Abstract
There is growing evidence of long-term effects of early dietary intervention in infancy on later obesity risk. Many studies showed reduced risk of obesity with breastfeeding in infancy, which could be related to the reduced protein intake with human milk compared to infant formula. In a randomized controlled trial (Childhood Obesity Project), we were able to show that infant formula with reduced protein content results in lower BMI both at 2 and 6 years. These effects seem to be mediated mainly by branched-chain amino acids which stimulate the insulin-like growth factor (IGF)-1 axis and insulin release. In this trial, we also showed an influence of high-protein diet on larger kidney size, which seems to be partly explained by a significant effect of free IGF-1 on kidney volume. The IGF-1 axis was shown to regulate early growth, adipose tissue differentiation and early adipogenesis in animals and in humans. Leptin and adiponectin can also be regarded as important endocrine regulators of obesity. These markers were tested in observational studies. Leptin seems to be closely correlated with BMI but changes in adiponectin require further exploration. Still, there is a lack of good data or some results are contradictory to indicate the role of either leptin or adiponectin in infancy for determining later obesity risk.

© 2016 Nestec Ltd., Vevey/S. Karger AG, Basel
Introduction

There is an increasing body of evidence that prenatal or early postnatal external factors, such as nutrition, modulate metabolic and epigenetic regulation of body development and function [1]. Many observational studies linked early nutrition with late health outcomes, but the processes by which disease risk is regulated remain to be elucidated. Part of this programming effect seems to be regulated by selected nutrients and hormones (fig. 1). Different organ systems have their own critical periods of development. Thus, short- and long-term consequences may differ depending on the time point of nutritional intervention and organ-specific responses [2]. Numerous studies associated breastfeeding with a decreased risk of obesity and other disorders in later life, as compared to infant formula feeding (FOF) [3, 4]. Several factors might be involved in such early programming effects of later obesity risk. We proposed high protein intake with FOF as a major causal factor [5]. Our hypothesis was built on earlier observations that dietary protein intake modulates blood concentrations of insulin-like growth factor (IGF)-1. The IGF-1 axis was shown to regulate early growth, adipose tissue differentiation and early adipogenesis in animals and in humans. The most sensitive window for programming effects is still uncertain and has been proposed to last from the first weeks of life [6] up to the first 2 years, since weight gain both in infancy and in the 2nd year of life have been demonstrated to impact on later obesity [1, 7]. We tested this hypothesis in the European Childhood Obesity Project (CHOP; see Appendix) trial, in which infants were randomized double blind to conventional FOF with a relatively high protein supply during the 1st year of life or to a

Fig. 1. Metabolic programming: process linking early nutrition with later health outcomes.
protein-reduced intervention formula with equal content of energy achieved by adjustment of total fat content and similar content of other nutrients. Feeding conventional formula in the 1st year induced faster weight gain and higher BMI up to 2 years without any difference in length growth compared to infants fed a reduced protein formula or breast-fed infants [8]. Data from the follow-up at 6 years of age confirmed the programming effect of nutritional intervention in the 1st year of life [9]. We investigated metabolic and endocrine mechanisms of obesity programming within CHOP and collected blood and urine samples in the participants of this study at the age of 6 months [10]. Several endocrine markers were studied to explore effects of early diet on later obesity risk, including insulin, IGF-1, leptin and adiponectin. These mediators are of particular interest as they have been previously proposed to be related to obesity and metabolic syndrome [1]. Our hypothesis was built on earlier observations that diet and mainly protein intake modulate blood concentrations of IGF-1 [11]. The IGF-1 axis was shown to regulate early growth, adipose tissue differentiation and early adipogenesis in animals and humans [12–14]. IGF-1 has a strong structural homology to insulin, which is also reflected in the binding motifs of the IGF-binding proteins (IGFBPs). Amino acids, and in particular the branched-chained amino acids, were proven to stimulate insulin secretion [15].

In the CHOP study, we found consistent changes in biochemical and endocrine markers which fit the hypothesis of metabolic programming of obesity: increased plasma levels of nonessential amino acids, especially branched-chained amino acids, in infants fed the high-protein diet, which were accompanied by increased concentrations of total and free IGF-1, increased urinary C-peptide levels (reflecting increased insulin secretion) and lowered serum glucose levels [9]. Compared to formula-fed children, breastfed children had generally lower plasma amino-acid levels, a less active IGF-1 axis and lower insulin production (fig. 2) [9].

