found that growth varied among different national and ethnic groups [5]. Means of height were generally within 0.5 of the standard deviation (SD) of the MGRS means, whereas weight varied by as

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much as 1.5 SD. Mean head circumference values also varied widely, with means in many groups consistently 0.5–1 SD above the MGRS mean. Of interest, head size in breastfed children at any age examined was found to be far closer to local norms than to the MGRS mean [5]. These data question whether the use of one single international growth standard is justified and call for further work aiming at a better understanding of the predictors and regulators of child growth.

Early growth faltering and malnutrition have long been known to induce adverse later outcomes, such as poor cognitive and motor function [6, 7]. In contrast, the adverse effects of excessive growth and in particular of rapid weight and body fat gain on long-term health have only recently received increased attention. It is now widely recognized that early growth and tissue development during the first 1,000 days of human life and beyond – from conception through to early childhood – are important predictors of long-term health and performance up to adulthood and old age *(Early Metabolic Programming of Lifelong Health, Developmental Origins of Adult Health)* [8–13]. Therefore, the study of child growth receives increasing interest.

Infant Diet, Early Growth and Later Obesity

A relationship between diet in early childhood, rapid early growth and later outcomes has been found in many studies. More than 1 decade ago, our group showed in a large cross-sectional study including more than 9,000 children in Germany that breastfed children have a markedly reduced later risk of obesity at school age than previously bottle (formula)-fed children, with an inverse dose-response relationship between breastfeeding duration and the adjusted odds ratios for obesity (fig. 1) [14]. This finding was subsequently confirmed in numerous cohort studies and meta-analyses [15–18], which has markedly influenced policy on breastfeeding promotion worldwide [12, 19–21]. We hypothesized that breastfeeding protects through reducing weight gain velocity during early childhood, resulting from a different substrate supply with breastfeeding compared to feeding conventional infant formula, in particular the markedly lower protein content in human milk than in infant formula [22, 23]. Indeed, rapid weight gain during the first 2 years of life is highly predictive of overweight at early school age [24], which was confirmed in numerous other studies: an increase in weight-for-age SD score >0.67 SD during the 1st and 2nd year of life predicts two- to threefold increased odds of
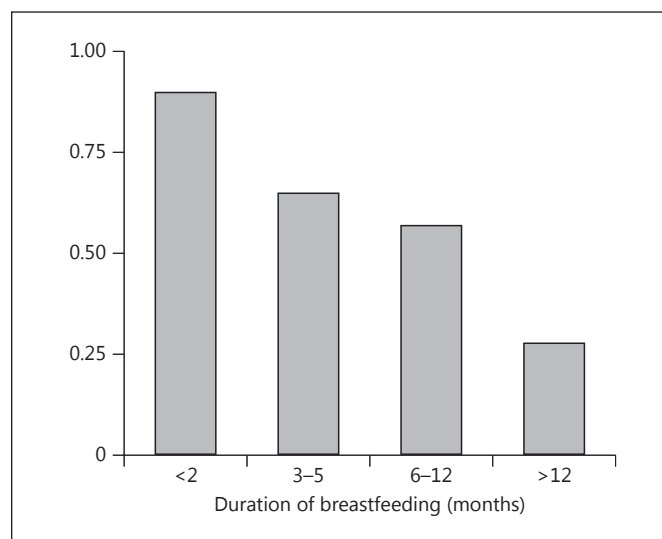

Fig. 1. Longer duration of breastfeeding is linked to a lower adjusted odds ratio of obesity at early school age. Data from a cross-sectional study in more than 9,000 children in Bavaria, Germany [14].

obesity in children, teenagers and adults [25]. A recent systematic review and meta-analysis of 15 studies examining body composition in healthy infants showed that breastfed infants had a lower body fat mass at age 1 year than formula-fed infants [26]. Accordingly, we recently found that weight gain velocity during the first 6 months of life is closely correlated to infant body fat mass, as assessed by isotope dilution (deuterium method), whereas lean body mass is not related to weight gain velocity [27]. It seems likely that variation in substrate supply is the causative factor. It is of key importance to elucidate the underlying mechanisms and which substrates are the major modulators of early growth.

