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Developmental Origins of Health and Disease: Exposures, Outcome, Mechanisms and Interventions

Marek Zygmunt, Hans Georg Bender and Wolfgang Künzel (Eds.)

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Developmental Origins of Health and Disease: Exposures, Outcome, Mechanisms and Interventions

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With 4 Figures and 6 Tables

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Preface

Marek Zygmunt (Greifswald), Hans Georg Bender ML (Düsseldorf) and
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The "Developmental Origins of Health and Disease" (DOHaD) hypothesis has evolved from innovative epidemiological studies on infant and adult mortality. One of the pioneers of DOHaD research was David Barker whose work revealed high geographic correlation between rates of infant mortality and certain causes of death as well as an association between birth weights and cardiovascular diseases in certain areas. Indeed, he was able to show that in cases of lower birth weight individuals tended to have a higher risk for ischemic heart failure in adulthood. These studies certainly point to the high relevance of perinatal and prenatal cues on postnatal development. As epidemiological and experimental research moved on, new data emerged. Fetal programming, that permanently shapes structure, function and metabolism of the body, has been linked to a number of adult disorders including ischemic heart disease, stroke, diabetes, hypertension and mental disorders. The concept that different environmental cues including diet, exercise or stress during pregnancy, and fetal adoptive response to those, built up the framework of DOHaD theory. Furthermore, mismatch between pre- and postnatal environments has been postulated to play a key role in those processes. The role of placenta and its function as a regulator of fetal development has been also recognized. At the same time the emerging science of epigenetics provides evidence on possible molecular mechanisms involved in the phenotypic plasticity proposed in the DOHaD approach.

In the "Leopoldina-Symposium" held in Greifswald, Germany, on September 4th and 5th, 2009 experts from different fields of medical sciences including epidemiologists, molecular biologists and behavioural biologists presented and discussed a wide range of current developments in the field of DOHaD. In addition, this symposium provided an excellent opportunity to define critical issues in the field of reproductive sciences.

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Introduction

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Population-Based Studies are Necessary to Study the Effect of Intrauterine Life

Wolfgang Hoffmann and Jochen René Thyrian (Greifswald)

With 1 Figure

Abstract

Epidemiological research is important to describe and explain the developmental origins of health and disease, including genetics, environment, behaviour and time. Systematic evidence underlines the importance and the interdependence of the determinants. However, the key challenge for public health is prevention. To achieve this goal the research agenda must identify (*i*) causal mechanisms, (*ii*) supporting or hindering factors of the environment and the individual, (*iii*) vulnerable/ sensitive phases of the individual and (*iv*) trajectories over time. In our view prospective population based cohort and intervention studies like the SNiP-study provide the most promising way to meet this complex challenge.

Zusammenfassung

Die epidemiologische Forschung ist von großer Bedeutung für die Beschreibung und Analyse der Entstehung von Gesundheit und Krankheit in der kindlichen Entwicklung, einschließlich Genetik, Umwelt, Verhalten und Zeit. Die Herausforderung für das Gesundheitssystem ist aber letztlich die Prävention. Voraussetzung dafür ist die Erforschung kausaler Mechanismen, fördernder Bedingungen und Barrieren des Individuums als auch der Umwelt sowie von vulnerablen Phasen und Entwicklungspfaden über die Zeit. Prospektive, populationsbasierte Kohortenstudien, wie z. B. die SNiP-Studie, bieten dazu einen viel versprechenden Ansatz.

Epidemiological research is playing an important role in describing, analysing and ultimately understanding the life-long effects of intrauterine life. While G. Dörner had shown the effect of environmental changes during pregnancy on outcomes later in life based on experimental research with animals (Dörner 1976), it was Barker who first introduced empirical data on humans (Barker and Osmond 1986). He proposed the fetal origins of adult disease hypothesis, which is sometimes referred to as "Barker's hypothesis", by stating that, "coronary heart disease, type 2 diabetes, stroke and hypertension originate(s) in developmental plasticity, in response to undernutrition during foetal life and infancy" (Barker 2004). He started out with analysing the association between mortality from selected diseases with infant mortality and childhood nutrition data in England (Barker et al. 1986). Initially this work was regarded as heretical which, however, only caused him to reinforce his research efforts. By personal involvement and down to earth epidemiological fieldwork, including copying handwritten documents and spending countless hours in registries, he generated a database for empirical research on prenatal risk factors for later disease (Barker et al. 1989). This inspired a considerable array of epidemiological studies which provided convincing empirical evidence that turned many critics to supporters (Gillman and Rich-Edwards 2000).

Classical empirical evidence stems from the Dutch famine during WWII (Ravelli et al. 1976) after which consistent associations between low birth weight and higher incidence of hy-

pertension and higher prevalence of adult-onset diabetes were observed. A higher birth weight is associated with decreased risk of coronary heart diseases and stroke (Morley 2006). Size at birth is considered a marker for fetal nutrition (Barker 2004), and birth weight is considered a summary measure of fetal growth. Determinants include genetic growth potential, gestation length, maternal size, maternal factors (metabolic and nutritional status, utero-placental perfusion and placental function) (Morley 2006). Recently, associations between exposure to maternal smoking and preterm delivery or lower birth weight (Thyrian et al. 2005), between the metabolic syndrome and preterm delivery (Chatzi et al. 2009), or maternal-fetal nutrition and intrauterine growth restriction were established (Sankaran and Kyle 2009).

In general, the determinants of the development of diseases in later adulthood under research are: genetics, the environment, and individual factors like behaviour and time. There is systematic evidence for the importance of all of them, and Figure 1 illustrates their complex relation and influences on disease over the course of life.

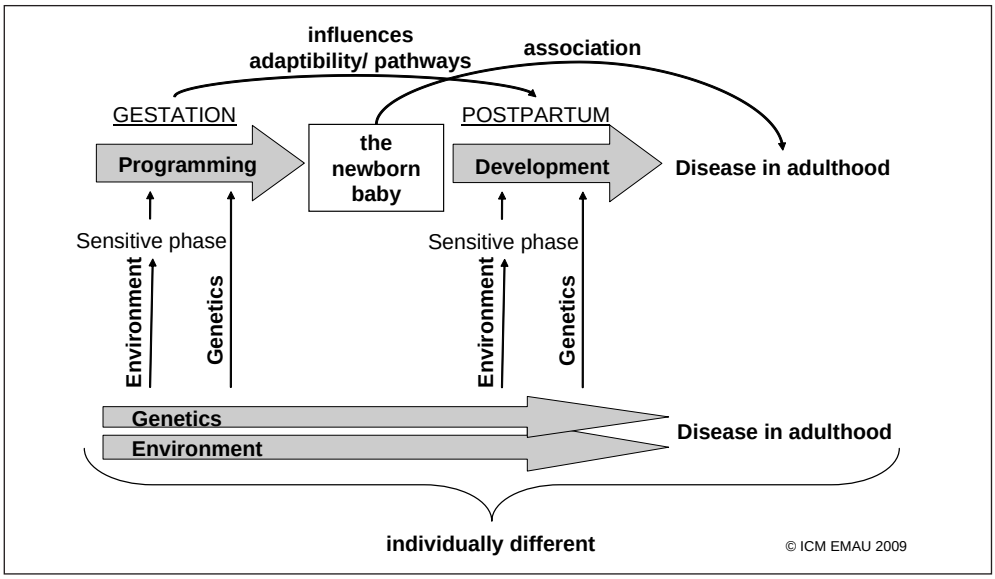

Fig. 1 The interaction of genetics, the environment, the individual and time on the development of diseases

The current model has been explained as, "when foetal environment is poor, there is an adaptive response, which optimizes the growth of the key organs to the detriment of others and leads to an altered postnatal metabolism, which is designed to enhance postnatal survival of intermittent or poor nutrition" (Hales and Barker 2001). Genetic epidemiology has shown associations between genes and obesity, asthma, allergies, blood pressure, bone mineralization, anxiety, depression etc. (Frayling et al. 2007, Golding et al. 2009), and there is extensive experimental support from animal research for the epidemiological observations in humans (Morley 2006).

The impact of the environment, however, does not stop at the time of birth. The WHO estimates the burden of disease from preventable, environmental exposures at a minimum of

13 million deaths per year (Pruss-Ustun et al. 2008). Environmental factors include environmental tobacco smoke (ETS) (Cook and Strachan 1999), contaminants in water and food (Suk et al. 2003), but also poverty, inadequate education, poor housing, and physical as well as mental health (Evans 2004). There are gene–environment interactions conditional on the presence of particular exposures (Eder et al. 2004) as well as specific genes (Choudhry et al. 2005).

These effects are not static over time since the susceptibility to an environmental factor may vary at sensitive phases during a child's development. Children are especially sensitive at different stages of gestation (Silbergeld and Patrick 2005), during infancy and in early childhood (Makri et al. 2004). ETS exposure before and after birth differs and Epstein-Barr virus infections differ with age at the time of infection (Macsween and Crawford 2003).

Epidemiologic research has provided interesting clues to possible mechanisms. There is evidence that the association between low birth weight and the increased incidence of hypertension is mediated by social class. Being born in the working class or lower middle class emphasizes this association (Barker 2004). The association between birth weight and type 2 diabetes later in life is mediated by the adiposity rebound (AR). The AR indicates the point in time when the normal decrease in body mass index (BMI) in early childhood turns into an increase (Rolland-Cachera et al. 1984). The earlier the AR in children with low birth weight, the higher the chance to be diagnosed with type 2 diabetes later in life (Barker 2004).

These results have been summarised into principles (Nathanielsz 2006) that can guide future research:

- Critical periods of vulnerability to suboptimal conditions occur at different times for different systems → research needs to address different time periods, the complexity requires assessment of more different variables.
- The placenta plays a key role → asservation of bio-materials is mandatory.
- Compensation carries a price. In suboptimal conditions the developing baby attempts to compensate, which might cause problems later on → effects may show up a considerable time distance from their origins requiring prospective cohort designs.
- Programming may cross generations → intergenerational, longitudinal, and epidemiologic research is needed.
- Males and females may be affected differently → research into fetal programming must be gender sensitive.

Various research methods have been applied, but all show serious limitations. Twin studies and adoption studies are restricted in their generalisability (Morley 2005). Twins show a general constraint of fetal growth, the biology of fetal growth is different, on average the gestation is shorter etc. Cross-sectional studies have yielded many associations, but do not allow individual or even causal pathways. Retrospective research is limited by hindsight bias and memory effects causing over- or underestimation of various associations. Studies using birth cohorts can control for many of these problems, but they may have a selection bias, for example with respect to socio-economic status (Kramer et al. 2009) or race/ethnicity (Savitz et al. 2005). Participants recruited in hospital settings were more likely to be black, younger, less educated, and unmarried compared to residents in the surrounding area (Savitz et al. 2005). Lower neighbourhood income was associated with preterm birth in the population, but not in the study cohort (Kramer et al. 2009). Hence the methodological 'gold standard' to control for most of these problems are population-based prospective cohort studies.

The Danish National Birth Cohort comprises 101,042 pregnant women between 1996 and 2002 (Olsen et al. 2001), and the Norwegian Mother and Child Cohort Study (MOBA) recruited 110,000 pregnant women from 1999 to 2008 (Magnus et al. 2006). More recently the USA National Children's Study has stated that its recruitment goal is 100,000 parents and children with prospective follow-up from birth to age 21 (Knox and Echeveria 2009). These cohorts include child, mother and father and collect biospecimen. They allow the analysis of complex associations. However, they are expensive (US > $400 million), face considerable loss due to follow-up and are restricted to an a priori set of hypotheses that may not cover aspects that are relevant in other regions or future time periods.

Some regional studies control for some of these problems. The Avon Longitudinal Study of Parents and Children (ALSPAC) was established in the 1990s with 14,541 pregnant women covering 85 % of the population in the study area (Golding 2004). The Generation R Study in Rotterdam started in 2002–2006 with a 61 % participation rate of 3,787 pregnant women (Hofman et al. 2004).

Just recently a prospective birth cohort was initiated with the Survey of Neonates in Pomerania (SNiP) by the University of Greifswald, which has so far enrolled 6,747 child-bearing women between 2003 and 2008 (Ebner et al. 2010). The population coverage was 95 % with 75 % of the participants providing informed consent to participate in a detailed assessment (Ebner et al. 2010), including maternal records of the pregnancy, hospital records of mother and child, face-to-face interviews, questionnaires and biological samples from the blood cord, the placenta and maternal mouth swabs. It has been shown that the SNiP region could serve as model region for future research (Thyrian et al. 2010).

The key challenge for future research into fetal programming is prevention. Based on the determinants illustrated in Figure 1 options are (*i*) to interfere on the genetic level, which is currently not – and probably never will be – feasible, (*ii*) to address maternal behaviour, which has been successful for example in supplemental food programs (Rush et al. 1988), (*iii*) to change the fetal environment, e.g. banning of smoking in public places or supporting folate supplements in pregnancy (Badovinac et al. 2007), and (*iv*) intervene into developmental pathways postpartum by behavioural and environmental interventions (Hannöver et al. 2009). Research priorities for evidence based prevention include: identification of (*i*) causal mechanisms, (*ii*) risk or protective factors of the environment and the individual, (*iii*) vulnerable/ sensitive phases of the individual, and (*iv*) trajectories over time (in individuals as well as the environment). Results need to be translated into well designed preventive interventions with population impact (Thyrian and John 2007). In our view the most promising way to meet the challenge is to conduct prospective population based cohort and intervention studies.

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References

Badovinac, R. L., Werler, M. M., Williams, P. L., Kelsey, K. T., and Hayes, C.: Folic acid-containing supplement consumption during pregnancy and risk for oral clefts: a meta-analysis. Birth Defects Res. A Clin. Mol. Teratol. *79*, 8–15 (2007)

Barker, D. J.: The developmental origins of adult disease. J. Amer. Coll. Nutr. *23*, 588S–595S (2004)

Barker, D. J., and Osmond, C.: Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet *1*, 1077–1081 (1986)

Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., and Simmonds, S. J.: Weight in infancy and death from ischaemic heart disease. Lancet *2*, 577–580 (1989)

Chatzi, L., Plana, E., Daraki, V., Karakosta, P., Alegkakis, D., Tsatsanis, C., Kafatos, A., Koutis, A., and Kogevinas, M.: Metabolic syndrome in early pregnancy and risk of preterm birth. Amer. J. Epidemiol. *170*, 829–836 (2009)

Choudhry, S., Avila, P. C., Nazario, S., Ung, N., Kho, J., Rodriguez-Santana, J. R., Casal, J., Tsai, H. J., Torres, A., Ziv, E., Toscano, M., Sylvia, J. S., Alioto, M., Salazar, M., Gomez, I., Fagan, J. K., Salas, J., Lilly, C., Matallana, H., Castro, R. A., Selman, M., Weiss, S. T., Ford, J. G., Drazen, J. M., Rodriguez-Cintron, W., Chapela, R., Silverman, E. K., and Burchard, E. G.: CD14 tobacco gene-environment interaction modifies asthma severity and immunoglobulin E levels in Latinos with asthma. Amer. J. Respir. Crit. Care Med. *172*, 173–182 (2005)

Cook, D. G., and Strachan, D. P.: Health effects of passive smoking-10: Summary of effects of parental smoking on the respiratory health of children and implications for research. Thorax *54*, 357–366 (1999)

Dörner, G.: Hormones and Brain Differentiation. Amsterdam, Oxford, New York: Elsevier 1976

Ebner, A., Thyrian, J. R., Lange, A., Lingnau, M. L., Scheler-Hofmann, M., Rosskopf, D., Zygmunt, M., Haas, J. P., Hoffmann, W., and Fusch, C.: Survey of Neonates in Pomerania (SNiP): a population-based birth study – objectives, design and population coverage. Paediatr. Perinat. Epidemiol. *24*/2, 190–199 (2010), doi: 10.1111/j.1365-3016.2009.01078.x. (2009)

Eder, W., Klimecki, W., Yu, L., Mutius, E. von, Riedler, J., Braun-Fahrlander, C., Nowak, D., Martinez, F. D., and *ALEX Study Team*: Toll-like receptor 2 as a major gene for asthma in children of European farmers. J. Allergy Clin. Immunol. *113*, 482–488 (2004)

Evans, G. W.: The environment of childhood poverty. Amer. Psychol. *59*, 77–92 (2004)

Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., Perry, J. R., Elliott, K. S., Lango, H., Rayner, N. W., Shields, B., Harries, L. W., Barrett, J. C., Ellard, S., Groves, C. J., Knight, B., Patch, A. M., Ness, A. R., Ebrahim, S., Lawlor, D. A., Ring, S. M., Ben-Shlomo, Y., Jarvelin, M. R., Sovio, U., Bennett, A. J., Melzer, D., Ferrucci, L., Loos, R. J., Barroso, I., Wareham, N. J., Karpe, F., Owen, K. R., Cardon, L. R., Walker, M., Hitman, G. A., Palmer, C. N., Doney, A. S., Morris, A. D., Smith, G. D., Hattersley, A. T., and McCarthy, M. I.: A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science *316*, 889–894 (2007)

Gillman, M. W., and Rich-Edwards, J. W.: The fetal origin of adult disease: from sceptic to convert. Paediatr. Perinat. Epidemiol. *14*, 192–193 (2000)

Golding, J.: The Avon Longitudinal Study of Parents and Children (ALSPAC) – study design and collaborative opportunities. Eur. J. Endocrinol. *151* Suppl 3, U119–U123 (2004)

Golding, J., Jones, R., Brune, M. N., and Pronczuk, J.: Why carry out a longitudinal birth survey? Paediatr. Perinat. Epidemiol. *23* Suppl 1, 1–14 (2009)

Hales, C. N., and Barker, D. J.: The thrifty phenotype hypothesis. Br. Med. Bull. *60*, 5–20 (2001)

Hannöver, W., Thyrian, J. R., Röske, K., Grempler, J., Rumpf, H. J., John, U., and Hapke, U.: Smoking cessation and relapse prevention for postpartum women: results from a randomized controlled trial at 6, 12, 18 and 24 months. Addict. Behav. *34*, 1–8 (2009)

Hofman, A., Jaddoe, V. W., Mackenbach, J. P., Moll, H. A., Snijders, R. F., Steegers, E. A., Verhulst, F. C., Witteman, J. C., and Büller, H. A.: Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr. Perinat. Epidemiol. *18*, 61–72 (2004)

Knox, S. S., and Echeveria, D.: Methodological issues related to longitudinal epidemiological assessment of developmental trajectories in children. J. Epidemiol. Community Health *63* Suppl. 1, i1–i3 (2009)

Kramer, M. S., Wilkins, R., Goulet, L., Seguin, L., Lydon, J., Kahn, S. R., McNamara, H., Dassa, C., Dahhou, M., Masse, A., Miner, L., Asselin, G., Gauthier, H., Ghanem, A., Benjamin, A., Platt, R. W., and *Montreal Prematurity Study Group*: Investigating socio-economic disparities in preterm birth: evidence for selective study participation and selection bias. Paediatr. Perinat. Epidemiol. *23*, 301–309 (2009)

Macsween, K. F., and Crawford, D. H.: Epstein-Barr virus-recent advances. Lancet. Infect. Dis. *3*, 131–140 (2003)

Magnus, P., Irgens, L. M., Haug, K., Nystad, W., Skjaerven, R., and Stoltenberg, C.: Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. *35*, 1146–1150 (2006)

Makri, A., Goveia, M., Balbus, J., and Parkin, R.: Children's susceptibility to chemicals: a review by developmental stage. J. Toxicol. Environ. Health B Crit. Rev. *7*, 417–435 (2004)

Morley, R.: Can we generalise from findings in twins? Paediatr. Perinat. Epidemiol. *19*, Suppl 1, 54–59 (2005)

Morley, R.: Fetal origins of adult disease. Semin. Fetal Neonatal. Med. *11*, 73–78 (2006)

Nathanielsz, P. W.: Animal models that elucidate basic principles of the developmental origins of adult diseases. ILAR. J. *47*, 73–82 (2006)

Olsen, J., Melbye, M., Olsen, S. F., Sorensen, T. I., Aaby, P., Andersen, A. M., Taxbøl, D., Hansen, K. D., Juhl, M., Schow, T. B., Sørensen, H. T., Andresen, J., Mortensen, E. L., Olesen, A. W., and Søndergaard, C.: The Danish National Birth Cohort – its background, structure and aim. Scand. J. Public Health *29*, 300–307 (2001)

Pruss-Ustun, A., Bonjour, S., and Corvalan, C.: The impact of the environment on health by country: a meta-synthesis. Environ. Health *7*, 7 (2008)

Ravelli, G. P., Stein, Z. A., and Susser, M. W.: Obesity in young men after famine exposure in utero and early infancy. New Engl. J. Med. *295*, 349–353 (1976)

Rolland-Cachera, M. F., Deheeger, M., Bellisle, F., Sempe, M., Guilloud-Bataille, M., and Patois, E.: Adiposity rebound in children: a simple indicator for predicting obesity. Amer. J. Clin. Nutr. *39*, 129–135 (1984)

Rush, D., Leighton, J., Sloan, N. L., Alvir, J. M., Horvitz, D. G., Seaver, W. B., Garbowski, G. C., Johnson, S. S., Kulka, R. A., Devore, J. W., et al.: The National WIC Evaluation: evaluation of the Special Supplemental Food Program for Women, Infants, and Children. VI. Study of infants and children. Amer. J. Clin. Nutr. *48*, 484–511 (1988)

Sankaran, S., and Kyle, P. M.: Aetiology and Pathogenesis of IUGR. Best Pract. Res. Clin. Obstet. Gynaecol. (2009)

Savitz, D. A., Dole, N., Kaczor, D., Herring, A. H., Siega-Riz, A. M., Kaufman, J., and Thorp, J. M. Jr.: Probability samples of area births versus clinic populations for reproductive epidemiology studies. Paediatr. Perinat. Epidemiol. *19*, 315–322 (2005)

Silbergeld, E. K., and Patrick, T. E.: Environmental exposures, toxicologic mechanisms, and adverse pregnancy outcomes. Amer. J. Obstet. Gynecol. *192*, S11–S21 (2005)

Suk, W. A., Murray, K., and Avakian, M. D.: Environmental hazards to children's health in the modern world. Mutat. Res. *544*, 235–242 (2003)

Thyrian, J. R., Hannöver, W., Röske, K., John, U., and Hapke, U.: [Smoking before, during and after pregnancy: longitudinal data from a population based sample]. Geburtshilfe und Frauenheilkunde *65*, 687–689 (2005)

Thyrian, J. R., and John, U.: Population impact – definition, calculation and its use in prevention science in the example of tobacco smoking reduction. Health Policy *82*, 348–356 (2007)

Thyrian, J. R., Lange, A., Lingnau, M. L., Fusch, C., Hoffmann, W., Zygmunt, M., and Haas, J. P.: Sociodemographie of primiparae and multiparae in a population based survey – the Survey of Neonates in Pomerania (SNiP)]. Z. Geburtshilfe Neonatol. *214*/1, 15–23 (2010)

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Exposures

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Oxidative Stress and Fetal Programming

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Abstract

The "fetal/developmental origins/programming" of certain chronic diseases has gained increasing recognition, but the mechanisms of the "programming" remain elusive.

Many known or suspected causes of, or conditions associated with, adverse fetal growth (poor or excessive) or preterm birth have been associated with oxidative stress. Plausibly, oxidative stress may be a common link unifying the superficial "programming" associations between adverse fetal growth or preterm birth, and elevated risks of type 2 diabetes, coronary heart disease, and certain other chronic disorders. Oxidative stress programming may act by directly modulating gene expression, or indirectly through the effects of certain oxidized molecules. Adverse programming may occur without affecting fetal growth, but may be more frequent among low birth weight infants merely because they experienced more frequently oxidative insults. We reviewed the evidence of oxidative stress programming in animal and human studies and presented some preliminary results in an ongoing pregnancy cohort study. Experimental investigations have well demonstrated the role of redox balance in modulating gene expression. Both the insulin functional axis and blood pressure could be sensitive targets for oxidative stress programming. Preliminary results from our ongoing cohort strongly support the concept that maternal oxidative stress can be transferred from the mother to the fetus. The study is ongoing to determine whether oxidative stress may program infant insulin sensitivity/resistance.

Zusammenfassung

Die „Fetal-/Entwicklungsursprungs/-programmierung" bestimmter chronischer Krankheiten hat eine wachsende Beachtung erhalten, aber die Mechanismen des „Programmierens" bleiben schwer nachvollziehbar.

Viele bekannte oder vermutete Ursachen oder Bedingungen für ungünstig verlaufendes Fetalwachstum (unzulänglich oder übermäßig) oder Frühgeburten wurden mit oxidativem Stress verbunden. In nachvollziehbarer Weise kann oxidativer Stress ein gemeinsames Bindeglied für die Programmverbindungen zwischen ungünstig verlaufenden Fetalentwicklungen bzw. Frühgeburten und erhöhten Risiken für Typ-2-Diabetes, koronare Herzerkrankungen und gewisse andere chronische Störungen sein. Solche Prägungen durch oxidativen Stress können direkt durch Veränderung der Genexpression oder indirekt durch die Wirkung gewisser oxidierter Moleküle vermittelt werden. Eine ungünstige Programmierung kann auftreten, ohne die Fetalentwicklung zu beeinträchtigen, sie tritt aber häufiger bei Kindern mit geringem Geburtsgewicht auf, da sie öfter oxidative Insulte erfahren haben. Wir haben die Anzeichen von Programmierung aufgrund von oxidativem Stress in Studien mit Tieren und Menschen überprüft und einige vorläufige Ergebnisse in einer fortlaufenden Kohortenstudie mit Schwangeren wurden vorgestellt. Experimentelle Untersuchungen haben die Rolle des Redoxgleichgewichts bei der Anpassung der Genexpression gut aufgezeigt. Sowohl die Insulinfunktionskette als auch der Blutdruck könnten sensitive Zielobjekte für die Programmierung durch oxidativen Stress sein. Vorläufige Ergebnisse aus unserer fortlaufenden Kohorte stützen stark die Vorstellung, dass

maternaler oxidativer Stress von der Mutter auf den Fetus übertragen werden kann. Die Studie wird weitergeführt, um festzustellen, ob oxidativer Stress Insulinempfindlichkeit/-resistenz beim Kind hervorrufen kann.

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Maternal "Junk Food" Diet and Post-Natal Development

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With 2 Tables

Abstract

Eating habits of Western societies have changed in the last few decades. People consume greater proportions of "away from home" foods and snacks which are rich in energy, saturated fat, sugar and salt but lack the micronutrients found in wholesome foods. These "junk food" diets are generally blamed for the current obesity epidemic, but the effects of exposure to such diets from fetal life through maternal nutrition in pregnancy and lactation are poorly characterised. We have developed an animal model to examine this issue in rats, and data show that offspring exposed to a "junk food" diet from fetal life are susceptible to aggravated obesity and related disorders by the end of adolescence. This work highlights the maternal diet as a contributing factor to obesity in offspring and emphasizes that healthy eating habits ought to be encouraged starting at the fetal stage of life.

Zusammenfassung

Die Essgewohnheiten haben sich in den westlichen Gesellschaften in den letzten Jahrzehnten verändert. Viele Menschen konsumieren eine größere Menge an nicht selbst zu Hause bereiteten Nahrungsmitteln, die reich an Energie, gesättigten Fettsäuren, Zucker und Salz sind, aber denen es an Spurenelementen, wie in gesunden Nahrungsmitteln vorhanden, mangelt. Die Ernährung mit ungesunder Fertigkost ist dafür bekannt, der Auslöser der derzeitigen Adipositasepidemie zu sein. Die Auswirkungen einer solchen „junk food diet" bei einer Belastung durch die maternale Ernährung in der Schwangerschaft und während der Laktation auf den Feten sind jedoch bisher nicht ausreichend beschrieben. Wir haben ein Tiermodell entwickelt, um die Auswirkungen am Beispiel der Ratte zu untersuchen, und die Daten zeigen, dass der Nachwuchs, der einer ungesunden Fertignahrung bereits während der Fetalzeit ausgesetzt war, anfällig für Übergewicht und verwandte Störungen am Ende der Adoleszenzzeit war. Diese Arbeit hebt hervor, dass die maternale Ernährung Einfluss auf ein potenzielles Übergewicht des Nachwuchses hat, und betont, dass man zu gesunden Ernährungsgewohnheiten bereits von der Fetalzeit an raten sollte.

1. Introduction

Obesity rates have risen sharply in Western countries over the last few decades, and the World Health Organization predicts that the number of obese adults worldwide will grow from 400 million in 2005 to over 700 million by 2015. Children are also affected at increasingly earlier ages with around 20 million children under the age of 5 classified as overweight in 2005.

Obesity generally occurs when energy intake exceeds energy expenditure, but evidence suggests that the recent obesity epidemic may not be fuelled only by people leading more sedentary lifestyles (Westerterp and Speakman 2008). Food energy supplies have increased in Western countries over the past few decades and these directly correlate with increased BMI (Silventoinen et al. 2004). Concurrently, eating habits have changed and Western nations consume greater proportions of "away from home foods", salty snacks and fizzy drinks which are rich in energy, fat, sugar and salt (Guthrie et al. 2002, Nielsen et al. 2002). Energy

dense diets with added fat and sugar are often lacking vitamins and micronutrients found in unrefined wholesome foods, but their palatability and low cost encourage consumers' consumption (Andrieu et al. 2006, Maillot et al. 2007, Levine et al. 2003, Glanz et al. 1998). Consequently, energy dense diets with low nutritional value and added fat, sugar and salt are often qualified as "junk foods" and are believed to contribute to the obesity epidemic (Anderson and Patterson 2005).

More recent evidence from both human and animal studies indicates that maternal obesity and nutrition in pregnancy and lactation also contribute to the development of obesity and the metabolic syndrome in offspring (Armitage et al. 2008). However, the influence of a maternal "junk food" diet rich in energy, fat, sugar and salt on the offspring is not fully characterised.

We have developed an animal model based on the "supermarket" (Sclafani and Springer 1976) and "cafeteria" (Rothwell and Stock 1978) diets to examine this issue in rats and focused our attention on appetite and food preferences, skeletal muscle development and function, glycaemia, insulinemia, lipidemia and adiposity as well as non-alcoholic fatty liver disease in progeny. This animal model is particularly relevant to the human Western diet since obesity-related disorders are attributed to complex interactions between various nutritional factors consumed in excess including refined sugars, oils and salt as opposed to excessive intake of a single dietary component such as saturated fat alone (Cordain et al. 2005). Our data published to date is summarised below.

2. Experimental Design

In all studies, pregnant dams and offspring were fed either a balanced rodent chow diet alone (C) or with "junk food" items (J), namely biscuits, sweets, full fat cheese, potato crisps, cakes etc, all given *ad libitum*, during pregnancy, lactation and/or post-weaning as described in detail in a previous publication (Bayol et al. 2007). Pups were kept with their own mothers throughout lactation to reflect human situations. Litters were selected such that the number of pups born in each litter was statistically the same across all nutritional groups to control for litter sizes during both gestation and lactation, and prevent uncontrolled maternal physiological adaptations caused by removal of un-identical numbers of pups at birth across the groups. At weaning, six offspring per litter (three males plus three females) were selected to cover the whole range of birth weights and take into account intra-litter variations in the statistical design. Offspring were either culled at weaning (21 days post-partum) or at 10 weeks post-partum, which corresponds to about the end of adolescence in rats (Quinn 2005). Group names consist of either 2 (weaning) or 3 letters (10 weeks), with each letter (either C or J) corresponding to the diet given during gestation, lactation and post-weaning, respectively. Data was analysed by hierarchical two-way ANOVA to take into account litter effects and intra-litter variations.

3. Appetite and Food Preferences

By the end of adolescence, offspring fed the "junk food" diet from fetal life (JJJ) consumed approximately 22.5 % more energy daily than offspring born to chow fed mothers and given

free access to "junk food" only after weaning (CCJ) (Tab. 1). The increased energy intake exclusively came from the "junk food" source and was characterised by a selective exacerbated intake of fat (including saturated fat), sucrose and sodium while protein consumption was comparable among all offspring weaned on the "junk food" diet (Tab. 1). The exacerbated hyperphagia was accompanied by a 15 % and 18 % increase in body mass in JJJ males and females, respectively, compared with the CCJ group and BMIs were also increased. The data showed that a maternal "junk food" diet in pregnancy and lactation promoted exacerbated hyperphagia, a greater taste for "junk food" and obesity in progeny. Offspring born to "junk food" fed mothers switched to chow at weaning (JJC) reduced their daily energy intake for the first two weeks from weaning before their appetite returned to control (CCC) level by post-partum week 10, suggesting that the exacerbated hyperphagia observed in JJJ offspring may be triggered by the hedonic aspect of appetite regulation rather than hunger as previously discussed (Bayol et al. 2007).

Tab. 1 Nutrient intake. Average daily nutrient intake consumed by dams during pregnancy and lactation and by offspring (males and females) during the 10th week post-partum. C and J indicate chow and "junk food" diets, respectively, during gestation, lactation and/or post weaning. Different letters indicate statistically significant differences ($P < 0.05$). Adapted from Bayol et al. 2007.

	Pregnancy		Lactation			Week 10 post-partum					
	C	J	CC	JC	JJ	CCC	CCJ	JCC	JCJ	JJC	JJJ
Energy (KJ)	413.9a	645.4b	1217.1a	1072.8b	1448.9c	399.9a	723.3b	400.6a	657.2b	399.4a	885.9c
Fat (g)	1.2a	13.2b	3.4a	3.0b	26.5c	1.2a	18.3b	1.1a	16.2b	1.1a	25.1c
Sucrose (g)	1.6a	7.3b	4.6a	4.1b	13.4c	1.6a	7.7b	1.5a	6.9b	1.5a	9.5c
Sodium (g)	0.09a	0.16b	0.26a	0.23b	0.36c	0.09a	0.18b	0.08a	0.17b	0.08a	0.23c
Protein (g)	6.1a	3.9b	17.9a	15.8b	11.2c	6.1a	4.5b	5.9a	4.0b	5.9a	5.1b

4. Skeletal Muscle Development and Function

Weanling pups born to mothers fed the "junk food" diet in pregnancy alone or during both pregnancy and lactation exhibited semitendinosus muscle atrophy (reduced whole muscle cross sectional area) with fibre hypoplasia and fewer nuclear counts per cross sectional area. This was accompanied by increased intramuscular fat in the JJ group as well as increased expression of peroxisome proliferator-activated receptor (PPAR)-γ mRNA (Bayol et al. 2005).

This data showed that a maternal "junk food" diet impaired skeletal muscle development in weanling offspring, thus we decided to test whether this would translate into impaired muscle function at the end of adolescence. A small study revealed that offspring born to mothers fed the "junk food" diet in pregnancy and lactation exhibited reduced specific twitch and tetanic tensions in the plantar group of muscles (gastrocnemius, plantaris and soleus) following electrical stimulation *ex-vivo*, regardless of the post-weaning diet (Bayol et al. 2009).

5. Serum Biochemistry and Abdominal Adiposity

Elevated serum glucose, insulin, triglyceride and cholesterol are associated with insulin resistance, type 2 diabetes and cardiovascular disease. Our data showed that male offspring fed the "junk food" diet from fetal life (JJJ group) exhibited increased serum insulin with normal glycaemia while females were hyperglycaemic with normal insulin levels. Serum triglycerides and cholesterol were raised in both male and female progeny from the JJJ group while glycemia, insulinemia and lipidemia were not affected in CCJ or JJC groups. This showed that offspring exposed to the "junk food" diet from fetal life on exhibited impaired serum glycaemia, insulinemia and lipidemia and were therefore at greater risk of cardiovascular disease and type 2 diabetes by the end of adolescence. Abdominal adiposity is also strongly associated with the metabolic syndrome (Phillips and Prins 2008). As a measure of abdominal adiposity, we studied the perirenal fat pad; a major abdominal fat present both in male and female rats (Rokling-Andersen et al. 2009, Belzung et al. 1993). Table 2 shows that all offspring fed the "junk food" diet at some stage in the study exhibited increased perirenal fat mass relative to body weight which was characterised by adipocyte hypertrophy while their numbers were only increased in offspring fed "junk food" during the post-weaning period. Abdominal adiposity was markedly enhanced in JJJ offspring compared with all other groups and was greater in female than male progeny across all nutritional groups. Gene expression analyses (mRNA) revealed sex differences in the molecular metabolic adaptation to diet-induced adiposity in JJJ offspring, which could be explained by the sex differences in serum insulin and glucose described above. Females showed a marked up-regulation of mRNA expression for insulin-like growth factor-1, insulin receptor substrate (IRS)-1, vascular endothelial growth factor (VEGF)-A, PPAR-γ , leptin, adiponectin, adipsin, lipoprotein lipase (LPL), glucose transporters (Glut)-1 and -3 indicating greater adipocyte proliferation and differentiation, as well as increased uptake of glucose and dietary lipids in abdominal adipose tissue compared with female offspring never given access to "junk food" (CCC). Male JJJ offspring up-regulated IRS-1, VEGF-A, Glut-4 and LPL mRNAs compared with the CCC group (Bayol et al. 2008). Data unpublished to date revealed that the increased abdominal adiposity was accompanied by aggravated signs of non-alcoholic fatty liver disease and hepatic oxidative stress in JJJ offspring with sex differences in the molecular hepatic adaptation to diet-induced obesity.

