

NUTRICIA RESEARCH



ABSTRACTS

10th World Congress
Developmental Origins of Health and Disease

2017
15 - 18 October
Rotterdam
The Netherlands

Nutrition in early life is crucial for growth and metabolic development and influences lifelong health

The early stages of life are a period of rapid growth and development. From the first day of pregnancy until 2 years of age, the so-called “first 1,000 days”, all organs and tissues are formed and become functional. Therefore, this period offers a critical window of opportunity to shape lifelong health. Nutrition plays a crucial role in this: imbalanced intake of nutrients in terms of quality and quantity (e.g. under- and overnutrition) can have profound effects on the development of the child, and may increase the risk of non-communicable diseases in later life.

Increasing scientific evidence shows that meeting the specific nutritional needs during the different developmental phases of these first 1,000 days and beyond, can positively influence short and long-term health outcomes. Human milk is the natural preferred nutrition for all infants to ensure optimal growth in early life. It also is our source of inspiration to develop adapted nutritional solutions that benefit the growth and early development for those infants that do not, or no longer receive human milk. For example, lipids provide the energy for growth and differences in lipid quality are associated with differences in growth trajectories in infants. Both quantity and quality of lipids affect how the body will digest, absorb, metabolize and use lipids for growth and development. As children are not just small adults, their nutritional needs change as they grow.

Some infants and children are more vulnerable and at an increased risk of developing undernutrition. Faltering growth is a common paediatric problem with many unfavourable effects both on the short and long term. Although the causes of faltering growth are multi-factorial, the driver most often is inadequate intake, especially of energy, protein and micronutrients. Nutritional intervention should be initiated by the healthcare professionals as early as possible to help prevent adverse consequences.

Pregnant and breastfeeding women, infants and young children, healthy or sick, have specific nutritional needs. Nutrition during this vulnerable period in early life impacts physical growth and body composition, cognitive development, immune maturation, development of the gastrointestinal system as well as the development of healthy and appropriate eating habits of the child. At Nutricia Research, we aim to deliver optimal nutrition along each stage of development to ensure an optimal start in life.

INDEX

- 1. Effect of dietary lipid structure in early life improves metabolic function of adult adipose tissue in mice** p.6
A. Baars, L. Schipper, M. Mischke, M. Klein Hazebroek, E. van Schothorst, E.M. van der Beek, A. Oosting

Abstract No. 171 – Oral Presentation
Parallel session PA1.10 - Early Nutrition
Van Capellen Zaal
Monday 16 October 2017, 14:20 - 15:30hrs
- 2. Differences in development of white adipose tissue depots in post-weaning C57BL/6j mice** p.7
A. Kodde, E. Engels, A. Oosting, K. van Limpt, E.M. van der Beek, J. Keijer

Abstract No. 226 - Oral Poster Presentation (Poster Walk)
Poster Session PO1.1: Adiposity – Early Life Factors
Jurriaanse kwartier
Monday, October 16 2017, 13:50 - 14:20hrs
- 3. Individual rearing of mice impairs adolescent growth and increases adult obesity risk** p.8
S. van Heijningen, L. Schipper, A. Baars, E. van der Beek, G. van Dijk

Abstract No. 207 – Poster Presentation
Poster Session PO2.1: Adiposity – Life course
Jurriaanse kwartier
Tuesday, October 17 2017, 13:50 - 14:20hrs
- 4. Synergistic effects of stress, the neuroimmune system and nutrition in early-life stress induced cognitive decline** p.9
K. Reemst, K. Yam, L. Hoeijmakers, E.F.G. Naninck, E.M. van der Beek, L. Schipper, A. Oosting, P.J. Lucassen, A. Korosi

Abstract No. 394 - Oral Poster Presentation (Poster Walk)
Poster Session PO3.6: Neurodevelopment - Nutrition
Jurriaanse kwartier
Wednesday, October 18 2017, 13:20 - 14:20hrs
- 5. Early-life stress reduces availability of essential micronutrients; a nutritional intervention strategy to prevent its lasting consequences on cognitive function** p.10
E.F.G. Naninck, K. Reemst, J. Dumay, J.B. van Goudoever, L. Schipper, E. M. Van der Beek, P.J. Lucassen, A. Korosi