The Early Protein Hypothesis

We explored 'The Early Protein Hypothesis' (fig. 2): high protein supply in infancy increases plasma and tissue concentrations of amino acids that stimulate an enhanced secretion of the growth factors insulin-like growth factor-1 (IGF-1) and insulin, with consecutively increased weight gain in the first 2 years of life, increased adipogenic activity, and increased long-term risk of later obesity and associated disorders [10, 28, 29]. We tested this Early Protein Hypothesis in a large, multicentre randomised controlled trial consecutively funded by the European Commission Framework Programmes, the Child-

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Table 1. Prevalence and relative risk of obesity at the age of 6 years in children participating in the multicentre CHOP trial who had been randomised to receive conventional formula with high protein content (control) or intervention formula with reduced protein but equal energy content

Intervention: lower protein	4.4%	
Control: higher protein	10%	
Unadjusted relative risk	2.43 95% CI: 1.12–5.27	p = 0.024
Adjusted relative risk	2.87 95% CI: 1.22–6.75	p = 0.016
Breastfed reference group	2.9%	

A group of breastfed children was followed for reference. Lower protein supply in infancy reduced the risk for obesity at early school age 2.43-fold (unadjusted) or 2.87-fold (adjusted), respectively [31].

hood Obesity Project (CHOP) Trial [30, 31]. In this trial, we enrolled 1,678 healthy term infants born appropriate for gestational age that were either exclusively breastfed for at least the first 3 months of life (by parental choice) or formula fed; the latter were randomised double blind to receive during the 1st year of life milk formulas with equal content of energy and most nutrients, but either (conventionally) high or reduced protein contents. Reducing protein supply with infant formula normalised growth measures at 2 years of age relative to the breastfed reference group and the WHO growth standards. Higher protein intake induced significantly increased weight for length and body mass index (BMI) in early childhood [32]. Follow-up of subjects up to school age demonstrates lasting large effects of early substrate supply and growth on later health. At 6 years of age, previously breastfed children have a much lower BMI than those fed conventional formula with high protein contents (fig. 3), which agrees with results of observational studies [33]. In contrast, children who had been double-blind randomised to receive experimental formula with reduced protein but equal energy content in the 1st year of life have a significantly lower BMI than those in the control group with conventional formula, and they achieve a BMI which is not different from the breastfed reference group [31]. There was a very large effect of early feeding and growth on the risk of obesity at age 6 years: Lower protein supply in infancy reduced the risk for obesity at early school age 2.43-fold (unadjusted) or 2.87-fold (adjusted), respectively [31] (table 1).
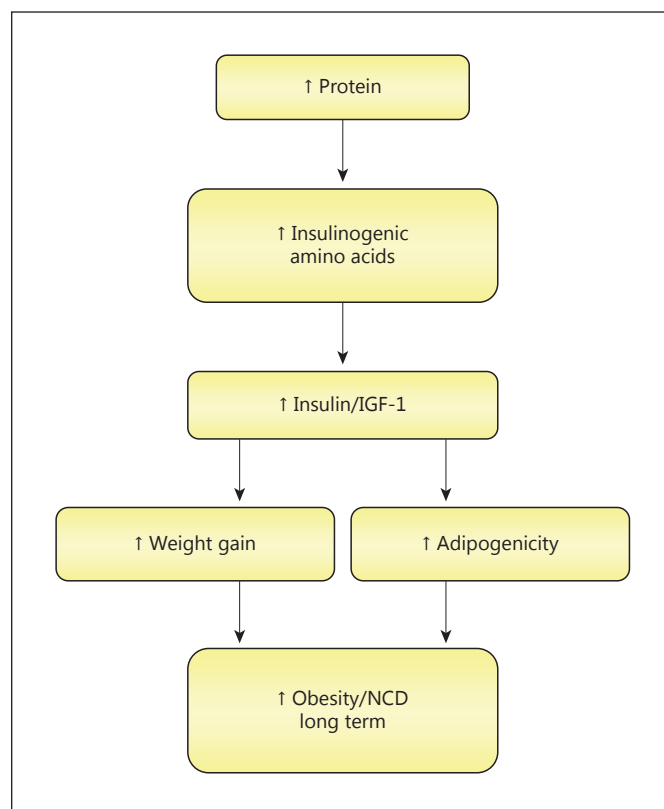