Tab. 2 Abdominal adiposity. Adiposity parameters in 10 week old male (M) and female (F) offspring fed a "junk food" diet at various stages of development and growth. C and J indicate chow and "junk food" diets, respectively, during gestation, lactation and/or post weaning. Different letters indicate statistically significant differences ($P<0.05$). Adapted from Bayol et al. 2008.

	Perirenal fat mass (g)		% perirenal fat mass to body mass		Average mature adipocyte area (μm²)		Adipocyte density × perirenal fat pad mass	
	M	F	M	F	M	F	M	F
CCC	5.3a	2.8a	1.4a	1.2a	3638.3a	2998.6a	456.3a	285.1a
CCJ	9.9b	8.0b	2.4b	3.0b	4725.8b	4539.2b	739.8b	643.7b
JJC	6.9c	3.8a	1.9c	1.7c	4385.6b	3787.3b	542.4a	331.9a
JJJ	15.2d	13.7c	3.2d	4.5d	9388.8c	8494.7c	684.8b	638.4b

6. Conclusions

The rapid rise in obesity rates among Western countries is attributed to changes in dietary habits with increased intake of foods prepared away from home, snacks and "junk foods" which are dense in energy, fat, sugar and salt. Our studies revealed that exposure to such "junk food" diets from fetal life through maternal nutrition in pregnancy and lactation leads to exacerbated hyperphagia, obesity, abdominal adiposity as well as hyperglycaemia, hyperinsulinemia and hyperlipidemia by the end of adolescence. Therefore, a maternal "junk food" diet puts progeny at greater risk of cardiovascular diseases and type 2 diabetes early in adult life compared with offspring born to mothers fed a balanced diet. These studies highlight the importance of a healthy maternal diet in pregnancy and lactation for the prevention of obesity in future generations.

References

Anderson, J. W., and Patterson, K.: Snack foods: comparing nutrition values of excellent choices and "junk foods". J. Amer. Coll. Nutr. *24*, 155–156; discussion 156–157 (2005)

Andrieu, E., Darmon, N., and Drewnowski, A.: Low-cost diets: more energy, fewer nutrients. Eur. J. Clin. Nutr. *60*, 434–436 (2006)

Armitage, J. A., Poston, L., and Taylor, P. D.: Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. Front. Horm. Res. *36*, 73–84 (2008)

Bayol, S. A., Farrington S. J., and Stickland, N. C.: A maternal "junk food" diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. Br. J. Nutr. *98*, 843–851 (2007)

Bayol, S. A., Macharia, R., Farrington, S. J., Simbi, B. H., and Stickland, N. C.: Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. Eur. J. Nutr. *48*, 62–65 (2009)

Bayol, S. A., Simbi, B. H., Bertrand, J. A., and Stickland, N. C.: Offspring from mothers fed a "junk food" diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. J. Physiol. *586*, 3219–3230 (2008)

Bayol, S. A., Simbi, B. H., and Stickland, N. C.: A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. J. Physiol. *567*, 951–961 (2005)

Belzung, F., Raclot, T., and Groscolas, R.: Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Amer. J. Physiol. *264*, R1111–1118 (1993)

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., O'Keefe, J. H., and Brand-Miller, J.: Origins and evolution of the Western diet: health implications for the 21st century. Amer. J. Clin. Nutr. *81*, 341–354 (2005)

Glanz, K., Basil, M., Maibach, E., Goldberg, J., and Snyder, D.: Why Americans eat what they do: taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. J. Amer. Diet. Assoc. *98*, 1118–1126 (1998)

Guthrie, J. F., Lin, B. H., and Frazao, E.: Role of food prepared away from home in the American diet, 1977–1978 versus 1994–1996: changes and consequences. J. Nutr. Edu. Behav. *34*, 140–150 (2002)

Levine, A. S., Kotz, C. M., and Gosnell, B. A.: Sugars and fats: the neurobiology of preference. J. Nutr. *133*, 831S–834S (2003)

Maillot, M., Darmon, N., Vieux, F., and Drewnowski, A.: Low energy density and high nutritional quality are each associated with higher diet costs in French adults. Amer. J. Clin. Nutr. *86*, 690–696 (2007)

Nielsen, S. J., Siega-Riz, A. M., and Popkin, B. M.: Trends in energy intake in U.S. between 1977 and 1996: similar shifts seen across age groups. Obes. Res. *10*, 370–378 (2002)

Phillips, L. K., and Prins, J. B.: The link between abdominal obesity and the metabolic syndrome. Curr. Hypertens. Rep. *10*, 156–164 (2008)

Quinn, R.: Comparing rat's to human's age: how old is my rat in people years? Nutrition *21*, 775–777 (2005)

Rokling-Andersen, M. H., Rustan, A. C., Wensaas, A. J., Kaalhus, O., Wergedahl, H., Rost, T. H., Jensen, J., Graff, B. A., Caesar, R., and Drevon, C. A.: Marine n-3 fatty acids promote size reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet. Br. J. Nutr. *102*, 995–1006 (2009)

Rothwell, N. J., and Stock, M. J.: A paradox in the control of energy intake in the rat. Nature *273*, 146–147 (1978)

Sclafani, A., and Springer, D.: Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol. Behav. *17*, 461–471 (1976)

Silventoinen, K., Sans, S., Tolonen, H., Monterde, D., Kuulasmaa, K., Kesteloot, H., and Tuomilehto, J.: Trends in obesity and energy supply in the WHO MONICA Project. Int. J. Obes. Relat. Metab. Disord. *28*, 710–718 (2004)

Westerterp, K. R., and Speakman, J. R.: Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. Int. J. Obes. (London) *32*, 1256–1263 (2008)

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Nutritional Programming of Renal Function

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Abstract

Large epidemiological studies suggest a clear relation between low birth weight and adverse renal outcomes evident as early as childhood. Such adverse outcomes may include glomerular disease, hypertension and renal failure. Data from autopsy material and from experimental models suggest that reduction in nephron number via diminished nephrogenesis may be a major mechanism, and factors that lead to this reduction are incompletely elucidated. Other mechanisms appear to be renal (e.g. via the intrarenal RAAS) and non-renal (e.g. changes in endothelial function). It also appears likely that the outcomes of fetal programming may be influenced postnatally, for example, by the amount of nutrients made available at critical times.

Zusammenfassung

Große epidemiologische Studien sprechen für einen klaren Zusammenhang zwischen niedrigem Geburtsgewicht und pathologischen Nierenveränderungen bereits in der Kindheit. Hierunter sind Glomerulopathien, Hypertonie und Nierenversagen. Einer der Mechanismen scheint in einer durch gestörte Nephrogenese bedingten reduzierten Nephronenzahl zu liegen, auch wenn die Pathogenese dieses Phänomens noch weitgehend unklar ist. Andere Mechanismen lassen sich in renal (z. B. über das intrarenale RAAS) oder nicht-renal (z. B. Veränderungen der endothelialen Funktion) einteilen. Wahrscheinlich lässt sich eine pathologische fetale Programmierung durch postnatale Interventionen, wie eine Reduktion der Nahrungszufuhr in einer kritischen Phase, günstig beeinflussen.

1. Introduction

Recent studies have demonstrated an increased prevalence of end stage renal failure in adults who were small for gestational age (SGA) at birth (Lackland et al. 2000, Vikse et al. 2008). However, whether low birth weight is a primary risk factor for renal dysfunction in later life requires further examination. Questions such as whether epidemiological evidence that low birth weight infants have a higher risk of renal dysfunction in later life is robust and whether a reduction in nephron number is involved must be more definitively answered. Whether specific mechanisms are involved and whether programming of renal disease ends with birth or continues with postnatal modification are questions requiring further study. The present review aims to address these issues.

2. Epidemiological and Experimental Evidence for Altered Renal Function after Low Birth Weight at Term

A relatively lower birth weight has been associated with many subsequent health problems. Approximately 10 years ago Lackland and co-workers reported that low birth weight was

associated with early onset end-stage renal failure in US residents from a variety of ethnic backgrounds (Lackland et al. 2000, 2001). Of 1230 cases with end-stage renal disease, 70 % of patients (858) were black, 72 % (892) were male, 19 % (233) had diabetes, 29 % (359) had hypertension, and 46 % (571) were "other." The odds ratio for renal failure was 1.4 (95 % confidence interval 1.1–1.8) for the entire group including patients with diabetes mellitus and hypertension. More recently, Li et al. (2008) reported that more men who self reported having had a relatively low and a relatively high birth weight also had evidence of chronic kidney disease when screened using estimated glomerular filtration rate (eGFR). In detail, there was a U-shaped association between birth weight and chronic kidney disease (CKD) in men. Compared with men whose birth weight was between 3000 and 3999 g, those whose birth weight was less than 2500 g had 1.65-fold odds (95 % confidence interval 1.24–2.20) of CKD and those with birth weight 4500 g and more had 1.41-fold odds (95 % confidence interval 1.06–1.88) of CKD.

One difficulty with such associational studies is that the primary effects of low birth weight on renal function cannot be separated from conditions that were also associated with small for gestational age (SGA) birth, such as maternal diabetes mellitus type 2 and arterial hypertension during gestation.

Focussing on end-stage renal disease, a hard clinical endpoint, a recent large population-based cohort study reported that of 2,183,317 children born in Norway between 1967 and 2004, 526 developed end-stage renal disease. Those with a birth weight below the 10th percentile (small for gestational age, SGA) had a higher risk of end-stage renal failure than those who were not SGA (relative risk 1.5, 95 % confidence interval 1.2–1.9). Further, the development of end-stage renal disease in former SGA patients compared to controls appeared to be more probable under 14 years of age than after age 15 (Vikse et al. 2008). Patients under the age of 14 are unlikely to have factors predisposing for chronic renal failure such as diabetes mellitus and hypertension. Thus, the reason for higher incident end-stage renal disease under age 15 is unclear.

In a very recent metaanalysis by White and co-workers (2009), 16 studies reported a significant association between low birth weight and risk of CKD and 16 observed a null result. The combination of weighted estimates from the 18 studies for which risk estimates were available (n = 46,249 plus 2,183,317 from the record linkage study) gave an overall odds ratio (OR) of 1.73 (95 % confidence interval, 1.44 to 2.08). Combined ORs were consistent in magnitude and direction for risks of albuminuria (OR, 1.81; 95 % confidence interval, 1.19 to 2.77), end-stage renal disease (OR, 1.58; 95 % confidence interval, 1.33 to 1.88), or low estimated glomerular filtration rate (OR, 1.79; 95 % confidence interval, 1.31 to 2.45).

3. Glomerular Disease in Childhood and Relation to Birth Weight

Retrospective clinical studies have demonstrated that children with a history of low birth weight who have idiopathic nephrotic syndrome have a higher incidence of relapses and steroid dependency (Sheu et al. 2001, Zidar et al. 1998). Children with such a course have a greater need for additional therapy, such as alkylating agents and cyclosporine A. Other, more recent, studies have confirmed a more severe course and a higher rate of steroid resistance in children with nephrotic syndrome with a history of having been SGA babies (Plank et al. 2007, Teeninga et al. 2008). However, the underlying mechanisms are not yet delineated.

A retrospective study of 62 children with IgA nephropathy reported a 3-fold greater number of sclerotic glomeruli among those children with IgA nephropathy who were born small for gestational age as compared to those who were normal weight (Zidar et al. 1998).

4. Intrauterine Growth Restriction and Later Morbidity: Animal Models

Most data originating from human studies are based on epidemiological associations. Although epidemiological methods minimize confounding factors as much as possible, such studies are associative and cannot prove causal relationships between an initial programming event such as intrauterine growth restriction and later morbidity. Therefore, animal studies have been utilized to demonstrate causal relationships. The most widely used models are a physical model of uterine artery ligation in the rat and protein restriction in the rat and other mammalian species.

The ligation of both uterine arteries reduces blood flow to the placentas of individual rat fetuses. This model is, therefore, reminiscent of placental insufficiency in humans. Uterine artery ligation is commonly used to examine metabolic disorders such as diabetes mellitus (Nüsken et al. 2008).

Protein restriction has been the most widely used method for demonstrating how intrauterine growth restriction (IUGR) affects the cardiovascular system and the kidney (Plank et al. 2006). Although a number of mammalian models of protein restriction have been employed, most studies have been carried out in rats. In such work, pregnant rats are fed an isocaloric but protein restricted diet, varying from 10 % to 40 % of normal protein intake. This model mimics protein restriction, which is thought to be a frequent cause for intrauterine growth retardation (IUGR) in developing countries.

Recently, the protein-restricted model was employed to examine susceptibility to acquired renal diseases. For example, using the protein restriction animal model we demonstrated that offspring of protein-restricted mothers who suffered IUGR had increased susceptibility to a more severe and potentially chronic course when an acute mesangioproliferative glomerulonephritis (injection of an anti-Thy-1.1 antibody) was induced in male rats (Plank et al. 2006). This suggests that the animal models have additional utility for addressing putative causal relationships between IUGR and kidney disease and may help to delineate the mechanisms of fetal programming of renal disease.

5. Low Nephron Number and Fetal Programming of Renal Function

Nephron number has been acknowledged as a determinant of susceptibility to renal disease (Brenner and Mackenzie 1997, Hoy et al. 2008) and possibly to the development of hypertension in both animal models (Woods et al. 2004) and human beings (Brenner et al. 1988, Keller et al. 2003, Hughson et al. 2006). During nephrogenesis both intrinsic and extrinsic factors with myriad interactions "program" nephron number, or what has been called "nephron endowment" (Kuure et al. 2000). Following the completion of nephrogenesis, no further nephrons are formed, but subsequent nephron loss due to aging or renal injury decreases nephron number.

6. Other Mechanisms Contributing to Fetal Programming of Renal Function

An important indicator of changes in the intrauterine milieu that might lead to fetal programming of renal disease is thought to be alteration in the renin-angiotensin-aldosterone system (RAAS). Experimental models of fetal programming have reported an increased renal renin expression in adult rats that had been born IUGR after maternal protein restriction (Langley-Evans et al. 1999, Bogdarina et al. 2007).

Another renal alteration that has been reported in models of maternal protein restriction is an alteration in the activity of 11β hydroxysteroid dehydrogenase (11βHSD). This enzyme, present in the cells of the distal renal tubule, converts active cortisol into inactive cortisone (Seckl and Meaney 2004). Under physiological circumstances this reaction protects the mineralocorticoid receptor from stimulation by cortisol. In the IUGR rat model, renal 11βHSD expression is reduced, allowing for increased mineralocorticoid activity (Bertram et al. 2001). Interestingly, a reduction of 11βHSD has been reported in the placenta of human pregnancies complicated by IUGR (Schoof et al. 2001, Struwe et al. 2007). These observations might imply that maternal cortisol, which is usually inactivated by the placental 11βHSD2, can pass to the fetus. As a consequence, cortisol may lead to growth restriction and potentially to a programming of renal 11βHSD in the unborn child (Bertram et al. 2001, Seckl and Meaney 2004).

Apart from renal mechanisms, programming of extrarenal tissues has been investigated with regard to potential roles in future renal and vascular disease. For example, the endothelium and its interaction with vascular smooth muscle cells may be important in determining future renal and vascular disease. Another extrarenal mechanism that has been discussed in the context of fetal programming of kidney disease is increased sympathetic nerve activity, since there is a relation between birth weight and basal heart rate in adulthood (Nuyt 2008). The hypothesis that increased sympathetic nerve activity is a consequence of a challenging intrauterine environment is supported by animal data reporting that denervation of renal sympathetic nerve supply leads to a normalization of blood pressure in IUGR rats (Alexander et al. 2005). Such a mechanism is almost certainly of importance for renal function, since sympathetic nerve activity regulates intrarenal renin synthesis and salt retention.

7. Postnatal Modification of Fetal Programming of Kidney Disease

One of the first potential strategies considered to prevent morbidity after IUGR is the avoidance of hyperalimentation. Details on the so called mismatch concept are depicted by Gluckman and co-workers (2008). There is considerable evidence that rapid increase in caloric and protein intake postnatally plays an important pathophysiological role in developmental origins of health and disease (Clayton et al. 2007). Low birth weight and premature infants grow at different rates, and rapid "catch up" growth may have adverse health consequences. For example, babies who had higher calorie and protein intake from infant formula increased from 284 to 301 kcal/100 ml and from 1.4 to 1.8 g/100 ml of protein had an increase of diastolic blood pressure by 3.5 mm Hg at the age of 6–8 years (Singhal et al. 2007).

8. Conclusion

Adverse outcomes related to low birth weight include glomerular disease, hypertension and renal failure. Reduction in nephron number may be a major mechanism, and factors that lead to this reduction must be elucidated. It also appears likely that the outcomes of fetal programming may be influenced postnatally, for example by the amount of nutrients given at critical times.

Thus, it is important to consider how much hyperalimentation should be provided to a newborn during ICU stay, or whether, in some circumstances, it should be avoided. Whether avoiding overnutrition might alter the adverse course of renal disease after premature or SGA birth remains to be investigated. We will have to wait to see whether this knowledge can be used for future concepts.

References

Alexander, B. T., Hendon, A. E., Ferril, G., and Dwyer, T. M.: Renal denervation abolishes hypertension in low-birth-weight offspring from pregnant rats with reduced uterine perfusion. Hypertension *45*, 754–758 (2005)

Bertram, C., Trowern, A. R., Copin, N., Jackson, A. A., and Whorwood, C. B.: The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. Endocrinology *142*, 2841–2853 (2001)

Bogdarina, I., Welham, S., King, P. J., Burns, S. P., and Clark, A. J.: Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. Circ. Res. *100*, 520–526 (2007)

Brenner, B. M., Garcia, D. L., and Anderson, S.: Glomeruli and blood pressure: Less of one, more of the other? Amer. J. Hypertens. *1*, 335–347 (1988)

Brenner, B. M., and Mackenzie, H. S.: Nephron mass as a risk factor for progression of renal disease. Kidney Int. Suppl. *63*, S124–127 (1997)

Clayton, P. E., Cianfarani, S., Czernichow, P., Johannsson, G., Rapaport, R., and Rogol, A.: Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. J. Clin. Endocrinol. Metab. *92*, 804–810 (2007)

Gluckman, P. D., Hanson, M. A., Cooper, C., and Thornburg, K. L.: Effect of in utero and early-life conditions on adult health and disease. New Engl. J. Med. *359*, 61–73 (2008)

Hoy, W. E., Bertram, J. F., Denton, R. D., Zimanyi, M., Samuel, T., and Hughson, M. D.: Nephron number, glomerular volume, renal disease and hypertension. Curr. Opin. Nephrol. Hypertens. *17*, 258–265 (2008)

Hughson, M. D., Douglas-Denton, R., Bertram, J. F., and Hoy, W. E.: Hypertension, glomerular number, and birth weight in African Americans and white subjects in the southeastern United States. Kidney Int. *69*, 671–678 (2006)

Keller, G., Zimmer, G., Mall, G., Ritz, E., and Amann, K.: Nephron number in patients with primary hypertension. New Engl. J. Med. *348*, 101–108 (2003)

Kuure, S., Vuolteenaho, R., and Vainio, S.: Kidney morphogenesis: cellular and molecular regulation. Mech. Dev. *92*, 31–45 (2000)

Lackland, D. T., Bendall, H. E., Osmond, C., Egan, B. M., and Barker, D. J.: Low birth weights contribute to high rates of early-onset chronic renal failure in the Southeastern United States. Arch. Intern. Med. *160*, 1472–1476 (2000)

Lackland, D. T., Egan, B. M., Fan, Z. J., and Syddall, H. E.: Low birth weight contributes to the excess prevalence of end-stage renal disease in African Americans. J. Clin. Hypertens. (Greenwich) *3*, 29–31 (2001)

Langley-Evans, S. C., Sherman, R. C., Welham, S. J., Nwagwu, M. O., Gardner, D. S., and Jackson, A. A.: Intrauterine programming of hypertension: the role of the renin-angiotensin system. Biochem. Soc. Trans. *27*, 88–93 (1999)

Li, S., Chen, S. C., Shlipak, M., Bakris, G., McCullough, P. A., Sowers, J., Stevens, L., Jurkovitz, C., McFarlane, S., Norris, K., Vassalotti, J., Klag, M. J., Brown, W. W., Narva, A., Calhoun, D., Johnson, B., Obialo, C., Whaley-Connell, A., Becker, B., Collins, A. J., and *Kidney Early Evaluation Program Investigators*: Low birth weight is associated with chronic kidney disease only in men. Kidney Int. *73*, 637–642 (2008)

Nüsken, K. D., Dötsch, J., Rauh, M., Rascher, W., and Schneider, H.: Uteroplacental insufficiency after bilateral uterine artery ligation in the rat: impact on postnatal glucose and lipid metabolism and evidence for metabolic programming of the offspring by sham operation. Endocrinology *149*, 1056–1063 (2008)

Nuyt, A. M.: Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. Clin. Sciences *114*, 1–17 (2008)

Plank, C., Östreicher, I., Hartner, A., Marek, I., Struwe, F. G., Amann, K., Hilgers, K. F., Rascher, W., and Dötsch, J.: Intrauterine growth retardation aggravates the course of acute mesangioproliferative glomerulonephritis in the rat. Kidney Int. *70*, 1974–1982 (2006)

Plank, C., Östreicher, I., Dittrich, K., Waldherr, R., Voigt, M., Amann, K., Rascher, W., and Dötsch, J.: Low birth weight, but not postnatal weight gain, aggravates the course of nephrotic syndrome. Ped. Nephrol. *22*, 1881–1889 (2007)

Schoof, E., Girstl, M., Frobenius, W., Kirschbaum, M., Dörr, H. G., Rascher, W., and Dötsch, J.: Reduced placental gene expression of 11β hydroxysteroid dehydogenase type 2 and 15-hydrodroxy prostaglandin dehydrogenase in patients with preeclampsia J. Clin. Endocrinol. Metabol. *86*, 1313–1317 (2001)

Seckl, J. R., and Meaney, M. J.: Glucocorticoid programming. Ann. New York Acad. Sci. *1032*, 63–84 (2004)

Sheu, J. N., and Chen, J. H.: Minimal change nephrotic syndrome in children with intrauterine growth retardation. Amer. J. Kidney Dis. *37*, 909–914 (2001)

Singhal, A., Cole, T. J., Fewtrell, M., Kennedy, K., Stephenson, T., Elias-Jones, A., and Lucas, A.: Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation *115*, 213–220 (2007)

Struwe, E., Berzl, D., Schild, R. L., Beckmann, M. W., Dörr, H. G., Rascher, W., and Dötsch, J.: Simultaneously reduced gene expression of cortisol-activating and cortisol-inactivating enzymes in placentas of small-for-gestational-age neonates. Amer. J. Obstet. Gynecol. *197*, 43.e1-6 (2007)

Teeninga, N., Schreuder, M. F., Bökenkamp, A., Delemarre-van de Waal, H. A., and van Wijk, J. A.: Influence of low birth weight on minimal change nephrotic syndrome in children, including a meta-analysis. Nephrol. Dial. Transplant. *23*, 1615–1620 (2008)

Vikse, B. E., Irgens, L. M., Leivestad, T., Hallan, S., and Iversen, B. M.: Low birth weight increases risk for end-stage renal disease. J. Amer. Soc. Nephrol. *19*, 151–157 (2008)

White, S. L., Perkovic, V., Cass, A., Chang, C. L., Poulter, N. R., Spector, T., Haysom, L., Craig, J. C., Salmi, I. A., Chadban, S. J., and Huxley, R. R.: Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Amer. J. Kidney Dis. *54*/2, 248–261 (2009)

Woods, L. L., Weeks, D. A., and Rasch, R.: Programming of adult blood pressure by maternal protein restriction: role of nephrogenesis. Kidney Int. *65*, 1339–1348 (2004)

Zidar, N., Cavic, M., Kenda, R. B., and Ferluga, D.: Unfavorable course of minimal change nephrotic syndrome in children with intrauterine growth retardation. Kidney Int. *54*, 1320–1323 (1998)

Zidar, N., Cavic, M. A., Kenda, R. B., Koselj, M., and Ferluga, D.: Effect of intrauterine growth retardation on the clinical course and prognosis of IgA glomerulonephritis in children. Nephron *79*, 28–32 (1998)

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Mechanisms

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Diabetes in Pregnancy – Consequences for the Offspring

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Abstract

It is clear that in the human fetal overgrowth (macrosomia), as well as intra-uterine growth, restrictions induce changes in the neuroendocrine system of the fetus and the newborn. Furthermore, abnormal fetal growth has consequences for disorders in later (adult) life. In rats, fetal macrosomia can be induced by mild maternal diabetes. This macrosomia is related to hyperplasia of the insulin producing B cells and hyperinsulinism. The offspring have a reduced capacity for insulin secretion in situations of increased demand such as pregnancy. When obesity is present in the offspring insulin resistance will deteriorate B cell function. You can see similar findings in growth-restricted offspring of rats. Obesity is becoming an epidemic problem in both the developed and underdeveloped world. Maternal obesity is not only a risk factor for maternal and perinatal outcomes, it also increases the risk of obesity and insulin resistance in the offspring. Indeed in rats it has been clearly demonstrated that maternal diet induces obesity and insulin resistance in the offspring. It may be concluded that the fetal period in human and animal life is crucial for later development.

Zusammenfassung

Es ist bewiesen, dass die humane Makrosomie wie auch die intrauterine Wachstumsretardierung (IUGR) zu Veränderungen im Neuroendokrinium des Feten sowie des Neugeborenen führen. Außerdem hat ein verändertes fetales Wachstum Auswirkungen auf die Entwicklung von Erkrankungen im späteren (adulten) Leben. Bei der Ratte kann eine fetale Makrosomie durch einen milden maternalen Diabetes hervorgerufen werden. Die Makrosomie steht im Zusammenhang mit einer Hyperplasie der B-Zellen des Pankreas und Hyperinsulinismus. Der Nachwuchs hat eine reduzierte Anpassungsfähigkeit der Insulinsekretion in Situationen des vermehrten Verbrauchs, wie z. B. der Schwangerschaft. Bei bestehender Fettleibigkeit des Nachwuchses wird Insulinresistenz die Funktion der B-Zellen verschlechtern. Ähnliche Ergebnisse zeigen wachstumsretardierte Nachkömmlinge im Rattenmodell. Adipositas wird nicht nur in den Industrieländern zum epidemischen Problem, auch die Entwicklungsländer leiden darunter. Maternale Fettleibigkeit ist nicht nur ein Risikofaktor für die maternale und perinatale Entwicklung, sondern steigert auch das Risiko von Adipositas und Insulinresistenz des Nachwuchs. Im Rattenmodell kann eindeutig gezeigt werden, dass die maternale Ernährung Fettleibigkeit und Insulinresistenz des Nachwuchses induzieren kann. Zusammenfassend kann man sagen, dass die Fetalzeit bei Menschen sowie Tieren entscheidend für die spätere Entwicklung ist.

1. Introduction

Even before the discovery of insulin, Dubreuil and Anderodias (1920) described giant islets in the pancreas of a human newborn of a diabetic mother. It was suggested that the increased glucose level in the mother had an effect on the increased size of the fetal islets. Indeed the increased transplacental transfer of glucose and other nutrients in the diabetic pregnancy produces B cell hyperplasia in the fetus. It is also important to mention that fetal B cell hyperplasia is only present in asymmetric macrosomia and not in symmetric macrosomia (genetic influence). Furthermore, an intact hypothalamo-hypophysial (HH) system was needed to develop

fetal B cell hyperplasia, hyperinsulinism and overgrowth, indicating the importance of the HH system in the regulation of the neuroendocrine function (Van Assche and Gepts 1971, Van Assche et al. 2001). We put forward the hypothesis at that time that the hyperactivity of the fetal B cells may result in a reduced capacity for insulin secretion in later life.

These observations were in accordance with the original observations of Gunther Doerner that the diabetic intra-uterine environment is an important determinant for the development of diabetes in the offspring. These observations were confirmed by several epidemiologic studies and recently summarised by Mclean et al. (2006): the excess of maternal transmission of diabetes is consistent with an epigenetic effect of hyperglycemia in pregnancy acting in addition to genetic factors to induce diabetes in the next generation.

2. Results

Animal models of diabetes during pregnancy may discover the specific effects of an exposure to an abnormal diabetic intra-uterine environment independent of inherited trails.

We have used rats as an experimental model. By inducing mild diabetes in pregnant rats, the fetal endocrine pancreas showed B cell hyperplasia together with macrosomia and hyperinsulinism, comparable with the findings in humans. Moreover, the first evidence of developmental programming came from animal research in 1979; it was demonstrated that mild diabetes in pregnant rats induced gestational diabetes in the second generation and as a consequence macrosomia, increased insulin secretion and B cell hyperplasia in the fetuses of the third generation (Aerts and Van Assche 1979). It was postulated that (over) stimulation of the insulin producing B cells *in utero* leads to a reduced capacity for insulin secretion in conditions of increased demand in later life, such as obesity and pregnancy. The reduced B cell function is not able to meet the increased insulin resistance.

The effect of a diabetic intra-uterine environment on the subsequent generations has been further explored and dysfunctional B cells in adult offspring of rats with gestational diabetes has been confirmed (Aerts and Van Assche 2006, Boloker et al. 2002). Various mechanisms of this dysfunction, originated *in utero*, have been proposed: B cell exhaustion after chronic overstimulation, direct glucose toxicity on the B cells, or a decrease in insulin gene promoter activity and binding of PDX-1 (pancreatic/duodenal homebox-1) to the insulin promoter, leading to defects in insulin secretion (De Vlieger et al. 2008).

Not only the diabetic intra-uterine environment and fetal overgrowth have consequences for later life; fetal undergrowth also induces consequences in the offspring. Obesity in pregnancy shows similar effects as diabetes in pregnancy. Obesity is an important health problem with epidemic proportions. In the EU countries more than half of the adult population is overweight, and between 20 and 30 % of the overweignt population is obese (*EU Document* 2006). Obesity in pregnancy is responsible for increased maternal and perinatal morbidity and mortality (Sibai et al. 1999, Jensen et al. 2003). Furthermore, the prevalence of congenital malformations in the offspring is increased in these pregnancies (Watkins et al. 2003). But even more, obesity in pregnancy has consequences for diseases in the offspring in later life with a transgenerational effect. By inducing obesity in rats with an obesogenic diet before pregnancy, it has been shown that when pregnant, these animals have insulin resistance and an abnormal glucose tolerance. Offspring of these pregnant obese rats remain obese in their later life and have insulin resistance (Holemans et al. 2004). Numerous studies in animals

have confirmed that maternal over-nutrition induces a deleterious effect during perinatal life, leading to a metabolic syndrome in the offspring (Holemans et al. 2004, Armitage et al. 2005, McMillen et al. 2005).

3. Conclusion

A diabetic intra-uterine environment induces a transgenerational effect of gestational diabetes. Also, diabetes in pregnancy has health consequences in the offspring. At the other end of the spectrum of fetal growth, it has also been shown that intra-uterine growth restriction is related to diseases in later life.

References

Aerts, L., and Van Assche, F. A.: Is gestational diabetes an acquired condition? J. Dev. Physiol. *1*, 219–225 (1979)

Aerts, L., and Van Assche, F. A.: Animal evidence for transgenerational development of diabetes mellitus. Int. J. Biochem. Cell Biol. *38*, 894–903 (2006)

Armitage, A. A., Taylor, P. D., and Poston L.: Experimental models of developmental programming. J. Physiol. *565*, 3–8 (2005)

Boloker, J., Gertz, S. J., and Simmons, R. A.: Gestational diabetes leads to the development of diabetes in adulthood in the rat. Diabetes *51*, 1499–1506 (2002)

De Vlieger, R., Casteels, K., and Van Assche, F. A.: Reduced adaptation of the pancreatic B cells during pregnancy. Acta Obst Gyn. Scand. *12*, 1266–1270 (2008)

Dubreuil, G., and Anderodias, J.: Ilots de Langerhans géants chez un nouveau-né issu de mère glycosurique. C. R. Soc. Biol. *23*, 1491 (1920)

EU Document. A6-0450 (2006)

Holemans, K., Caluwaerts, S., Poston, L., and Van Assche, F. A.: Diet induced obesity in the rat. Amer. J. Obstet. Gynecol. *190*, 858–865 (2004)

Jensen, D. M., Damm, P., Sørensen, B., Mølsted-Pedersen, L., Westergaard, J. G., Ovesen, P., and Beck-Nielsen, H.: Pregnancy outcome and pre-pregnancy body mass index in 2459 glucose tolerant Danish women. Amer. J. Obstet. Gynecol. *189*, 239–244 (2003)

Mc Lean, M., Chipps, D., and Cheung, N. W.: Mother to child transmission of diabetes mellitus: does gestational diabetes program type 2 diabetes in the next generation? Diabetic med. *23*, 1213–1215 (2006)

Mcmillen, I. C., and Robinson, J.: Developmental origin of the metabolic syndrome. Physiol. Rev. *85*, 471–633 (2005)

Sibai, B. M., Gordon, T., Thom, E., Caritis, S. N., Klebanoff, M., McNellis, D., and Paul, R. H.: Risk factors for preeclampsia in healthy nulliparous women. Amer. J. Obstet. Gynecol. *172*, 642–658 (1999)

Van Assche, F. A., and Gepts, W.: The cytological composition of the foetal endocrine pancreas in normal and pathological conditions. Diabetologia *7*, 434–444 (1971)

Van Assche, F. A., Holemans, K, and Aerts, L.: Longterm consequences for offspring of diabetes during pregnancy. Br. Med. Bull. *60*, 173–182 (2001)

Watkins, M. L., Rasmussen, S. A., Honein, M. A., Botto, L. D., and Moore, C. A.: Maternal obesity and risk of birth defects. Pediatrics *111*, 323–328 (2003)

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Medicine at the Interface between Science and Ethics

Leopoldina-Symposium

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Naturwissenschaft und Theologie/Ethik versuchen mit unterschiedlichen Konzepten, ein Weltbild zu erfassen, das die *conditio humana* besser zu verstehen erlaubt. Die Fragen sind weit gefasst; endgültige Antworten wird man nicht leicht finden. Gemeinsame Diskussionen über diese Probleme könnten beiden Gebieten Anregungen geben und der Biomedizin im Umgang mit der sehr kritischen Öffentlichkeit helfen. Voraussetzung ist Offenheit gegenüber der anderen Denkweise. Der vorliegende Band behandelt daher aus der Perspektive von Naturwissenschaftlern und Ethikern so verschiedene Themen wie die neuen Herausforderungen an Moral- und Ethikdiskurse durch die jüngsten Fortschritte der Biowissenschaften, die Grenzen der ethischen Reflexion bei den neueren Entwicklungen der Molekularbiologie, die Geschichte der Auffassungen vom „Gen“ und seiner Bedeutung in der Humanbiologie, aber auch die Missverständnisse zwischen den beiden Kulturen der Naturwissenschaften und der Geisteswissenschaften in der Forschung über Lebensprozesse. Dazu kommen Beiträge zur Stammzellproblematik, der Verwendung von Tiermodellen in der Translationsmedizin, über Würde von Zellen in Kultur, Fragen der Pluripotenz von Zellen und der Reprogrammierung von Zellkernen sowie der Bedeutung von Methylierungsmustern für die Epigenetik. Die Beiträge sind in englischer oder deutscher Sprache verfasst.

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Outcome

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Intrauterine Programming of Bone Development

Stuart Lanham, Carol Roberts, and Richard O. C. Oreffo
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Abstract

Epidemiological evidence suggests bone development and risk of osteoporosis in later life in the offspring is influenced by maternal nutrition. We have explored this connection in a number of animal models using a range of maternal nutritional challenges.

Maternal diet has deleterious effects on adult offspring bone. Adult offspring on a high fat diet from mothers fed the high fat diet showed increased adiposity in the femur compared to high fat fed offspring from control fed mothers. Female offspring from low protein fed mothers showed altered bone structure and strength indicative of osteoporosis. Changes to bone structure occur before birth. Newborn offspring from low protein fed mothers showed sex specific differences in bone structure and differential expression of specific genes essential for bone developmental. Analysis of these genes showed altered DNA methylation at specific sites around these genes. These data shows that the maternal diet can alter bone structure in the offspring.

Zusammenfassung

Epidemiologische Daten weisen daraufhin, dass die Knochenentwicklung und das Risiko von Osteoporose im späteren Leben der Nachkommen durch die Ernährung der Mutter beeinflusst wird. Wir haben in diesem Zusammenhang in einer Reihe von Tiermodellen den Einfluss von unterschiedlich zusammengestellter mütterlicher Nahrung untersucht.