Abstract No. 452 - Oral Poster Presentation (Poster Walk)
Poster Session PO1.9: Nutrition – micronutrients
Jurriaanse kwartier
Monday, October 16 2017, 13:50 - 14:20hrs

- 6. A transient gestation-specific mouse model of hyperglycemia offers unique opportunities for nutritional and pharmacological interventions in dams and offspring** p.11
H.Y. Li, Y.X. Liu, L. Harvey, S. Shafaeizadeh, E. van der Beek, W.P. Han

Abstract No. 400 - Oral Presentation
Parallel session PA3.9: New intervention strategies
Grote Zaal
Wednesday, October 18 2017, 10:30 - 12:00hrs
- 7. Role of breastmilk microbiome in colonisation of the infant gut: a systematic review** p.12
C. Edwards, A. Gil, E.M. van der Beek, M.H. Schoemaker, S. Ozanne, C. Belzer, C. Stanton, C.A. Van Loo-Bouwman, V. Marinello, R. Rueda

Abstract No. 232 - Oral Poster Presentation (Poster Walk)
Poster Session PO3.05: Microbiome
Jurriaanse kwartier
Wednesday, October 18 2017, 13:20 - 14:20hrs
- 8. Role of placental, amniotic and cord blood microbiome in the colonisation of the infant gut: a systematic review** p.13
C. Edwards, A. Gil, E.M. van der Beek, M.H. Schoemaker, S. Ozanne, C. Belzer, C. Stanton, C.A. Van Loo-Bouwman, V. Marinello, R. Rueda

Abstract No. 236 - Oral Slam Session
Lunch meeting LM1.2 - Slam session Maternal and fetal health
Arcadis Zaal
Monday, October 16 2017, 12:40 - 13:40hrs
- 9. Increased risk of overweight during school age in children born to mothers with GDM** p.14
S. Shafaeizadeh, L. Muhardi, E.M. van der Beek

Abstract No. 271 - Poster Presentation
Poster Session PO1.4: Gestational Diabetes
Jurriaanse kwartier
Monday, October 16 2017, 13:50 - 14:20hrs
- 10. The sex-specific risk on body composition and metabolic outcomes in term born children with extreme birth weights: a narrative review** p.15
G.C. Tanner, K.L. Hanna, E.M. Van Der Beek, L. Muhardi

Abstract No. 275 - Oral Slam Session
Lunch meeting LM2.2 - Slam session Fetal and childhood outcomes
Arcadis Zaal
Tuesday, October 17 2017, 12:40 - 13:40hrs

11. **Prospective associations of age at weaning and exclusive breastfeeding duration with relative weight at 5-6 years within different risk groups** p.16
O. Sirkka, T.G.M. Vrijkotte, J. Halberstadt, M. Abrahamse-Berkeveld, T. Hoekstra, J.C. Seidell, M.R. Olthof

Abstract No. 467 - Oral Poster Presentation (Poster Walk)
Poster Session PO3.1: Adiposity – Nutritional and physical activity
Jurriaanse kwartier
Wednesday, October 18 2017, 13:20 - 14:20hrs
12. **Growth in early life and the risk of obesity among children in Aruba: a retrospective population-wide cohort study** p.17
A.J. Oudshoorn, D.E. Bakker, J. van Heijningen, J.H. van der Lee, R.M. van Elburg

Abstract No. 520 - Oral Poster Presentation (Poster Walk)
Poster Session PO2.1: Adiposity – Life Course
Jurriaanse kwartier
Tuesday, October 17 2017, 13:50 - 14:20hrs
13. **Effects of enteral glutamine supplementation on neurodevelopmental outcomes in very preterm children at thirteen years of age** p.18
S. Twilhaar, J.F. de Kieviet, J. Oosterlaan, R.M. van Elburg

Abstract No. 820 – Poster Presentation
Poster Session PO3.9: Interventions
Jurriaanse kwartier
Wednesday, October 18 2017, 13:20 - 14:20hrs