Fig. 2. The Early Protein Hypothesis stipulates that a high infant protein supply in excess of metabolic requirements increases plasma and tissue concentrations of amino acids that stimulate an enhanced secretion of the growth factors IGF-1 and insulin, with consecutively increased weight gain in the first 2 years of life, increased adipogenic activity and increased long-term risk of later obesity and associated non-communicable diseases (NCD) such as diabetes mellitus type 2 [10].

These data demonstrate that metabolic modulation of early growth has a very large impact on later obesity prevalence, which indicates major opportunities for disease prevention and public health promotion. Therefore, it is of paramount importance to investigate and understand the underlying mechanisms and key drivers through which early metabolic exposure modulates child growth and long-term health, which also requires meaningful description of the kinetics of child growth.

Metabolic Mechanisms

In our randomised CHOP Trial, a higher formula protein supply to infants induced markedly elevated plasma concentrations of the branched-chain amino ac-

Fig. 3. Evolution of mean BMI from early infancy to the age of 6 years in children with different infant feeding regimens participating in the multicentric CHOP trial. Previously breastfed children have a lower BMI than those fed conventional formula with high protein contents. In contrast, children who had been double-blind randomised to receive experimental formula with reduced protein but equal energy content (lower protein) in the 1st year of life have a significantly lower BMI than those in the control group with conventional formula, and they achieve a BMI equal to that of the breastfed reference group [31].
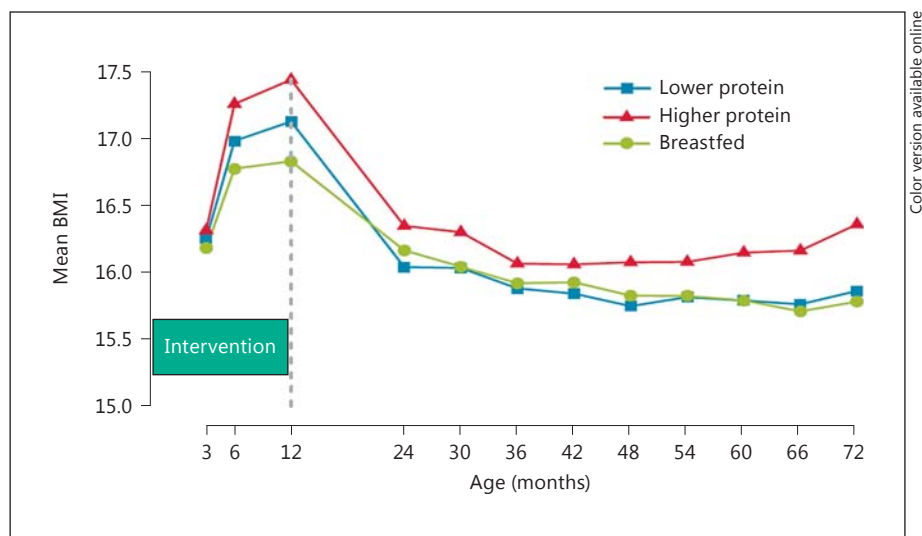
ids leucine, isoleucine and valine, along with slight elevations in other indispensable amino acids [34]. In contrast, high protein supply did not change the plasma concentrations of most other amino acids, and even reduced the plasma concentrations of glutamine and glycine. We hypothesise that enhanced concentrations of branched-chain amino acids in response to protein supply may be causative for inducing excessive weight gain and higher body fat mass in formula-fed compared to breastfed subjects. Experimental observations provide support for a potential key role of amino acids in growth regulation [25]. Verification of this hypothesis would allow new avenues to early obesity prevention by modifying specifically the amino acid composition of feed, rather than reducing further the total protein intake, which has considerable limitations for practical and safety reasons.