Die Ernährung der Mutter hat schädliche Auswirkungen auf die Knochen volljähriger Kinder. Adulte Nachkommen, die mit einer fettreichen Ernährung gefüttert wurden und von Müttern stammten, die eine Diät mit hohem Fettanteil erhalten hatten, zeigten eine Zunahme der Adipositas im Oberschenkel im Vergleich zu den Nachkommen, die eine fettreiche Ernährung erhielten, jedoch von Müttern abstammten, die zur Kontrollgruppe gehörten. Weibliche Nachkommen von Müttern, die eine Diät mit wenig Protein erhielten, zeigten eine veränderte Knochenstruktur und -stärke, die auf eine Osteoporose hinwiesen. Änderungen der Knochenstruktur treten vor der Geburt auf. Nachkommen von Müttern, die eine proteinreduzierte Diät erhielten, zeigten geschlechtsspezifische Unterschiede in der Knochenstruktur und eine differentielle Expression spezifischer Gene, die wichtig für die Knochenentwicklung sind. Die Analyse dieser Gene ergab eine veränderte DNA-Methylierung in unmittelbarer Nachbarschaft dieser Gene. Diese Daten zeigen, dass die Ernährung der Mutter die Knochenstruktur bei den Nachkommen verändern kann.

The principle that maternal nutrition can influence the appearance of disease in the offspring is now scientifically accepted. This so-called “Barker hypothesis” has been shown to influence the incidence of, amongst others, heart disease, stroke, and type 2 diabetes. The geographical maps of the incidence rates of heart disease and osteoporosis are very similar. This observation leads to our hypothesis that osteoporosis and the development of bone structure may also be influenced by maternal nutrition during pregnancy. We have examined the affect maternal diet during pregnancy has on offspring bone structure using a range of animal models, a range of dietary models, and a range of age groups.

Initially we have looked at older offspring to determine if maternal nutrition has any long-lasting affect on the bone structure of the offspring. We used a mouse model where mothers

were fed either a control or a high fat diet during pregnancy, and the offspring continued on the high fat diet (Lanham et al. 2009b). At 30 weeks of age, the offspring fed a high fat diet showed increased bone mineral density at the proximal and distal end of the femur, regardless of their mother's diet. Similarly, high fat fed male offspring, from mothers fed a control or a high fat diet, showed similar increases in body mass of around 50 %, a reduced femur length, and increased femoral midshaft diameter, but little variation in trabecular structure when compared to control offspring. Similar alterations were seen in high fat fed female offspring from high fat fed mothers. In contrast, high fat fed female offspring from control fed mothers showed over 100 % increase in body mass, maintenance of femur length, and increases in bone volume and trabecular spacing. These data indicates some form of maternal programming in the female offspring. It appears that mothers fed a high fat diet during pregnancy can alter the phenotype of their female offspring, such that the offspring are better adapted to live on a high fat diet. In this case, the female offspring show a reduced weight gain and modification of bone structure to cope with the expected mass increase, but without the need to increase calcium uptake.

We have also used another animal model with a different maternal dietary restriction. Here we used a rat model of maternal low protein during pregnancy to assess the bone structure in their offspring at 75 weeks of age. Male offspring were found to be unaffected in regard to their bone structure and strength, whereas female offspring showed lower bone density and altered bone structure in the femoral head indicative of osteoporosis (Lanham et al. 2008). In contrast, the lumbar vertebra from the same animals showed increased bone density and altered bone structure, producing a bone with increased strength to fracture. These differences were found in areas containing trabeculae; the scaffold structure that gives bones its strength. However, when we analysed the midshaft tibia, an area of purely cortical bone with no trabeculae, we again found increased bone mineral density, but here the bone fractured with a lower load. Here the cortical bone appeared to be more brittle.

Romano et al. (2009) used a bilateral uterine artery and vein ligation on day 18 of a 22 day gestation, as a model of total nutrient insufficiency to the offspring. All offspring were weaned by control females to remove any affect weaning may have on offspring growth. Compared to controls, nutritionally restricted male offspring showed reduced body mass at 6 months of age (when all samples were analysed), whereas nutritionally restricted female offspring displayed "catch-up" growth, whereby they were born smaller than controls, but showed no differences in mass at 6 months of age. In addition, all nutritionally restricted offspring had shorter femora than control animals. Male nutritionally restricted offspring also had lower bone mineral density, reduced cortical thickness, reduced femoral midshaft diameter, and reduced bone strength. However, correcting for mass removed all the differences seen in the male offspring. The group also determined the affect of weaning by keeping nutrient restricted offspring with their mothers. Reduced intrauterine growth induces poor milk quality in the mother. Firstly, it was noted that female offspring in this uterine and weaning nutritionally restricted group did not display catch-up growth as was seen in the uterine restricted group. Secondly, male offspring characteristics in this uterine and weaning restricted group were found to be similar to the previously described *in utero* only restricted group. Thirdly, female offspring showed intermediate results between female *in utero* only restricted (which were not significant from controls) and male results. Hence, it was concluded that improved lactation could attenuate the differences seen in the female offspring.

Next, we looked to see if maternal nutrition could influence bone structure in early age. We used a mini pig model to test the affect of a maternal low protein diet during the third

trimester on the bone structure of the fetus on day 113 of a 115 day gestation (Lanham et al. 2009a). Fetuses from mothers fed a low protein diet showed increased bone density in the proximal femur. The same area also showed increased porosity of the bone. These two characteristics were found to produce a bone with a weaker structure. We also found that the cartilage surrounding the femoral head had a reduced stiffness due to the structure being less mineralised than that in control fetuses. The lumbar vertebra of the fetuses from the low protein fed mothers did not display any alteration in bone mineral density, but did show increased porosity, and increased trabecular spacing with the result that the bone fractured at a lower load than controls. Hence, bone structural and strength differences were evident before birth in these fetuses.

We have also assessed the bone structure in newborn offspring in the rat maternal low protein diet model described above. Female offspring from low protein fed mothers showed increased trabecular thickness and reduced porosity in the vertebra, whereas no differences were found in the male offspring from the same mothers. We also cultured bone marrow cells from the offspring and used microarrays to determine gene expression differences between those from low protein diet fed mothers and control diet fed mothers. We discovered that female offspring from low protein diet fed mothers over-expressed gene "X", whereas the male offspring from the same mothers under-expressed gene "Y". The Barker hypothesis phenomenon has been found to be due to alterations in epigenetic markers affecting gene expression, such as DNA methylation and histone acetylation, etc. We assessed the level of DNA methylation around genes X and Y to determine if their expression levels were influenced by this. In both cases, we found altered methylation around the gene. Hence, we now have evidence that the maternal diet during pregnancy can alter the DNA methylation status of genes that may alter bone structure and development in the offspring.

References

Lanham, S. A., DuPriest, E., Kupfer, P., Roberts, C., Cooper, C., Bagby, S. P., and Oreffo, R. O. C.: Altered vertebral and femoral bone structure in fetal offspring of microswine subject to nutritional challenge. Bone. Submitted (2009a)

Lanham, S. A., Roberts, C., Hollingworth, T., Sreekumar, R., Elahi, M. M., Cagampang, F. R., Hanson, M. A., and Oreffo, R. O. C.: Maternal high-fat diet: effects on offspring bone structure. Osteoporos. Int. *21*/10, 1703–1714 (2009b)

Lanham, S. A., Roberts, C., Perry, M., Cooper, C., and Oreffo, R. O. C.: Intrauterine programming of bone. Part 2: Alteration of skeletal structure. Osteoporos. Int. *19*, 157–167 (2008)

Romano, T., Wark, J. D., Owens, J. A., and Wlodek, M. E.: Prenatal growth restriction and postnatal growth restriction followed by accelerated growth independently program reduced bone growth and strength. Bone *45*, 132–141 (2009)

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Metabolism Meets Virulence

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Das Wissen über mikrobielle Virulenzfaktoren ist mittlerweile sehr detailliert, während das Verständnis, wie sich Bakterien während einer Infektion ernähren und wie sie ihren Stoffwechsel an den eukaryotischen Wirt anpassen, erst am Anfang steht. Wissenschaftler mit den Arbeitsschwerpunkten pathogene Bakterien, bakterielle Physiologie, Regulation, Analyse des Metaboloms und Symbiose von Mikrobe und Wirt berichten in dem vorliegenden Band über Ansätze zur Analyse des Stoffwechsels während Infektionen sowie die *In-silico*-Modellierung von metabolischen Netzwerken. Behandelt werden allgemeine Aspekte des bakteriellen Stoffwechsels, die globale Regulation des Bakterienstoffwechsels, die RNA-Biologie in ihrer Bedeutung für den bakteriellen Stoffwechsel und die Virulenz, die metabolischen Anpassungen von Pathogenen an extrazelluläre oder intrazelluläre Lebensweisen, die Rolle von Biofilmen in der bakteriellen Kommunikation und der Übergang von der parasitischen zur endosymbiontischen Lebensweise. Die *Keynote-Lecture* hielt Frederick Neidhardt (Ann Arbor, MI, USA) zum Thema „Growth meets virulence: Confluence of two paths of microbiology“. Zu den eingeladenen Rednern gehörten u. a. Tyrrell Conway (Oklahoma, OK, USA), Richard H. ffrench-Constant (Exeter, Großbritannien), Werner Goebel (Würzburg), Roy Gross (Würzburg), Regine Hengge (Berlin), Tina Henkin (Columbus, OH, USA), Wolfgang Hillen (Erlangen), Steven Lory (Boston, MA, USA), Andres Moya (Valencia, Spanien), Richard A. Procter (Madison, WI, USA), Eliora Ron (Tel Aviv, Israel), Milton H. Saier (La Jolla, CA, USA), Vanessa Sperandio (Dallas, TX, USA), Jörg Stülke (Göttingen) und Jörg Vogel (Berlin). Alle Beiträge sind in englischer Sprache verfasst.

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Developmental Origins of Cardiovascular Disease: A Role for Endothelial Dysfunction

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Abstract

There is considerable evidence that cardiovascular and metabolic diseases have their origins partly in the developmental environment. One finding that is consistent to both epidemiology and animal models is the propensity for endothelial dysfunction. The endothelium plays a variety of roles, including modulating platelet aggregation and controlling vascular tone through the release of various factors such as nitric oxide. The importance of a healthy endothelium is seen in disease states, where endothelial dysfunction is associated with atherosclerosis, hypertension and the metabolic syndrome. As a primary step in the pathogenesis of cardiovascular disease, damage to the endothelium as a consequence of a poor developmental environment may be a pivotal step in the progression of cardiovascular disease and therefore an important target for intervention.

Zusammenfassung

Es gibt bedeutende Daten, die zeigen, dass die Entwicklung von kardiovaskulären und metabolischen Erkrankungen ihren Ursprung teilweise in der Entwicklung hat. Ein Ergebnis, das die epidemiologischen sowie die Tiermodelle zeigen, ist die Neigung zur endothelialen Dysfunktion. Das Endothel hat verschiedene Aufgaben, u. a. die Modulation der Plättchenaggregation und die Kontrolle des Gefäßtonus während der Freisetzung diverser Faktoren wie Stickstoffmonoxid (NO). Die Bedeutung eines gesunden Endothels verdeutlichen Erkrankungen, die mit geschädigtem Endothel in Verbindung mit Arteriosklerose, Bluthochdruck und dem metabolischen Syndrom in Zusammenhang gebracht werden. Wenn der Endothelschaden einer der ersten Schritte in der Pathogenese der kardiovaskulären Erkrankungen als Konsequenz aus einer mangelhaften Entwicklung ist, könnte hier auch die Ursache der Progression der kardiovaskulären Erkrankungen liegen und das ein entscheidender Faktor für die Intervention sein.

1. Introduction

There is considerable evidence that cardiovascular and metabolic diseases have their origins, in part, in the developmental environment (Gluckman et al. 2008). Alongside the epidemiological cohorts, the development of a wide range of animal models provides mechanistic insight into the processes underlying such of developmental origins. As a key feature of much cardiovascular and metabolic disease, endothelial dysfunction is also widely noted in many studies of development origins. This brief overview puts forward some of the evidence linking endothelial dysfunction with the concept of developmental origins.

2. The Vascular Endothelium and Nitric Oxide

The healthy vascular endothelium plays a critical role in the maintenance of cardiovascular homeostasis; controlling platelet and leukocyte adhesion, thrombogenesis and inflamma-

tory responses, as well as influencing the tone of the underlying vascular smooth muscle (Hunt and Jurd 2002). This regulation is maintained by the synthesis and release of both pro-thrombotic, vasoconstrictor factors such as thromboxane and endothelin, as well as the anti-thrombotic, vasodilatory factors such as nitric oxide (NO), prostacyclin (PGI_2) and endothelial-derived hyperpolarising factor (EDHF; Busse and Fleming 2006).

The NO pathway has been studied in great detail and it is now recognised as a crucial regulator of vascular homeostasis. NO is synthesised by the enzyme nitric oxide synthase of which there are 3 isoforms identified: two constitutively expressed isoforms (nNOS/type I and eNOS/type III) which are regulated by Ca^{2+}, as well as an inducible form (iNOS/type II) which is regulated by cytokine stimulation (Napoli et al. 2006). The produced NO plays a variety of roles regulating vascular tone, proliferation of vascular smooth muscle and platelet aggregation (Napoli et al. 2006). Due to this pivotal role in the vasculature, disruption of the NO pathway and dysfunction of the endothelium is an important factor in cardiovascular and metabolic disease.

2.1 Endothelial Dysfunction in Human Cardiovascular Disease

The importance of a healthy endothelium is apparent in cardiovascular and metabolic diseases, where endothelial function impaired. Endothelial dysfunction is associated with insulin resistance (Hsueh et al. 2004) and essential hypertension (Taddei et al. 2001). Furthermore, the activation of the endothelium and subsequent inflammation are seen as the primary step in the development of atherosclerosis (Landmesser et al. 2004). Taken together, is it now clear that endothelial dysfunction is a recognised marker of cardiovascular disease (Juonala et al. 2004).

An important factor in the underlying endothelial dysfunction is a reduction in the bioavailability of NO, in part due the overproduction of reactive oxygen species (Delles et al. 2008, Napoli and Ignarro 2009). This oxidative stress can lead to the depletion of the co-factor tetrahydrobiopterin, the uncoupling of eNOS and the production of peroxynitrite (Landmesser et al. 2003, Delles et al. 2008), all of which ultimately reduce the availability of NO.

In humans, endothelial function is usually assessed using ultrasound to assess flow-mediated dilatation (FMD), this being less invasive than standard organ bath techniques. This utilises the NO-dependent dilatation induced by mechanical shear stress on the artery as blood flow increases. Briefly, a rapid increment in flow is achieved by the inflation and subsequent deflation of a cuff on the lower arm. This produces a hyperaemic response and increased flow in the brachial artery, the resultant change in the diameter of which can be measured by high resolution ultrasound. This type of measurement not only correlates well to other more invasive measures of endothelial function but also to cardiovascular risk (Juonala et al. 2004, Antoniades et al. 2009).

2.2 Evidence of Early Endothelial Dysfunction in DOHaD

Given the relationships which exist between birth weight and cardiovascular risk and endothelial dysfunction and cardiovascular disease; is there a relationship between birth weight and endothelial dysfunction? And if so, does it precede the onset of other risk factors such as hypertension?

In otherwise healthy young adults (19–25 year olds), reduced endothelial function has been reported in those who were smaller at birth (Goodfellow et al. 1998, Leeson et al. 2001). While this association was lost in those who had the highest risk profile, in subjects with the lowest risk profile the effect of a 1 kg decrease in birth weight was equivalent to 4.5 pack years of smoking (Leeson et al. 2001). Similar associations have also been reported in prepubescent children, where low birth weight was also associated with reduced endothelial function. This was true whether assessed by FMD (Leeson et al. 1997, Franco et al. 2007) or acetylcholine (Martin et al. 2000b). Furthermore, capillary recruitment in response to either heating or acetylcholine is reduced in children of low birth weight (Ijzerman et al. 2002).

Perhaps the most striking information comes from studies on the very young, where endothelial dependent vasodilatation has found to be impaired in low birth weight infants as young as 3 months (Goh et al. 2001) and 3 days of age (Martin et al. 2000a). In addition, this effect is not simply a consequence of prematurity as no relationship was seen when compared with premature infants of appropriate weight for their gestationel age (Norman and Martin 2003). This very early appearance of altered endothelial function, long before the appearance of any additional risk factors, suggests that the endothelium is crucially important and particularly vulnerable to stress during development.

3. Animal Models in DOHaD

To date, several animal models have been developed to investigate the mechanisms underlying the phenomenon of developmental origins. Initially, many of these were adapted from models of intra-uterine growth restriction (IUGR), such as total calorific or protein restriction and placental insufficiency, but more recently these models include those of maternal obesity and stress, as well as xenobiotics. Many of these have been reviewed extensively elsewhere (Armitage et al. 2004, McMillen and Robinson 2005). What is striking about this data is that the offspring phenotypes are fairly consistent, despite variations in the nature, severity, timing and duration of these maternal environmental exposures. One such consistent finding is that of endothelial dysfunction and furthermore it is true for a number of different species, up to and including humans.

Evidence of Endothelial Dysfunction in Animal Models of DOHaD

In animal models, endothelial dysfunction has been assessed in mice, rats and sheep in response to a wide range of maternal stresses ranging from maternal undernutrition, over-nutrition and hypoxia, as well as placental insufficiency and glucocorticoid administration. The vast majority of these studies have utilised the classical organ bath approach and assessed endothelial function in response to acetylcholine, which is known to act through the release of NO, PGI_2 and EDHF (Busse and Fleming 2006).

In rats, evidence from the offspring of fat-fed dams points to alterations in the EDHF pathway underlying the endothelial dysfunction (Taylor et al. 2004). In contrast, the models of maternal undernutrition, whether protein or total calorific intake, appear to suggest alterations in the NO pathway (Lamireau et al. 2002, Franco et al. 2004, Torrens et al. 2006, 2009a), linked to altered oxidative stress (Franco et al. 2004, Langley-Evans and Sculley 2005, Rodford et al. 2008).

The timing of the challenge is important in having an effect on the endothelium. Using a model of nutrient restriction solely in late gestation, Williams et al. (2005) reported no effect on endothelial function, while in sheep, undernutrition or maternal cortisol in the first 30 days (term ~147 days) is sufficient to alter vascular function in the offspring (Roghair et al. 2005, Khan et al. 2005, Cleal et al. 2007) and is even apparent at the fetal stage (Nishina et al. 2003). All of which fits with the evidence from the Dutch Hunger Winter, where those exposed early in gestation were at greater risk of cardiovascular disease (Ravelli et al. 1999). Indeed, in both mice and sheep effects of vascular function in offspring can be seen if the mother undergoes a period of nutrient restriction immediately prior to conception (Watkins et al. 2008, Torrens et al. 2009b).

4. Conclusion

Endothelial dysfunction is an important factor in the progression of cardiovascular disease. It is apparent in adults and, importantly, children of low birth weight and is a common finding in animal models utilised to study the mechanisms of the developmental origins hypothesis. The early appearance of endothelial dysfunction makes it an important risk factor and provides a novel target for paediatric prevention strategies (Leeson 2007).

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References

Antoniades, C., Mussa, S., Shirodaria, C., Lee, J., Diesch, J., Taggart, D. P., Channon, K. M., and Leeson, P.: Relation of preoperative radial artery flow-mediated dilatation to nitric oxide bioavailability in radial artery grafts used in off-pump coronary artery bypass grafting. Amer. J. Cardiol. *103*, 216–220 (2009)

Armitage, J. A., Khan, I. Y., Taylor, P. D., Nathanielsz, P. W., and Poston, L.: Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J. Physiol. *561*, 355–377 (2004)

Busse, R., and Fleming, I.: Vascular endothelium and blood flow. In: Handb. Exp. Pharmacol.; pp. 43–78. 2006

Cleal, J. K., Poore, K. R., Boullin, J. P., Khan, O., Chau, R., Hambidge, O., Torrens, C., Newman, J. P., Poston, L., Noakes, D. E., Hanson, M. A., and Green, L. R.: Mismatched pre- and postnatal nutrition leads to cardiovascular dysfunction and altered renal function in adulthood. Proc. Natl. Acad. Sci. USA *104*, 9529–9533 (2007)

Delles, C., Miller, W. H., and Dominiczak, A. F.: Targeting reactive oxygen species in hypertension. Antioxid. Redox Signal *10*, 1061–1077 (2008)

Franco, M. C., Fortes, Z. B., Akamine, E. H., Kawamoto, E. M., Scavone, C., Britto, L. R. de, Muscara, M. N., Teixeira, S. A., Tostes, R. C., Carvalho, M. H., and Nigro, D.: Tetrahydrobiopterin improves endothelial dysfunction and vascular oxidative stress in microvessels of intrauterine undernourished rats. J. Physiol. *558*, 239–248 (2004)

Franco, M. C., Higa, E. M., D'Almeida, V., Sousa, F. G. de, Sawaya, A. L., Fortes, Z. B., and Sesso, R.: Homocysteine and nitric oxide are related to blood pressure and vascular function in small-for-gestational-age children. Hypertension *50*, 396–402 (2007)

Gluckman, P. D., Hanson, M. A., Cooper, C., and Thornburg, K. L.: Effect of in utero and early-life conditions on adult health and disease. New Engl. J. Med. *359*, 61–73 (2008)

Goh, K. L., Shore, A. C., Quinn, M., and Tooke, J. E.: Impaired microvascular vasodilatory function in 3-month-old infants of low birth weight. Diabetes Care *24*, 1102–1107 (2001)

Goodfellow, J., Bellamy, M. F., Gorman, S. T., Brownlee, M., Ramsey, M. W., Lewis, M. J., Davies, D. P., and Henderson, A. H.: Endothelial function is impaired in fit young adults of low birth weight. Cardiovasc. Res. *40*, 600–606 (1998)

Hsueh, W. A., Lyon, C. J., and Quinones, M. J.: Insulin resistance and the endothelium. Amer. J. Med. *117*, 109–117 (2004)

Hunt, B. J., and Jurd, K. M.: The endothelium in health and disease. In: Hunt, B. J., Poston, L., Schachter, M., and Halliday, A. (Eds.): An Introduction to Vascular Biology. Cambridge University Press 2002

Ijzerman, R. G., van Weissenbruch, M. M., Voordouw, J. J., Yudkin, J. S., Serne, E. H., Delemarre-van de Waal, H. A., and Stehouwer, C. D.: The association between birth weight and capillary recruitment is independent of blood pressure and insulin sensitivity: a study in prepubertal children. J. Hypertens. *20*, 1957–1963 (2002)

Juonala, M., Viikari, J. S., Laitinen, T., Marniemi, J., Helenius, H., Ronnemaa, T., and Raitakari, O. T.: Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. Circulation *110*, 2918–2923 (2004)

Khan, O. A., Torrens, C., Noakes, D. E., Poston, L., Hanson, M. A., Green, L. R., and Ohri, S. K.: Effects of pre-natal and early post-natal undernutrition on adult internal thoracic artery function. Eur. J. Cardiothorac. Surg. *28*, 811–815 (2005)

Lamireau, D., Nuyt, A. M., Hou, X., Bernier, S., Beauchamp, M., Gobeil, F. Jr., Lahaie, I., Varma, D. R., and Chemtob, S.: Altered vascular function in fetal programming of hypertension. Stroke *33*, 2992–2998 (2002)

Landmesser, U., Dikalov, S., Price, S. R., McCann, L., Fukai, T., Holland, S. M., Mitch, W. E., and Harrison, D. G.: Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J. Clin. Invest. *111*, 1201–1209 (2003)

Landmesser, U., Hornig, B., and Drexler, H.: Endothelial function: a critical determinant in atherosclerosis? Circulation *109*, II27–II33 (2004)

Langley-Evans, S. C., and Sculley, D. V.: Programming of hepatic antioxidant capacity and oxidative injury in the ageing rat. Mech. Ageing Dev. *126*, 804–812 (2005)

Leeson, P.: Pediatric prevention of atherosclerosis: targeting early variation in vascular biology. Pediatrics *119*, 1204–1206 (2007)

Leeson, C. P., Kattenhorn, M., Morley, R., Lucas, A., and Deanfield, J. E.: Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. Circulation *103*, 1264–1268 (2001)

Leeson, C. P., Whincup, P. H., Cook, D. G., Donald, A. E., Papacosta, O., Lucas, A., and Deanfield, J. E.: Flow-mediated dilation in 9- to 11-year-old children: the influence of intrauterine and childhood factors. Circulation *96*, 2233–2238 (1997)

Martin, H., Gazelius, B., and Norman, M.: Impaired acetylcholine-induced vascular relaxation in low birth weight infants: implications for adult hypertension? Pediatr. Res. *47*, 457–462 (2000a)

Martin, H., Hu, J., Gennser, G., and Norman, M.: Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation *102*, 2739–2744 (2000b)

McMillen, I. C., and Robinson, J. S.: Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol. Rev. *85*, 571–633 (2005)

Napoli, C., and Ignarro, L. J.: Nitric oxide and pathogenic mechanisms involved in the development of vascular diseases. Arch. Pharm. Res. *32*, 1103–1108 (2009)

Napoli, C., Nigris, F. de, Williams-Ignarro, S., Pignalosa, O., Sica, V., and Ignarro, L. J.: Nitric oxide and atherosclerosis: an update. Nitric Oxide *15*, 265–279 (2006)

Nishina, H., Green, L. R., McGarrigle, H. H., Noakes, D. E., Poston, L., and Hanson, M. A.: Effect of nutritional restriction in early pregnancy on isolated femoral artery function in mid-gestation fetal sheep. J. Physiol. *553*, 637–647 (2003)

Norman, M., and Martin, H.: Preterm birth attenuates association between low birth weight and endothelial dysfunction. Circulation *108*, 996–1001 (2003)

Ravelli, A. C., Der Meulen, J. H., Osmond, C., Barker, D. J., and Bleker, O. P.: Obesity at the age of 50 y in men and women exposed to famine prenatally. Amer. J. Clin. Nutr. *70*, 811–816 (1999)

Rodford, J. L., Torrens, C., Siow, R. C., Mann, G. E., Hanson, M. A., and Clough, G. F.: Endothelial dysfunction and reduced antioxidant protection in an animal model of the developmental origins of cardiovascular disease. J. Physiol. *586*, 4709–4720 (2008)

Roghair, R. D., Lamb, F. S., Miller, F. J. Jr., Scholz, T. D., and Segar, J. L.: Early gestation dexamethasone programs enhanced postnatal ovine coronary artery vascular reactivity. Amer. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R46–R53 (2005)

Taddei, S., Virdis, A., Ghiadoni, L., Sudano, I., and Salvetti, A.: Endothelial dysfunction in hypertension. J. Cardiovasc. Pharmacol. *38*/2, S11–S14 (2001)

Taylor, P. D., Khan, I. Y., Hanson, M. A., and Poston, L.: Impaired EDHF-mediated vasodilatation in adult offspring of rats exposed to a fat-rich diet in pregnancy. J. Physiol. *558*, 943–951 (2004)

Torrens, C., Brawley, L., Anthony, F. W., Dance, C. S., Dunn, R., Jackson, A. A., Poston, L., and Hanson, M. A.: Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. Hypertension *47*, 982–987 (2006)

Torrens, C., Kelsall, C. J., Hopkins, L. A., Anthony, F. W., Curzen, N. P., and Hanson, M. A.: Atorvastatin restores endothelial function in offspring of protein restricted rats in a cholesterol-independent manner. Hypertension *53*, 661–667 (2009a)

Torrens, C., Snelling, T. H., Chau, R., Shanmuganathan, M., Cleal, J. K., Poore, K. R., Noakes, D. E., Poston, L., Hanson, M. A., and Green, L. R.: Effects of pre- and periconceptional undernutrition on arterial function in adult female sheep are vascular bed dependent. Exp. Physiol. *94*, 1024–1033 (2009b)

Watkins, A. J., Wilkins, A., Cunningham, C., Perry, V. H., Seet, M. J., Osmond, C., Eckert, J. J., Torrens, C., Cagampang, F. R., Cleal, J., Gray, W. P., Hanson, M. A., and Fleming, T. P.: Low protein diet fed exclusively during mouse oocyte maturation leads to behavioural and cardiovascular abnormalities in offspring. J. Physiol. *586*, 2231–2244 (2008)

Williams, S. J., Hemmings, D. G., Mitchell, J. M., McMillen, I. C., and Davidge, S. T.: Effects of maternal hypoxia or nutrient restriction during pregnancy on endothelial function in adult male rat offspring. J. Physiol. *565*, 125–135 (2005)

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Perinatal Programming and the Metabolic Syndrome

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With 2 Figures and 1 Table

Abstract

In parallel with the general 'diabesity' epidemic, diabetes during pregnancy and the number of overweight pregnant women reach dramatic prevalences. Consequently, mean birth weight and frequencies of 'fat babies' rise. Neonatal excess weight, however, predisposes one to be overweight in later life, which is the main risk factor of the metabolic syndrome. Experimental data indicate that fetal and neonatal overfeeding lead to hormonally induced, epigenetic malprogramming of key regulatory systems of body weight and metabolism resulting in permanent disposition to obesity, diabetes, and the metabolic syndrome. Therefore, universal screening and respective therapy of all types of diabetes during pregnancy, avoidance of maternal excess weight and overnutrition during pregnancy as well as avoidance of neonatal and infant overfeeding, especially by promotion of breastfeeding, may be effective approaches for a primary prevention of 'diabesity' and its cardiovascular endpoints.

Zusammenfassung

Parallel zu der mittlerweile globalen Adipositas- und Diabetesepidemie steigen die Prävalenzen von Diabetes und Übergewicht bei schwangeren Frauen dramatisch, und infolgedessen auch das mittlere Geburtsgewicht und die Häufigkeit von Übergewicht bei Geburt. Neonatales Übergewicht prädisponiert aber zu späterem Überwicht, welches wiederum den Hauptrisikofaktor für das metabolische Syndrom darstellt. Experimentelle Daten zeigen, dass fetale und neonatale Überernährung zu einer hormonell induzierten, epigenetischen Fehlprogrammierung entscheidender Regelsysteme von Körpergewicht und Stoffwechsel führt, woraus eine permanente Disposition zu Adipositas, Diabetes und metabolischem Syndrom resultieren kann. Deshalb sind ein generelles Screening und eine entsprechende Therapie jedweder Form von Glukosetoleranzstörung während der Schwangerschaft, die Vermeidung eines mütterlichen Übergewichts und/oder einer Überernährung während der Schwangerschaft sowie die Vermeidung neonataler und frühkindlicher Überernährung, insbesondere durch Förderung des Stillens, als effektive, genuine Maßnahmen zur Primärprävention der Adipositas-/Diabetes-Epidemie und ihrer kardiovaskulären Endpunkte dringend zu empfehlen.

1. Introduction

The impact of the intrauterine and early postnatal environment on lasting determination of fundamental processes of life, health or disease, is becoming more and more accepted. Investigations and hypotheses by the groups of Hales and Barker led to the postulation of a so-called 'small-baby-syndrome', which was explained by a 'thrifty phenotype' acquired by poor fetal nutrition (Hales and Barker 1992). This concept has contributed to worldwide attention to the phenomenon of early conditioning and terms like 'nutritional programming' or 'imprinting' were proposed to describe it. Recently, Gluckman and Hanson aimed to formulate a general theoretical framework for phenomena of 'developmental origins of health and diseases' by putting forward the 'match-mismatch' hypothesis (Gluckman and Hanson 2004).

To the best of my knowledge, however, it was Günter Dörner who was the first (1975) to postulate a general etiological concept on epigenetic, perinatal programming of the lifetime function of fundamental regulatory systems of the organism and, thereby, the possibility of perinatal prophylaxis (Dörner 1975, 1976, 1980). In the early 1970s in a series of clinical as well as experimental studies Dörner demonstrated and postulated that especially hormones are environment-dependent organizers of the neuro-endocrine system, which is the definitive regulator of all fundamental processes of life. When present in non-physiological concentrations, induced by alterations of the intrauterine and/or early postnatal environment, hormones can also act as 'endogenous functional teratogens' by 'malprogramming' the 'neuro-endocrine-immune system (NEIS)', leading to developmental disorders and diseases throughout life.

2. Diabetes in Pregnancy, Perinatal Hyperinsulinism, and Perinatal Programming

Against the backdrop of a long-term conceptual and semantic history (Tab. 1), mechanistic evidence for the existence of the biomedical phenomenon of fetal programming originated from the fields of reproductive behaviour and stress (Dörner 1975, 1976, 1980, Meaney et al. 1996, Francis and Meaney 1999), with research addressing the significance of altered concentrations of the respective steroid hormones (sexual steroids, or gluco- and mineralocorticoids) during critical periods of perinatal development for a permanent malprogramming of the affected subsystems of the NEIS. However, the results of clinical investigations and animal experiments on the long-term effects of maternal diabetes and, thereby, fetal overfeeding during pregnancy for the development of the offspring, have for a long time provided key support for the concept of fetal programming of disposition to later diseases.

Table 1 Historical milestones in the establishment of the concept of perinatal, environmental (epigenetic) programming of ontogenesis, health and disease.

Author(s)	Year	Term or Concept
Lamarck	1809	Heredity of acquired conditions
Saint-Hilaire	1837	Teratology (structural, i.e., teratomorphogenesis)
Lorenz	1935	Behavioural imprinting
Waddington	1942	Epigenetics
Dubos	1966	Biological Freudianism
Dörner	1975	'Pre- and neonatal pre-programming', 'Functional Teratology'
Freinkel and Metzger	1979	Fuel-mediated teratogenesis
Lucas	1991	Nutritional programming
Hales and Barker	1992	Fetal programming, 'small-baby-syndrome'
Gluckman and Hanson	2004	Match-mismatch hypothesis

2.1 Clinical Observations

Pregnancy is a diabetogenic situation *per se*. Women with gestational diabetes (GD), just as pre-gravid diabetic women, are classed as risk pregnancies, and their offspring show increased perinatal morbidity and mortality. The disturbances manifested during the neonatal period, apart from a tendency for hypoglycaemia, hyperbilirubinaemia, neonatal respiratory

distress syndrome etc., are characterised above all by an increased prevalence of macrosomia. This is caused by the virtually pathognomic fetal and perinatal hyperinsulinism, which arises because of the materno-fetal hyperglycaemia and consequent overstimulation of the fetal pancreatic B-cells.

By the 1970s it had been shown in a cohort of 4,000 diabetic patients that type 2 diabetes was 'inherited' more frequently through the mother than the father (Dörner and Mohnike 1976). The offspring of a mother with diabetes during pregnancy shows an increased tendency to become overweight or obese in childhood (Plagemann et al. 1997a) accompanied by disturbances of glucose tolerance, insulin secretion, and insulin sensitivity (Silverman et al. 1995, Plagemann et al. 1997b).

It is particularly noticeable that these alterations may occur even independently from genetic influences and the type of maternal gestational hyperglycaemia (Plagemann et al. 1997a, b, Pettitt et al. 1983, Dabelea et al. 2000). However, they do show marked correlations with fetal metabolic alterations of the affected children, namely the degree of fetal and perinatal hyperinsulinism (Kohlhoff and Dörner 1990, Silverman et al. 1995, Plagemann et al. 1997b, Harder et al. 2001). In particular, a positive correlation was found between the level of amniotic fluid insulin or perinatal hyperinsulinaemia and the increase in relative body weight and the risk of impaired glucose tolerance (IGT) in later life for children of diabetic mothers (Silverman et al. 1995, Plagemann et al. 1997b). The latter has to be interpreted as indication of a persistent influence of the diabetic intrauterine milieu and consequent hyperinsulinism for the long-term outcome, in the sense of a hormonally initiated malprogramming. Moreover, by the 1980s it could be shown by our group that the long-term deleterious consequences of exposure to a diabetic intrauterine environment respond to primary prevention, even over successive generations (Dörner et al. 1984, 1985, 1987). Against these data, accompanied by respective experimental observations, in 1987 we proposed that "[…] an epigenetic transmission of acquired conditions appears to be possible over several generations (epigenetic transmission rule) […]" (Dörner et al. 1987).

2.2 Experimental Observations

Animal experiments have confirmed early that maternal gestational hyperglycaemia may lead to overweight, impaired glucose tolerance, hyperinsulinaemia and insulin resistance in the juvenile and adult offspring, irrespective of any genetic disposition (Aerts and Van Assche 1979, Aerts et al. 1990, Dörner et al. 1988, Oh et al. 1991). Remarkably, moreover, female F1 offspring of gestationally diabetic F0 dams spontaneously develop gestational hyperglycaemia. In the F2 offspring thereby exposed *in utero* this can then in turn lead to diabetogenic disturbances in later life again, and therefore an epigenetic materno-fetal transmission of increased disposition to diabetes is possible through a number of subsequent generations even without any genetic predisposition (Aerts and Van Assche 1979, Aerts et al. 1990, Dörner et al. 1988, Dörner and Plagemann 1994, Plagemann 2004, 2005a, 2005b, 2008).