Satellite Symposium – Tuesday October 17

Sponsored by Nutricia Research, Utrecht, The Netherlands

17.40 Short introduction

Prof Dr Eline van der Beek

Nutricia Research, Utrecht / UMCG Groningen, the Netherlands

17.45 Innate immune adaptation and disease

Prof Dr Mihai Netea

Radboud University Nijmegen, the Netherlands

18:30 When things go wrong

Prof Dr Aletta Kraneveld

Utrecht University, Utrecht, the Netherlands

1. Effect of dietary lipid structure in early life improves metabolic function of adult adipose tissue in mice

A. Baars¹, L. Schipper¹, M. Mischke¹, M. Klein Hazebroek², E. van Schothorst², E.M. van der Beek^{1,3}, A. Oosting¹

¹Nutricia Research, Utrecht, the Netherlands; ²Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands; ³Department of Pediatrics, University Medical Center Groningen, University of Groningen

Background

The incidence of childhood obesity is increasing worldwide and is associated with adult obesity and metabolic disease. Environmental factors in early life, including nutrition, have shown to impact lifelong health (1). For instance, breastfeeding is associated with a reduced risk of childhood obesity and adult metabolic disease (2-3). We developed an infant milk formula with a complex lipid matrix (Nuturis[®]) more closely resembling the lipid structure of human milk (4). Early life exposure to Nuturis[®] has consistently resulted in a beneficial effect on adult fat mass accumulation in mice (5-7). In this study we investigated the potential underlying mechanism in adipose tissue in more detail.

Methods

A diet containing either Nuturis[®] or a standard infant milk formula (CTRL) was provided to mice from postnatal day (PN) 15 to 42, a time period corresponding to infancy and childhood. Subsequently, mice were challenged with a moderate Western style diet (WSD; 40 En%) or fed a standard rodent chow during adolescence and adulthood until dissection at PN112. Body composition (BC) was monitored by EchoMRI bi-weekly from PN42 to PN98. Adipocyte cell size and macrophage content were determined by epididymal white adipose tissue (epiWAT) staining and epiWAT gene expression analysis by means of microarrays at PN112.

Results

BC analysis confirmed reduced adiposity in the Nuturis[®] mice after a WSD challenge, whereas no differences were found between Nuturis[®] and CTRL mice after the standard chow. Adipocyte cell size was smaller in Nuturis[®] compared to CTRL mice independent of the adult diet. Nuturis[®] mice showed a trend towards reduced crown-like structures and individual macrophages infiltration in WAT after the WSD challenge, suggesting a reduced inflammation risk. In addition, expression of genes related to metabolic function and energy metabolism were beneficially affected in Nuturis[®] mice.

Conclusion

Our study showed that the reduced fat accumulation in mice after a WSD in adult life was associated with selective changes in adipocyte size, inflammatory markers and markers related to metabolic function and energy metabolism. These changes indicate that early modulation of epiWAT function may contribute to the protective effects of Nuturis[®] on later life health.

2. Differences in development of white adipose tissue depots in post-weaning C57BL/6j mice

A. Kodde¹, E. Engels¹, A. Oosting¹, K. van Limpt¹, E.M. van der Beek¹, J. Keijer²

¹Nutricia Research, Utrecht, the Netherlands; ²Wageningen University, Wageningen, The Netherlands

Background

Different white adipose tissue (WAT) depots have a distinct contribution to metabolic health, depending on their location in the body. Distribution of fat over WAT depots and the metabolic profile of these depots may be programmed by nutritional exposure in early (postnatal) life. Yet, little information is available on early life development of the metabolic function of different WAT depots. We evaluated post-weaning development of three WAT depots focusing on markers for mitochondrial content, oxidative capacity and WAT browning.

Method

C57BL/6j male mice were fed AIN93G until day 42 and AIN93M from day 42 to 98. Inguinal (ING), epididymal (EPI) and retroperitoneal (RP) WAT of 21, 42 and 98 days old mice was dissected, weighed and investigated for markers for adiposity and preadipocyte number (*Lep*, *Mest* and *Pref1* gene expression), mitochondrial content (citrate synthase activity) and oxidative capacity (OXPHOS protein expression) and WAT browning (*Ucp1* and *Cidea* gene expression).