Many hormones are differently secreted by males and females, which is usually the case first during puberty but can be also observed in early life. Sexual dimorphism has been observed in many physiologic situations. We analyzed the IGF-1 axis response to high-protein feeding in regard to sex within the CHOP study. Total and free IGF-1 and IGFBP-3 concentrations were higher in girls than in boys. We observed a similar effect of the high-protein formula on the IGF-1 axis; still, the effects tended to be stronger in girls than in boys. The leptin concentration was higher in girls than in boys and was correlated to the IGF-1 axis parameters. We concluded that the endocrine response to a high-protein diet early in life may be modulated by gender [16].
Within the CHOP trial, we also showed an influence of high-protein diet on larger kidney size which seems to be partly explained by a significant effect of free IGF-1 on kidney volume [17]. Thus, IGF-1 is involved in protein-induced kidney growth in healthy infants [18]. We analyzed also genetic regulation of IGF-1 secretion and were able to show that there is predominant nutritional regulation of the IGF-1 axis compared to the small influence of single nucleotide polymorphisms [19].

Insulin and IGF-1 were also investigated in other studies. Renault et al. [20] studied the EDEN mother-child cohort (n = 342 subsample) from pregnancy to 1 year of age. Maternal glycemia was correlated with birth weight of their children, and this relationship seemed to be mediated by fetal insulin and fetal
IGF-1. Moreover, high fetal insulin correlated inversely with growth during the 1st year of life in girls, which could be explained by partial insulin resistance in girls.

Leptin was also investigated in infants whose obesity was assessed in later life. Savino et al. [21] investigated 237 healthy term infants in whom leptin levels were determined at 8 months of age and followed them up to the age of 8.8 years when BMI was measured. In this cohort, breastfed infants had significantly higher serum leptin levels than formula-fed ones. Children who were formula fed in infancy had a significantly higher BMI at the follow-up. Interestingly, the authors found 2.7 ng/ml leptin as cutoff value (median serum leptin level in breastfed infants) below which infants had a higher BMI in childhood, and they concluded that a higher leptin level in infancy may be inversely associated with BMI in childhood. As this is an observational study, it is difficult to infer causality. Moreover, the contribution of leptin in breast milk to serum levels was not addressed in this study. Volber et al. [22] described leptin and adiponectin trajectories from birth to 9 years of age and concluded that there are developmental differences in leptin and adiponectin throughout childhood. Adiponectin showed weak correlations between birth and later values, with closer correlations observed between later ages. Similar results were obtained for leptin (significant correlations for children aged 2 and 5; 2 and 9, and 5 and 9 years). The authors identified several potential risk factors for altered childhood adipokine levels, including increased maternal sugar-sweetened beverage consumption during pregnancy and increased child birth weight. Leptin was closely and positively correlated with BMI, but changes in adiponectin require further exploration as the correlations were weaker. The positive correlation of BMI with leptin is not in line with what was described by Savino et al. [21] but the influence of other factors like breastfeeding or different time points of sampling can be considered. Interestingly, maternal leptin during pregnancy is also significantly related to infant birth weight. This effect was shown by Misra et al. [23] only in overweight and obese women, in whom an increase in the rate of change in maternal serum leptin in the second half of pregnancy was significantly associated with a decrease in infant birth weight, adjusted for gestational age at delivery. The authors found this effect to be distinct from that of maternal body weight. Further interesting observations come from the EPOCH study, which examined the association between cord blood leptin levels and BMI growth velocity from birth to 12 months of age among infants exposed and not exposed to overnutrition in utero. The authors found a negative correlation between cord blood leptin levels and the rate of change in BMI during the 1st year of life. They tried to explain this relationship by a feedback mechanism during the early postnatal period, with lower baseline leptin levels promoting increased BMI growth velocity [24].
Interesting data were obtained from a randomized trial in small-for-gestational-age (SGA) infants, where a similar concept as in the CHOP trial was tested. The control population was a group of breastfed infants born appropriate for gestational age. SGA infants were breastfed or received FOF. FOF infants were randomized to receive either a standard (FOF1) or protein-rich formula (FOF2). Endocrine markers were assessed at birth and 4 months of age. At 4 months, circulating high-molecular-weight (HMW) adiponectin and IGF-1 seemed to be influenced by nutrition but not the gestational age at birth: the circulating levels of HMW adiponectin and IGF-1 in SGA breastfed infants were comparable with those in breastfed controls who were appropriate for gestational age, but they were elevated in SGA-FOF infants. Moreover, HMW adiponectin levels were higher in SGA-FOF1 than in SGA-FOF2 infants, whereas IGF-1 levels were higher in SGA-FOF2 than in SGA-FOF1 infants. Therefore, it seems that a high-protein diet has a different effect on adiponectin and IGF-1 – it decreased HMW adiponectin and increased IGF-1 levels [25]. The results in SGA infants concerning IGF-1 correspond well with the results of the CHOP study.