A permanent influence on the function of pancreatic B-cells has been proposed, on the one hand, as an aetiopathogenetic mechanism of this prenatally acquired malprogramming, in particular hyperplasia and hyperactivity leading in the long-term to impairment of insulin secretion in the offspring (Aerts et al. 1990). On the other hand, studies have shown that permanent alterations of the programming of neuroendocrine and vegetative functional systems play a key aetiopathogenetic role (Dörner et al. 1988, Dörner and Plagemann 1994,

Plagemann et al. 1998, 1999a, Plagemann 2004, 2005a, b, 2008). Thus, the experimental induction of gestational hyperglycaemia not only leads to perinatal hyperinsulinaemia but also to increased insulin concentrations within the immature brain, especially the hypothalamus, i.e., the key regulator of body weight, food intake, and metabolism, followed by morphological characteristics of permanent, i.e., lifelong dysplasia of central nervous control centres for metabolism and body weight. In particular, this affects the ventromedial hypothalamic nucleus (VMN), which develops a permanent dysplasia, neuronal hypotrophy, and disturbed function as a result of the exposure to increased insulin concentrations during critical periods of development (Dörner et al. 1988, Dörner and Plagemann 1994, Plagemann et al. 1999a, Heidel et al. 1999, Davidowa and Plagemann 2001). Furthermore, as an expression of perinatally acquired hypothalamic resistance to the peripheral satiety signals insulin and leptin, there is a permanent disorganisation and malfunction of specific neuropeptidergic neurones in the arcuate nucleus (ARC). Particularly important seems to be a lifelong increased activity and number of neurones which express the orexigenic peptides, galanin and neuropeptide Y (Plagemann et al. 1998, 1999b), while the number and function of anorexigenic neuropeptidergic neurons becomes permanently decreased (Davidowa et al. 2003, Franke et al. 2005). All this is accompanied by and correlated to a permanently increased disposition to diabetes and obesity, characterised by hyperphagia, overweight, basal hyperinsulinaemia, insulin resistance, and IGT. Similar findings have recently been described occuring in offspring of rat dams with diet-induced obesity during pregnancy (Kirk et al. 2009). It should be emphasised that both clinically and experimentally the disturbances occur even independently of the birth weight and can also be observed in animals treated neonatally with insulin experimentally applicated either peripherally or only intrahypothalamically (Dörner et al. 1988, Dörner and Plagemann 1994, Plagemann et al. 1992a, 1992b, Plagemann 2008). All of this points towards a hormonally and nutritionally induced complex malprogramming of the neurovegetative control of body weight and metabolism, which may occur independent of the genetic background and irrespective of birth weight *per se*.

2.3 Conclusions

Taking together epidemiological, clinical, and experimental observations, it seems obvious that fetal hyperinsulinism induced by maternal hyperglycaemia/overweight has 'functionally teratogenic' significance for a permanently increased disposition to obesity, diabetes, the metabolic syndrome, and subsequent cardiovascular diseases in the offspring (Fig. 1). Given that gestational diabetes has meanwhile reached a prevalence in excess of 10 % in the developed industrialised countries, while maternal overweight affects more than one third of pregnancies, it seems urgently necessary that all pregnant women should be screened for glucose intolerance and adequately treated as a measure of primary prevention.

3. Birth Weight, Neonatal Nutrition, and Lasting Programming

The widely discussed data and hypotheses of the working groups around Barker and Hales have led the postulation of a 'small baby syndrome' according to which fetal undernutrition and 'low birth weight' predispose one to the later development of alterations of metabolism,

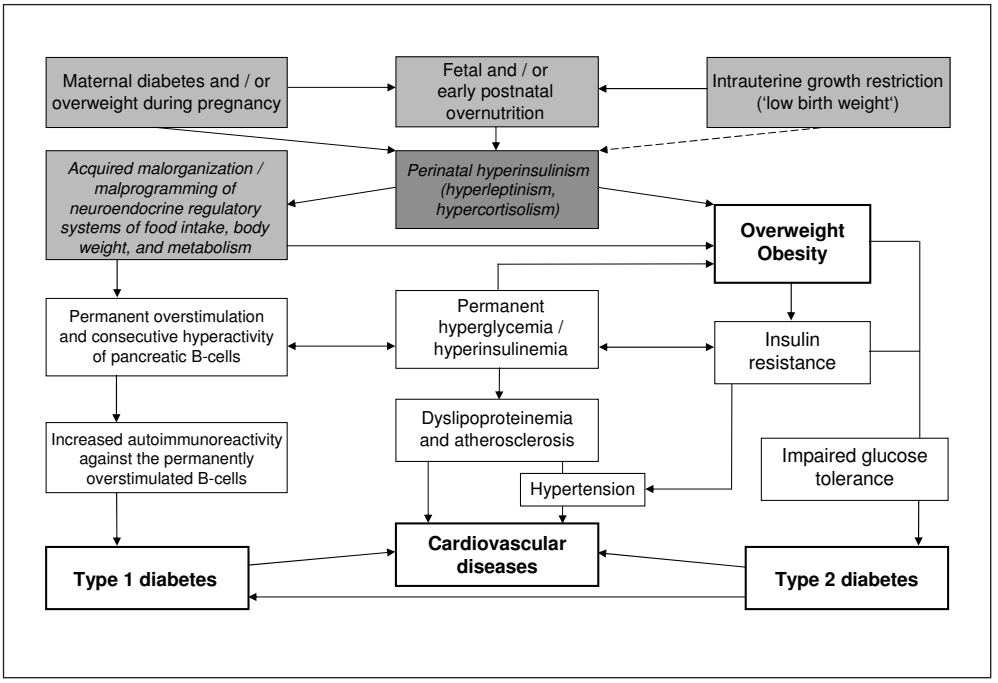

Fig. 1 Proposal of a pathogenetic framework, mechanisms and consequences of perinatal malprogramming, showing the etiological significance of perinatal overfeeding and hyperinsulinism for excess weight gain, obesity, diabetes mellitus (type 2 as well as type 1) and subsequent cardiovascular diseases (CVD) in later life. Adapted from and modified to Dörner and Plagemann (1994).

body weight and the cardiovascular system in terms of type 2 diabetes, metabolic syndrome, and cardiovascular diseases (Hales and Barker 1992, Barker 1998).

3.1 Clinical Observations

Since the mid 1990s, an impressive number of studies of various populations have been published which show clearly a phenomenologically persuasive link between a 'low birth weight' and a subsequently increased risk of aspects of the metabolic syndrome. Even in the Pima Indian study, a long-term investigation in a North American population with a particularly high disposition to diabetes and obesity, it was shown that type 2 diabetes associated with overweight in adulthood was more prevalent in patients who had been overweight at birth, but also in patients who were neonatally underweight (McCance et al. 1994). By means of meta-analysis of all respective studies beyond that on the Pima Indians we could confirm these observations (Harder et al. 2007a). This leads to the postulation that, in fact, not a linear-inverse, but a U-shaped relationship exists between birth weight and subsequent diabetes, obesity, and metabolic syndrome.

Whereas the pathogenetic context seems more or less obvious for fetal overfeeding and neonatal overweight (see above), no clear aetiopathogenetic link has been established for reduced perinatal weight (Dörner and Plagemann 1994, Lucas et al. 1999, Plagemann

2005a, b, 2008). In particular, it should be emphasised that no independent causal link has been established between low birth weight and a subsequently increased disposition to obesity, i. e., the pathophysiological key for diabetes and cardiovascular diseases (CVD) in terms of the metabolic syndrome. It therefore remains to be clarified whether fetal growth restriction and underweight at birth, or rather the quality and quantity of early postnatal nutrition and weight gain in early infancy have pathophysiological significance for the prospective risk.

The central pathogenetic importance of later overweight is clear, especially within the context of the metabolic syndrome, but though a positive correlation has frequently been demonstrated between weight at birth and healthy weight or overweight in later life, an independent inverse relationship has never been shown (Harder et al. 2007b). Increased weight gain in neonatal life, on the other hand, leads to increased disposition for obesity in later life (Dörner et al. 1977, Stettler et al. 2002). It also seems remarkable that increased weight gain in early childhood, in particular in underweight newborns, leads to early manifestation of insulin resistance (Crowther et al. 2000, Fewtrell et al. 2000). Finally, it has been variously shown that increased weight gain in early childhood is a predictive factor for disposition to metabolic syndrome and cardiovascular risk in adulthood, in particular in the case of low birth weight (Vanhala et al. 1999, Eriksson et al. 1999, Forsen et al. 1999).

3.2 Experimental Observations

Against the backdrop of the 'thrifty phenotype hypothesis', investigations on the 'small baby syndrome' frequently use animal models of maternal underfeeding during gestation and lactation, which experimentally leads to intrauterine and neonatal growth restriction in the offspring (Petry et al. 1997). However, it should be noted that even under extreme famine conditions in humans birth weight is hardly affected (Stanner et al. 1997, Stein et al. 2004). Moreover, examination of the results on the long-term effects obtained with animal models also showed no consistent congruence with the observations after low birth weight in humans.

Thus, for example, animal experiments of Hales' group have shown that offspring born to rat dams that were malnourished during gestation and lactation do not become overweight later on, but rather show a life-long persistence of low weight. This is associated with a permanently reduced food intake (Petry et al. 1997). The animals predominantly show increased instead of decreased glucose tolerance. In contrast to the metabolic syndrome in humans, hyperinsulinaemia and insulin resistance do not occur, but rather lower insulin secretion. All these findings persist even after dietary provocation in later life (Petry et al. 1997, Moura et al. 1997). Moreover, an increased life span was even observed in this animal model (Ozanne and Hales 2004).

In contrast, it has been previously postulated that transition from fetal malnutrition to early postnatal overfeeding could play a key role in the aetiopathogenesis of the 'small baby syndrome' (Dörner and Plagemann 1994), especially since it seems quite possible that low weight neonates, also in the epidemiological studies, had been 'overfed' and possibly even 'fattened' in neonatal life. Similar hypotheses on the possible significance of early postnatal nutrition for the long-term outcome of underweight neonates have since been formulated by many other authors, including Hales and Barker themselves (Fewtrell et al. 2000, Eriksson et al. 1999). Meanwhile, rapid neonatal weight gain, which mainly is attributable

to absolute or relative overnourishment, is an established risk factor to explain the 'small baby syndrome' (Stettler et al. 2002, Monteiro and Victora 2005), thereby confirming our earlier data and concepts (Dörner and Plagemann 1994).

The influence of early postnatal overnutriton on the later outcome of metabolism and body weight has often been investigated using the 'small litter model'. Rats which are overfed in the early postnatal period show phenotypic alterations through juvenile age into adulthood, such as overweight, hyperphagia, glucose intolerance, hyperinsulinaemia, dyslipidaemia and increased blood pressure, which correspond in critical aspects to those of the metabolic syndrome in humans (Plagemann et al. 1992b, 1999c, Boullu-Ciocca et al. 2005). This is all the more remarkable because clinical findings suggest that early postnatal overfeeding in humans also predisposes for an increased risk of metabolic syndrome in later life (see above). But here too the pathophysiological causes and mechanisms are not clear.

As already mentioned, neuropeptidergic hypothalamic centres play a key role in the regulation of food intake, body weight, and metabolism. Note that very similar to offspring of diabetic dams, neonatally overfed 'small litter rats' show persisting disorganization and malprogramming of these regulatory systems, including malfunction of the VMN and resistance of the ARC to the circulating satiety signals insulin and leptin, which may explain their neonatally acquired long-term risk in terms of a neuro-vegetative malprogramming of the regulation of body weight and metabolism (Dörner et al. 1988, Dörner and Plagemann 1994, Plagemann et al. 1999c, 1999d, Plagemann 2004, 2005a, b, 2008). Moreover, recently our group could show that this neonatally acquired hypothalamic leptin and insulin resistance might be, at least in part, due to a nutritionally induced altered DNA methylation pattern of the hypothalamic promoter region of the gene encoding proopiomelanocortin (POMC), which is the most important anorexigenic neuropeptide (Plagemann et al. 2009). In particular, we observed that rats raised in small litters showed hypermethylation of essential transcription factor binding sites of the POMC promoter, leading to a lack of up-regulation of POMC expression despite marked hyperleptinaemia and hyperinsulinaemia. The extent of hypermethylation of these binding sequences was shown to depend upon neonatal blood glucose levels (Plagemann et al. 2009). Similar was observed for the insulin receptor promoter (Plagemann et al. 2010). Neonatally acquired alterations were again strongly correlated to hyperglycaemic conditions in neonatal life, supporting the concept of (over-)nutrition-dependent epigenetic programming during critical windows of early development.

3.3 Conclusions

From an epidemiological point of view there is a clear *phenomenological* link between reduced birth weight and subsequently elevated risk in terms of the metabolic syndrome. The critical integration of epidemiological, clinical, and experimental observations, however, casts doubt on a causal relationship. Rather, neonatal overfeeding and rapid early weight gain with increased fat deposition and its hormonal consequences (hyperinsulinaemia, hyperleptinaemia etc.) could be of lifelong pathophysiological importance especially for underweight newborns (Fig. 1). Therefore, prophylactic recommendations should focus on the recognition, avoidance, and optimal treatment of the causes of intrauterine growth restriction (nicotine, alcohol, stress, gestosis, etc.), and also on the avoidance of neonatal overfeeding.

4. Synopsis

Globally, diabetes mellitus, obesity, and the metabolic syndrome are life-shortening diseases, and the continual dramatic increase in their prevalence represents a health problem of the greatest relevance, so that there is an urgent need for prevention strategies.

Generally, complex pathogenetic processes, in particular those relating to the so-called 'diseases of civilisation', originate from an impaired interaction or an imbalance between environmental factors and the genetic matrix. From a practical clinical viewpoint it is therefore extremely important to characterise early environmental, epi-genetic risk factors with long-term malprogramming effects which can be influenced by preventive measures in critical periods of early development.

At least every tenth pregnancy in the developed industrialised countries is affected by a disturbed glucose tolerance. The great majority of cases go unrecognised, and thus untreated, because there is no universal screening for glucose intolerance of all pregnant women. Moreover, at least one third of pregnant women in the westernized world are overweight, leading to similar consequences for the fetus as gestational diabetes.

Fetal or early postnatal hyperinsulinism (and also hyperleptinism), induced as a result of maternal gestational hyperglycaemia, overweight and/or early postnatal overfeeding, may act as a hormonal 'functional teratogen' during critical periods of differentiation and maturation of the complex NEIS. This can lead to irreversible, lifelong malprogramming of fundamental control systems and the central nervous regulation of metabolism, food intake, and body weight. The result is a disposition to become overweight, and the development of associated metabolic disturbances such as hyperinsulinaemia, insulin resistance, IGT, type 2 and even type 1 diabetes, and the metabolic syndrome, including critical clinical endpoints such as cardiovascular diseases (Fig. 1).

Moreover, even irrespective of the quality and quantity of the neonatal nutrition of underweight newborns, it is probable that they are exposed to a further form of hormone-dependent malprogramming. As a consequence of fetal malnourishment they show considerable alterations of the fetal and perinatal glucocorticoid levels, at least in the form of temporary hypercortisolism during critical windows of early development, with the potential consequence of glucocorticoid-induced malprogramming of the hypothalamic-pituitary-adrenal (HPA) axis and neuro-vegetative stress system, in general. This, in turn, may substantially contribute to long-term risk of central obesity due to increased HPA activity and accompanying metabolic and cardiovascular disorders, in a similar sense as proposed above according to exposure to fetal hyperinsulinism and hyperleptinism (Fig. 1).

5. Prospects

The aspects presented here have a model character, and show by way of example the long-term pathophysiological significance of abnormal nutritive, metabolic, and most of all hormonal, conditions during critical fetal and perinatal periods of development, implying at the same time that *primary* prophylactic management is possible by optimising the fetal and early postnatal environmental conditions. In this context, the general aetiopathology should be extended to include developmentally acquired epigenetic dispositions which may act for the long-term, as exemplarily illustrated in Figure 2. Molecular causes could lie, for example,

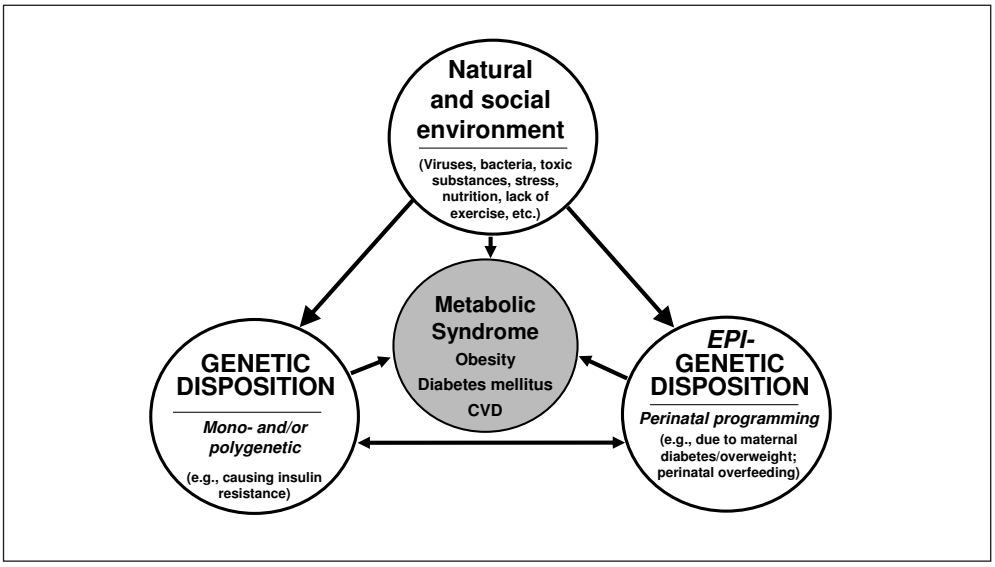

Fig. 2 Fundamental concept on the multi-etiological origin of obesity, diabetes mellitus, the metabolic syndrome, and subsequent cardiovascular diseases (CVD), pre-programmed by pre- and perinatal conditions. Adapted from and modified to Plagemann (2004, 2005a, 2005b, 2008).

in perinatally acquired alterations of the DNA methylation pattern of promoters of genes encoding for receptors and/or neuropeptides which are involved at a cybernetically key position in the regulation of the NEIS. All this may be of critical importance for the development and lifelong functioning, or permanent malfunctioning, of fundamental regulatory systems and life processes, and in future should therefore be taken much more into account in research into aetiopathogenesis and first of all preventive medicine. This may open enormous chances and challenges of perinatal medicine to effectively prevent increasing risk of 'modern diseases' for the long term and, thereby, reduce the increasing burden for individuals and societies.

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References

Aerts, L., and Van Assche, F. A.: Is gestational diabetes an acquired condition? J. Dev. Physiol. *1*, 219–225 (1979)

Aerts, L., Holemans, K., and Van Assche, F. A.: Maternal diabetes during pregnancy: Consequences for the offspring. Diabetes Metab. Rev. *6*, 147–167 (1990)

Barker, D. J. P.: In utero programming of chronic disease. Clinical Science *95*, 115–128 (1998)

Boullu-Ciocca, S., Dutour, A., Guillaume, V., Achard, V., Oliver, C., and Grino, M.: Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. Diabetes *54*, 197–203 (2005)

Crowther, N. J., Trusler, J., Cameron, N., Toman, M., and Gray, I. P.: Relation between weight gain and beta-cell secretory activity and non-esterified fatty acid production in 7-year-old african children: results from the birth to ten study. Diabetologia *43*, 978–985 (2000)

Dabelea, D., Hanson, R. L., Lindsay, R. S., Pettitt, D. J., Imperatore, G., Gabir, M. M., Roumain, J., Bennett, P. H., and Knowler, W. C.: Intrauterine exposure to diabetes conveys risks for type II diabetes and obesity: A study of discordant sibships. Diabetes *49*, 2208–2211 (2000)

Davidowa, H., and Plagemann, A.: Inhibition by insulin of hypothalamic VMN neurons in rats overweight due to postnatal overfeeding. Neuroreport *12*, 3201–3204 (2001)

Davidowa, H., Li, Y., and Plagemann, A.: Altered responses to orexigenic (AGRP, MCH) and anorexigenic (alpha-MSH, CART) neuropeptides of paraventricular hypothalamic neurons in early postnatally overfed rats. Eur. J. Neurosci. *18*, 613–621 (2003)

Dörner, G.: Perinatal hormone levels and brain organization. In: Stumpf, W. E., and Grant, L. D. (Eds.): Anatomical Neuroendocrinology; pp. 245–252. Basel: Karger 1975

Dörner, G.: Hormones and Brain Differentiation. Amsterdam, Oxford, New York: Elsevier 1976

Dörner, G.: Die Ontogenese des neuroendokrinen Systems als kinetischer Prozess. Nova Acta Leopoldina NF Bd. *51*, Nr. 237, 279–291 (1980)

Dörner, G., and Mohnike, A: Further evidence for a predominantly maternal transmission of maturity-onset type diabetes. Endokrinologie *68*, 121–124 (1976)

Dörner, G., and Plagemann, A.: Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm. Metab. Res. *26*, 213–221 (1994)

Dörner, G., Grychtolik, H., und Julitz, M.: Überernährung in den ersten drei Lebensmonaten als entscheidender Risikofaktor für die Entwicklung von Fettsucht und ihrer Folgeerkrankungen. Dt. Gesundheitswesen *32*, 6–9 (1977)

Dörner, G., Plagemann, A., Rückert, J., Götz, F., Rohde, W., Stahl, F., Kürschner, U., Gottschalk, J., Mohnike, A., and Steindel, E.: Teratogenetic maternofoetal transmission and prevention of diabetes susceptibility. Exp. Clin. Endocrinol. *91*, 247–258 (1988)

Dörner, G., Plagemann, A., and Reinagel, H.: Familial diabetes aggregation in type 1 diabetics: gestational diabetes an apparent risk factor for increased diabetes susceptibility in the offspring. Exp. Clin. Endocrinol. *89*, 84–90 (1987)

Dörner, G., Steindel, E., Kohlhoff, R., Reiher, H., Anders, B., Verlohren, H. J., and Hielscher, K.: Further evidence for a preventive therapy of insulin-dependent diabetes mellitus in the offspring by avoiding maternal hyperglycaemia during pregnancy. Exp. Clin. Endocrinol. *86*, 129–140 (1985)

Dörner, G., Steindel, E., Thoelke, H., and Schliack, V.: Evidence for decreasing prevalence of diabetes mellitus in childhood apparently produced by prevention of hyperinsulinism in the fetus and newborn. Exp. Clin. Endocrinol. *84*, 134–142 (1984)

Dubos, R., Savage, D., and Schaedler, R.: Biological Freudianism: lasting effects of early environmental influences. Pediatrics *38*, 789–800 (1966)

Eriksson, J. G., Forsén, T., Winter, P. D., Osmond, C., and Barker, D. J. P.: Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ *318*, 427–431 (1999)

Fewtrell, M. S., Doherty, C., Cole, T. J., Stafford, M., Hales, C. N., and Lucas, A.: Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9–12 years. Diabetologia *43*, 714–717 (2000)

Forsén, T., Eriksson, J. G., Tuomilehto, J., Osmond, C., and Barker, D. J. P.: Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ *319*, 1403–1407 (1999)

Francis, D. D., and Meaney, M. J.: Maternal care and the development of stress response. Curr. Opin. Neurobiol. *9*, 128–134 (1999)

Franke, K., Harder, T., Aerts, L., Melchior, K., Fahrenkrog, S., Rodekamp, E., Ziska, T., Van Assche, F. A., Dudenhausen, J. W., and Plagemann, A.: 'Programming' of orexigenic and anorexigenic hypothalamic neurons in offspring of treated and untreated diabetic mother rats. Brain Res. *1031*, 276–283 (2005)

Freinkel, N., and Metzger, B. E.: Pregnancy as a tissue culture experience: the critical implications of maternal metabolism for fetal development. In: Pregnancy Metabolism, Diabetes, and the Fetus; pp. 3–23. Ciba Foundation Symposium *63*. Amsterdam: Excerpta Medica 1979

Gluckman, P. D., and Hanson, M. A.: Living with the past: evolution, development, and patterns of disease. Science *305*, 1733–1736 (2004)

Hales, C. N., and Barker, D. J. P.: Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia *35*, 595–601 (1992)

Harder, T., Kohlhoff, R., Dörner, G., Rohde, W., and Plagemann, A.: Perinatal 'programming' of insulin resistance in childhood: Critical impact of neonatal insulin and low birth weight in a risk population. Diabetic Med. *18*, 634–639 (2001)

Harder, T., Rodekamp, E., Schellong, K., Dudenhausen, J. W., and Plagemann, A.: Birth weight and subsequent risk of type 2 diabetes: A meta-analysis. Amer. J. Epidemiol. *165*, 849–857 (2007a)

Harder, T., Schellong, K., Stupin, J., Dudenhausen, J. W., and Plagemann, A.: Where is the evidence that low birth weight leads to subsequent obesity? (Letter.) Lancet 369, 1859 (2007b)

Heidel, E., Plagemann, A., and Davidowa, H.: Increased response to NPY of hypothalamic VMN neurons in postnatally overfed juvenile rats. Neuroreport *10*, 1827–1831 (1999)

Kirk, S. L., Samuelson, A. M., Argenton, M., Dhonye, H., Kalamatianos, T., Poston, L., Taylor, P. D., and Coen, C. W.: Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. PLoS One *4*, e5870 (2009)

Kohlhoff, R., and Dörner, G.: Perinatal hyperinsulinism and perinatal obesity as risk factors for hyperinsulinaemia in later life. Exp. Clin. Endocrinol. *96*, 105–108 (1990)

Lamarck, J. B.: Philosophie zoologiques. Paris 1809

Lorenz, K.: Der Kumpan in der Umwelt des Vogels: der Artgenosse als auslösendes Moment sozialer Verhaltensweisen. J. Ornithol. S 83 (1935)

Lucas, A.: Programming by early nutrition in man. In: The Childhood Environment and Adult Disease; pp. 38–55. Ciba Foundation Symposium *156*. Chichester: Wiley 1991

Lucas, A., Fewtrell, M. S., and Cole, T. J.: Fetal origins of adult disease – the hypothesis revisited. BMJ *319*, 245–249 (1999)

McCance, D. R., Pettitt, D. J., Hanson, R. L., Jacobsson, L. T. H., Knowler, W. C., and Bennett, P. H.: Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? BMJ *308*, 942–945 (1994)

Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C. H., Sharma, S., Seckl, J. R., and Plotsky, P. M.: Early environmental regulation of forebrain glucocorticoid receptor gene expression: Implications for adrenocortical responses to stress. Dev. Neurosci. *18*, 49–72 (1996)

Monteiro, P. O. A., and Victora, C. G.: Rapid growth in infancy and childhood and obesity in later life – a systematic review. Obes. Rev. *6*, 143–154 (2005)

Moura, A. S., De Souza, C. F. J., De Freitas, M. P. C., and De Sa, C. C. N. F.: Insulin secretion impairment and insulin sensitivity improvement in adult rats undernourished during early lactation. Res. Comm. Mol. Pathol. Pharmacol. *96*, 179–192 (1997)

Oh, W., Gelardi, N. L., and Cha, C. J. M.: The cross-generation effect of neonatal macrosomia in rat pups of streptozotocin-induced diabetes. Pediatr. Res. *29*, 606–610 (1991)

Ozanne, S. E., and Hales, C. N.: Lifespan: catch-up growth and obesity in male mice. Nature *427*, 411–412 (2004)

Petry, C. J., Ozanne, S. E., Wang, C. L., and Hales, C. N.: Early protein restriction and obesity independently induce hypertension in 1-year-old rats. Clin. Sci. *93*, 147–152 (1997)

Pettitt, D. J., Baird, H. R., Aleck, K. A., Bennett, P. H., and Knowler, W. C.: Excessive obesity in offspring of pima indian women with diabetes during pregnancy. New Engl. J. Med. *308*, 242–245 (1983)

Plagemann, A.: 'Fetal programming' and 'functional teratogenesis': on epigenetic mechanisms and prevention of perinatally acquired lasting health risks. J. Perinat. Med. *32*, 297–305 (2004)

Plagemann, A.: Fetale Programmierung und funktionelle Teratologie. In: Ganten, D., und Ruckpaul, K. (Eds.): Molekularmedizinische Grundlagen von fetalen und neonatalen Erkrankungen. S. 325–346. Berlin, Heidelberg, New York: Springer 2005a

Plagemann, A.: Perinatal programming and functional teratogenesis: Impact on body weight regulation and obesity. Physiol. Behav. *86*, 661–668 (2005b)

Plagemann, A.: A matter of insulin: developmental programming of body weight regulation. J. Matern. Fetal. Neonatal. Med. *21*, 143–148 (2008)

Plagemann, A., Harder, T., Brunn, M., Harder, A., Roepke, K., Wittrock-Staar, M., Ziska, T., Schellong, K., Rodekamp, E., Melchior, K., and Dudenhausen, J. W.: Hypothalamic POMC promoter methylation be-

comes altered by early overfeeding: An epigenetic model of obesity and the metabolic syndrome. J. Physiol. *587*, 4963–4976 (2009)

Plagemann, A., Harder, T., Janert, U., Rake, A., Rittel, F., Rohde, W., and Dörner, G.: Malformations of hypothalamic nuclei in hyperinsulinaemic offspring of gestational diabetic mother rats. Dev. Neurosci. *21*, 58–67 (1999a)

Plagemann, A., Harder, T., Kohlhoff, R., Rohde, W., and Dörner, G.: Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes. Int. J. Obes. *21*, 451–456 (1997a)

Plagemann, A., Harder, T., Kohlhoff, R., Rohde, W., and Dörner, G.: Glucose tolerance and insulin secretion in infants of mothers with pregestational insulin-dependent diabetes mellitus or gestational diabetes. Diabetologia *40*, 1094–1100 (1997b)

Plagemann, A., Harder, T., Melchior, K., Rake, A., Rohde, W., and Dörner, G.: Elevation of hypothalamic neuropeptide Y-neurons in adult offspring of diabetic mother rats. NeuroReport *10*, 3211–3216 (1999b)

Plagemann, A., Harder, T., Rake, A., Melchior, K., Rittel, F., Rohde, W., and Dörner, G.: Hypothalamic insulin and neuropeptide Y in the offspring of gestational diabetic mother rats. NeuroReport *9*, 4069–4073 (1998)

Plagemann, A., Harder, T., Rake, A., Voits, M., Fink, H., Rohde, W., and Dörner, G.: Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome X-like alterations in adulthood of neonatally overfed rats. Brain Res. *836*, 146–155 (1999c)

Plagemann, A., Harder, T., Rake, A., Waas, T., Melchior, K., Ziska, T., Rohde, W., and Dörner, G.: Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. J. Neuroendocrinol. *11*, 541–546 (1999d)

Plagemann, A., Heidrich, I., Götz, F., Rohde, W., and Dörner, G.: Lifelong enhanced diabetes susceptibility and obesity after temporary intrahypothalamic hyperinsulinism during brain organization. Exp. Clin. Endocrinol. *99*, 91–95 (1992a)

Plagemann, A., Heidrich, I., Götz, F., Rohde, W., and Dörner, G.: Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. Exp. Clin. Endocrinol. *99*, 154–158 (1992b)

Plagemann, A., Roepke, K., Harder, T., Brunn, M., Harder, A., Wittrock-Staar, M., Ziska, T., Schellong, K., Rodekamp, E., Melchior, K., and Dudenhausen, J. W.: Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J. Perinat. Med. *38*, 393–400 (2010)

Saint-Hilaire, E. G.: Histoire generale et particuliere des anomalies de l'organisation chez l'homme et les animaux ou traite de teratologie. Brussels 1837

Silverman, B. L., Metzger, B. E., Cho, N. H., and Loeb, C. A.: Impaired glucose tolerance in adolescent offspring of diabetic mothers. Diabetes Care *18*, 611–617 (1995)

Stanner, S. A., Bulmer, K., Andres, C., Lantseva, O. E., Borodina, V., Poteen, V. V., and Yudkin, J. S.: Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. BMJ *315*, 1342–1348 (1997)

Stein, A. D., Zybert, P. A., van de Bor, M., and Lumey, L. H.: Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. Int. J. Epidemiol. *33*, 831–836 (2004)

Stettler, N. S., Zemel, B. S., Kumanyika, S., and Stallings, V. A.: Infant weight gain in a multicenter, cohort study. Pediatrics *109*, 194–199 (2002)

Vanhala, M. J., Vanhala, P. T., Keinänen-Kiukaanniemi, S. M., Kumpusalo, E. A., and Takala, J. K.: Relative weight gain and obesity as a child predict metabolic syndrome as an adult. Int. J. Obes. *23*, 656–659 (1999)

Waddington, C. H.: The canalization of development and the inheritance of acquired characters. Nature *150*, 563–565 (1942)

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Preterm Birth – A Risk Factor for Chronic Adult Disease?

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With 1 Table

Abstract

Approximately 5 to 13 % of all newborns are born preterm, before 37 weeks of gestation. Recent studies have suggested that in particular those born smallest, for example at very low birth weight (<1500 g, 0.8 to 1.5 % of all newborns), have as young adults increased risk factors for chronic adult disease including higher blood pressure, impaired glucose regulation and lower bone mineral density, and that they exercise less than their peers born at term. Emerging evidence suggests that these risk factors extend to people born moderately preterm and argues for a "dose-response" relationship between the degree of prematurity and many of these later in life outcomes, although detailed studies are lacking.

Zusammenfassung

Ungefähr 5 bis 13 % der Neugeborenen werden zu früh geboren, das bedeutet vor der 37. Schwangerschaftswoche. Aktuelle Studien haben ergeben, dass besonders die „Kleinstgeborenen", z. B. mit sehr niedrigem Geburtsgewicht (<1500g, 0,8 bis 1,5 % aller Neugeborenen), als junge Erwachsene erhöhte Risikofaktoren haben für chronische Erkrankungen im Erwachsenenalter wie erhöhter Blutdruck, gestörte Glukosetoleranz und verminderte Knochendichte. Außerdem treiben sie weniger Sport als Gleichaltrige, die am regelrechten Geburtstermin zur Welt kamen. Neueste Daten zeigen, dass diese Risikofaktoren sich auch auf moderat zu früh Geborene beziehen, so dass eine „Dosis-Wirkungs-Beziehung" zwischen dem Grad der Frühgeburtlichkeit und den Auswirkungen auf das spätere Erwachsenenleben diskutiert wird. Hierfür fehlen bislang jedoch detaillierte Studien.

1. Introduction

Conditions during fetal life and infancy have a substantial impact on the risk of chronic adult disease, including cardiovascular disease, type 2 diabetes, osteoporosis, mental health disorders and cognitive impairments. Most research has focused on term birth. Although preterm birth (before 37 weeks of gestation) is common, ranging from 5 to 13 % in different countries where statistics are available *(Euro-Peristat* 2008, *NCHS* 2009, *Public Health Agency of Canada* 2008, *STAKES* 2007), there is relatively little published data about the long-term consequences of preterm birth. The aim of this mini-review is to summarise what is known on the consequences of preterm birth on physical health outcomes in adult life. Most of the existing data come from studies of adults born severely preterm who constitute the main focus of the review. This review will also recapitulate the emerging data that suggest that many of the associations are graded and extend over the entire range of preterm births. The style is narrative: a number of excellent systematic reviews have recently been published on the long-term outcomes of severely preterm infants (Hack 2006, 2009, Saigal and Doyle 2008). This review focuses on physical characteristics. Behavioural outcomes of severely preterm

birth are reviewed elsewhere in this issue (Ref. Saigal in this issue). However, it should be noted that although some behavioural characteristics may confer increased risks of disease, other characteristics such as the personality trait of conscientiousness (Pesonen et al. 2008) and lower levels of risk-taking behaviours (Hack et al. 2002, Strang-Karlsson et al. 2008, Kajantie et al. 2010) may actually limit adverse physical and mental health outcomes.

2. Definitions

Preterm birth can be broadly divided in two categories: severely and moderately preterm birth. Definitions of severely preterm birth vary. They may be based on either gestational age or weight at birth. In settings where gestational age is not systematically estimated by ultrasound during early pregnancy, definition by birth weight may be more accurate. However, it is of note that groups defined by birth weight are by design biased towards small-for-gestational age (SGA) infants because infants born at a later gestational age are included only if SGA. Common definitions include very low birth weight (VLBW; <1500 g; 0.8 % to 1.5 % of all births in high-income countries [*NCHS* 2009, *Public Health Agency of Canada* 2008]), extremely low birth weight (ELBW; <1000 g; approximately 0.4 % to 0.7 % [*Public Health Agency of Canada* 2008, *STAKES* 2007]) and below 32 weeks of gestation (approximately 1 % [*Euro-Peristat* 2008]). Severely preterm birth is characterised by extensive hospital treatment after birth and prematurity-associated illness. Advances in neonatal intensive care have resulted in remarkable increases in the survival of VLBW infants in high-income countries: in the 1960s approximately 40 % of these infants were discharged alive from hospital; in the 1980s 70 %; today 89 % (Lee et al. 1995, *STAKES* 2007), although VLBW infants still account for a substantial proportion of neonatal mortality.