In fact, amino acids have been shown to be more potent stimulators of IGF-1 release than glucose in fetal rat islets [35]. Studies in 4-week-old rats showed that feeding a diet with 15 instead of 5% protein for only 1 week increased serum IGF-1 more than fourfold [36]. Amino acids also markedly influence insulin secretion with key regulatory roles for anabolic pathways and lipid deposition during early growth [37, 38]. Glucose is a key driver of insulin secretion, but glucose-induced insulin secretion is markedly attenuated by low protein supply. Also, leucine and most likely also other amino acids enhance insulin secretion via both acute effects, such as activated glutamate dehydrogenase activity, as well as chronic effects, such as gene transcription and regulation of β cell

metabolism [39]. One pathway through which amino acids and the growth factors insulin and IGF-1 could effectively modulate metabolic response and growth in children is the mammalian target of rapamycin (mTOR), a highly conserved Ser/Thr kinase present in two structurally and functionally distinct complexes (fig. 4) [40]. The growth factors insulin and IGF-1 stimulate mTORC2 via an unknown pathway, and mTORC1 via phosphoinositide 3-kinase (PI3K) and Akt inducing the mTORC1 activator Rheb. Amino acids enhance ATP loading of RAG proteins and RAG-GTPases, which interact with Rheb and activate mTORC1 [40]. Of importance, full activation of mTORC1 is only achieved through the synergistic action of both growth factors and amino acids, while a low energy supply downregulates mTORC1 [40]. Thus, this pathway represents an elaborate sensor system by which nutritional supply regulates metabolism and growth. The enormous power of this system is demonstrated, for example, in mice with knockout of raptor in adipose tissue, which leads to disruption of mTORC1. These mice are lean and resistant to diet-induced obesity, and they have improved metabolic characteristics, such as better glucose tolerance and insulin sensitivity, as well as resistance to diet-induced hypercholesterolaemia [41]. These observations lead us to the conclusion that regulation of mTORC1 signalling by amino acids may control whole body energy metabolism, body weight and body fat deposition. This hypothesis needs to be tested by detailed metabolic characterisation of prospective cohorts in which precise phenotyping of growth has been performed and in which informative biomarkers of nutritional expo-

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Fig. 4. Simplified scheme of nutrient sensing by mTOR and its regulatory signalling for metabolism and growth. Amino acids and the growth factors insulin and IGF-1 modulate metabolic response and growth through mTOR. Insulin and IGF-1 stimulate mTORC2 via an unknown pathway, and mTORC1 via PI3K and protein kinase B (Akt) inducing the mTORC1 activator Rheb (GTP-binding protein Rheb). Amino acids enhance ATP loading of RAG proteins and RAG-GTPases, which interact with Rheb and activate mTORC1. A low energy supply downregulates mTORC1. mTORC1 effects the regulators of protein translation 4E-BP and S6K. Activation of mTORC2 induces phosphorylation of the AGC protein kinases Akt, serum- and glucocorticoid-induced kinase (SGK1) and protein kinase C (PKC), which modulate cell survival, metabolic response and cytoskeletal organisation.
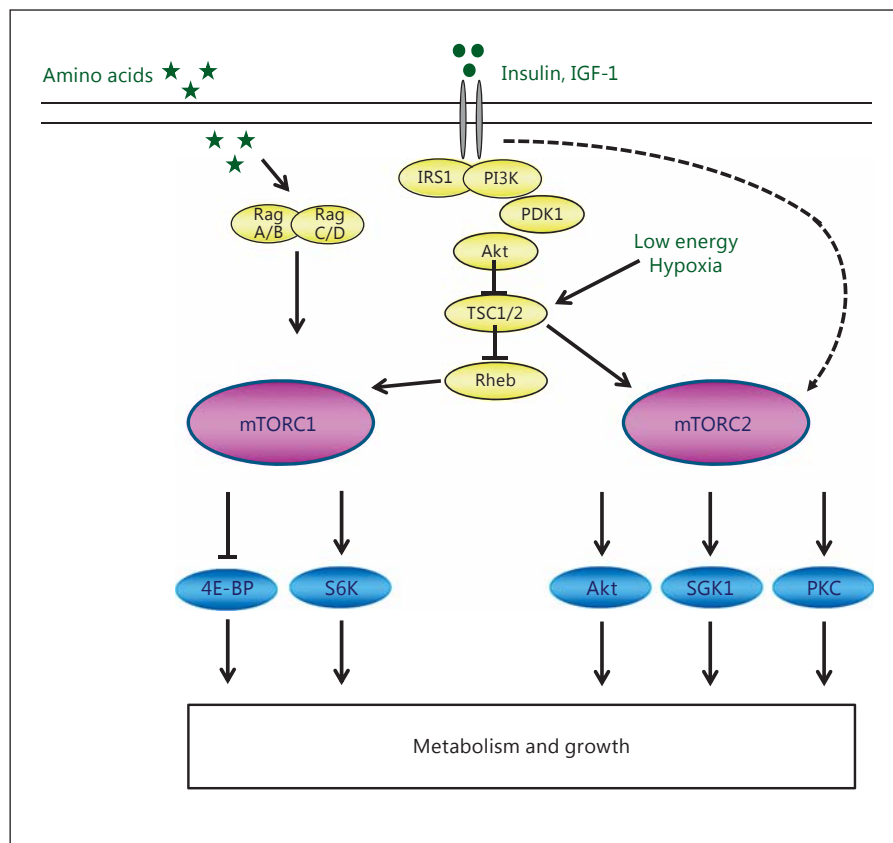
sure, status, function, growth and other effects are determined [42].