Results

WAT depot weight increased over time. In line with this, gene expression of adiposity markers *Lep* and *Mest* increased in EPI ($p < 0.05$) and RP WAT ($p = 0.06$ for *Lep* and $p < 0.05$ for *Mest*), but levels were stable in ING WAT. Preadipocyte numbers as measured by *Pref1* gene expression declined over time in all three depots ($p < 0.001$). Citrate synthase activity was highest in ING WAT and decreased in all three depots ($p < 0.01$), indicating higher mitochondrial numbers in ING WAT and a decline in mitochondrial number over time, being most pronounced between day 21 and 42 in ING WAT (-56%). Protein expression of OXPHOS complexes I-III were also highest in ING WAT and declined over time in this depot ($p < 0.01$), but remained stable over time in EPI and RP WAT. The ATP synthase subunit (ATP5A) of OXPHOS showed an opposite pattern, with levels being stable over time in ING WAT but declining in EPI WAT ($p < 0.05$). The latter may suggest that activity of the electron transport chain was not coupled to ATP synthase activity in ING WAT. Together with the substantial decrease in gene expression of *Ucp1* and *Cidea* in ING WAT ($p < 0.01$), this could indicate that ING WAT transforms from a browner phenotype to a pure white depot during early post-weaning.

Conclusion

Together these data showed that developmental trajectory of ING WAT during the post-weaning period in C57BL/6j mice is distinctly different from that in EPI and RP WAT, indicating that postnatal programming of adipose tissue function is depot specific.

3. Individual rearing of mice impairs adolescent growth and increases adult obesity risk

A. Baars², L. Schipper^{1,2}, S. van Heijningen¹, E.M. van der Beek^{2,3}, G. van Dijk¹

¹ GELIFES, Groningen Institute for Evolutionary Life Sciences, University of Groningen, the Netherlands; ² Nutricia Research, Utrecht, the Netherlands; ³ Department of Pediatrics, University Medical Center Groningen, University of Groningen, the Netherlands

Background

Mice are widely used to study the neurobiology of energy balance, metabolic homeostasis and how environmental challenges at different life-stages can influence this regulation. For these purposes and to assess individual caloric intake and energy expenditure, many studies apply solitary housing conditions. The downside is that social isolation can produce chronic psychological stress in mice, and affect thermoregulatory processes. This causes changes in neuroendocrine functioning, baseline anxiety and food intake regulation. Abovementioned processes may be highly relevant during adolescent life, when fast body growth, affective development and maturation of metabolic organs are established. We therefore hypothesized that individual rearing from weaning onwards alters metabolic development and contributes to later in life vulnerability to obesity and metabolic disease.

Methods

Directly after weaning at postnatal (P) day 21, male C57BL/6J mice were housed in either individual or social (n=2 siblings/cage) housing conditions at 21 °C and kept on semi-synthetic (AIN93G based) rodent chow. At P43, one third of the mice from the individually and socially housed groups were sacrificed and the remaining groups were split and either exposed to a moderate western-style diet (WSD, 40En% as fat) or kept on control diet (AIN-93-M, 20En% as fat) until P126. Bodyweight was monitored and energy intake and expenditure were determined using indirect calorimetry during adolescence (P40-42) and adulthood (P105-107). In addition, mice were subjected to a sucrose preference test to assess food reward mechanisms during adulthood (P74-79) and to an elevated plus maze test (EPM) to assess baseline levels of anxiety during adolescence (P39) and adulthood (P92). After dissection at P43 or P126 plasma adipokines, corticosterone and Insulin-like Growth Factor-1 (IGF-1) were measured and body composition was determined by carcass analysis.

Results

Compared to socially housed mice, individually housed animals showed reduced body weight gain during adolescence, while energy intake and energy expenditure were increased. At P43 these mice had reduced lean body mass (LBM), but significantly higher white adipose tissue mass (WAT) compared to socially housed mice. In adulthood, the bodyweight gain of individually housed animals exceeded that of socially housed mice, with elevations in both energy intake and expenditure. At P126 the individually housed mice showed higher adiposity, accompanied by a reduced plasma adiponectin/leptin ratio. Adult exposure to WSD amplified these changes and reduced plasma levels of corticosterone and IGF1. Individually housed mice that were exposed to WSD showed the lowest sucrose preference index, suggesting that food reward mechanisms were blunted in this group. Baseline anxiety levels in EPM were reduced by individual housing and by adult exposure to WSD.