Moderately preterm birth is commonly defined as between 32 and 37 weeks of gestation (*Euro-Peristat* 2008). This group includes births between 34 and 37 weeks, referred to as late preterm (Engle et al. 2007). Approximately 80 to 90 % of preterm infants are born moderately preterm (*Euro-Peristat* 2008, *NCHS* 2009, *Public Health Agency of Canada* 2008, *STAKES* 2007). This group has only recently received attention. Moderately preterm birth is also associated with prolonged hospital care and a 6-fold higher risk of death during infancy (Swamy et al. 2008), and thus constitutes an important public health issue (Kramer et al. 2000). Moreover, while the number of severely preterm births has remained stable, in many countries the number of moderately preterm births has been increasing steadily over the past decade (*Euro-Peristat* 2008, *Public Health Agency of Canada* 2008).

A number of causes can lead to a baby being born preterm, including preterm labour, premature rupture of membranes, or medically indicated preterm delivery (Goldenberg et al. 2008). Common medical indications include pre-eclampsia and intrauterine growth restriction, which often occur together. It is important to take the reason for premature birth into consideration when studying its long-term effects.

3. Blood Pressure

Of all physical outcomes related to preterm birth, blood pressure has been most widely studied (Doyle et al. 2003, Hack et al. 2005, Hovi et al. 2007, 2010 Kistner et al. 2000, Ro-

GERS et al. 2005, STEVENSON et al. 2001). While there is some heterogeneity between studies (Tab. 1), it is safe to conclude that adults born severely preterm have higher blood pressure than their peers born at term. The differences are considerable given that, at the general population level, already a 2 mmHg reduction in blood pressure among adults translates to a 4.3 % reduction in deaths due to coronary heart disease and 6.5 % reduction in deaths due to stroke (STAMLER 1991).

Tab. 1 Studies comparing systolic blood pressure between VLBW adults or adolescents and their peers born at term.

Reference		Number of VLBW subjects and controls born at term	Mean age (years)	Mean difference (mmHg)	95 % confidence interval or p value
Office blood pressure					
KISTNER et al.	2000	15+17 [1]	26	13	p<0.001
STEVENSON et al.	2001	128+128	15	3.2	(0.4 to 6.0)
DOYLE et al.	2003	156+38	18.6	10.6 [2]	(5.8 to 15.5)
HACK et al.	2005	195+208	20.1	3.5 [2]	(1.4 to 5.6)
ROGERS et al.	2005	53+31 [3]	17.5	"Not statistically significant"	
HOVI et al.	2007	163+169	22.4	4.8 [2]	(2.3 to 7.2)
Ambulatory blood pressure					
KISTNER et al.	2000	15+17	26	4	p=0.2
DOYLE et al.	2003	156+38	18.6	5.2 [2]	(2.1 to 8.3)
HOVI et al.	2010	118+120	22	3.1 [2]	(0.6 to 5.5)

It is likely that multiple mechanisms contribute to higher blood pressure in people born severely preterm, including renal function, vascular resistance and sympathetic activity. Two studies have assessed renal function in VLBW adults. KEIJZER-VEEN and colleagues showed in the Dutch POPS study smaller kidney volume and lower glomerulal filtration rate and effective renal plasma flow were lower in very preterm SGA subjects (but not very preterm AGA) as compared with controls born at term (KEIJZER-VEEN et al. 2007). No difference was found in another study, although its impact was limited (KISTNER et al. 2000). The vulnerability of the kidney is further supported by a case-control study that suggested increased risks of focal segmental glomerulosclerosis in people born at VLBW (HODGIN et al. 2009). As to vascular function, increased peripheral vascular resistance is suggested by an observation of abnormal retinal vascularisation in VLBW adults (KISTNER et al. 2002). However, no association was seen with endothelial function as measured by flow-mediated arterial dilatation in the Helsinki Study of Very Low Birth Weight Adults (HeSVA) (HOVI et al. 2011) or in another study of 13- to 16-year-olds (SINGHAL et al. 2004).

1 Subjects born at less than 32 weeks of gestational age.
2 Adjusted for current body size.
3 Subjects with birth weight 800 g or less and controls born at term.

The possibility of increased sympathetic activity deserves special attention. VLBW adults have a higher resting heart rate than their peers born at term (Hovi et al. 2007). Differences in blood pressure are stronger for clinical than for ambulatory blood pressures. For example, adjusted differences were 10.6 mmHg for clinical and 5.2 mm Hg for mean 24-hour systolic pressure in a cohort in Victoria, Australia (Doyle et al. 2003), and 4.8 and 3.1 mm Hg in the HeSVA (Hovi et al. 2007, 2010) (Tab. 1). This may suggest increased blood pressure responses to stress in people born severely preterm. This was indeed shown to be the case in a subgroup of the HeSVA who underwent the Trier Social Stress Test (TSST), although the difference was statistically significant only for diastolic blood pressure (Pyhälä et al. 2009). Although the finding awaits further confirmation, it is potentially important because blood pressure stress reactivity is a strong independent predictor of cardiovascular disease (Kajantie and Räikkönen 2010, Treiber et al. 2003).

Whether the differences in blood pressure are modified by sex of the patient is not clear. In the Victoria study, the differences were similar in women and men (Doyle et al. 2003). In the HeSVA, differences for clinical and 24-hour blood pressure tended to be stronger among women (Hovi et al. 2010); a sex difference was, however, not apparent among the subgroup who underwent the TSST (Pyhälä et al. 2009). The higher blood pressures seem to be similar among VLBW adults born SGA and AGA (Doyle et al. 2003, Hovi et al. 2007, 2010) and do not seem to be attributable to maternal pre-eclampsia or hypertension in pregnancy (Doyle et al. 2003, Hovi et al. 2010).

4. Glucose Tolerance

We recently showed that young adults born at VLBW show higher glucose and insulin concentrations in an oral glucose tolerance test than their peers born at term (Hovi et al. 2007). The difference was not attributable to the VLBW adults' lower lean body mass and again was similar in VLBW subjects born SGA than in those born AGA. A study in the Dutch POPS cohort suggested that this risk is greatest among those very preterm subjects who grew rapidly during the first three months post term; that study included no term controls (Finken et al. 2006a, b). A previous study of prepubertal children born at 32 weeks or less, using an intravenous glucose tolerance test, suggested that the difference in glucose tolerance difference is due to reduced insulin sensitivity rather than impaired insulin release (Hofman et al. 2004). These findings suggest that adults born at VLBW may be more vulnerable to type 2 diabetes later in life, which is consistent of recent observations in older adults born with a lesser degree of prematurity (Kaijser et al. 2009, Lawlor et al. 2006).

5. Plasma Lipids

Although plasma lipids are well established risk factors of cardiovascular disease, they have been examined in few studies. We found no difference in serum total or HDL cholesterol or triglyceride concentrations between VLBW adults and a comparison group born at term (Hovi et al. 2007). A study among the adults born very preterm in the Dutch POPS cohort showed no association of perinatal factors or postnatal growth with these lipids or apolipoprotein A1 and B concentrations; there were no term controls in that study (Finken et al. 2006a, b).

6. Bone

We recently found that VLBW adults have a lower bone mineral density than their peers born at term (Hovi et al. 2009). The difference is substantial: for lumbar spine, for example, 0.51 SD. In our study the difference remained statistically significant after adjustment for the VLBW adults' smaller body size, which is contrary to a study of 25 VLBW adults and 25 controls (Weiler et al. 2002). The finding is important because bone mineral density tracks throughout adulthood: individuals' scores show a correlation of 0.93 between individual measurements at age 25 and 44 (Emaus et al. 2005). In adults, a 1-unit decrease in bone mineral density z score is associated with a doubling or even tripling of fracture risk (Cummings et al. 1990, 1993, Marshall et al. 1996).

7. Physical Activity

Higher blood pressure (Doyle et al. 2003, Hack et al. 2005, Hovi et al. 2007, 2010, Pyhälä et al. 2009), impaired glucose regulation (Hofman et al. 2004, Hovi et al. 2007) and lower bone mineral density (Hovi et al. 2009) may in later life lead to a substantial burden of chronic disease. Physical activity is effective in reducing the risks associated with these factors (Kohrt et al. 2004, Orozco et al. 2008, Pescatello et al. 2004). Recent observations suggest that lower of physical activity in adolescents and adults born severely preterm. Most of available evidence concerns leisure-time conditioning physical activity.

Adults born at VLBW report lower frequency, duration and intensity of leisure-time conditioning physical activity than their peers born at term (Strang-Karlsson et al. 2008). They also gain lower scores in Child Health and Illness Profile Physical Activity subscale (Hack et al. 2007). It has been shown that ELBW adolescents report less sports participation than their peers born at term (Rogers et al. 2005, Saigal et al. 2007). The reasons are not known, but may include poorer motor coordination (Evensen et al. 2009, Rogers et al. 2005) leading to lower physical self-confidence and perceived physical ability (Saigal et al. 2007), possibly further aggravated by poor visual acuity (Evensen et al. 2009), and taken together making physical activity less rewarding. This may lead to a self-perpetuating effect which not only leads to lower degrees of physical activity (Hovi et al. 2007, Rogers et al. 2005, Saigal et al. 2007, Smith et al. 2008) but also aggravates the lower exercise capacity (Kilbride et al. 2003, Rogers et al. 2005, Smith et al. 2008) and lower lean body mass (Hovi et al. 2007) which are present from childhood onwards. This evidence suggests that promotion of physical activity should deserve more attention in the follow-up of healthy children born preterm. In the absence of other evidence, levels of physical activity specified in guidelines targeting the general population of children could serve as a logical aim.

8. Moderately Preterm Birth

Although 80 to 90 % of all newborns born preterm are born moderately preterm, often defined as after 32 weeks of gestation, much less is known about the long-term prognosis of these newborns. Because of their large number, even lower individual risks could be as significant at the population level as are risks associated with severely preterm birth. The little data that

exists from young adult cohorts points to a graded "dose-response" relationship between the degree of prematurity and many outcomes including blood pressure (Dalziel et al. 2005, Irving et al. 2000, Johansson et al. 2005, Järvelin et al. 2004), cognitive functioning (Lundgren et al. 2003), education, income and parenting a child (Moster et al. 2008, Swamy et al. 2008). Alarmingly, recent epidemiological studies in older populations have suggested that these outcomes may also translate to risks of common chronic disorders in later life. A large Swedish cohort study showed an increased risk of type 2 diabetes in people born preterm (Kaijser et al. 2009). This increased risk was also found in a study in Aberdeen, Scotland (Lawlor et al. 2006). Despite a relatively high sample size, the Swedish cohort study found no increased risk of coronary heart disease (Kaijser et al. 2008) and hypertension (Bonamy et al. 2008) associated with preterm birth *per se*. However, hypertension was assessed only through the national hospital discharge register, which is likely to identify only a small proportion of people with hypertension. Moreover, in these studies the effects of preterm birth and birth weight SDs score (birth weight in relation to the length of gestation) were carefully separated whereas people born preterm frequently have suffered from intrauterine growth restriction which was found to be associated with an increased risk of all these outcomes. Accordingly, another Swedish study has shown that people born before 35 weeks of gestation have a 3-fold risk of death from stroke when compared with people born at term (Koupil et al. 2005). These risks remain after adjustment for socio-economic status and other confounders. While people who survived preterm birth over fifty years ago are likely to differ from people born preterm today, these findings highlight the importance of all degrees of prematurity as a predictor of adult chronic disease.

References

Bonamy, A. K., Norman, M., and Kaijser, M.: Being born too small, too early, or both: does it matter for risk of hypertension in the elderly? Amer. J. Hypertens. *21*, 1107–1110 (2008)

Cummings, S. R., Black, D. M., Nevitt, M. C., Browner, W., Cauley, J., Ensrud, K., Genant, H. K., Palermo, L., Scott, J., and Vogt, T. M.: Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet. *341*, 72–75 (1993)

Cummings, S. R., Black, D. M., Nevitt, M. C., Browner, W. S., Cauley, J. A., Genant, H. K., Mascioli, S. R., Scott, J. C., Seeley, D. G., Steiger, P., et al.: Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group. JAMA *263*, 665–668 (1990)

Dalziel, S. R., Walker, N. K., Parag, V., Mantell, C., Rea, H. H., Rodgers, A., and Harding, J. E.: Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. Lancet *365*, 1856–1862 (2005)

Doyle, L. W., Faber, B., Callanan, C., and Morley, R.: Blood pressure in late adolescence and very low birth weight. Pediatrics *111*, 252–257 (2003)

Emaus, N., Berntsen, G. K. R., Joakimsen, R. M., and Fønnebø, V.: Longitudinal changes in forearm bone mineral density in women and men aged 25–44 years: the Tromso study: a population-based study. Amer. J. Epidemiol. *162*, 633–643 (2005)

Engle, W. A., Tomashek, K. M., and Wallman, C.: "Late-preterm" infants: a population at risk. Pediatrics *120*, 1390–1401 (2007)

Euro-Peristat: European perinatal health report. Available at: http://www.europeristat.com/publications/european-perinatal-health-report.shtml. Accessed 19th April 2009. (2008)

Evensen, K. A., Lindqvist, S., Indredavik, M. S., Skranes, J., Brubakk, A. M., and Vik, T.: Do visual impairments affect risk of motor problems in preterm and term low birth weight adolescents? Eur. J. Paediatr. Neurol. *13*, 47–56 (2009)

Finken, M. J., Inderson, A., Van Montfoort, N., Keijzer-Veen, M. G., van Weert, A. W., Carfil, N., Frolich, M., Hille, E. T., Romijn, J. A., Dekker, F. W., and Wit, J. M.: Lipid profile and carotid intima-media

thickness in a prospective cohort of very preterm subjects at age 19 years: effects of early growth and current body composition. Pediatr. Res. *59*, 604–609 (2006a)

Finken, M. J., Keijzer-Veen, M. G., Dekker, F. W., Frolich, M., Hille, E. T., Romijn, J. A., and Wit, J. M.: Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life Insulin resistance 19 years after preterm birth. Diabetologia *49*, 478–485 (2006b)

Goldenberg, R. L., Culhane, J. F., Iams, J. D., and Romero, R.: Epidemiology and causes of preterm birth. Lancet *371*, 75–84 (2008)

Hack, M.: Adult outcomes of preterm children. J. Dev. Behav. Pediatr. *30*, 460–470 (2009)

Hack, M.: Young adult outcomes of very-low-birth-weight children. Semin. Fetal. Neonatal. Med. *11*, 127–137 (2006)

Hack, M., Cartar, L., Schluchter, M., Klein, N., and Forrest, C. B.: Self-perceived health, functioning and well-being of very low birth weight infants at age 20 years. J. Pediatr. *151*, 635–641, 641 e631–632 (2007)

Hack, M., Flannery, D. J., Schluchter, M., Cartar, L., Borawski, E., and Klein, N.: Outcomes in young adulthood for very-low-birth-weight infants. New Engl. J. Med. *346*, 149–157 (2002)

Hack, M., Schluchter, M., Cartar, L., and Rahman, M.: Blood pressure among very low birth weight (<1.5 kg) young adults. Pediatr. Res. *58*, 677–684 (2005)

Hodgin, J. B., Rasoulpour, M., Markowitz, G. S., and D'Agati, V. D.: Very low birth weight is a risk factor for secondary focal segmental glomerulosclerosis. Clin. J. Amer. Soc. Nephrol. *4*, 71–76 (2009)

Hofman, P. L., Regan, F., Jackson, W. E., Jefferies, C., Knight, D. B., Robinson, E. M., and Cutfield, W. S.: Premature birth and later insulin resistance. New Engl. J. Med. *351*, 2179–2186 (2004)

Hovi, P., Andersson, S., Eriksson, J. G., Järvenpää, A. L., Strang-Karlsson, S., Mäkitie, O., and Kajantie, E.: Glucose regulation in young adults with very low birth weight. New Engl. J. Med. *356*, 2053–2063 (2007)

Hovi, P., Andersson, S., Järvenpää, A. L., Eriksson, J. G., Strang-Karlsson, S., Kajantie, E., and Mäkitie, O.: Decreased bone mineral density in adults born with very low birth weight. PLoS Med. *6*, e1000135 (2009)

Hovi, P., Andersson, S., Räikkönen, K., Strang-Karlsson, S., Järvenpää, A. L., Eriksson, J. G., Pesonen, A. K., Heinonen, K., Pyhälä, R., and Kajantie, E.: Ambulatory blood pressure in young adults with very low birth weight. J. Pediatr. *156*/1, 54–59.e1 (2010)

Hovi, P., Turanlahti, M., Strang-Karlsson, S., Wehkalampi, K., Järvenpää, A. L., Eriksson, J. G., Kajantie, E., and Andersson, S.: Intima-media thickness and flow-mediated dilatation in the Helsinki Study of very low birth weight adults. Pediatrics *127*, e304–311 (2011)

Irving, R. J., Belton, N. R., Elton, R. A., and Walker, B. R.: Adult cardiovascular risk factors in premature babies. Lancet *355*, 2135–2136 (2000)

Järvelin, M. R., Sovio, U., King, V., Laurén, L., Xu, B., McCarthy, M. I., Hartikainen, A. L., Laitinen, J., Zitting, P., Rantakallio, P., and Elliott, P.: Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. Hypertension *44*, 838–846 (2004)

Johansson, S., Iliadou, A., Bergvall, N., Tuvemo, T., Norman, M., and Cnattingius, S.: Risk of high blood pressure among young men increases with the degree of immaturity at birth. Circulation *112*, 3430–3436 (2005)

Kaijser, M., Bonamy, A. K., Akre, O., Cnattingius, S., Granath, F., Norman, M., and Ekbom, A.: Perinatal risk factors for diabetes in later life. Diabetes *58*, 523–526 (2009)

Kaijser, M., Bonamy, A. K., Akre, O., Cnattingius, S., Granath, F., Norman, M., and Ekbom, A.: Perinatal risk factors for ischemic heart disease: disentangling the roles of birth weight and preterm birth. Circulation *117*, 405–410 (2008)

Kajantie, E., and Räikkönen, K.: Early life predictors of the physiological stress response later in life. Neurosci. Biobehav. Rev. *35*, 23–32

Kajantie, E., Strang-Karlsson, S., Hovi, P., Räikkönen, K., Pesonen, A. K., Heinonen, K., Järvenpää, A. L., Eriksson, J. G, and Andersson, S.: Adults born at very low birth weight exercise less than their peers born at term. J. Pediatr. *157*, 610–616 (2010)

Keijzer-Veen, M. G., Kleinveld, H. A., Lequin, M. H., Dekker, F. W., Nauta, J., Rijke, Y. B. de, and van der Heijden, B. J.: Renal function and size at young adult age after intrauterine growth restriction and very premature birth. Amer. J. Kidney Dis. *50*, 542–551 (2007)

Kilbride, H. W., Gelatt, M. C., and Sabath, R. J.: Pulmonary function and exercise capacity for ELBW survivors in preadolescence: effect of neonatal chronic lung disease. J. Pediatr. *143*, 488–493 (2003)

Kistner, A., Celsi, G., Vanpee, M., and Jacobson, S. H.: Increased blood pressure but normal renal function in adult women born preterm. Pediatr. Nephrol. *15*, 215–220 (2000)

Kistner, A., Jacobson, L., Jacobson, S. H., Svensson, E., and Hellström, A.: Low gestational age associated with abnormal retinal vascularization and increased blood pressure in adult women. Pediatr. Res. *51*, 675–680 (2002)

Kohrt, W. M., Bloomfield, S. A., Little, K. D., Nelson, M. E., and Yingling, V. R.: American College of Sports Medicine Position Stand: physical activity and bone health. Med. Sci. Sports Exerc. *36*, 1985–1996 (2004)

Koupil, I., Leon, D. A., and Lithell, H. O.: Length of gestation is associated with mortality from cerebrovascular disease. J. Epidemiol. Community Health *59*, 473–474 (2005)

Kramer, M. S., Demissie, K., Yang, H., Platt, R. W., Sauvé, R., and Liston, R.: The contribution of mild and moderate preterm birth to infant mortality. JAMA *284*, 843–849 (2000)

Lawlor, D. A., Davey Smith, G., Clark, H., and Leon, D. A.: The associations of birthweight, gestational age and childhood BMI with type 2 diabetes: findings from the Aberdeen Children of the 1950s cohort. Diabetologia *49*, 2614–2617 (2006)

Lee, K. S., Kim, B. I., Khoshnood, B., Hsieh, H. L., Chen, T. J., Herschel, M., and Mittendorf, R.: Outcome of very low birth weight infants in industrialized countries: 1947–1987. Amer. J. Epidemiol. *141*, 1188–1193 (1995)

Lundgren, E. M., Cnattingius, S., Jonsson, B., and Tuvemo, T.: Birth characteristics and different dimensions of intellectual performance in young males: a nationwide population-based study. Acta Paediatr. *92*, 1138–1143 (2003)

Marshall, D., Johnell, O., and Wedel, H.: Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ *312*, 1254–1259 (1996)

Moster, D., Lie, R. T., and Markestad, T.: Long-term medical and social consequences of preterm birth. New Engl. J. Med. *359*, 262–273 (2008)

Orozco, L. J., Buchleitner, A. M., Gimenez-Perez, G., Roque, I. F. M., Richter, B., and Mauricio, D.: Exercise or exercise and diet for preventing type 2 diabetes mellitus. Cochrane Database Syst. Rev. CD003054 (2008)

Pescatello, L. S., Franklin, B. A., Fagard, R., Farquhar, W. B., Kelley, G. A., and Ray, C. A.: American College of Sports Medicine position stand. Exercise and hypertension. Med. Sci. Sports Exerc. *36*, 533–553 (2004)

Pesonen, A. K., Räikkönen, K., Heinonen, K., Andersson, S., Hovi, P., Järvenpää, A. L., Eriksson, J. G., and Kajantie, E.: Personality in young adults with very low birth weight – Helsinki Study of Very Low Birth Weight Adults. J. Child Psychol. Psychiatr. *49*, 609–617 (2008)

Pyhälä, R., Räikkönen, K., Feldt, K., Andersson, S., Hovi, P., Eriksson, J. G., Järvenpää, A. L., and Kajantie, E.: Blood pressure response to psychosocial stress in young adults with very low birth weight – The Helsinki Study of Very Low Birth Weight Adults. Pediatrics *123*, 731–734 (2009)

Rogers, M., Fay, T. B., Whitfield, M. F., Tomlinson, J., and Grunau, R. E.: Aerobic capacity, strength, flexibility, and activity level in unimpaired extremely low birth weight (<or=800 g) survivors at 17 years of age compared with term-born control subjects. Pediatrics *116*, e58–65 (2005)

Saigal, S., and Doyle, L. W.: An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet *371*, 261–269 (2008)

Saigal, S., Stoskopf, B., Boyle, M., Paneth, N., Pinelli, J., Streiner, D., and Goddeeris, J.: Comparison of current health, functional limitations, and health care use of young adults who were born with extremely low birth weight and normal birth weight. Pediatrics *119*, e562–573 (2007)

Singhal, A., Cole, T. J., Fewtrell, M., Deanfield, J., and Lucas, A.: Is slower early growth beneficial for long-term cardiovascular health? Circulation *109*, 1108–1113 (2004)

Smith, L. J., van Asperen, P. P., McKay, K. O., Selvadurai, H., and Fitzgerald, D. A.: Reduced exercise capacity in children born very preterm. Pediatrics *122*, e287–293 (2008)

STAKES: Parturients, Deliveries and Births 2006. Statistical Summary 21/2007, 2.11.2007. Official Statistics of Finland, Health 2007 (2007)

STAKES: Parturients, Deliveries and Births 2006. Statistical Summary 21/2007, 2.11.2007. Official Statistics of Finland, Health 2007 (2007)

Stamler, J.: Blood pressure and high blood pressure. Aspects of risk. Hypertension *18*, I95–107 (1991)

Stevenson, C. J., West, C. R., and Pharoah, P. O.: Dermatoglyphic patterns, very low birth weight, and blood pressure in adolescence. Arch. Dis. Child Fetal. Neonatal Ed. *84*, F18–22 (2001)

Strang-Karlsson, S., Räikkönen, K., Pesonen, A. K., Kajantie, E., Paavonen, E. J., Lahti, J., Hovi, P., Heinonen, K., Järvenpää, A. L., Eriksson, J. G., and Andersson, S.: Very-low-birth-weight and behavioral symptoms of ADHD in young adulthood – The Helsinki Study of Very Low Birth Weight Adults. Amer. J. Psychiatry *165*, 1345–1353 (2008)

Swamy, G. K., Østbye, T., and Skjærven, R.: Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. JAMA *299*, 1429–1436 (2008)

Treiber, F. A., Kamarck, T., Schneiderman, N., Sheffield, D., Kapuku, G., and Taylor, T.: Cardiovascular reactivity and development of preclinical and clinical disease states. Psychosom. Med. *65*, 46–62 (2003)

Weiler, H. A., Yuen, C. K., and Seshia, M. M.: Growth and bone mineralization of young adults weighing less than 1500 g at birth. Early Hum. Dev. *67*, 101–112 (2002)

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Climate Change and Infectious Diseases

International Conference

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in Zusammenarbeit mit der Indian National Science Academy, dem Alfried Krupp Wissenschaftskolleg Greifswald und dem Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit

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Krankheitserreger können sich heute binnen kurzer Zeit weltweit verbreiten und so sowohl gesundheitliche als auch wirtschaftliche Schäden verursachen, wenn sie auf geeignete Umweltbedingungen treffen. Zoonosen, vom Tier auf den Menschen übertragbare Krankheiten, gewinnen dabei zunehmend an Bedeutung. Der Band enthält die Beiträge eines Symposiums, das die neuesten Erkenntnisse zum Klimawandel und zum Einfluss klimatischer Faktoren auf Infektionskrankheiten von Experten der Klimaforschung, der Biologie und der Infektionsmedizin zusammenführte. Erörtert werden auch Einflüsse von klimatischen Veränderungen auf die Evolution und die Biodiversität. Die Bedeutung des Klimawandels für das Auftreten von Infektionskrankheiten wird anerkannt, auch wenn konkrete Auswirkungen bisher nur schwer zu dokumentieren sind. Insbesondere der Überwachung von Vektor- und Reservoirpopulationen sowie der entsprechenden Erreger kommt daher eine große Bedeutung zu, wobei die Untersuchungen langfristig angelegt sein müssen, um Dynamiken in längeren Zeiträumen zu erfassen.

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Impact of Fetal Insulin-Effect on Imprinting of Glucose Homeostasis

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Abstract

Hypothalamic neuron populations play a central role in the control of energy homeostasis. Among them, the Proopiomelanocortin (POMC) expressing neurons are of critical importance for normal body weight and glucose homeostasis. POMC neurons are so-called first order neurons, as they sense circulating concentrations of peripheral hormones such as leptin and insulin to adapt food intake, energy expenditure and glucose production maintaining adequate energy homeostasis.

It is known that offspring of mothers who are obese during pregnancy are more susceptible to obesity and obesity-related diseases. However, the molecular mechanism(s) underlying this phenomenon remain ill-defined. Here, data is presented on the effects of maternal feeding status on hypothalamic POMC neuron synapse input and function, impacting on adult body weight defense and glucose homeostasis. Moreover, the critical role for insulin signaling in this neuronal circuit is elucidated using genetic tools. These results provide novel insights into the molecular mechanism(s) involved in fetal metabolic programming.

Zusammenfassung

Hypothalamische Neuronenpopulationen spielen eine wichtige Rolle bei der Kontrolle der Energiehomöostase. Darunter sind die Proopiomelanocortin (POMC)-exprimierenden Neuronen von entscheidender Bedeutung für normales Körpergewicht und Glucosehomöostase. POMC-Neuronen sind sogenannte Neurone erster Ordnung, da sie die zirkulierenden Konzentrationen peripherer Hormone, wie Leptin und Insulin, erfassen, um Nahrungsaufnahme, Energieaufwand und Glucoseproduktion zur Aufrechterhaltung einer angemessenen Energiehomöostase anzupassen.

Es ist bekannt, dass der Nachwuchs von Müttern, die während der Schwangerschaft adipös sind, anfälliger für Fettsucht und damit verbundene Krankheiten ist. Dennoch bleibt der molekulare Mechanismus (bzw. die Mechanismen) dieses Phänomens unklar. Hier werden Daten zu den Auswirkungen des maternalen Versorgungszustands auf den Input und die Funktion der hypothalamischen POMC-Neuronensynapse vorgestellt, die die Erhaltung des Gewichts und der Glucosehomöostase des erwachsenen Körpers beeinflussen. Darüber hinaus wird die entscheidende Rolle des Insulinsignals in diesem neuronalen Schaltkreis mit genetischen Methoden aufgeklärt. Diese Ergebnisse liefern neue Einsichten in die molekularen Mechanismen, die an der fetalen Stoffwechselprogrammierung beteiligt sind.

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Der Begriff der Natur
Wandlungen unseres Naturverständnisses und seine Folgen

Gaterslebener Begegnung 2009

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Das Verhältnis des Menschen zur „Natur“ ist in seiner Geschichte durch unterschiedliche Beziehungen geprägt. Seit der Aufklärung wird die Natur dem Menschen zu seiner Nutzung untergeordnet und zunehmend ausgebeutet. Natur wurde zum Objekt technischen, ökonomischen und politischen Handelns. Spätestens seit Mitte des vorigen Jahrhunderts wissen wir um die akute Gefährdung natürlicher Lebensräume.

Die Gaterslebener Begegnung 2009 widmete sich daher dem Thema „Der Begriff der Natur“ und untersuchte Wandlungen des Naturverständnisses sowie die Folgen der gegenwärtigen Auffassungen von Natur. Behandelt werden unser Bild vom Leben, die Frage „Was ist Natur?“ aus verschiedenen Perspektiven und die philosophische Analyse der Stellung des Menschen in der Natur. Beiträge zum Naturverständnis in der Gegenwartskunst und zum Problemkomplex Naturrecht und Bioethik sowie eine Diskussion „Frieden mit der Natur“ ergänzen den Band.

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Psychopathology, Prematurity and Very Low Birth Weight

Saroj Saigal (Hamilton, Canada)

Abstract

Advances in perinatal care have resulted in improved survival rates of very premature infants. However, the survivors, particularly those who are very immature, face many challenges with higher rates of neurodevelopmental impairments, school difficulties, and behavioural and emotional problems. During infancy and early childhood, very low birth weight (VLBW) infants are particularly vulnerable to problems related to inattention and hyperactivity that may persist to school age. VLBW adolescents, especially females, may suffer from a higher prevalence of anxiety and depression. Many investigators have shown a dose response with the most immature infants having a higher prevalence and severity of behavioural problems. By adulthood there is a decline in attentional problems, although depression may become more apparent. The purpose of this review is to highlight the type, severity and associated factors that contribute to psychopathology in very premature infants.

Zusammenfassung

Fortschritte in der perinatalen Versorgung haben dafür gesorgt, dass das Überleben von Frühgeborenen mit sehr niedrigem Geburtsgewicht verbessert wurde. Die Überlebenden, besonders die sehr Unreifen, sind mit vielen Herausforderungen wie erhöhten Raten von neurologischen Einschränkungen, Schulproblemen und Verhaltens- sowie emotionalen Auffälligkeiten konfrontiert. Im Säuglings- und frühen Kindesalter entwickeln besonders Frühgeborene mit sehr niedrigem Geburtsgewicht häufiger Auffälligkeiten, wie Hyperaktivität und Unaufmerksamkeit, die bis zum Schulalter persistieren können. Zumeist weibliche Frühgeborene mit sehr niedrigem Geburtsgewicht leiden im Erwachsenenalter an einer erhöhten Prävalenz von Depression und Angststörungen. Viele Untersucher konnten eine Dosis-Wirkungsbeziehung zeigen, d. h., dass die meisten sehr unreifen Frühgeborenen eine erhöhte Prävalenz und ein gesteigertes Ausmaß an Verhaltensstörungen aufweisen. Im Erwachsenenalter nimmt die Häufigkeit der Aufmerksamkeitsstörungen ab, Depressionen manifestieren sich jedoch vermehrt. Der Anspruch dieser Arbeit ist die Typen, den Schweregrad und die assoziierten Faktoren, die bei der Psychopathologie sehr unreifer Frühgeborenen mitwirken, zu beleuchten.

1. Introduction

It is widely known that infants who are born very premature are at increased risk for neurosensory problems, cognitive deficits and learning disabilities (Saigal et al. 1991, 2000, Bhutta et al. 2002). Until recently, when a broader approach was adopted, little attention was paid to behavioural and mental health issues in these children. In the presentation at the Leopoldina Symposium, longitudinal data from infancy to adulthood on the prevalence and type of behavioural problems in infants born with very low birth weight (VLBW) from the literature was reviewed.

2. Methodological Problems

As the review will show, there are disparate reports about the prevalence, severity, and type of psychiatric problems, and the role of small for gestational age (SGA). Methodological problems may, in part, explain some of the differences between studies. These include small sample size, single centre studies, differences in social class and health care delivery, type of measurement tools (questionnaires *versus* psychiatric interviews), and different respondents.

2.1 Preschool and Mid-Childhood

During infancy and early childhood, VLBW children are particularly vulnerable to problems related to inattention, and to a lesser extent, associated hyperactivity, that persists to school age (Gray et al. 2004, Farooqi et al. 2007). These problems have been reported by birth weight (BW) in VLBW (<1500 g BW), and extremely low birth weight (ELBW <1000 g BW) infants (Szatmari et al. 1990), and more recently by gestational age (GA) in very premature (VP <32 weeks GA) and extremely premature (EP <28 weeks GA) infants (Delobel-Ayoub et al. 2006, Samara et al. 2008, Farooqi et al. 2007). In one meta-analysis, the risk of attention deficit hyperactivity disorder (ADHD) was 2.65 fold at school-age in children born preterm (Bhutta et al. 2002). The odds for clinically significant behaviours were 2–9 times higher for EP children than those born at term. A recent study from the UK (Samara et al. 2008) has shown a worrisomely higher prevalence of pervasive behavioural problems (defined as concurrence between parent and teacher report) at age 6 compared to previous studies (EP 19.4 % versus 3.4 % controls). The concern here is that these early attentional difficulties are known precursors of educational and vocational disadvantage, and subsequent psychosocial and antisocial behaviours (Ferguson et al 1997).

Most studies show a dose response relationship between the degree of prematurity and behavioural problems (Anderson et al 2003, Delobel-Ayoub et al. 2006), and it is also strongly related to psychosocial risk. Despite cross-cultural differences, these types of behaviours were shown to be strikingly similar in a comparative study of North American and European school-aged children, with mean scores 0.5 to 1.2 SD higher than country-specific norms (Hille et al. 2001). Conduct disorders appear to be less frequent, at least in mid-childhood.

2.2 Adolescence

There is some evidence to suggest that VLBW adolescents suffer from a higher prevalence of anxiety and depression compared to normal birth weight (NBW) controls. Several investigators have shown a higher prevalence of psychiatric problems, particularly attention deficit hyperactivity disorder (ADHD), that was nearly 4 times more frequent among VLBW adolescents. Others have shown no differences in self-perception of behavioural problems between ELBW and controls (Saigal et al. 2003).

2.3 Young Adulthood

In general, ADHD declines with age, and in several studies no differences in attentional problems were noted by self-report between VLBW and NBW young adults. Hack et al. (2004)

found more self-reported internalizing problem behaviours, but not attentional problems, among VLBW young women compared to control women at age 20. Parents not only agreed with their daughters, but also identified more attentional and thought problems in both VLBW men and women compared with control participants. Similarly, the Helsinki study of VLBW YA aged 18–27 years found no increase in attentional problems by self-report (Strang-Karlsson et al. 2008). However, they found that within the VLBW group, the SGA young adults reported more executive dysfunction and more emotional instability than did their appropriate for gestational age (AGA) peers and term controls. Strang-Karlsson et al. (2008) state that SGA is a more important predictor for subsequent ADHD-related behavioural traits than is prematurity *per se*. In fact, where depression was concerned, although VLBW young adults reported less depression overall than term controls, this was restricted to the AGA group; those born VLBW SGA reported more depressive symptoms, used antidepressants more frequently, and were more likely to report a diagnosis of depression compared to both VLBW AGA group and term controls (Räikkönen et al. 2008). Boyle et al. (2010) reported that depression, anxiety and avoidant personality problems (internalizing behaviours) are elevated among ELBW survivors at young adulthood. In addition, a pattern of shyness, withdrawn behaviours and less impulsivity have been described in both sexes at adolescence and young adulthood (Allin 2006, Schmidt et al. 2008).

2.4 Social Functioning and Adaptation

Premature children are more prone to difficulties in social interactions, making friends, and participating in group activities. These difficulties may be related to ADHD, impulsive behaviour, poorer communication skills, or a lack of self-confidence. These factors, and the well-recognised associated cognitive deficits, result in the need for remedial assistance that is 3–4 times more common among premature children than in those born at term. However, most studies show that despite these problems, a similar proportion of premature children participate in music camps, sports and other group activities (Saigal et al. 1991).