This approach has now become feasible with established high-throughput, precise analytical tools for targeted metabolomic profiling from minimal sample volumes using flow injection analysis with triple-quad mass spectrometry, which is extremely powerful [43–49]. Application of these sophisticated tools bears the very promising potential to detect relevant but as yet unidentified regulatory substrates involved in the modulation of growth and body composition.

Do Epigenetic Mechanisms Regulate Growth?

Epigenetic modifications might be the missing link between the metabolic environment and alterations in gene expression inducing persistent later effects. Epigenetics is the study of heritable changes in gene expression not caused by changes in the DNA sequence, but by biochemical modifications of DNA that determine whether or not genes are expressed. Epigenetic mechanisms control

modifications in chromatin, regulate its accessibility to transcription factors and thus contribute to determine the level of expression of different genes. Mechanisms of epigenetic modification include the addition of methyl groups to DNA cytosine bases [50], the addition of methyl and acetyl groups to proteins (histones) around which DNA is folded [51] and interfering microRNA [52, 53]. Recently, interest in epigenetic research in relation to disease, development and aging has increased [9, 54, 55]. The available evidence for epigenetic effects of nutrition in animal models and first human studies are based on alterations in DNA methylation, where powerful analytical methodology for human genome-wide analysis has now become available.

Addition of a methyl group to the 5′ carbon of a cytosine base in the context of CpG is the most frequent and stable form of epigenetic modification that does not affect primary DNA sequence, but affects secondary interactions, which play a critical role in the regulation of gene expression. Normally, genes are expressed when transcriptions factors bind to DNA and activate the gene. DNA methylation prevents transcription factor

binding while it favours the binding of transcription-inhibiting proteins and is therefore mostly associated with switching genes 'off'. The degree of activation of a given gene generally depends upon its degree of methylation. Accumulating evidence from animal studies and from some first albeit limited studies in humans point to the possibility that differences in DNA methylation patterns, and potentially other epigenetic processes, are the 'missing link' in the 'Early Origins of Later Disease Hypothesis'.