Conclusion

Individual housing from weaning onwards results in impaired adolescent lean mass development and increased adult adiposity. The latter effects are exacerbated by adult exposure to an obesogenic diet. These effects are associated with mild alterations in reward sensitivity and reduced baseline levels of anxiety, indicating modest consequences for affective functioning. These results suggest that individual rearing of mice can be proposed as a model for programmed adult obesity.

4. Synergistic effects of stress, the neuroimmune system and nutrition in early-life stress induced cognitive decline

K. Reemst¹, K. Yam¹, L. Hoeijmakers¹, E.F.G. Naninck¹, E.M. van der Beek^{2,3}, L. Schipper², A. Oosting², P.J. Lucassen¹, A. Korosi¹

¹Swammerdam Institute for Life Sciences - Center for Neuroscience- Structural and functional plasticity of the nervous system, University of Amsterdam, Amsterdam, The Netherlands. ²Nutricia Research, Utrecht, the Netherlands; ³Department of Pediatrics, University Medical Centre Groningen, The Netherlands

Early-life stress effects the brain for life. Clinical and pre-clinical evidence shows that early-life stress (ES), malnutrition and infection can all lead to cognitive impairments and increased vulnerability to develop psychopathologies later in life. We investigate the synergistic effects of stress, nutrition and the neuroimmune system early in life. Given the high nutritional demand of the brain during development, early nutrition is critical for brain programming. Long-chain polyunsaturated fatty acids (LCPUFA's) have immunomodulatory roles and the ratio between omega-6 and omega-3 fatty acids is critical for brain development and function. We propose that an early dietary intervention with an improved lipid content might be protective against ES-induced functional deficits, and that the neuroimmune system is involved in mediating these effects. We have previously shown that chronic ES exposure induces cognitive decline in mice, which correlates with a reduction in hippocampal neurogenesis in adulthood. Also, ES also affects the neuroimmune cells of the brain, as ES-exposed mice display more IBA1 and CD68 expressing hippocampal microglia compared to control mice.

We investigated if a dietary intervention with essential LCPUFA's can modulate ES-induced effects and which are the neurobiological substrates for the beneficial effects of the diet. We found that early dietary intervention (P2 - P42) with a reduced n-6/n-3 ratio is able to prevent ES-induced cognitive decline. This rescue was accompanied by restoration of ES-induced reductions in hippocampal newborn cell survival and increased hippocampal CD68 expression, suggesting that the beneficial effect of the diet is mediated by modulating hippocampal neurogenesis and microglia functioning. The exact role of altered microglia activity in this context is currently under investigation.

Concluding, ES-induced cognitive decline can be prevented by nutritional intervention with improved lipid content, possibly by affecting the neuroimmune system. This gives new insights for the development of targeted dietary interventions for vulnerable populations.

5. Early-life stress reduces availability of essential micronutrients; a nutritional intervention strategy to prevent its lasting consequences on cognitive function

E.F.G. Naninck¹, K. Reemst¹, J. Dumay¹, J.B. van Goudoever², L. Schipper³,
E. M. Van der Beek^{3,4}, T. Plosch⁵, P.J. Lucassen¹, A. Korosi¹

¹Swammerdam Institute for Life Sciences - Center for Neuroscience- Brain Plasticity group, University of Amsterdam, Amsterdam, The Netherlands; ²Department of Mother & Child, Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands; ³Nutricia Research, Utrecht, the Netherlands; ⁴Department of Pediatrics, University Medical Centre Groningen, The Netherlands; ⁵Department of Obstetrics & Gynecology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Background

Stressful experiences in the early postnatal period can have long-lasting effects on cognition and the risk to develop psychopathology later in life. Stress during this critical period often affects both the lactating mother and her child. Because prevention of such stress exposure is generally difficult, adequate intervention or support strategies are needed to protect against the permanent detrimental effects of early-life stress (ES) on the offspring. Elucidating the underlying mechanisms will enable the development of targeted strategies for intervention. So far, the role of early nutrition in brain programming has been largely ignored. Since the diet provides the building blocks for brain development and is required for many biochemical processes, we investigated the possible role of nutrition in programming later cognition in the context of ES.