2.5 Role of Small for Gestational Age

The role of SGA in psychopathology is debated. The Helsinki study showed that SGA, rather than VLBW *per se*, posed a greater risk for behavioural and emotional instability and depression at young adults, whereas other studies have not supported this finding. The literature is not consistent in whether developmental and mental health problems can be modified by fetal growth restriction. Hack et al. (2004) found lower mean scores on anxious/depressed, and internalizing behaviours, and lower rates on withdrawn behaviour among VLBW SGA *versus* VLBW AGA groups. Indredavik et al. (2004) conducted in-depth psychiatric interviews at adolescence and found a higher prevalence of attentional and anxiety disorders and deficits in social skills among the VLBW adolescents compared to term controls, but no increase in psychiatric problems among the *term* SGA and comparison group. Dahl et al. (2006) showed that SGA was an independent early predictor of parent reported behavioural problems and social competencies at adolescence. Boyle et al. (2010) showed that although internalizing problems are elevated at young adulthood among ELBW survivors, this effect is relatively small overall but noticeably lager among ELBW survivors born SGA. Only the Helsinki study showed that intrauterine growth restriction, rather than prematurity *per se*, posed a

greater risk of behavioural and emotional instability and depression (Strang-Karlsson et al. 2008, Räikkönen et al. 2008).

2.6 Role of Gender

Gender plays a significant role with a higher prevalence of attentional and delinquent behaviours in the earlier years among males, and a higher prevalence of anxiety and depression emerging at adolescence among females (Saigal et al. 2003, Hille et al. 2001, Botting et al. 1998).

2.7 Role of Environmental and Social Factors

In one study, low birth weight was shown to be a significant risk factor for ADHD that was not accounted for by environmental, socioeconomic, genetic, prenatal or maternal factors (Mick et al. 2002). However, most studies show that environmental factors such as low socioeconomic status, low parental education, single parent, substance abuse, and residence in a deprived neighbourhood can further compound the impact of low birth weight and prematurity in both the management and subsequent outcome (McCormick et al. 1996, Farooqi et al. 2007, Gross 2001, Hack et al. 1992).

2.8 Differences Among Informants

The prevalence, severity and type of behavioural problems may vary across informants. Parents report a higher prevalence of behavioural problems than children. Parents of both VLBW and control children report significantly greater psychopathology than adolescents in depressive disorders, ADHD and social competencies (Saigal et al. 2003, Dahl et al. 2006, Gardner et al. 2004). Although the adolescents perceived no differences between groups, both groups reported higher scores in emotional problems than did their parents (Saigal et al. 2003, Dahl et al. 2006).

3. Conclusions

Despite differences in the reported prevalence and type of behavioural problems and the associated factors contributing to the same, it is clear that children who survive extreme prematurity suffer from a spectrum of behavioural, adaptive and social challenges.

This vulnerability requires ongoing surveillance of newer survivors, and in addition, management and parental guidance to ameliorate these problems and improve the quality of life of parents and children. Ongoing surveillance for psychopathology of VLBW children to young adulthood and middle-age is of the utmost importance. Future research should focus on investigating and modifying potentially mediating perinatal, child and family factors that contribute to psychopathology.

References

Allin, M., Rooney, M., Griffiths, T., Cuddy, M., Wyatt, J., Rifkin, L., and Murray, R.: Neurological abnormalities in young adults born preterm. J. Neurol. Neurosurg. Psychiatry *77*, 495–499 (2006)

Anderson, P., and Doyle, L. W.: Victorian Infant Collaborative Study Group. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. JAMA *289*, 3264–3272 (2003)

Bhutta, A. T., Cleves, M. A., Casey, P. H., Cradock, M. M., and Anand, K. J.: Cognitive and behavioural outcomes of school-aged children who were born preterm: a meta-analysis. JAMA *288*, 728–737 (2002)

Botting, N., Powls, A., Cooke, R. W., and Marlow, N.: Cognitive and educational outcome of very-low-birthweight children in early adolescence. Dev. Med. Child Neurol. *40*, 652–660 (1998)

Boyle, M. H., Miskovic, V., Van Lieshout, R., Duncan, L., Schmidt, L. A., Hoult, L., Paneth, N., and Saigal, S.: Pychopathology in young adults born at extremly low birth weight. Psychological Medicine 2010; doi: 10.1017/S0033291710002357

Dahl, L. B., Kaaresen, P. I., Tunby, J., Handegård, B. H., Kvernmo, S., and Rønning, J. A.: Emotional, behavioral, social, and academic outcomes in adolescents born with very low birth weight. Pediatrics *118*, e449–e459 (2006)

Delobel-Ayoub, M., Kaminski, M., Marret, S., Burguet, A., Marchand, L., N'Guyen, S., Matis, J., Thiriez, G., Fresson, J., Arnaud, C., Poher, M., Larroque, B., and *EPIPAGE Study Group*: Behavioral outcome at 3 years of age in very preterm infants: the EPIPAGE study. Pediatrics *117*, 1996–2005 (2006)

Farooqi, A., Hägglöf, B., Sedin, G., Gothefors, L., and Serenius, F.: Mental health and social competencies of 10- to 12-year-old children born at 23 to 25 weeks of gestation in the 1990s: a Swedish national prospective follow-up study. Pediatrics *120*, 118–133 (2007)

Fergusson, D. M., Lynskey, M. T., and Horwood, L. J.: Attentional difficulties in middle childhood and psychosocial outcomes in young adulthood. J. Child Psychol. Psychiat. *38*, 633–644 (1997)

Gardner, F., Johnson, A., Yudkin, P., Bowler, U., Hockley, C., Mutch, L., Wariyar, U., and *Extremely Low Gestational Age Steering Group*: Behavioral and emotional adjustment of teenagers in mainstream school who were born before 29 weeks' gestation. Pediatrics *114*, 676–682 (2004)

Gray, R. F., Indurkhya, A., and McCormick, M. C.: Prevalence, stability, and predictors of clinically significant behaviour problems in low birth weight children at 3, 5, and 8 years of age. Pediatrics *114*, 736–743 (2004)

Gross, S. J., Mettelman, B. B., Dye, T. D., and Slagle, T. A.: Impact of family structure and stability on academic outcome in preterm children at 10 years of age. J. Pediatr. *138*, 169–175 (2001)

Hack, M., Breslau, N., Aram, D., Weissman, B., Klein, N., and Borawski-Clark, E.: The effect of very low birth weight and social risk on neurological cognitive abilities at school-age. J. Dev. Behav. Pediatrics. *13*, 412–420 (1992)

Hack, M., Youngstrom, E. A., Cartar, L., Schluchter, M., Taylor, H. G., Flannery, D., Klein, N., and Borawski, E.: Behavioral outcomes and evidence of psychopathology among very low birth weight infants at age 20 years. Pediatrics *114*, 932–940 (2004)

Hille, E. T. M., den Ouden, A. L., Saigal, S., Wolke, D., Lambert, M., Whitaker, A., Pinto-Martin, J. A., Hoult, L., Meyer, R., Feldman, J. F., Verloove-Vanhorick, S. P., and Paneth, N.: Behavioural problems in children who weigh 1000 g or less at birth in four countries. Lancet *357*, 1641–1643 (2001)

Indredavik, M. S., Vik, T., Heyerdahl, S., Kulseng, S., Fayers, P., and Brubakk, A. M.: Psychiatric symptoms and disorders in adolescents with low birth weight. Arch. Dis. Child. Fetal. Neonatal. Ed. *89*, F445–F450 (2004)

McCormick, M. C., Workman-Daniels, K., and Brooks-Gunn, J.: The behavioral and emotional well-being of school-age children with different birth weights. Pediatrics *97*, 18–25 (1996)

Mick, E., Biederman, J., Prince, J., Fischer, M. J., and Faraone, S. V.: Impact of low birth weight on attention deficit hyperactivity disorder. J. Dev. Behav. Pediatr. *23*, 16–22 (2002)

Räikkönen, K., Pesonen, A. K., Heinonen, K., Kajantie, E., Hovi, P., Järvenpää, A. L., Eriksson, J. G., and Andersson, S.: Depression in young adults with very low birth weight: the Helsinki study of very low-birthweight adults. Arch. Gen. Psychiatry *65*, 290–296 (2008)

Saigal, S., Hoult, L., Streiner, D., Stoskopf, B. L., and Rosenbaum, P. L.: School difficulties at adolescence in a regional cohort of children who were extremely low birthweight. Pediatrics *105*, 325–331 (2000)

Saigal, S., Pinelli, J., Hoult, L., Kim, M. M., and Boyle, M.: Psychopathology and social competencies of adolescents who were extremely low birth weight. Pediatrics *111*, 969–975 (2003)

Saigal, S., Szatmari, P., Rosenbaum, P., Campbell, D., and King, S.: Cognitive abilities and school performance of extremely low birth weight children and matched term control children at age 8 years: a regional study. J. Pediatr. *118*, 751–760 (1991)

Samara, M., Marlow, N., Wolke, D., and *EPICure Study Group*: Pervasive behaviour problems at 6 years of age in a total-population sample of children born at ≤ 25 weeks of gestation. Pediatrics *122*, 562–573 (2008)

Schmidt, L. A., Miskovic, V., Boyle, M. H., and Saigal, S.: Shyness and timidity in young adults who were born at extremely low birth weight. Pediatrics *122*, e181–187 (2008)

Strang-Karlsson, S., Räikkönen, K., Pesonen, A. K., Kajantie, E., Paavonen, E. J., Lahti. J., Hovi, P., Heinonen, K, Järvenpää, A. L., Eriksson, J. G., and Andersson, S.: Very low birth weight and behavioral symptoms of attention deficit hyperactivity disorder in young adulthood: the Helsinki study of very-low-birth-weight adults. Amer. J. Psychiatry *165*, 1345–1353 (2008)

Szatmari, P., Saigal, S., Rosenbaum, P., Campbell, D., and King, S.: Psychiatric disorders at five years among children with birthweights <1000g: a regional perspective. Dev. Med. Child Neurol. *32*, 954–962 (1990)

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Maternal Toxic Substance Use and Childhood Outcome

Wolfgang E. Paulus (Ravensburg)

Abstract

Teratology is the branch of medical science that studies the contribution of the environment to abnormal prenatal growth and morphological or functional development. It deals specifically with the biological, genetic, biochemical and behavioural causes of maldirected development and spans the period from germ-cell differentiation to the termination of functional development in the postpartum individual.

Zusammenfassung

Teratologie ist der Zweig der Medizinwissenschaft, der den Beitrag der Umwelt zu Störungen im pränatalen Wachstum und in der morphologischen oder funktionellen Entwicklung untersucht. Sie beschäftigt sich speziell mit den biologischen, genetischen, biochemischen und verhaltensbezogenen Ursachen fehlgeleiteter Entwicklung und erstreckt sich über den Zeitraum von der Keimzelldifferenzierung bis zum Abschluss der funktionellen Entwicklung im postpartalen Individuum.

The term 'teratogen' broadly includes any reproductive and/or developmental toxicant that induces structural malformations, metabolic or functional deficits, growth retardation or psychological/behavioural anomalies in the offspring, whether at birth or in any defined postnatal period. Functional deficits refer to physiological or systems deficits that could result in hormonal, immunological or neurological/behavioural anomalies. Epigenetic influences (i.e. all the external environmental variables that regulate gene activity) modulate normal developmental processes. Harmful epigenetic influences can derail development by triggering intrinsic gene defects (mutations) or adversely modifying normal gene expression resulting in impaired growth and development.

Although a few pioneering studies were conducted in the early 20th century, the interest of the medical and public media burgeoned only in the wake of the thalidomide tragedy in the late 1950s and early 1960s, and once evidence emerged that rubella was responsible for a well-defined phenotype of embryopathy. Thalidomide definitely demonstrated that the environment plays a significant role in determining congenital malformations. The practical consequences were an increase in basic, clinical, and epidemiological research on environmental teratogenesis, and the creation of multiple controls aimed at reducing the introduction of teratogenic agents into the human environment.

Indeed, as physicians and patients became more and more aware of the possible teratogenic risks related to exposure to certain drugs, chemicals, physical or infectious agents during pregnancy, it also became clear that pertinent data was not available or difficult to interpret.

In fact, medical reports are often contradictory, anecdotal or limited to animal studies, while media messages to the general public are alarming and misleading.

Every year, the pharmaceutical industry markets new drugs with unknown embryofetal risks and labels warning against their use during pregnancy. As many pregnancies are not planned, women may therefore expose their embryos to environmental agents before realizing they are pregnant.

In 1990, members of 13 European programs and institutions engaged in counseling pregnant women at risk, evaluating and following up pregnancy outcomes, founded the European Network of Teratology Information Services (ENTIS). ENTIS has developed and promoted collaboration with the Organization of Teratology Information Services in the USA.

Results obtained in animal studies cannot automatically be applied to humans. Moreover, since most teratogenic agents are not identifiable (probably due to their low teratogenicity) and the mechanisms by which they produce their pathogenic effects are largely unknown, the possibility of a complete primary prevention through avoidance of hazardous prenatal exposures is unfortunately quite remote. At the same time, maternal diseases must be treated, as most are in themselves teratogenic (e.g. diabetes mellitus), and therapy may reduce the probability of fetal damage.

Moreover, Teratology Information Services (TIS) can collect prospective data on pregnancy exposure before the pregnancy outcome is known, thus assembling large unselected cohorts of specific exposures during pregnancy over a defined period. Their activity also has many practical results: reassuring women, reducing the rate of unnecessary pregnancy terminations, and advising on using the right drug instead of avoiding necessary therapy in both acute and chronic illnesses. TIS units employ the most unbiased method for postmarketing surveillance of drugs with respect to their use in pregnancy because they acquire data prospectively. The teratogenic potential of drugs such as valproic acid, phenprocoumon or mycophenolate mofetil can be better evaluated by multicentre studies of TIS.

As a basic rule, when planning pharmacological treatment in pregnant women, tried and tested substances should be given preference over new drugs. If a pregnant woman has been exposed to an insufficiently tested substance as a result of ignorance of her pregnancy, recognized centers with appropriate data bases (e. g. www.reprotox.de) should be consulted. As we also know of the severe and lifelong effects of maternal drug abuse (e. g. alcohol, amphetamines) during pregnancy, women in fertile age should be informed of these risks by special campaigns.

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Intervention

Nova Acta Leopoldina NF *112*, Nr. 382, 85 – 89 (2011)

Epigenetic Mechanisms in the Development of Type 2 Diabetes

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Abstract

Type 2 diabetes (T2D) is a disorder of complex genetics influenced by interactions between susceptible genetic loci and environmental perturbations. Intrauterine growth retardation (IUGR) is one such environmental perturbation that has been linked to development of T2D in adulthood. An abnormal metabolic intrauterine milieu affects the development of the fetus by permanently modifying expression of key genes regulating B-cell development (*Pdx1*) and glucose transport (*glut4*) in muscle. Epigenetic regulation of gene expression is one mechanism by which genetic susceptibility and environmental insults can lead to T2D. Therapeutic agents targeting epigenetic gene regulation may ultimately be used to treat T2D, but there is much to be learned about genome-wide epigenetic programming of health and disease before these therapies can be used in patient care.

Zusammenfassung

T2D ist eine Fehlanordnung von komplexen genetischen Strukturen, die beeinflusst wird von Wechselwirkungen zwischen empfänglichen Genloci und Umweltweinflüssen. Die intrauterine Wachstumsretardierung (IUGR) ist ein bekannter Umwelteinfluss, der in Zusammenhang mit der Expression von T2D im Erwachsenenalter steht. Ein verändertes intrauterines Milieu beeinflusst die Entwicklung des Feten durch die permanente Modifikation von Schlüsselgenen, die für die Regulation der B-Zellentwicklung (*Pdx1*) sowie des Glukosetransports in die Muskelzellen (*glut4*) verantwortlich sind. Epigenetische Regulation der Genexpression ist ein Mechanismus, mit dem durch genetische Prädisposition und Umwelteinflüsse T2D entstehen kann. Therapeutische Substanzen, die auf die epigenetische Genregulation abzielen, könnten schließlich direkt T2D blockieren. Bevor jedoch solche Substanzen für den Patienten zur Verfügung stehen können, müssen wir noch einiges über die genomweite epigenetische Programmierung von Krankheiten und Gesundheit lernen.

1. Introduction

One of the key environmental perturbations associated with T2D is exposure to an adverse intrauterine milieu, such as intrauterine growth retardation (IUGR). An adverse intrauterine milieu affects fetal development by modifying gene expression of both pluripotent cells, that are rapidly replicating and terminally differentiated cells that replicate poorly. Whether the effect of an exposure to an altered intrauterine milieu extends into adulthood depends on whether the cells are undergoing differentiation, proliferation, and/or functional maturation at the time of the exposure.

Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene expression from one generation of cells to the next. Epigenetic states can be modified by environmental factors, which may contribute to the development of abnormal phenotypes. There are at least two distinct mechanisms through which epigenetic information can be inherited: histone modifications and DNA methylation.

In eukaryotes, the nucleosome is formed when DNA is wrapped around an octameric complex of two molecules of each of the four histones: H2A, H2B, H3, and H4. The amino termini of histones can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation, and ADP ribosylation. The most common histone modifications involve acetylation and methylation of lysine residues in the amino termini of H3 and H4. Increased acetylation induces transcription activation, whereas decreased acetylation usually induces transcription repression. Methylation of histones, on the other hand, is associated with both transcription repression and activation. Moreover, lysine residues can be mono-, di-, or trimethylated *in vivo*, providing an additional mechanism of regulation.

2. DNA Methylation

The second class of epigenetic regulation is DNA methylation in which a cytosine base is modified by a DNA methyltransferase at the C5 position of cytosine, a reaction that is carried out by various members of a single family of enzymes. Approximately 70 % of CpG dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands. CpG islands are CG-rich sequences located near coding sequences, and serve as promoters for their associated genes. Approximately half of mammalian genes have CpG islands. The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors to DNA binding sites. In normal cells, most CpG islands remain unmethylated; however, under circumstances such as cancer and oxidative stress, they can become methylated *de novo*. This aberrant methylation is accompanied by local changes in histone modification and chromatin structure, such that the CpG island and its embedded promoter take on a repressed conformation that is incompatible with gene transcription. It is not known why particular CpG islands are susceptible to aberrant methylation.

DNA methylation is commonly associated with gene silencing and contributes to X-chromosomal inactivation, genomic imprinting, as well as transcriptional regulation of tissue-specific genes during cellular differentiation. It is not known why some genes are able to undergo aberrant DNA methylation, however, a study by Feltus et al. suggests that there is a "DNA sequence signature associated with aberrant methylation." Of major significance to T2D is their finding that *Pdx*-1, a pancreatic homeobox transcription factor, was one of only 15 genes (of 1749 examined) with CpG islands within the promoter that were methylation-susceptible (which was induced by over-expression of a DNA methyltransferase). This study demonstrates that genes essential to pancreatic development, like *Pdx-1*, are susceptible to epigenetic modifications, which could ultimately affect gene expression.

Histone methylation can influence DNA methylation patterns and *vice versa*. For example, methylation of lysine 9 on histone 3 (H3) promotes DNA methylation, while CpG methylation stimulates methylation of lysine 9 on H3. Recent evidence indicates that this dual relationship between histone methylation and DNA methylation might be accomplished by direct interactions between histone and DNA methyltransferases. Thus, chromatin modifications induced by adverse stimuli are self-reinforcing and can propagate.

3. Epigenetics in Intrauterine Growth Restriction

A number of studies suggest that uteroplacental insufficiency, the most common cause of IUGR in the developed world, induces epigenetic modifications in offspring. Fetal growth retardation can be induced by bilateral uterine artery ligation in pregnant rats. Pups are born spontaneously and have decreased levels of glucose, insulin, IGF-1, and amino acids. In this model, diabetes develops in animals at approximately 15–26 weeks of age with underlying B-cell secretory defects and insulin resistance, the salient features of most forms of T2D in humans. Epigenetic modifications affecting processes important to glucose regulation and insulin secretion, characteristics essential to the pathophysiology of T2D, have been described in the IUGR liver, pancreatic B-cells and muscle. The following sections describe specific epigenetic modifications induced in the IUGR model and their relationship to the development of T2D.

4. Chromatin Remodeling in the B-cell of IUGR Rats

Pdx-1 is a homeodomain-containing transcription factor that plays a critical role in the early development of both the endocrine and exocrine pancreas, and in the later differentiation and function of the B-cell. As early as 24 h after the onset of growth retardation, *Pdx-1* mRNA levels are reduced by more than 50% in IUGR fetal rats. Suppression of *Pdx*-1 expression persists after birth and progressively declines in the IUGR animal, implicating an epigenetic mechanism.

Changes in histone acetylation are the first epigenetic modification found in B-cells of IUGR animals. Islets isolated from IUGR fetuses show a significant decrease in H3 and H4 acetylation at the proximal promoter of *Pdx-1*. These changes in H3 and H4 acetylation are associated with a loss of binding of USF-1 to the proximal promoter of *Pdx-1*. USF-1 is a critical activator of *Pdx-1* transcription, and its decreased binding markedly decreases *Pdx-1* transcription. After birth, histone deacetylation progresses and is followed by a marked decrease in H3K4 trimethylation and a significant increase in dimethylation of H3K9 in IUGR islets. H3K4 trimethylation is usually associated with active gene transcription while H3K9 dimethylation is usually a repressive chromatin mark. Progression of these histone modifications parallels the progressive decrease in *Pdx-1* expression that manifests as a deterioration in glucose homeostasis and increased oxidative stress in the aging IUGR animals. Nevertheless, at 2 weeks of age, the silencing histone modifications in the IUGR pup are responsible for suppression of *Pdx-1* expression since there is no appreciable methylation of CpG islands in mice at this age. Reversal of histone deacetylation in IUGR islets at 2 weeks of age, is sufficient to nearly normalize *Pdx-1* mRNA levels permanently, perhaps due to active B-cell replication present in the neonatal rodent.

In IUGR, *Pdx-1* is first silenced due to recruitment of co-repressors, including histone deacetylase 1 (HDAC1) and mSin3A. These repressors catalyze histone deacetylation. Binding of these deacetylases facilitates loss of trimethylation of H3K4, further repressing *Pdx-1* expression. We found that inhibition of HDAC activity by trichostatin A (TSA) treatment normalizes H3K4me3 levels at *Pdx-1* in IUGR islets. These data suggest that the association of HDAC1 at *Pdx-1* in IUGR islets likely serves as a platform for the recruitment of a demethylase, which catalyzes demethylation of H3K4.

The molecular mechanism responsible for DNA methylation in IUGR islets is likely dependent on the methylation status of lysine 9 on H3 (H3K9). Previous studies have shown that changes in methylation of H3K9 precede changes in DNA methylation. It has also been suggested that DNA methyltransferases may act only on chromatin that is methylated at H3K9. Histone methyltransferases, specifically DNA methyltransferase 3A (DNMT3A) and DNA methyltransferase 3B (DNMT3B), bind to DNA methylases, thereby initiating DNA methylation.

These results demonstrate that IUGR induces a self-propagating epigenetic cycle in which the mSin3A/HDAC complex is first recruited to the *Pdx-1* promoter, histone tails are subjected to deacetylation and *Pdx-1* transcription is repressed. At the neonatal stage, this epigenetic process is reversible and may define an important developmental window for therapeutic approaches. However, as dimethylated H3K9 accumulates, DNMT3A is recruited to the promoter and initiates *de novo* DNA methylation, which locks in the silenced state in the IUGR adult pancreas resulting in diabetes.

How do these epigenetic events lead to diabetes? Targeted homozygous disruption of *Pdx*-1 in mice results in pancreatic agenesis, and homozygous mutations yield a similar phenotype in humans. Milder reductions in *Pdx*-1 protein levels, as occurs in the *Pdx*+/– mice, allow for the development of a normal mass of B-cells, but result in the impairment of several events in glucose-stimulated insulin secretion. These results indicate that *Pdx*-1 plays a critical role in the normal function of B-cells in addition to its role in B-cell lineage development. This may be the reason that humans with heterozygous missense mutations in *Pdx*-1 exhibit early and late onset forms of T2D.

5. Summary

The discovery of a critical developmental stage during which aberrant epigenetic modifications may be reversed represents a therapeutic window for the use of novel agents that could prevent common diseases with late-onset phenotypes. T2D is one such disease, where predisposed individuals could be treated with agents that normalize the epigenetic programming of key genes, thus providing protection against development of the adult diabetic phenotype.

The studies described above clearly show that environmental effects can induce epigenetic alterations, ultimately effecting expression of key genes linked to the development of T2D including genes critical for pancreatic development and B-cell function, peripheral glucose uptake, insulin resistance and atherosclerosis. Recent progress in understanding epigenetic programming of gene function has lead to the development of novel therapeutic agents with epigenetic targets in diseases such as cancer. Understanding the role of developmental programming of genes crucial to the development of T2D may unveil a critical window during which epigenetic therapeutic agents could be used as a means to prevent the later development of a disease. Prior to the use of such therapeutic agents there remains much to be learned about the programming of the epigenetic code, especially on a genome-wide scale. Much of the recent progress in understanding epigenetic phenomena is directly attributable to technologies that allow researchers to pinpoint the genomic location of proteins that package and regulate access to the DNA. The advent of DNA microarrays and inexpensive DNA sequencing has allowed many of those technologies to be applied to the whole genome. It is now possible that epigenetic profiling of CpG islands in the human genome can be used as a tool to identify genomic loci that are susceptible to DNA methylation. Aberrant methyla-

tion may be then be used as a biomarker for disease. The genome-wide mapping of histone modifications by ChIP-chip and ChIP-seq has led to important insights regarding the mechanism of transcriptional and epigenetic memory, and how different chromatin states are propagated through the genome in yeast and in mammalian cells. Although Chip-Seq experiments are currently being performed in human tissue, obstacles such as intrinsic human epigenetic variability (including age-related changes), and tissue-specific epigenetic variability must be characterized and mapped in the healthy, non-diseased state before this information can be applied to diseases such as T2D. Eventually genome wide epigenetic characterization will lead to specific therapies with epigenetic targets and will also allow monitoring of genome wide epigenetic consequences of these therapies once they are applied.

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Altern in Deutschland

Die Deutsche Akademie der Naturforscher Leopoldina und die Deutsche Akademie für Technikwissenschaften acatech gründeten im Mai 2005 eine gemeinsame interdisziplinäre Akademiengruppe „Altern in Deutschland“, die auf der Grundlage der besten verfügbaren wissenschaftlichen Evidenz öffentliche Empfehlungen erarbeitete, um die Chancen der im letzten Jahrhundert erheblich gestiegenen Lebenserwartung – die „gewonnenen Jahre“ – vernünftig zu nutzen und mit den Herausforderungen des demographischen Alterns klug umzugehen.

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ART and Fetal Programming

Marek Zygmunt and Herbert Fluhr (Greifswald)

Abstract

In 1978 Louise Brown was the first baby to be successfully carried to term and born following *in vitro* fertilization. Since then more than 4.5 million children have been born around the world using *in vitro* fertilization (IVF). Today reproductive medical procedures are used in 1–3 % of pregnancies in industrial countries. In the past few decades further advances and developments have been made in reproductive technology. The cornerstones of these treatments include pharmacological and surgical methods. Hormonal inactivation of the pituitary glands, stimulation of the ovaries, harvesting, *in vitro* maturation and preservation of gametes are some examples of things currently being tied to ART-related problems such as deformity, epigenetic alterations and perinatal "outcomes". Individual points will be more precisely explained in the following article.

Zusammenfassung

Louise Brown war 1978 das erste Kind, das nach künstlicher Befruchtung erfolgreich ausgetragen und geboren wurde. Seitdem sind weltweit mehr als 4,5 Millionen Kinder nach *In-vitro*-Fertilisation (IVF) geboren worden. Heutzutage sind bereits bei 1–3 % der Geburten in den Industrieländern reproduktionsmedizinische Verfahren beteiligt. In den letzten Jahrzenten wurden die reproduktionsmedizinischen Techniken erweitert und weiterentwickelt. Zu den Säulen der Behandlung gehören sowohl pharmakologische als auch chirurgische Methoden. Insbesondere die hormonelle Ausschaltung der Hypophyse, Stimulation der Ovarien, Gewinnung, *In-vitro*-Reifung und Konservierung von Gameten werden im Augenblick mit ART-assoziierten Problemen wie Fehlbildungen, epigenetischen Veränderungen und dem perinatalen „Outome" in Zusammenhang gebracht. Im nachfolgenden Beitrag werden die einzelnen Punkte genauer dargestellt.

1. Introduction

Since the birth of Louise Brown – the first baby delivered after *in vitro* fertilization (IVF) in 1978 (Steptoe and Edwards 1978) – over 4.5 million IVF babies have been born worldwide. Nowadays, in 1–3 % of all births in developed countries some kind of assisted reproductive technology (ART) is involved. Due to the high demand – 10–15 % of all couples in Europe have fertility problems – the methods used in ART have been changed and improved over last few decades. Reproductive medicine is based on several different procedures including the pharmacological treatment of intentional hormonal pituitary down-regulation or ovarian stimulation. The *in vitro* manipulation of gametes and embryos is an important tool in ART. Indeed, *in vitro* maturation of oocytes, the use of immature sperm, methods of intracytoplasmatic sperm injection (ICSI), and the *in vitro* culture of pre-implanted embryos have been established. However, recent epidemiologic data indicate changes in perinatal outcome associated with ART.

2. Perinatal Outcome

Studies focussing on the perinatal outcome of babies delivered after IVF and ICSI, revealed higher risk of obstetrical complications. Indeed, there are increased rates of obstetrical interventions including labour induction or elective Caesarean deliveries following procedures of assisted conception. In our opinion this information should definitely be part of the medical information provided to couples planning to conceive with reproductive assistance. In addition, information about increased risk of multiple pregnancies and the associated higher rates of perinatal complications should be the focus of a detailed medial education of patients. Depending on the number of embryos transferred during ART, the risk of multiple pregnancies increases. According to a CDC report 2000 published in 2002, transfer of three embryos resulted in 53.5 % singleton, 37.4 % twin, and 9.1 % triplet pregnancies. In addition, singleton ART-pregnancies are often associated with gestational diseases and complications. Studies concerning the health of ART pregnant women revealed higher rates of hypertension, preeclampsia, glucose intolerance and diabetes. Women pregnant after ART are in need of intensive medical care including in-patient services and psychological support resulting in dramatically increased medical expenses as compared to women conceiving spontaneously.

3. Risk of Malformation

Literature concerning increased risk of malformation after ART is rather limited showing in part controversial results. However, there are reports showing higher rates of malformations after ART. Even if the numbers are low, the high variability in different reports need to be further addressed. To start with, the reported rates depend on the system of classification, and the study design.

A recently published multi-centre study by Bonduelle et al. 2004 reports compelling data on the prevalence of congenital malformations after ICSI/IVF. On the one hand, the rate of minor neonatal malformations after ICSI and IVF increased, but on the other hand the prevalence of major malformations was comparable in all subgroups. In accordance with those findings it has been demonstrated that children born after ICSI or IVF are more susceptible for infections of the urogenital tract. However, those children also suffer more often from dermatological and gastroenterological illnesses and definitely need more medical assistance as measured by the number of hospital admissions.

4. Imprinting Defects

Another issue recently addressed by several research groups is the epigenetic phenomenon of genetic imprinting. Gene imprinting is a mechanism of structural adaptation of chromosomal regions to register, signal or perpetuate altered activity states of specific genes without changes in the nucleic acid sequence. The major mechanisms involved are DNA-methylation and histone modification. It is assumed that gene imprinting is a dynamic process, which can be erased and re-established. Importantly, the profile of gene imprinting is gender-specific resulting in a monoallelic expression of genes depending on the parental origin.

In contrast to sperm where imprinting is completed earlier, the remethylation of the oocyte takes place after ovulation. Therefore epigenetic vulnerability can be assumed at this point. In addition, the early embryo undergoes a genome-wide demethylation process after fertilization. Additionally placenta specific imprinting has been described recently. On the one hand, maternally expressed and paternally imprinted genes including PHLDA 2 and/or IGF-2R have been shown to restrict fetal growth. On the other hand, paternally expressed and maternally imprinted genes, including IGF-2, MEST, MEG-2, GATM, GNAS and PLAGL, promote fetal growth.

Definition of states of high epigenetic vulnerability during ART procedures may include the following processes: Mechanical injury of the oocyte during ICSI may potentially lead to aneuploidy. At the same time incomplete chromosome de-condensation can delay the DNA replication of the paternal genome. The introduction of sperm and media components into the oocytes cytoplasm as well as the inclusion of spermatoid transcripts into oocytes may eventually cause interference in epigenetic reprogramming.

During the *in vitro* preimplantation period, effects of culture medium components on methylation patterns of imprinted genes have to be considered (Celtin et al. 2003). In recent years rare syndromes which have been linked with imprinting defects such as Beckwith-Wiedemann syndrome, Angelman syndrome and retinoblastoma have been described. Especially ICSI procedures have been associated with those syndromes.

5. Fetal Programming

The impact of the perinatal period on the long-term outcome of an individual is summarized in the current hypothesis of *fetal programming*. The introduction of epigenetic modification as long-term memory of environmental influences gives a biological and detectible equivalent to examine the impact of ART on the postnatal period. The impact of epigenetic modifications on disease development and progression is currently the focus of several research programs on cancer and other complex diseases. However, a link between procedures used in ART, genetic imprinting and changes in *fetal programming* is the subject of ongoing studies. Findings from several animal studies clearly support the notion that there is an impact of ART procedures on epigentics. Importantly, recent findings even show that ART interference with imprinting reprogramming is transduced into the second or even third generation of offspring, therefore opening a field of inheritance apart from the classic sequence-based genetic traits.

It has been shown using a superovulated mouse model that hormonal stimulation by itself increases the incidence of abnormal methylation patterns especially in regard to PEG1 and H19. Additionally, *in vitro* maturation as well as embryo manipulation procedures influence DNA methylation in liver and muscle of bovine fetus causing specific endocrine changes and fetal overgrowth. In those cases abnormal methylation involves IGF-2 and H19. Similar results have been obtained by performing studies in humans. Although no evidence was provided that the use of immature sperm causes imprinting defects, 17 % of men with oligozoospermia and 30 % of men with severe oligozoospermia have altered H19 methylation profiles.

Above listed imprinting changes can have major impacts on further development of the fetus and may cause endocrine, metabolic diseases, or even behavioural changes.

6. Conclusion

In summary, ART summarizes a variety of methods used in reproductive medicine. Current data reveal a 30 % increase in rates of malformation in babies conceived after ICSI as compared to standard IVF procedures. There is also an association between ART procedures and imprinting disorders where a risk increase of up to 100-fold has been reported. It remains unclear whether the increased risks are attributed to the underlying infertility, characteristics of the infertile couples or the use of ART itself. Large prospective cohort studies are needed to address those questions.

References

Bonduelle, M., Bergh, C., Niklasson, A., Palermo, G. D., and Wennerholm, U. B.: Medical follow-up study of 5-year-old ICSI children. Reprod. Biomed. Online *9*/1, 91–101 (2004)

CDC Report 2000: National Vital Statistics Report *50*/15 (2002) http://www.cdc.gov/nchs/data/nvsr/nvsr50/nvsr50_15.pdf

Cetin, I., Cozzi, V., and Antonazzo, P.: Fetal development after assisted reproduction – a review. Placenta *24*, Suppl. 2, S104–S113 (2003)

Steptoe, P. C., and Edwards, R. G.: Birth after the reimplantation of a human embryo. Lancet *12*/2, 366 (1978)

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Postnatal Obesity – What Can be Done in the Early Life Period?

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Abstract

The obesity epidemic has seen the incidence of obesity and overweight almost double in Western societies, and the trend is mirrored in developing nations that are transitioning to first-world economies. Metabolic disease results from a complex interaction of many factors, including genetic, physiologic, behavioural, and environmental influences. The recent rate at which these diseases have increased suggests that environmental and behavioural influences, rather than genetic causes, are fuelling the present epidemic. In this context, the developmental origins of health and disease (DOHaD) hypothesis has highlighted the link between the periconceptual, fetal and early infant phases of life and the subsequent development of adult obesity and related metabolic disorders. Although the mechanisms are yet to be elucidated, this programming has generally been considered an irreversible change in developmental trajectory. Recent work suggests that, at least in animal models, developmental programming of postnatal obesity is potentially reversible by nutritional or targeted therapeutic interventions during the period of developmental plasticity.

Zusammenfassung

Die Adipositas-Epidemie zeigt in den westlichen Ländern nahezu eine Verdopplung des Auftretens von Adipositas und Übergewicht, und dieser Trend spiegelt sich auch in den Entwicklungsländern wider, die zu den westlichen Volkswirtschaften aufschließen. Stoffwechselerkrankungen sind auf eine komplexe Wechselwirkung verschiedener Faktoren zurückzuführen, darunter genetische, physiologische, verhaltensbezogene und umweltbedingte Einflüsse. Die jüngste Zuwachsrate bei diesen Erkrankungen deutet darauf hin, dass umweltbedingte und verhaltensbezogene Einflüsse mehr als genetische Ursachen die gegenwärtige Epidemie verstärken. In diesem Zusammenhang hat die Hypothese des entwicklungsbedingten Ursprungs von Gesundheit und Krankheit die Verbindung zwischen perikonzeptioneller, fetaler und Säuglingslebensphase und der nachfolgenden Entwicklung von Adipositas und damit verbundener Stoffwechselstörungen im Erwachsenenalter aufgezeigt. Obwohl die Mechanismen noch aufzuklären sind, ist diese Programmierung generell als eine irreversible Veränderung in der Entwicklungsbahn betrachtet worden. Jüngste Arbeiten deuten darauf hin, dass zumindest in Tiermodellen die entwicklungsbedingte Programmierung von postnataler Adipositas möglicherweise durch ernährungsbedingte oder gezielte therapeutische Interventionen während der Periode der Entwicklungsplastizität reversibel ist.