It has been shown that differentially methylated regions (DMR) are often the result of perturbations of the environment in sensitive or critical early periods of life (periconceptional and postnatal periods, and puberty), which can lead to alternative pathways of cell and organ development ('developmental plasticity') [9, 56, 57], and that can enhance the susceptibility of later diseases (e.g. obesity, diabetes or cancer). Methylation is principally reversible [58], e.g. by nutritional intervention. For example, folate or genistein supplementation can counteract bisphenol-A-induced DNA hypomethylation and the change in the coat colour phenotype in mice [59]. Such nutritional reprogramming of genetic and metabolic expression may even be induced transgenerationally, e.g. by paternal diet in combination with a low-protein diet of the offspring mice [60]. At this time, it is not known how stable nutrient-induced alterations are over time, i.e. whether and to which extent they show alterations in humans followed up over several years. Thus, exploring epigenetic markers over time may be a very important path for exploring future strategies of individual disease prevention and perhaps even treatment.

Nutritional effects on DMR so far have been mostly studied in animal models with a focus on folate supply and on caloric restrictions as determinants, but only to a very limited extent in humans [61, 62]. A relation to adiposity was recently reported in a single study that assessed methylation status of 68 CpG sites from five candidate genes in umbilical cord tissue DNA from healthy neonates using Sequenom MassARRAY, which explored maternal pregnancy diet and child's adiposity at age 9 years in two cohorts [63]. In both cohorts, higher retinoid X receptor-α chr9:136355885+ methylation was associated with childhood fat mass, and in one cohort also with lower maternal carbohydrate intake in early pregnancy. Regression analyses including sex and neonatal epigenetic markers explained >25% of the variance in childhood adiposity. Thus, programming effects of early nutrition and metabolic exposure may be mediated through altered DMR, which thus needs to be explored in greater detail and with the much more powerful genome-wide methods that are now available.

There is only limited information on the effects of protein intake on DMR from animal studies. A recent review on epigenetic programming of diabetes and obesity [64] identified 14 animal studies from 1999 to 2011 focussing on protein exposure during the fetal or neonatal period, and the impact on the obesity and diabetes risk mediated by epigenetic modifications. Of importance, the dietary interventions used in these animal studies generally tend to be extreme and are hardly comparable to the types of exposures observed in contemporary human populations. Several animal studies in rat and mouse models showed that the effects of very low maternal protein intake in pregnancy on later obesity of the offspring in puberty or adulthood, or even trans-generationally, are mediated by hypo- or hypermethylation mostly in promoter regions of different genes (e.g. PPARα, IGF2/H19 locus, Leptin or PEPCK) or gene receptors (GR, LXR or IR).

So far, there are only very few genome-wide DNA methylation studies in relation to protein intake in animals [65], while there is accumulating evidence that protein intake during pregnancy and in the postnatal period affects the methylation status of various genes and may indeed be a diet-induced mediating factor for later obesity [60, 66–76]. However, so far, there is not a single study in humans on protein intake and DNA methylation, nor are there any studies on the mediating effects of DMR regarding protein intake and growth or body composition.

Limited existing studies explored mediating effects of DNA methylation on rapid growth and obesity in adolescence [77]. In three studies, mediating effects of maternal folate intake in early pregnancy on birth weight were explored [78, 79]. Fryer et al. [78] performed a genome-wide methylation study. A further genome-wide study analysed the effect of the FTO gene on obesity mediated by DMR [80]. Some epigenetic studies analysed mediating effects of DMR in placental tissue regarding intrauterine growth restriction and fetal growth [81].

Conclusions

The description and analysis of growth patterns and their regulation through diet, and the potentially underlying metabolic and epigenetic mechanisms are of major relevance for public health and policy, and have the po-

tential to contribute greatly to promoting health and well-being in the population [82]. Hence, it is important to address these questions in large prospective cohort studies with detailed phenotyping of growth, markers of body composition as well as available biosamples that allow assessment of markers with state-of-the-art methodology. Groundbreaking research with the use of unconventional, new and sophisticated methodology is needed that goes significantly beyond the state of the art. Research collaboration of academic investigators and researchers from industry in this field has the potential to increase outputs and success [83]. If successful, the results of such research should provide answers to key questions on the regulation of growth, with major benefit for scientific understanding, opportunities for future research, promotion of public health, nutrition recommendations and development of improved food products.

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Disclosure Statement

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