Method

We focused on essential one-carbon metabolism associated micronutrients (1-CMAM; i.e. methionine and B-vitamins), important for development and epigenetic modifications. We investigated if ES alters micronutrient availability in milk, plasma and brain and studied the epigenetic, structural and behavioral consequences of ES in an established ES mouse model. Subsequently we tested if early 1-CMAM-supplementation can protect against the ES-induced changes in the offspring.

ES was induced in C57Bl/6 mice from postnatal day (P) 2-9, while dams received either control or 1-CMAM supplemented diet. Nutrient content was measured at P9 in offspring's stomach milk, plasma and brain. Next, we studied effects of 1-CMAM supplementation on ES-induced alterations in maternal behavior and the offspring's HPA-axis activity, neurogenesis, DNA methylation levels (global and Nr3C1 specific) and DNA methyltransferase expression in the hippocampus. All readouts were measured in male offspring, both at P9 as well as in adulthood.

Results

We found that ES reduced methionine levels in offspring's plasma and brain at P9. Importantly, 1-CMAM supplementation restored methionine levels in the offspring and ameliorated the ES-induced cognitive impairments in adulthood, abolishing ES induced deficits in object recognition and acquisition in the Morris water maze. The beneficial effects of the 1-CMAM supplementation do not involve changes in maternal care, hippocampal neurogenesis or DNA methylation, but appear to be mediated by preventing the ES-induced HPA-axis hyperactivity.

Conclusion

In conclusion, we show that a short nutritional intervention with essential micronutrients in early life can prevent ES-induced lasting effects on hippocampal function. To take these findings from bench to bedside we are currently setting up a human cohort to assess if and how stress affects the nutritional composition of breastmilk, and how this relates to history of stress, stress hormones and food intake of the mother. These findings might open new avenues for early nutritional intervention in humans to support infant development in vulnerable individuals, which is non-invasive and easily applicable.

6. A transient gestation-specific mouse model of hyperglycemia offers unique opportunities for nutritional and pharmacological interventions in dams and offspring

H.Y. Li¹, Y.X. Liu¹, L. Harvey², S. Shafaeizadeh², E. van der Beek^{3,4}, W.P. Han¹

¹Laboratory of Metabolic Medicine, Singapore Bioimaging Consortium, A*STAR, Singapore; ²Nutricia Research, Singapore; ³Nutricia Research, Utrecht, The Netherlands; ⁴Department of Pediatrics, University Medical Centre Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

Background

Global prevalence of gestational diabetes mellitus (GDM) is estimated at 14%, whilst some countries, such as Singapore, report prevalence closer to 25%. Though temporary, women who have had GDM have an increased risk for developing type 2 diabetes mellitus (T2DM) within 10 years post pregnancy. Infants of women with GDM have an increased risk for overweight/ obesity as young children and increased risk for developing T2DM in later life. Though the specific pathophysiology of GDM and T2DM are not fully understood, they share common risk factors and complications. Preliminary data suggest healthy diet and physical activity may prevent/delay the onset of diabetes. The transitory nature of GDM and recruitment prior to manifestation of the disease makes clinical studies to investigate prevention or treatment difficult. Thus, generation of animal models capturing the subtleties of GDM is necessary but success has been limited.

Method

Our objective was to develop a mouse model of transient and mild hyperglycemia during gestation resulting in clear diet induced obesity in the offspring of GDM mice. Six week old female mice were treated with high fat diet (HFD) and repeated low dose streptozotocin (STZ) injections prior to mating. Pre-treatment resulted in normoglycemic mice (8.8 vs. 7.2 mM random blood glucose [RBG]) prior to mating. Dams developed hyperglycemia in the second half of pregnancy (13.6 vs. 7.0 mM RBG @ E14, $p < 0.01$ Figure 1; and 6.2 vs. 5.2 mM fasting blood glucose [FBG] @ E15, $p < 0.01$), yet recovered from the characteristic hyperglycemia following parturition (8.5 vs. 7.1 mM RBG, $p < 0.01$, Figure 1) and post-weaning (8.0 vs. 7.1 mM RBG, Figure 1; and 7.8 vs. 6.7 mM FBG).