1. Introduction

Obesity is a serious health issue in the developed world and is becoming increasingly important on a global scale. Furthermore, the marked increases in childhood obesity will translate to a further increase in adult obesity in the near future and it has been ranked as a critical public health threat (Koplan et al. 2005). It is a widely held view that the development of an obesogenic environment, due to ease of access to highly calorific food and reduced energy expenditure in work and leisure activities, is the primary cause of obesity in the general population. Multiple systems regulate energy homeostasis, and there is strong evidence for a

genetic component to human obesity with the identification of a number of genes associated with human obesity (Bell et al. 2005). However, the genetic component of this condition cannot account for the dramatic increase in the prevalence of obesity in recent years. Of relevance, epidemiological and experimental studies have highlighted a relationship between the periconceptual, fetal and early infant phases of life and the subsequent development of adult obesity (Ravelli et al. 1999, Godfrey and Barker 2000, Breier et al. 2001). The terms "developmental programming" and the "Developmental Origins of Adult Health and Disease" are preferentially used to describe these associations. The mechanisms underlying developmental programming and the relative role of genetic *versus* environmental factors remain speculative. One general thesis is that in response to an adverse intrauterine environment the fetus adapts its physiological development to maximise its immediate chances for survival. These adaptations may include resetting of metabolic homeostasis and endocrine systems and the down-regulation of growth, commonly reflected in an altered birth phenotype. More recently the "predictive adaptive response" (PARs) hypothesis proposes that the degree of mismatch between the pre- and postnatal environments is a major determinant of subsequent disease (Gluckman and Hanson 2004a, Gluckman et al. 2008). Thus, it is thought that whilst these changes in fetal physiology may be beneficial for short term survival *in utero* they may be maladaptive in postnatal life, contributing to poor health outcomes when offspring are exposed to catch-up growth, diet-induced obesity and other factors (Gluckman and Hanson 2004b, Gluckman et al. 2008).

2. Developmental Programming of Obesity – Evidence from Epidemiology

Barker and colleagues demonstrated a relationship between low birth weight and an increased risk of hypertension, obesity, insulin resistance and dislipidemia (Barker et al. 1989, 1990, Osmond et al. 1990). From these initial observations, the importance of maternal nutrition and, in particular, the effect of poor nutrition on birth weight and development of adult disease was addressed in studies of famine exposure. The most widely reported of these being the Dutch Hunger Winter of 1944–1945 (Ravelli et al. 1976, 1999, Roseboom et al. 1999, 2001) where the timing of the exposure was a major determinant in phenotypic outcomes. During the last trimester of pregnancy and the first months of life, famine exposure produced significantly lower obesity rates. This result is consistent with the inference that nutritional deprivation affected a critical period of development for adipose-tissue cellularity. During the first half of pregnancy, however, famine exposure resulted in significantly higher obesity rates (Ravelli et al. 1976, 1999). However, the data derived from those exposed to famine during the siege of Leningrad did not show any relationship between birth weight and adult metabolic sequalae (Stanner and Yudkin 2001). Thus while fetal exposure to a substrate limited environment at most stages of development appears to lead to adult dysregulation of metabolism, the precise mechanisms responsible may vary with the timing of exposure. The disparity between the Dutch and the Leningrad studies may be explained by the PARs hypothesis – in the Dutch offspring, nutrition was plentiful following the famine and thus was mismatched to the predicted environment. In the Leningrad cohort, nutritional status was poor both before and after the period of famine and thus the PAR may have been appropriate for the postnatal environment experienced.

In historically undernourished, recently urbanised populations such as India, where individuals of low birth weight are exposed to a high-fat Western diet, the incidence of obesity

and type 2 diabetes is reaching epidemic proportions (Yajnik 2000). Work by Yajnik and colleagues have shown that although Indian babies are born of low birth weight, they exhibit relatively increased visceral adiposity. This is consistent with other studies of small babies, showing a disproportionate abdominal fat mass during adult life, despite a lower body mass index (BMI). Although there is considerable debate whether catch-up growth in early postnatal life is beneficial or not, most studies suggested that postnatal "catch-up" growth is associated with adverse outcomes in later life (Eriksson et al. 1999, Ong et al. 2000). Interestingly, work by Parsons et al. (2001) found that men with a lower birth weight who then exhibited catch-up growth to achieve a greater proportion of their adult height by age 7, had a risk of obesity comparable to that for men with higher birth weights. Recent work by Eriksson et al. (2003) has also demonstrated that ponderal index at birth was a reliable good predictor of later obesity and also found that an early adiposity rebound in babies born of low birth weight was associated with obesity in adult life.

3. Developmental Programming of Obesity – Evidence from Animal Studies

Animal models have been extensively used to study the basic physiological principles of the DOHaD hypothesis and are essential to the search for the mechanistic links between prenatal and postnatal influences and obesity risk in later life. Although epidemiological data suggest that developmental programming occurs within the normal range of birth size (Barker 2007a, b), most experimental work has tended to focus on significant restriction of fetal growth in the assumption that the nature of the insults that impair fetal growth are likely to be those that trigger developmental programming. Several approaches have been developed to induce early growth restriction in animals in an attempt to elucidate its relationship with adult onset disease and provide a framework for investigating the underlying mechanisms. In rats, obesity and metabolic disorders have been induced in offspring by maternal global undernutrition (McArdle et al. 2006, Vickers et al. 2000, 2001, 2002, Woodall et al. 1996a, b), a low protein diet (Langley Evans et al. 1999), maternal uterine artery ligation (Rajakumar et al. 1998, Simmons et al. 2001), maternal dexamethasone (DEX) treatment (Nyirenda et al. 1998), maternal anaemia (Lewis et al. 2001) or prenatal cytokine exposure (Dahlgren et al. 2001). In this context intrauterine growth restriction (IUGR) is not essential to developmental programming, but is merely a surrogate for evidence that fetal development may have been affected.

3.1 Maternal Undernutrition

The early work of Barker and colleagues highlighted the role of fetal nutrition as the primary factor involved in the developmental origins of adult disease. Within the laboratory, fetal undernutrition can most commonly be achieved through maternal dietary restriction during pregnancy. At present, rodent models investigating the mechanistic links between maternal undernutrition and adult disease generally utilise one of two dietary protocols; global undernutrition or isocaloric low protein diets. The maternal low protein (MLP) diet during pregnancy and lactation is one of the most extensively utilised models of nutritional programming (Snoeck et al. 1990, Langley and Jackson 1994, Desai et al. 1996, Ozanne et al. 1999,

Petry et al. 2001). This model involves *ad-libitum* feeding to pregnant rats a low protein diet containing 5–8% (w/w) protein (casein), generally a little under half the protein content but equivalent in energy of a control diet containing 18–20% (w/w) protein (Langley-Evans 2000). Offspring from protein restricted mothers are around 15–20% lighter at birth (Desai et al. 1996). Maintenance of a MLP diet during lactation enhances this weight difference and permanently limits later growth. If MLP offspring are cross-fostered to mothers fed a control diet, they exhibit rapid catch-up growth (Desai et al. 1996). This catch-up growth appears to have a detrimental effect on life span, resulting in premature death which is associated with accelerated loss of kidney telomeric DNA (Jennings et al. 1999). Altered insulin sensitivity of adipocytes in MLP offspring has also been well documented; the findings of these studies show that enhanced activation of insulin receptor substrate-1 (IRS-1) associated phosphoinositol 3-kinase (PI3K) activity may be the key to improvements in insulin sensitivity (Ozanne et al. 2001). However, alterations in PI3K subunit expression indicate that the adipocytes of MLP offspring may be resistant to insulin's antilipolytic effects.

Experimental observations in the MLP diet model of developmental programming highlight many potential mechanisms that may be involved in the pathogenesis of obesity and diabetes. These mechanisms include both physical and functional changes to various organ and endocrine systems. For example, recent work has examined adipose tissue gene expression profiling of offspring from MLP rats (Guan et al. 2005). Gene ontogeny analysis of visceral adipose tissue (VAT) revealed a global up-regulation of genes involved in carbohydrate, lipid and protein metabolism in offspring of MLP animals. Thus VAT in the MLP model is marked by dynamic changes in the transcriptional profile of key metabolic genes.

Global undernutrition at various times during pregnancy is another widely used approach to induce nutritional programming of obesity. Various different models have been developed with different levels of undernutrition during different periods of pregnancy. A moderate nutritional restriction (70% of normal intake) in the first 18 days of pregnancy in rats results in offspring with significant IUGR that catch up in body weight to that of controls by postnatal day 20 (Ozaki et al. 2000). These abnormalities increase with age and are most pronounced in male offspring.

We have developed rodent models of developmental programming using global maternal undernutrition throughout pregnancy (Woodall et al. 1996a, b, Vickers et al. 2000). When dams are fed at 30% of *ad-libitum* intake throughout pregnancy, i.e. a severe level of undernutrition, offspring birth weights and placental weights are 25–30% lower than offspring of control fed mothers. These offspring display increased adiposity, hypertension, hyperinsulinemia, hyperleptinemia, reduced locomotor activity, leptin resistance and hyperphagia in adult life (Vickers et al. 2000, 2003, Krechowec et al. 2006). When the degree of undernutrition is reduced to a more moderate level, i.e. 50% of *ad-libitum*, offspring still display a significant level of obesity in postnatal life. Of note, if pre-weaning catch-up growth in offspring is prevented by maintaining the mothers on the restricted diet throughout lactation, offspring do not develop an obese phenotype (author's unpublished observations). This is similar to reports in the MLP model where continuation of the LP diet into lactation prevents the development of the metabolic phenotype, once again highlighting the possible adverse consequences of catch-up growth (Desai et al. 2005).

3.2 Maternal Nutritional Excess

Epidemiological studies have demonstrated that fetal growth restriction correlates with adult disease, implying that fetal nutritional deprivation is a strong stimulus for programming (Armitage et al. 2005). Thus, experimental animal models were developed using controlled maternal caloric intake or protein or macronutrient deficiency. However, in many developed societies, maternal and postnatal caloric intake is either sufficient or excessive. A number of studies have shown that programmed obesity may represent a U-shaped curve with a higher prevalence of adult obesity occurring in individuals who were on either low or high planes of maternal nutrition (Armitage et al. 2005, McMillen et al. 2005, Samuelsson et al. 2008, Morris and Chen 2009, Howie et al. 2009). Further, in pregnancies which have been complicated by maternal diabetes, gestational diabetes or impaired glucose tolerance, offspring have been shown to be at enhanced risk of developing obesity (Holemans et al. 2004). Thus, following the predictive adaptive response hypothesis, in response to a given *in utero* or early postnatal nutritional plane (either high or low), cellular processes are invoked to cope with the predicted environment. This hypothesis suggests that disease only manifests when the actual nutritional environment diverges from that which was predicted. It is notable that there is evidence for the programming of obesity and several other features of the metabolic syndrome from both nutrient restriction (caloric, protein, iron) and fat-feeding studies, possibly suggestive of a commonality of mechanism (Armitage et al. 2004).

4. Critical Windows for Intervention

Until recently, developmental programming was seen to be an irreversible change in developmental trajectory and the consequences of which had to be managed, e.g. obesity and type 2 diabetes. To date, few studies have addressed the possibility of reversibility or prevention of the postnatal programmed phenotype.

It has been proposed that deficiencies in the obesity hormone leptin during critical windows of development could lead to a hardwiring of obesity (Horvath and Bruning 2006). In adult mammals, leptin acts on the brain to reduce food intake by regulating the activity of neurons in the arcuate nucleus of the hypothalamus (ARH). Bouret et al. have shown that neural projection pathways from the ARH are permanently disrupted in leptin-deficient (Lep^{ob}/Lep^{ob}) mice (Bouret et al. 2004a, b). Treatment of Lep^{ob}/Lep^{ob} neonates with exogenous leptin rescues the development of ARH projections, and leptin promotes neurite outgrowth from ARH neurons *in vitro*. It is well established that children small for gestational age (SGA) are hypoleptinemic and cord blood leptin concentrations are significantly diminished (Iniguez et al. 2004). These children often go on to develop obesity and leptin resistance in adult life, and this can be mimicked experimentally in the rat (Vickers et al. 2000). Thus perturbations in perinatal nutrition that alter leptin levels may have enduring consequences for the formation and function of circuits that regulate food intake and body weight (Elmquist et al. 1998, Bouret et al. 2004a, b, c). Recent work investigating neonatal systemic leptin treatment in female Wistar rats born following maternal undernutrition has found that leptin prevented the development of diet-induced obesity and associated metabolic sequalae in adult life (Vickers et al. 2005). Leptin treatment normalised caloric intake, locomotor activity, body weight, fat mass, fasting plasma glucose, insulin, c-pep-

tide and leptin concentrations; suggesting that any effect is not restricted solely to a central mechanism. Moreover, the effects were specific to animals born of low birth weight, with leptin having no effect in animals born to control mothers. Whether this effect of leptin is central or peripheral is unclear – one possibility is that the period of developmental plasticity is still open, and the high leptin levels reverse the cuing effects of prenatal undernutrition (Gluckman et al. 2007). The next piece to the puzzle is the question of the neonatal leptin surge – while the surge is well characterised in rodents (Ahima et al. 1998) and may inform a window of intervention – the presence or absence of a leptin surge in humans is uncertain. The translation of findings across animal models itself bodes well for defining the role of leptin during this critical window of development.

Work in rodents has also shown that both growth hormone (GH) and insulin-like growth factor (IGF)-I can resolve several aspects of the metabolic phenotype in developmentally programmed offspring. Utilising a model of maternal undernutrition to induce fetal growth restriction, offspring were fed either a chow or high fat diet postnatally. These offspring were hypertensive, obese, hyperphagic, hyperinsulinemic and hyperleptinemic; the effects of which were markedly amplified in the presence of a postnatal high fat diet (Vickers et al. 2000). Treatment of the adult phenotype with GH normalised systolic blood pressure and reduced fat mass. However, the hyperinsulinemia was exacerbated as a result of the diabetogenic actions of GH (Vickers et al. 2002). A further study in adult females with IGF-I infusion led to a complete normalisation of adiposity, appetite, fasting plasma insulin and leptin concentrations in developmentally programmed offspring (Vickers et al. 2001). These studies highlight the role of the somatotropic axis in programmed metabolic disturbances although the longer term efficacy of such treatment regimes are not known. Trials with GH in small for gestation age children have shown a normalisation in systolic blood pressure which was maintained for the six year duration of treatment (Sas et al. 2000).

Treatment of neonatal rats with the glucagon-like peptide (GLP)-1 analog Exendin 4 (EX-4) can reverse the adverse consequences of developmental programming and prevents the development of diabetes in adulthood (Stoffers et al. 2003, Park et al. 2008, Raab et al. 2009). This occurs because neonatal Ex-4 prevents the progressive reduction in insulin-producing B-cell mass that is observed in IUGR rats over time and expression of pancreatic duodenal homeobox (PDX), a critical regulator of pancreas development and islet differentiation, is restored to normal levels. Although adiposity was not examined in this study, glucagon-like peptides are known to modify food intake, increase satiety, delay gastric emptying, and suppress glucagon release. Therefore further studies are warranted.

The role of possible direct nutritional interventions has been highlighted in a study by Wyrwoll et al. (2006). Pregnant rats were treated with DEX from d13 to term, and offspring were cross-fostered to mothers on either a standard diet or a diet high in omega-3 fatty acids and remained on these diets post-weaning. Maternal DEX reduced birth weight and delayed the onset of puberty in offspring. Hyperleptinemia and increased fat mass developed in offspring by 6 months of age in DEX-exposed animals fed a standard diet, but these effects were completely ameliorated by a high omega-3 diet. These results demonstrated for the first time that direct manipulation of postnatal diet can limit adverse outcomes of developmental programming.

5. Discussion

Numerous epidemiological studies have described a relationship between adverse prenatal factors and the development of metabolic disease and obesity in later life. Both prospective clinical studies and experimental research have clearly shown that the propensity to develop increased adiposity in later life is increased when early life development has been adversely affected. The pathogenesis is not based on genetic defects, but on altered genetic expression as a consequence of an adaptation to environmental changes during early life development. However, little is known about the interaction between the pre-and postnatal nutritional environment on either amplification or resolution of the programming phenotype depending on the degree of nutritional match/mismatch. Thus, experiments to examine the predictive adaptive response hypothesis are required in conjunction with transgenerational work to further the DOHaD paradigm.

The molecular mechanisms underlying developmental programming are only recently beginning to be investigated. Epigenetics has now become a model that is fundamental to research into DOHaD. The two most studied epigenetic mechanisms identified as having a role in the adaptive developmental programming of metabolic disorders are DNA methylation and histone modifications. Availability of dietary methyl donors and cofactors during a critical window of fetal development may influence DNA methylation patterns. Thus it has been proposed that early methyl donor malnutrition (i.e. excess nutrition or undernutrition) could effectively lead to premature epigenetic aging and thereby conferring an enhanced susceptibility to adult disease in later life (Waterland and Jirtle 2004).

Developmental programming research offers a novel approach to investigate the mechanistic basis of obesity and related metabolic disorders which in human populations predominantly arises from environmental factors and lifestyle choices. It is notable that the variety of different insults in early life (caloric, protein, iron, fat-fed) produce the same detrimental consequences that occur in adult life, suggestive of a common mechanism underlying the developmental early-life programming of adult disease. A recent emerging focus has been on studies aimed at reversing the programmed phenotype; such studies offer an exciting potential for new advances in our understanding of critical determinants and mechanisms for human obesity and metabolic disorders.

References

Ahima, R. S., Prabakaran, D., and Flier, J. S.: Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J. Clin. Invest. *101*, 1020–1027 (1998)

Armitage, J. A., Khan, I. Y., Taylor, P. D., Nathanielsz, P. W., and Poston, L.: Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J. Physiol. *561*, 355–377 (2004)

Armitage, J. A., Taylor, P. D., and Poston, L.: Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. J. Physiol. *565*, 3–8 (2005)

Barker, D. J.: Obesity and early life. Obes. Rev. *8*/1, 45–49 (2007a)

Barker, D. J.: The origins of the developmental origins theory. J. Intern. Med. *261*, 412–417 (2007b)

Barker, D. J., Bull, A. R., Osmond, C., and Simmonds, S. J.: Fetal and placental size and risk of hypertension in adult life. BMJ (Clinical research ed.) *301*, 259–262 (1990)

Barker, D. J., Osmond, C., Golding, J., Kuh, D., and Wadsworth, M. E.: Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ (Clinical research ed.) *298*, 564–567 (1989)

Bell, C. G., Walley, A. J., and Froguel, P.: The genetics of human obesity. Nature Rev. Genet. *6*, 221–234 (2005)

Bouret, S. G., Draper, S. J., and Simerly, R. B.: Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. J. Neurosci. *24*, 2797–2805 (2004a)

Bouret, S. G., Draper, S. J., and Simerly, R. B.: Trophic action of leptin on hypothalamic neurons that regulate feeding. Science *304*, 108–110 (2004b)

Bouret, S. G., and Simerly, R. B.: Minireview: Leptin and development of hypothalamic feeding circuits. Endocrinology *145*, 2621–2626 (2004c)

Breier, B. H., Vickers, M. H., Ikenasio, B. A., Chan, K. Y., and Wong, W. P.: Fetal programming of appetite and obesity. Mol. Cell. Endocrinol. *185*, 73–79 (2001)

Dahlgren, J., Nilsson, C., Jennische, E., Ho, H. P., Eriksson, E., Niklasson, A., Bjorntorp, P., Albertsson Wikland, K., and Holmang, A.: Prenatal cytokine exposure results in obesity and gender-specific programming. Amer. J. Physiol. Endocrinol. Metab. *281*, E326–334 (2001)

Desai, M., Crowther, N. J., Lucas, A., and Hales, C. N.: Organ-selective growth in the offspring of protein-restricted mothers. Br. J. Nutr. *76*, 591–603 (1996)

Desai, M., Gayle, D., Babu, J., and Ross, M. G.: Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. Amer. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R91–96 (2005)

Elmquist, J. K, Ahima, R. S., Elias, C. F., Flier, J. S., and Saper, C. B.: Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. Proc. Natl. Acad. Sci. USA *95*, 741–746 (1998)

Eriksson, J., Forsen, T., Osmond, C., and Barker, D.: Obesity from cradle to grave. Int. J. Obes. Relat. Metab. Disorders – J. Int. Assoc. Study Obes. *27*, 722–727 (2003)

Eriksson, J. G., Forsen, T., Tuomilehto, J., Winter, P. D., Osmond, C., and Barker, D. J.: Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ (Clinical research ed.) *318*, 427–431 (1999)

Gluckman, P. D., Beedle, A. S., Hanson, M. A., and Vickers, M.: Leptin reversal of the metabolic phenotype: evidence for the role of developmental plasticity in the development of the metabolic syndrome. Horm. Res. *67*, 115–120 (2007)

Gluckman, P. D., and Hanson, M. A.: Developmental origins of disease paradigm: a mechanistic and evolutionary perspective. Pediatr. Res. *56*, 311–317 (2004a)

Gluckman, P. D., and Hanson, M. A.: Living with the past: evolution, development, and patterns of disease. Science *305*, 1733–1736 (2004b)

Gluckman, P. D., Hanson, M. A., Beedle, A. S., and Spencer, H. G.: Predictive adaptive responses in perspective. Trends Endocrinol. Metab. 2008

Godfrey, K. M., and Barker, D. J.: Fetal nutrition and adult disease. Amer. J. Clin. Nutr. *71*, 1344S–1352S (2000)

Guan, H., Arany, E., van Beek, J. P., Chamson-Reig, A., Thyssen, S., Hill, D. J., and Yang, K.: Adipose tissue gene expression profiling reveals distinct molecular pathways that define visceral adiposity in offspring of maternal protein-restricted rats. Amer. J. Physiol. Endocrinol. Metab. *288*, E663–673 (2005)

Hofbauer, K. G.: Molecular pathways to obesity. Int. J. Obes. Relat. Metab. Disord. *26*/2, S18–27 (2002)

Holemans, K., Caluwaerts, S., Poston, L., and Van Assche, F. A.: Diet-induced obesity in the rat: a model for gestational diabetes mellitus. Amer. J. Obstet. Gynecol. *190*, 858–865 (2004)

Horvath, T. L., and Bruning, J. C.: Developmental programming of the hypothalamus: a matter of fat. Nature Med. *12*, 52–53; discussion 53 (2006)

Howie, G. J., Sloboda, D. M., Kamal, T., and Vickers, M. H.: Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. J. Physiol. *587*, 905–915 (2009)

Iniguez, G., Soto, N., Avila, A., Salazar, T., Ong, K., Dunger, D., and Mericq, V.: Adiponectin levels in the first two years of life in a prospective cohort: relations with weight gain, leptin levels and insulin sensitivity. J. Clin. Endocrinol. Metab. *89*, 5500–5503 (2004)

Jennings, B. J., Ozanne, S. E., Dorling, M. W., and Hales, C. N.: Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett. *448*, 4–8 (1999)

Koplan, J. P., Liverman, C. T., and Kraak, V. I.: Preventing childhood obesity: health in the balance: executive summary. J. Amer. Diet. Assoc. *105*, 131–138 (2005)

Krechowec, S. O., Vickers, M., Gertler, A., and Breier, B. H.: Prenatal influences on leptin sensitivity and susceptibility to diet-induced obesity. J. Endocrinol. *189*, 355–363 (2006)

Langley, S. C., and Jackson, A. A.: Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin. Sci. (Lond.) *86*, 217–222; discussion: 121 (1994)

Langley-Evans, S. C.: Critical differences between two low protein diet protocols in the programming of hypertension in the rat. Int. J. Food Sci. Nutr. *51*, 11–17 (2000)

Langley-Evans, S. C, Welham, S. J., and Jackson, A. A.: Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci. *64*, 965–974 (1999)

Lewis, R. M., Petry, C. J., Ozanne, S. E., and Hales, C. N.: Effects of maternal iron restriction in the rat on blood pressure, glucose tolerance, and serum lipids in the 3-month-old offspring. Metabolism *50*, 562–567 (2001)

McArdle, H. J., Andersen, H. S., Jones, H., and Gambling, L.: Fetal programming: causes and consequences as revealed by studies of dietary manipulation in rats – a review. Placenta *27*, Suppl. A, S56–60 (2006)

McMillen, I. C., Adam, C. L., and Muhlhausler, B. S.: Early origins of obesity: programming the appetite regulatory system. J. Physiol. *565*, 9–17 (2005)

Morris, M. J., and Chen, H.: Established maternal obesity in the rat reprograms hypothalamic appetite regulators and leptin signaling at birth. Int. J. Obes. (Lond.) *33*, 115–122 (2009)

Nyirenda, M. J., Lindsay, R. S., Kenyon, C. J., Burchell, A., and Seckl, J. R.: Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J. Clin. Invest. *101*, 2174–2181 (1998)

Ong, K. K., Ahmed, M. L., Emmett, P. M., Preece, M. A., and Dunger, D. B.: Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ (Clinical research ed.) *320*, 967–971 (2000)

Osmond, C., Barker, D. J., and Slattery, J. M.: Risk of death from cardiovascular disease and chronic bronchitis determined by place of birth in England and Wales. J. Epidemiol. Commun. Health *44*, 139–141 (1990)

Ozaki, T., Hawkins, P., Nishina, H., Steyn, C., Poston, L., and Hanson, M. A.: Effects of undernutrition in early pregnancy on systemic small artery function in late-gestation fetal sheep. Amer. J. Obstet. Gynecol. *183*, 1301–1307 (2000)

Ozanne, S. E., Dorling, M. W., Wang, C. L., and Nave, B. T.: Impaired PI 3-kinase activation in adipocytes from early growth-restricted male rats. Amer. J. Physiol. Endocrinol. Metab. *280*, E534–539 (2001)

Ozanne, S. E., Wang, C. L., Dorling, M. W., and Petry, C. J.: Dissection of the metabolic actions of insulin in adipocytes from early growth-retarded male rats. J. Endocrinol. *162*, 313–319 (1999)

Park, J. H., Stoffers, D. A., Nicholls, R. D., and Simmons, R. A.: Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J. Clin. Invest. *118*, 2316–2324 (2008)

Parsons, T. J., Power, C., and Manor, O.: Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. BMJ (Clinical research ed.) *323*, 1331–1335 (2001)

Petry, C. J., Dorling, M. W., Pawlak, D. B., Ozanne, S. E., and Hales, C. N.: Diabetes in old male offspring of rat dams fed a reduced protein diet. Int. J. Exp. Diabetes Res. *2*, 139–143 (2001)

Raab, E. L., Vuguin, P. M., Stoffers, D. A., and Simmons, R. A.: Neonatal exendin-4 treatment reduces oxidative stress and prevents hepatic insulin resistance in intrauterine growth retarded rats. Amer. J. Physiol. Regul. Integr. Comp. Physiol. *297*/6, R1785–1794 (2009)

Rajakumar, P. A., He, J., Simmons, R. A., and Devaskar, S. U.: Effect of uteroplacental insufficiency upon brain neuropeptide Y and corticotropin-releasing factor gene expression and concentrations. Pediatr. Res. *44*, 168–174 (1998)

Ravelli, A. C., van Der Meulen, J. H., Osmond, C., Barker, D. J., and Bleker, O. P.: Obesity at the age of 50 y in men and women exposed to famine prenatally. Amer. J. Clin. Nutr. *70*, 811–816 (1999)

Ravelli, G. P., Stein, Z. A., and Susser, M. W.: Obesity in young men after famine exposure in utero and early infancy. New Engl. J. Med. *295*, 349–353 (1976)

Roseboom, T. J., van der Meulen, J. H., Ravelli, A. C., Osmond, C., Barker, D. J, and Bleker, O. P.: Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol. Cell. Endocrinol. *185*, 93–98 (2001)

Roseboom, T. J., van der Meulen, J. H., Ravelli, A. C., van Montfrans, G. A., Osmond, C., Barker, D. J., and Bleker, O. P.: Blood pressure in adults after prenatal exposure to famine. J. Hypertens. *17*, 325–330 (1999)

Samuelsson, A. M., Matthews, P. A., Argenton, M., Christie, M. R., McConnell, J. M., Jansen, E. H., Piersma, A. H., Ozanne, S. E., Twinn, D. F., Remacle, C., Rowlerson, A., Poston, L., and Taylor, P. D.: Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension *51*, 383–392 (2008)

Sas, T., Mulder, P., and Hokken-Koelega, A.: Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J. Clin. Endocrinol. Metab. *85*, 3786–3792 (2000)

Simmons, R. A., Templeton, L. J., and Gertz, S. J.: Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. Diabetes *50*, 2279–2286 (2001)

Snoeck, A., Remacle, C., Reusens, B., and Hoet, J. J.: Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. Biol. Neonate *57*, 107–118 (1990)

Stanner, S. A., and Yudkin, J. S.: Fetal programming and the Leningrad Siege study. Twin Res. *4*/5, 287–292 (2001)

Stoffers, D. A., Desai, B. M., DeLeon, D. D., and Simmons, R. A.: Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes *52*, 734–740 (2003)

Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L., and Gluckman, P. D.: Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Amer. J. Physiol. Endocrinol. Metab. *279*, E83–87 (2000)

Vickers, M. H., Breier, B. H., McCarthy, D., and Gluckman, P. D.: Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Amer. J. Physiol. Regul. Integr. Comp. Physiol. *285*, R271–273 (2003)

Vickers, M. H., Gluckman, P. D., Coveny, A. H., Hofman, P. L., Cutfield, W. S., Gertler, A., Breier, B. H., and Harris, M.: Neonatal leptin treatment reverses developmental programming. Endocrinology *146*, 4211–4216 (2005)

Vickers, M. H., Ikenasio, B. A., and Breier, B. H.: Adult growth hormone treatment reduces hypertension and obesity induced by an adverse prenatal environment. J. Endocrinol. *175*, 615–623 (2002)

Vickers, M. H., Ikenasio, B. A., and Breier, B. H.: IGF-I treatment reduces hyperphagia, obesity, and hypertension in metabolic disorders induced by fetal programming. Endocrinology *142*, 3964–3973 (2001)

Waterland, R. A., and Jirtle, R. L.: Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. Nutrition *20*, 63–68 (2004)

Woodall, S. M., Breier, B. H., Johnston, B. M., and Gluckman, P. D.: A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. J. Endocrinol. *150*, 231–242 (1996a)

Woodall, S. M., Johnston, B. M., Breier, B. H., and Gluckman, P. D.: Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. Pediatr. Res. *40*, 438–443 (1996b)

Wyrwoll, C. S., Mark, P. J., Mori, T. A., Puddey, I. B., and Waddell, B. J.: Prevention of programmed hyperleptinemia and hypertension by postnatal dietary omega-3 fatty acids. Endocrinology *147*, 599–606 (2006)

Yajnik, C.: Interactions of perturbations in intrauterine growth and growth during childhood on the risk of adult-onset disease. The Proceedings of the Nutrition Society *59*, 257–265 (2000)

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Behavioural Outcomes and Perinatal Development

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Abstract

This study investigated the effects of optimized neonatal parent-infant transactions on parenting stress and joint attention performance in both term and preterm infants. Surviving infants <2000 g from a geographically defined area were randomly assigned to a preterm intervention ($n = 71$) or preterm control group ($n = 69$). Comparisons were made between preterm groups, secondary with a term group ($n = 75$). The Parenting Stress Index was administered to the mothers at 6, 12, and 24 months' corrected age and to the fathers at 12 and 24 months' corrected age. Joint attention was measured at 12 months corrected age using the Early Social Communication Scales. Both parents in the intervention group reported significantly lower stress across time, and preterm intervention infants scored significantly higher than preterm control infants on various aspect of joint attention. An intervention implemented during the neonatal period can both lower parenting stress and be of advantage for certain aspects of joint attention performance in preterm infants.

Zusammenfassung

Diese Studie untersuchte die Wirkung optimierter neonataler Eltern-Säuglings-Transaktionen auf elterlichen Stress und die damit verbundene Zuwendung bei am Termin Geborenen und Frühgeborenen. Frühgeborene überlebende Säuglinge < 2000 g einer bestimmten Region wurden randomisiert einer Interventionsgruppe ($n = 71$) und einer Kontrollgruppe ($n = 69$) zugewiesen. Diese wurden untereinander und mit einer Gruppe am Termin geborener Säuglinge ($n = 75$) verglichen. Der „Parenting Stress Index" wurde im Alter von 6, 12 und 24 Monaten (korrigiertes Alter der Säuglinge) bei den Müttern und im Alter von 12 und 24 Monaten (korrigiertes Alter der Säuglinge) bei den Vätern erhoben. Die Zuwendung wurde im Alter von 12 Monaten (korrigiertes Alter) mit Hilfe der „Early Social Communication Scales" erhoben. Beide Elternteile der Interventionsgruppe gaben signifikant weniger Stress zu den jeweiligen Messzeitpunkten an, und die frühgeborenen Säuglinge der Interventionsgruppe erreichten signifikant höhere Werte als die frühgeborenen Säuglinge der Kontrollgruppe in Bezug auf verschiedene Dimensionen der Zuwendung. Eine Intervention innerhalb der neonatalen Phase kann elterlichen Stress senken und sich vorteilhaft auf bestimmte Dimensionen der Zuwendung auswirken.

1. Introduction

Biological and environmental risk factors act in interactive, synergistic relationships to shape preterm children's adaptation (Feldman 2007). This perspective implies that it is the ongoing, daily transactions between preterm infants and their effective environments that may be associated with various outcomes. The question of whether a brief intervention in an early phase of development could influence subsequent transactions has both practical and theoretical implications. The main aim of the study was to investigate one of the few early intervention programs that have shown long-term beneficial effects in cognitive performance as well as in behaviour, the Mother-Infant Transaction Program (MITP) (Achenbach et al. 1993, Rauh et al. 1990). The MITP is clearly rooted in the transactional model of development, but the timing of the intervention attracts notice due to its confined action in an early phase of development. Positive results of the intervention could thus not only be ascribed to optimizing

continuing infant-caregiver transactions from an early phase, but also to the improvement of functional aspects of preterm infants' central nervous system in a period with rapid development and plasticity. Thus, the intervention may target parents' effectiveness in handling their premature offspring as well as the actual behaviour of the infant. This will be illustrated by presenting results on parenting stress and infants' joint attention performance.

2. Method

2.1 Subjects

All surviving infants with a weight at birth lower than 2000 g treated at the University Hospital of North Norway (UNN) between March 1999 and August 2002, without congenital anomalies and whose mothers' native language was Norwegian, were eligible for inclusion in the preterm group. Triplets were excluded due to the character of the intervention. A total of 203 infants with a birth weight (BW) < 2000 g were treated, representing 96 % of all infants with a birth weight < 2000 g who were born in the counties of Troms and Finnmark during the recruitment period; 63 of these were not included (14 died, 13 had non-Norwegian speaking parents, six were triplets, one had Down syndrome, six had serious sequelae, one was not asked, and 22 were parental refusals). The consent rate was 89.2 %. The study sample comprised 140 preterm infants and 75 term infants (of whom 73 were preterm males and 40 were term males). Gestational age (GA) was determined by ultrasound scanning at 16 – 18 weeks, which was performed in 208 of the infants; otherwise, the date of the last menstrual period was used. GA at birth is given as number of completed weeks.

2.2 Randomization

One hundred and forty-six infants were randomly assigned to a preterm intervention or preterm control group. Six infants were withdrawn due to serious sequelae (one from the intervention group, and 5 from the control group), leaving 71 infants in the preterm intervention and 69 in the preterm control group eligible for assessment at 12 months. Randomization was arranged in random blocks of four and six using computer-generated random numbers and stratified by gestation (< 28 weeks and ≥ 28 weeks). Twins were always randomized to the same group. The allocation was made by sealed opaque envelopes, identified by stratification group and consecutively numbered, which were opened after the parents completed various questionnaires. A full report of the randomization procedure can be found in Kaaresen et al. (2006). The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

2.3 Intervention

The intervention was a modified version of the "Vermont Intervention Program for Low Birthweight Infants" (Rauh 1979), translated by Smith, and modified by Rønning and Ulvund (unpublished manuscript). The modifications included an initial session in which the parents could express experiences from the hospital stay and possibly their feelings of

grief. Second, a more active participation from both the mother and the father in evaluating and handling the infants and their cues was adopted. Emphasizing the transactional nature of development, the aims of the intervention was to (*i)* enable the parents to appreciate their baby's specific behavioural and temperamental characteristics; (*ii*) to sensitize parents to the infant's cues, especially those that signal stimulus overload, distress and readiness for interaction; and (*iii*) to teach parents to respond appropriately to those cues in order to facilitate mutually satisfying interactions. One of eight specially trained neonatal nurses implemented the intervention through one-hour daily sessions with the parents and infant on seven consecutive days, starting one week before planned discharge at a gestation age of at least 34 weeks. Four home visits lasting one hour each followed the daily sessions by the same intervention nurse, at three, fourteen, thirty, and ninety days after discharge. There was no emphasis on "extra" stimulation; the focus is on transactions that occur naturally in the course of daily care. Each mother moved at her own pace, according to her own behavioural progression. Techniques of interaction (arousal, engagement, and consolation) are based on the stages of behavioural organization as proposed by Als et al. (1976) and Gorski et al. (1979).