Currently, it is difficult to interpret results related to leptin and adiponectin as they come mainly from observational studies. The role of these hormones in predicting obesity is not clear. Breastfeeding effects may be mediated by leptin, but this could not be proven directly. Leptin seems to be closely correlated with BMI at different ages, but there are conflicting data in infancy, while such a relationship has not been shown for adiponectin. Whether leptin and adiponectin responses are mediated by specific nutrients in infancy is unclear and requires further studies.

We conclude that evidence from clinical trials firmly demonstrates strong long-term effects of early diet in infancy on later obesity risk. These effects are related to altered plasma concentrations of nonessential amino acids, and secretion of IGF-1 and insulin. Better understanding of the underlying mechanisms of early-life nutritional programming on later health will open new opportunities for prevention.

Appendix

The European CHOP Study Group
Philippe Goyens, Clotilde Carlier, Pascale Poncelet, Elena Dain and Joana Hoyos (Free University of Brussels, Brussels, Belgium); Françoise Martin, Annick Xhonneux, Jean-Paul Langhendries and Jean-Noel Van Hees (Centre Hospitalier Chrétien St Vincent, Liège, Belgium); Ricardo Closa-Monasterolo, Joaquin Escribano, Veronica Luque, Georgina Mendez, Natalia Ferre and Marta Zaragoza-Jordana (Universitat Rovira I Virgili,
Tarragona, Spain); Marcello Giovannini, Enrica Riva, Carlo Agostoni, Silvia Scaglioni, Elvira Verduci, Fiammetta Vecchi and Alice Re Dionigi (University of Milan, Milan, Italy); Jerzy Socha, Anna Dobrzańska, Dariusz Gruszfeld, Piotr Socha, Anna Stolarczyk, Agnieszka Kowalik, Roman Janas and Ewa Pietraszek (Children’s Memorial Health Institute, Warsaw, Poland); Emmanuel Perrin (Danone Research Center for Specialized Nutrition, Schiphol, The Netherlands); Helfried Groebe, Anna Reith, and Renate Hofmann (Klinikum Nurnberg Sued, Nurnberg, Germany); Berthold Koletzko, Veit Grote, Martina Weber, Sonia Schiess, Jeannette Beyer, Michaela Fritsch, Uschi Handel, Ingrid Pawlek, Sabine Verwied-Jorky, Iris Hannibal, Hans Demmelmaier, Gudrun Haile, Wolfgang Peissner, Ulrike Harder, Franca F. Kirchberg, Melissa Theurich, Peter Rzehak, Christian Hellmuth and Olaf Uhl (Dr. von Hauner Children’s Hospital, Ludwig Maximilian University of Munich, Munich Germany), and Rüdiger von Kries (Institute for Social Pediatrics and Adolescent Medicine, University of Munich, Munich, Germany).

Acknowledgments

Work reported herein is carried out with partial financial support from the Commission of the European Communities, the 7th Framework Program, contract FP7-289346-EarlyNutrition and the European Research Council Advanced Grant ERC-2012-AdG – No. 322605 META-GROWTH. This paper does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area.

Disclosure Statement

For this specific topic the authors do not have any conflict of interest.

References

Socha et al.