A detailed log was written up for every intervention session. The log was regularly reviewed and supervised by the coordinating nurse and a senior child psychologist (JAR) in order to maintain the consistency of the intervention. The department's standard protocol for discharge of preterm infants followed the preterm control group. This included an examination and offer for training in baby massage from the department's physical therapist, a clinical examination including visual and auditory screening, and a discharge consultation by one of the senior pediatricians (LBD). The term control group underwent a routine clinical examination on the third day after birth. No other interventions were offered.

2.4 Measures

The results on parenting stress at 6, 12 and 24 months have been presented in previous publications (Kaaresen et al. 2006, 2008). Briefly, a self-report questionnaire, the Parenting Stress Index (PSI) (Abidin 1995), was used to measure stress in the parent-child relationship. The PSI consists of 101 items that are scored on a 5-point Likert scale, and comprise a child domain and a parent domain (including various subscales), as well as total stress. Overall scores for the domains are obtained by summing the scores on the subscales, and a combination of the domain scores gives the total stress score. Higher scores indicate more stress.

Joint attention performance in preterm and term infants were assessed at 12 months corrected age, and the results have previously been published (Olafsen et al. 2006). A videotaped, structured observational measure of non-verbal communication skills was used (Early Social Communication Scales; Mundy et al. 2003). The recordings enabled observers to classify children's behaviour into one of three mutually exclusive categories of early social communication behaviours. *Joint attention behaviours* refer to the infant's skill in using non-verbal behaviours to share the experience of objects or events with others. *Behavioural requests* refer to the infant's skill in using non-verbal behaviours to elicit aid in obtaining objects or events. *Social interaction behaviours* refer to the capacity of the child to engage in playful, affectively positive turn-taking interactions with others. Behaviours are also classified as to whether they are child-initiated bids or responses from the child to a tester's bid.

3. Results

3.1 Parenting Stress

At 24 months, the overall PSI response rate was 97 % for the mothers and 85 % for the fathers. The PSI was administered to only mothers at 6 months, but fathers were included at two subsequent measurement points. Compared with the preterm control group, the preterm intervention group had significantly lower stress scores at 6, 12, and 24 months. The level of stress among the preterm intervention group was comparable to their term peers. At 6 months, there was a 6.8 point difference between mothers in the control group and the intervention group in the child domain (95 % CI: 1.6 to 12.0, $p = 0.01$). For the parent domain, there was 8.4 point difference (95 % CI: 1.2 to 15.6, $p = 0.02$), whereas for the total stress domain there was a 16.9 point difference (95 % CI: 5.2 to 28.5, $p = 0.005$). At 12 months, the scores for mothers were as follows: In the child domain, there was a 5.0 point difference between intervention and control group (95 % CI: –0.6 to 10.7, $p = 0.08$). There was a 8.7 point difference in the parent domain (95 % CI: 1.5 to 15.9, $p = 0.02$), and a 13.7 point difference for the total stress score (95 % CI: 1.6 to 25.9, $p = 0.03$). For fathers, there was a 6.7 point difference between intervention and control group in the child domain (95 % CI: 1.0 to 12.3, $p = 0.02$), and a 8.2 point difference in the parent domain (95 % CI: 0.5 to 15.9, $p = 0.02$). For fathers' total stress score, there was a 14.8 point difference (95 % CI: 2.1 to 27.6, $p = 0.02$). There were no significant differences in mean scores between mothers and fathers at 12 months.

The comparisons between mothers in the preterm control group and mothers in the preterm intervention group at 24 months showed that there were differences in the child domain (10.2 point difference; 95 % CI: 4.2 to 16.1, $p = 0.001$), in the parent domain (8.6 point difference; 95 % CI: 1.4 to 15.7, $p = 0.02$), and in the total stress score (19.1 point difference; 95 % CI: 7.2 to 31.0, $p = 0.002$). For fathers, there was a 7.0 point difference in the child domain (95 % CI: 1.1 to 13.0, $p = 0.02$). There was no significant difference in the parent domain (5.4 point difference; 95 % CI: –2.0 to 13.7, $p = 0.20$), and a borderline significant difference in the total stress score (12.6 point difference; 95 % CI: –1.1 to 26.2, $p = 0.07$).

3.2 Joint Attention

There were overall differences between all groups in initiating joint attention (IJA) (preterm control, preterm intervention, term) ($F = 4.44$, $p < 0.05$), in responding to joint attention (RJA) ($F = 14.03$, $p < 0.0005$), in initiating object request (IOR) ($F = 4.00$, $p < 0.05$), and in responding to social interaction (RSI) ($F = 11.05$, $p < 0.0005$). The preterm intervention group outperformed the preterm control group on the dimensions IJA and RSI, where the mean scores were at the same level as the term group. These treatment effects were consistent across weight categories within the preterm group (≤ 1000 g, 1001 – 1500 g, 1501 – 2000 g). The term group outperformed both of the preterm groups on the dimensions RJA and IOR. Both prematurely and term born girls had significantly higher scores than boys from both birth conditions [IJA: $F = 7.28$, $p < 0.01$; RJA: $F = 19.42$, $p < 0.0005$; IOR: $F = 8.75$, $p < 0.01$; responding to requesting (RR): $F = 4.51$, $p < 0.05$; RSI: $F = 9.90$, $p < 0.01$].

4. Comment

This study indicated that early optimized interactions reduced parenting stress in both mothers and fathers during the two first years after a preterm birth, as well as increased premature infants' joint attention behaviours at 12 months corrected age. The intervention program was focused on enabling the parents to interpret and act according to their infant's cues, thereby strengthening the parent-infant relationship and making them feel more competent in handling their preterm infant. As parenting stress may be a risk factor for later behavioral problems, the reduction in parenting stress during the infant's first years of life may be an important factor in reducing long-term behavioural problems in these infants (Saylor et al. 2003).

Increasing preterm infants' joint attention behaviours may contribute to a reduction in behaviour problems, as the aspects of social communication are associated with social, linguistic, and cognitive growth, and thus considered a major milestone in development (Adamson and Russel 1999, Schertz and Odom 2004, Sheinkopf et al. 2004, Smith and Ulvund 2003). Due to the developmental significance of joint attention, the results may contribute to a better understanding of processes that might be associated with long-term outcomes for preterm children. This includes an understanding of differential outcomes for prematurely born girls and boys.

References

Abidin R. R.: Parenting Stress Index. Professional Manual. 3rd ed. Odessa, FL: Psychological Assessment Resources 1995

Achenbach, T. M., Howell, C. T., Aoki, M. F., and Rauh, V. A.: Nine-year outcome of the Vermont Intervention Program for Low Birth Weight Infants. Pediatrics *91*, 45–55 (1993)

Adamson, L. B., and Russel, C.: Emotion regulation and emergence of joint attention. In: Rochat, P. (Ed.): Early Social Cognition: Understanding Others in the First Months of Life*;* pp. 281–297. Mahwah, NJ: Erlbaum 1999

Als, H., Tronick, E., and Adamson, L.: The behavior of the full-term yet under weight newborn infant. Devolopmental Medicine and Child Neurology *18*, 590–602 (1976)

Feldman, R.: Parent-infant synchrony and the construction of shared timing: physiological precursors, developmental outcomes, and risk conditions. Journal of Child Psychology and Psychiatry *48*, 329–354 (2007)

Gorski, P. A., Davison, M. F., and Brazelton, T. B.: Stages of behavioral organization in the high-risk neonate. Theoretical and clinical considerations. Seminare in Perinatology *3*, 61–72 (1972)

Kaaresen, P. I., Rønning, J. A., Ulvund, S. E., and Dahl, L. B.: A randomized controlled trial of the effectiveness of an early-intervention program in reducing parenting stress after preterm birth. Pediatrics *118*, 9–19 (2006)

Kaaresen, P. I., Rønning, J. A., Tunby, J., Nordhov, S. M., Ulvund, S. E., and Dahl, L. B.: A randomized controlled trial of an early intervention program in low birth weight children: Outcome at 2 years. Early Human Development *84*, 201–209 (2008)

Mundy, P., Delgado, C., Block, J., Venezia, M., Hogan, A., and Seibert, J.: Manual for the early social communication scales (ESCS) (2nd ed.). Psychology Department, University of Miami, www.psy.miami.edu/faculty/pmundy/ESCS.pdf (2003)

Olafsen, K. S., Rønning, J. A., Kaaresen, P. I., Ulvund, S. E., Handegård, B. H., and Dahl, L. B.: Joint attention in term and preterm infants at 12 months corrected age: The significance of gender and intervention based on a randomized controlled trial. Infant Behavior and Development *29*, 554–563 (2006)

Rauh, V. A.: Vermont Intervention Program for Low Birthweight Infants. Translated into Norwegian by L. Smith, modified by J. A. Rønning and S. E. Ulvund. Unpublished manuscript, University of Tromsø, Norway (1979)

Rauh, V. A., Nurcombe, B., Achenbach, T., and Howell, C.: The Mother-Infant Transaction Program. The content and implications of an intervention for the mothers of low-birthweight infants. Clinics of Perinatology *17*, 31–45 (1990)

Saylor, C. F., Boyce, G. C., and Price, C.: Early predictors of school-age behavior problems and social skills in children with intraventricular hemorrhage (IVH) and/or extremely low birthweight (ELBW). Child Psychiatry and Human Development *33*, 175–192 (2003)

Schertz, H. H., and Odom, S.: Joint attention and early intervention with autism: A conceptual framework and promising approaches. Journal of Early Intervention *27*, 42–54 (2004)

Sheinkopf, S. J., Mundy, P., Claussen, A. H., and Willoughby, J.: Infant joint attention skill and preschool behavioral outcomes in at-risk children. Development and Psychopathology *16*, 273–291 (2004)

Smith, L., and Ulvund, S. E.: The role of joint attention in later development among preterm children: Linkages between early and middle childhood. Social Development *12*, 222–234 (2003)

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Prenatal Famine Exposure and Long Term Consequences for Adult Health

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With 1 Figure and 2 Tables

Abstract

An adverse prenatal environment may predispose to later disease. This paper describes the effects of prenatal exposure to the Dutch famine of 1944–1945 on adult health. The findings of the Dutch famine birth cohort study show that maternal undernutrition during gestation has important effects on health in later life and that the effects on health depend on its timing during gestation. Early gestation seems to be an especially vulnerable period. Adequate dietary advice to women before and during pregnancy seems a promising strategy in preventing chronic diseases in future generations.

Zusammenfassung

Eine ungünstige pränatale Umgebung kann eine Prädisposition für Erkrankungen im späteren Leben sein. Diese Arbeit beschreibt den Zusammenhang zwischen pränatalen Auswirkungen des niederländischen Hungerwinters 1944–1945 und der Gesundheit im Erwachsenenalter. Die Ergebnisse der „Niederländischen Hungerwinter-Geburtskohortenstudie" zeigen, dass eine maternale Unterernährung in der Schwangerschaft Einfluss auf die Gesundheit der Nachkommen hat, insbesondere der Zeitpunkt der Unterernährung (Gestationsalter) spielt eine große Rolle. Besonders die frühen Schwangerschaftswochen scheinen eine empfindliche Periode zu sein. Eine adäquate Ernährungsberatung von Frauen vor und während der Schwangerschaft sollte eine erfolgsversprechende Strategie für die Prävention von chronischen Erkrankungen künftiger Generationen zu sein.

1. Introduction

Coronary heart disease remains a major burden on public health in the Western world, and is taking on epidemic proportions in the developing countries (Murray et al. 1997). Small babies go on to develop more coronary heart disease in adult life (Barker 1995). Restricted intrauterine growth has been identified as an important contributor to later coronary heart disease and its biological risk factors. Developing organ systems adapt in response to the reduced availability of nutrients, particularly during periods of rapid development, the so-called critical periods (Hoet and Hanson 1999). These adaptations, while advantageous for short-term survival, can be detrimental to health in later life. Animal experiments indicate that substantial changes in cardiovascular function can result from maternal or fetal undernutrition without necessarily affecting birth weight (Harding 2001). However, most studies in humans only have access to indirect measures of intrauterine growth, such as birth weight. In this chapter we describe the effects of restricted prenatal nutrition during different periods of gestation on health in later life. In the past 15 years, we have studied the health of people born around the time of the Dutch famine of 1944–1945, and this has provided more insight into fetal origins of adult disease.

1.1 The Dutch Famine as an Experimental Study Design

While famine is sadly not uncommon in many parts of the world, studying the effects of undernutrition during specific periods of pregnancy is hampered by the fact that undernutrition is usually not restricted to pregnancy alone and the effects of chronic undernutrition and the accompanying problems of infection complicate the situation. What is unusual about the Dutch famine is; first, that the famine was imposed on a previously well-nourished population; second, there was both a sudden onset and relief from the famine; and, third, despite the adversities of the war, midwives and doctors continued to offer professional obstetric care and kept detailed records of the course of pregnancy, the delivery, and the size and health of the baby at birth. Furthermore, detailed information is available on the weekly rations for the population of Amsterdam and because birth records were kept we were able to trace those born around the time of the famine allowing us to study the long-term effects of famine. All these characteristics make the Dutch famine a unique counterpart for animal models that study the effects of restricted maternal nutrition during different stages of gestation.

1.2 The Dutch Famine

After weeks of heavy fighting following the invasion on the 6th of June 1944, the Allied forces finally broke through German lines. With lightning speed the Allied troops took possession of much of France, Luxembourg and Belgium. By the 4th of September 1944 the Allies had the strategic city of Antwerp in their hands, and on the 14th they entered the Netherlands. Everyone in the Netherlands expected that the German occupation would soon be over. The advance went so quickly that the commanders of the Allied forces also thought it would be only a matter of days before the Germans would surrender. But the advance of the Allies to the north of the Netherlands came to a halt when attempts to gain control of the bridge across the river Rhine at Arnhem (operation 'Market Garden') failed.

In order to support the Allied offensive, the Dutch government in exile called for a strike of the Dutch railways. As a reprisal, the Germans banned all food transports. This embargo on food transports was lifted in early November 1944, when food transport by water was permitted again. By then, it had become impossible to bring in food from the rural east to the urban west of the Netherlands because most canals and waterways were frozen due to the extremely severe winter of 1944–1945, which had started unusually early. Consequently, food stocks in the urban west of the Netherlands ran out rapidly.

As a result, the official daily rations for the general adult population – which had decreased gradually from about 1800 calories in December 1943 to 1400 calories in October 1944 – fell abruptly to below 1000 calories in late November 1944. At the height of the famine from December 1944 to April 1945, the official daily rations varied between 400 and 800 calories. Children under the age of 1 were relatively protected because their official daily rations never fell below 1000 calories and the specific nutrient components were always above the standards used by the Oxford Nutritional Survey (Burger et al. 1948). Pregnant and lactating women were entitled to an extra quota of food, but at the peak of the famine these extra supplies could not be provided. In addition to the official rations, food came from church organisations, central kitchens, the black market and foraging trips to the countryside. After the liberation of the Netherlands in early May 1945, the food situation improved swiftly. By June 1945, the rations had risen to more than 2000 calories (Burger et al. 1948).

There was a serious shortage of fuel during the war which caused a gradual decrease and finally a complete shut down of the production of gas and electricity, and in several places even the water supply, while the authorities were unable to provide fuel for stoves and furnaces in homes. Throughout the winter of 1944–1945 the population had to live without light, without gas, without heat, laundries ceased operating, soap for personal use was unobtainable, and adequate clothing and shoes were lacking in most families. In hospitals, there was serious overcrowding as well as lack of medicines. Above all, hunger dominated all misery.

The famine had a profound effect on the general health of the population. In Amsterdam, the mortality rate in 1945 had more than doubled compared to 1939, and it is very likely that most of this increase in mortality was attributable to malnutrition (Banning 1946). But even during this disastrous famine women conceived and gave birth to babies, and it is in these babies that the effects of maternal malnutrition during different periods of gestation on health in adult life can be studied. Because of its unique experimental characteristics, it is not surprising that people born around the time of the Dutch famine have been studied by many investigators.

1.3 Dutch Famine Studies

The period of starvation ended early in May 1945 immediately after the final surrender of the Germans. In addition to the immediate provision of food after the war, medical aid was a top priority for the Netherlands. Doctors from the UK and US were sent to survey medical needs. Clement Smith from Harvard Medical School was among the first to witness the effects of the famine on the health of the Dutch population. He immediately saw the opportunity to obtain information that would help resolve important questions on how poor maternal nutrition affects pregnancy and the development of the fetus before birth. Using obstetric records from Rotterdam and The Hague, he studied the effects of prenatal exposure to famine on pregnancy and the fetus (Smith 1947). He found that babies born during the famine (and thus exposed to famine in late gestation) were about 200 g lighter at birth. Later studies focussed on mental performance, following the increasing awareness in the late 1960s that early nutritional deprivation might cause irreversible damage to the brain (Stein et al. 1972). This study of military conscripts did not demonstrate any effect of starvation during pregnancy on adult mental performance. However, men exposed to famine in early gestation were more likely to be obese, whereas those exposed in late gestation were less likely to be obese (Ravelli et al. 1976). More recently, it has been shown that people conceived during the famine, and thus exposed in early gestation, had a two-fold increase in risk of schizophrenia (Hoek et al. 1996) and anti-social personality disorder (Neugebauer et. al. 1999). In men, the risk of congenital neural defects was also increased (Stein et al. 1972) which suggests that permanent changes in the central nervous system might be involved. Lambert Lumey (1992) studied intergenerational effects of exposure to the Dutch famine and found that women who had spent the first six months of their own fetal life during the famine had slightly smaller babies than women who had not been exposed to famine *in utero*. Later results, however, were inconsistent with these findings and showed that first-born babies of women who – as a fetus – had been exposed to the famine in early gestation were somewhat heavier at birth (Lumey et al. 1995).

2. Methods: The Dutch Famine Birth Cohort Study

We traced a group of 2414 term singletons born between November 1943 and February 1947 in the Wilhelmina Gasthuis in Amsterdam for whom we had detailed birth records. At ages 50 and 58, we invited the cohort to come to the clinic for detailed investigations. We defined exposure according to the daily official food rations for the general population older than 21 years in Amsterdam. A person was considered to be exposed if the average daily ration during any thirteen week period of gestation was below 1000 calories. The rations for babies never fell below 1000 calories a day, therefore people born before the famine (as well as those conceived after it) are considered as unexposed. We defined periods of 16 weeks each to differentiate between those who were exposed to famine in late gestation (born between January 7th and April 28th 1945), mid gestation (born between April 29th and August 18th 1945) and in early gestation (born between August 19th and December 8th 1945)(Fig. 1).

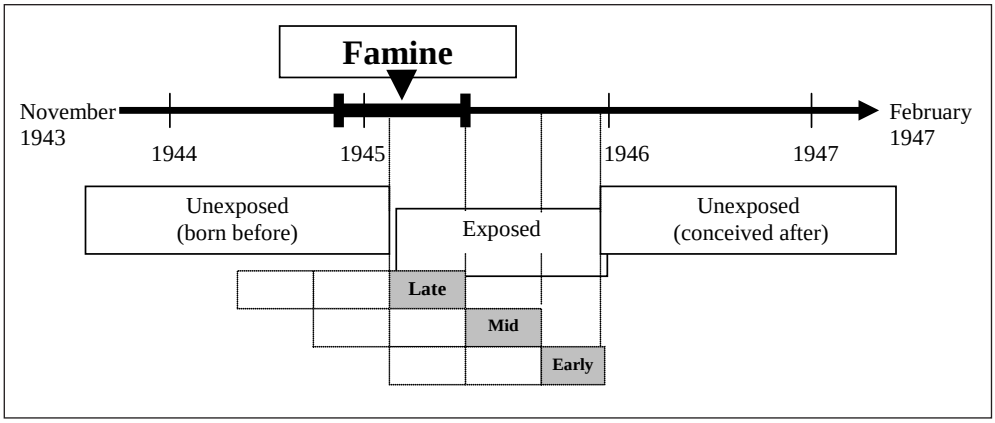

Fig. 1 The Dutch famine birth cohort: famine exposure and birth in relation to the timing of the Dutch famine

3. Results: Long-term Consequences of Prenatal Exposure to the Dutch Famine

The Dutch famine study provided the first direct evidence in humans that undernutrition during gestation increases the risk of many diseases that plague our society, such as heart disease, diabetes, airways disease, obesity, renal disease and cancer. The effect size is striking: compared to controls, men and women conceived during the Dutch famine have twice the level of cardiovascular disease, and women exposed to famine during gestation even had a 5-fold increase in breast cancer risk.

The effects of famine appeared to depend on its timing during gestation, and the organs and tissues undergoing critical periods of development at that time. Early gestation appeared to be the most vulnerable period. People who were conceived during the famine – and who had been exposed to famine in early gestation – had a more atherogenic plasma lipid profile (Roseboom et al. 2000a), altered blood coagulation (Roseboom et al. 2000c), unhealthy lifestyle choices (Lussana et al. 2008), were more responsive to stress (Painter et al. 2006a) and had a doubled risk of coronary heart disease (Roseboom et al. 2000b, Painter et al.

Tab. 1 Maternal and infant characteristics according to the timing of prenatal exposure to the Dutch famine.

	Exposure to famine						n
	Born before	Late gestation	Mid gestation	Early gestation	Conceived after	All (SD)	
Number	764	307	297	217	829	2414	
Proportion of men	53 %	49 %	49 %	50 %	53 %	52 %	2414
Maternal characteristics							
Age (years)	29	30	28	28	28	28 (6.4)	2414
Primiparous	40 %	30 %	37 %	39 %	39 %	38 %	2414
Not married	13.2 %	9.8 %	20.2 %	25.8 %	16.3 %	15.8 %	2414
Weight at last antenatal visit (kg)	66.7	61.8[1]	63.5[1]	67.9	69.1	66.6 (8.7)	2133
Weight gain 3rd trimester (kg)	3.2	0.0[1]	4.9[1]	5.7[1]	4.3	3.5 (3.2)	1682
Infant characteristics							
Birth weight (g)	3373	3133[1]	3217[1]	3470[1]	3413	3346 (477)	2414
Birth length (cm)	50.5	49.4[1]	49.8[1]	50.9[1]	50.5	50.3 (2.2)	2382
Head circumference (cm)	32.9	32.3[1]	32.1[1]	32.8	33.2	32.8 (1.6)	2397
Ponderal index (kg/m3)	26.1	25.8[1]	26.0[1]	26.2	26.5	26.2 (2.4)	2382
Placental area (cm2)	370	339[1]	346	340[1]	350	353 (83)	2056
Gestational age (days)	285	283[1]	285	287	286	285 (12)	2044

Mean and standard deviation. [1] p corrected for gender <0.05 compared to unexposed (born before or conceived after the famine).

Tab. 2 Adult characteristics (at the age of 58) according to timing of prenatal exposure to the Dutch famine.

	Exposure to famine						n
	Born before	Late gestation	Mid gestation	Early gestation	Conceived after	All (SD)	
Adult characteristics							
Proportion of men	48 %	43 %	39 %	43 %	51 %	46 %	783
Plasma glucose 120 min[1] (mmol/l)	5.8	6.2	6.2	6.2	5.9	6.0 (1.4)	678
Plasma insulin 120 min[1] (pmol/l)	242	263	254	269	240	249 (2.1)	672
Type 2 diabetes	15.1 %	14.2 %	13.8 %	18.9 %	12.6 %	14.4 %	783
LDL/HDL cholesterol[1]	2.3	2.5	2.3	2.6[1]	2.4	2.4 (1.4)	783
Sports	55 %	61 %	58 %	61 %	51 %	56 %	781
BMI (kg/m^2)	28.0	28.0	27.8	27.5	28.7	28.1 (1.2)	783
Coronary heart disease	6.3 %	5.7 %	3.9 %	8.2 %	6.9 %	6.2 %	783
Breast cancer (%)	2.8 %	3.7 %	3.9 %	8.7 %	0.8 %	3.2 %	475

Mean and standard deviation. [1] geometric mean.

2006c). Women in this group also tended to have the highest body mass index, and had an increased risk of breast cancer (Painter et al. 2006b). It is of interest to note that people exposed to famine in early gestation were not small at birth, but did have the most health problems in later life. Although many of these diseases (such as coronary heart disease) were linked to small size at birth, the effects of famine were independent of this. Based on the size of these babies at birth, one would not have predicted these health effects. The transition from poor nutrition in early gestation to better nutrition later on may have lasting consequences for health in later life.

We found that undernutrition during any period of gestation was associated with reduced glucose tolerance and raised insulin concentrations at age 50 and 58. Importantly, this effect

was larger than could be explained by the lower birth weights of babies born during the famine and by the low weight gain of their mothers (Ravelli et al. 1998, de Rooij et al. 2006). Also, exposure to famine in mid gestation was linked to a 3.2 fold increase in occurrence of microalbuminurea in adulthood and a 10 % increase in creatinine clearance, neither of which can be explained by cardiovascular confounders (Painter et al. 2005). We propose that mid gestational exposure to famine – the period of rapid increase in nephron number – may prevent formation of sufficient glomeruli and thus increase the risk for microalbuminurea and possibly affect renal function in adulthood. This supports the hypothesis that intrauterine conditions during distinct, organ-specific periods of sensitivity may permanently determine health outcomes in later life. Another finding in our study also supports this concept: we found that people who had been exposed to famine in mid gestation had an increased prevalence of obstructive airways disease (Lopuhaä et al. 2000). These observations were not paralleled by reduced lung function or increased serum concentrations of IgE. This suggests that the increased prevalence of symptoms and disease may be attributable to increased bronchial reactivity rather than to irreversible airflow obstruction or atopic disease. Because the bronchial tree grows most rapidly in mid gestation, our findings support the hypothesis that fetal undernutrition permanently affects the structure and physiology of the airways during 'critical periods' of development that coincide with periods of rapid growth.

Women – but not men – who were exposed to the Dutch famine of 1944–1945 *in utero* were more reproductively successful than women who were not exposed to famine during their fetal development; they had more offspring, more twins, were less likely to remain childless and started reproducing at a younger age (Painter et al. 2008b). The increased reproductive success of these women is unlikely to be explained by genes which favor fertility and are passed from mothers to their daughters. *In utero* exposure to famine did not affect the reproductive success of males. These findings suggest that poor nutrition during fetal development, followed by improved nutrition after birth can give rise to a female phenotype characterized by greater reproductive success.

The constellation of reproductive and metabolic adaptations during fetal development in response to undernutrition *in utero* may be part of a thrifty phenotype which is associated with enhanced reproduction. Post-war Holland provided a postnatal environment of food abundance, which was unlike the conditions anticipated by the environment *in utero*. This disadaptation of a thrifty phenotype may be important in the later occurrence of chronic disease. We have shown that people exposed to famine during gestation have an increased risk of cardiovascular disease, metabolic disease, breast cancer and obesity rates. In conclusion, our findings are consistent with the theory of life history regulation, which proposes that the two traits – fertility and body maintenance – are mutually balanced; investments in one are offset by reduction in investment in the other (Stearns 1992). Our findings show that the balance in phenotypic traits underpinning life history regulation may be set by the environmental conditions during fetal development.

We found preliminary evidence that grand-maternal exposure to famine for a brief period during gestation is associated with increased neonatal adiposity and poorer health in the grand-offspring (Painter et al. 2008a). These findings constitute the first direct evidence in humans that the detrimental effects on health in later life resulting from poor maternal nutrition during gestation are passed down to subsequent generations. This may imply that improved maternal nutrition during gestation may benefit the health of many generations to come. Also, these findings indicate that the transgenerational effects of famine exposure *in*

utero on health in later life differ depending on the exposed parent's sex. Increased neonatal adiposity was only found among the children of prenatally exposed women, whereas poor health was reported among the offspring of both men and women who had been conceived in famine. Importantly, this indicates that effects on health in later life are also passed down transgenerationally through the male line. Transgenerational effects of a poor intrauterine environment may thus affect the generation parented by exposed individuals.

4. Study Limitations

In interpreting our findings, a number of matters need to be considered. Women were less fertile during the famine. The women who did conceive during the famine, whose offspring were exposed to famine in early gestation, may have been of a different constitution. However, correcting for markers of maternal constitution or fertility, such as maternal weight, age and parity, and socio-economic status did not affect the outcome.

The high rates of infant mortality during the famine most affected the groups born before the famine and exposed in late gestation. The two groups with the largest contrast in early mortality (those born before the famine and those conceived after it) are homogeneous in terms of adult health outcomes, indicating that selective survival cannot have a large confounding effect on outcome in later life.

Selective participation of people that were fit enough to attend the clinic, and prior excess mortality among the most seriously affected people may have led to an underestimation of the effect of prenatal famine on subsequent disease.

Finally, there are limitations in pinpointing the exact timing of famine exposure during gestation and associated outcomes in later life, due to the relatively small sample size on the one hand, and partial overlap between the three famine exposed groups on the other. However, the famine exposure period does give an estimate of the timing of the focus of effect.

5. Interpretation

The findings of the Dutch famine birth cohort study strongly support the fetal origins hypothesis. Chronic diseases originate in the womb through adaptations made by the fetus in response to undernutrition. The effects of undernutrition, however, depend upon its timing during gestation and the organs and systems developing during that critical time window. Furthermore, our findings suggest that maternal malnutrition during gestation may permanently affect adult health without affecting the size of the baby at birth. This gives the fetal origins hypothesis a new dimension. This may imply that adaptations that enable the fetus to continue to grow may nevertheless have adverse consequences for health in later life. Coronary heart disease may be viewed as the price paid for adaptations made to an adverse intra-uterine environment. It also implies that the long-term consequences of improved nutrition of pregnant women will be underestimated if these are solely based on the size of the baby at birth.

The Dutch famine study has established the importance of maternal nutrition during early pregnancy for the offspring's cardiovascular risk. The nutritional experience of babies who were exposed to famine in early gestation may resemble that of babies in developing countries whose mothers are undernourished in early pregnancy and receive supplementation later on,

but also of babies in developed countries whose mothers suffer from severe morning sickness. Morning sickness is common in the first trimester, and severe morning sickness is associated with metabolic changes in the mother which are similar to those seen during starvation. Since the results of our study consistently show that the effects of undernutrition are independent of size at birth, the assumption that the long-term consequences of hyperemesis gravidarum will be limited because of the normal size of the babies at birth no longer holds. The consequences of hyperemesis gravidarum for the health of the offspring need to be investigated. We also need to study the consequences of dieting before pregnancy and unbalanced diets or fasting during pregnancy. Although the Dutch famine was an exceptional situation, the nutritional experience of these babies may resemble that of babies developing today. We need to use this information to optimize maternal nutrition before and during pregnancy in order to prevent chronic degenerative diseases in generations to come.

References

Banning, C.: Food shortage and public health, first half of 1945. American Academy of Political and Social Sciences *245*, 93–110 (1946)

Barker, D. J. P.: Fetal origins of coronary heart disease. BMJ *311*, 171–174 (1995)

Burger, G. C. E., Sandstead, H. R., and Drummond, J. C. (Eds.): Malnutrition and Starvation in Western Netherlands, September 1944 to July 1945. Part I and II. The Hague: General State Printing Office 1948

Harding, J.: The nutritional basis of the fetal origins of adult disease. Int. J. Epidemiol. *30*, 15–23 (2001)

Hoek, H. W., Susser, E. Z., Buck, K., Lumey, L. H., Lin, S. P., and Gorman, J. M.: Schizoid personality disorder after prenatal exposure to famine. Amer. J. Psych. *153*, 1637–1639 (1996)

Hoet, J. J., and Hanson, M.: Intrauterine nutrition: its importance during critical periods for cardiovascular and endocrine development. J. Physiol. *514*, 617–627 (1999)

Lopuhaä, C. E., Roseboom, T. J., Osmond, C., Barker, D. J. P., Ravelli, A. C. J., Bleker, O. P., van der Zee, J. S., and van der Meulen, J. H. P.: Atopy, lung function and obstructive airways disease after prenatal exposure to famine. Thorax *55*, 555–561 (2000)

Lumey, L. H.: Decreased birth weight in infants after maternal *in utero* exposure to the Dutch famine of 1944–1945. Paed. Perinat. Epi. *6*, 240–253 (1992)

Lumey, L. H., Steyn, A. D., and Ravelli, A. C. J.: Timing of prenatal starvation in women and birth weight in their first and second born offspring: the Dutch famine birth cohort study. Eur. J. Obs. Gyn. *61*, 23–30 (1995)

Lussana, F., Buller, H., Bossuyt, P. M., Ocke, M., and Roseboom, T. J.: Prenatal exposure to the Dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. Amer. J. Clin. Nutr. *88*/6, 1648–1652 (2008)

Murray, C., and Lopez, A.: Global mortality, disability, and the contribution of risk factors: global burden of disease study. Lancet *349*, 1436–1442 (1997)

Neugebauer, R., Hoek, H. W., and Susser, E.: Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood. JAMA *282*, 455–462 (1999)

Painter, R. C., Osmond, C., Phillips, D. I. W., Hanson, M. A., and Roseboom, T. J.: Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. Br. J. Obstet. and Gynaecol. *115*, 1243–1249 (2008a)

Painter, R. C., Rooij, S. R. de, Bossuyt, P. M., Phillips, D. I., Osmond, C., Barker, B. J., Bleker, O. P., and Roseboom, T. J.: Blood pressure response to psychological stressors in adults after prenatal exposure to the Dutch famine. J. of Hypertens. *24*, 1771–1778 (2006a)

Painter, R. C., Rooij, S. R. de, Roseboom, T. J., Bossuyt, P. M. M., Simmers, T. A., Osmond, C., Barker, D. J. P., and Bleker, O. P.: Early onset of coronary heart disease after prenatal exposure to the Dutch famine. Amer. J. Clin. Nutr. *84*/2, 271–272 (2006c)

Painter, R. C., Rooij, S. R. de, Bossuyt, P. M. M., Osmond, C., Barker, D. J. P., Bleker, O. P., and Roseboom, T. J.: A possible link between prenatal exposure to famine and breast cancer – a preliminary study. Amer. J. Hum. Biol. *18*, 853–856 (2006b)

Painter, R. C., Roseboom, T. J., van Montfrans, G. A., Bossuyt, P. M. M., Krediet, R. T., Osmond, C., Barker, D. J., and Bleker, O. P.: Microalbuminurea in adults after prenatal exposure to the Dutch famine. J. Amer. Soc. Nephrol. *16*/1, 189–194 (2005)

Painter, R. C., Westendorp, R. G. J., Rooij, S. R. de, Osmond, C., Barker, D. J. P., and Roseboom, T. J.: Increased reproductive success after prenatal exposure to famine. Human Reproduction: doi10.1093/humrep/den274 (2008b)

Ravelli, G. P., Stein, Z. A., and Susser, M. W.: Obesity in young men after famine exposure in utero and early pregnancy. NEJM *7*, 349–354 (1976)

Ravelli, A. C. J., van der Meulen, J. H. P., Michels, R. P. J., Osmond, C., Barker, D. J. P., Hales, C. N., and Bleker, O. P.: Glucose tolerance in adults after in utero exposure to the Dutch famine. Lancet *351*, 173–177 (1998)

Rooij, S. R. de, Painter, R. C., Roseboom, T. J., Phillips, D. I., Osmond, C., Barker, D. J., Tanck, M. W., Michels, R. P., Bossuyt, P. M., and Bleker, O. P.: Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. Diabetologia *49*/4, 637–643 (2006)

Roseboom, T. J., van der Meulen, J. H. P., Osmond, C., Barker, D. J. P., Ravelli, A. C. J., and Bleker, O. P.: Plasma lipid profile in adults after prenatal exposure to the Dutch famine. Amer. J. Clin. Nutr. *72*, 1101–1106 (2000a)

Roseboom, T. J., van der Meulen, J. H. P., Osmond, C., Barker, D. J. P., Ravelli, A. C. J., Schroeder-Tanka, J. M., van Montfrans, G. A., Michels, R. P. J., and Bleker, O. P.: Coronary heart disease after prenatal exposure to the Dutch famine 1944–1945. Heart *84*/6, 595–598 (2000b)

Roseboom, T. J., van der Meulen, J. H. P., Ravelli, A. C. J., Osmond, C., Barker, D. J. P., and Bleker, O. P.: Plasma fibrinogen and factor VII concentrations in adults after prenatal exposure to famine. Br. J. Haematol. *117*, 111–112 (2000c)

Smith, C.: The effects of wartime starvation in Holland on pregnancy and its product. Amer. J. Obs. Gyn. *53*, 599–608 (1947)

Stearns, S. C.: The Evolution of Life Histories. Oxford University Press 1992

Stein, Z., Susser, M., Saenger, G., and Marolla, F.: Nutrition and mental performance. Science *178*, 706–713 (1972)

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