Results

A two-month Western diet challenge (low fat diet [LFD] as control) in the offspring started during adolescence (postnatal day 42). Compared to controls, GDM offspring exhibited greater fat mass gain compared to similar lean mass gain, resulting in a shift in overall fat percentage. In addition, GDM offspring had increased fasting insulin level without overt hyperglycemia or glucose intolerance, which resulted in increased homeostatic model assessment-insulin resistance and beta cell function. An experimental diet administered early in life significantly ameliorated the insulin resistance parameters, attenuated the body weight increase and prevented excessive fat mass accumulation in offspring later in life when compared with a group receiving a standard diet.

Conclusion

This new mouse model captures the transient gestation-specific hyperglycemia characteristic of GDM in dams as well as susceptibility to obesity and possibly other disorders in offspring. It provides windows of opportunity for various nutritional and pharmacological interventions in GDM mothers and offspring, making it suitable for mechanistic studies of GDM development and associated complications in the offspring, and unique opportunities for exploration of nutritional and pharmacological interventions to improve outcomes.

7. Role of breastmilk microbiome in colonisation of the infant gut: a systematic review

C.A. Edwards¹, A. Gil², E. M. van der Beek^{3,4}, M.H. Schoemaker⁵, S. Ozanne⁶, C. Belzer⁷, C. Stanton⁸, C.A. van Loo-Bouwman⁹, V. Marinello¹, R. Rueda¹⁰

¹Human Nutrition, University of Glasgow, United Kingdom; ²Dept. Biochemistry & Molecular Biology, University of Granada, Spain; ³Nutricia Research, Utrecht, The Netherlands; ⁴Dept Pediatrics, Univ Med Ctr Groningen The Netherlands; ⁵Mead Johnson, Pediatric Nutrition Institute, Nijmegen, The Netherlands; ⁶University of Cambridge, United Kingdom; ⁷University of Wageningen, The Netherlands; ⁸APC Microbiome Institute, Teagasc, Ireland; ⁹Yili Innovation Center Europe, The Netherlands; ¹⁰Abbott Nutrition, Spain.

Background

Characterisation of the human gut microbiome has led to much interest in its role in the development of chronic diseases from obesity and cardiovascular disease to inflammatory and neurological diseases. Early colonisation of the infant gastrointestinal tract is likely to be a key determinant in the establishment of the gut microbiome in later life. The breastmilk microbiome may contribute significantly but it is unclear how the bacteria detected in human milk are derived and if they are transferred to and survive in the infant gut. A systematic review was carried out to evaluate the published evidence.

Methods

Searches were carried out using PUBMED, OVID, LILACS and PROQUEST and search terms included: ((“Microbiota”[Mesh] OR “Metagenome”[Mesh] OR “Dysbiosis”[Mesh]) AND “Anti-Bacterial Agents”[Mesh]) AND (“Infant”[Mesh] OR “Infant, Extremely Premature”[Mesh] OR “Infant, Extremely Low Birth Weight”[Mesh] OR “Infant, Low Birth Weight”[Mesh] OR “Infant, Very Low Birth Weight”[Mesh] OR “Infant, Small for Gestational Age”[Mesh] OR “Infant, Premature”[Mesh] OR “Infant, Postmature”[Mesh] OR “Infant, Newborn”[Mesh] OR “Infant, Premature, Diseases”[Mesh])and (microbiota OR bacteria OR microflora OR microbes) AND (dysbiosis) AND (infant OR neonate OR baby) AND (health) AND (disease) AND (birth OR parturition) AND (breastmilk OR breast milk OR human milk) AND (breastfeeding OR breast feeding OR breastfed) AND (formula fed OR infant formula OR bottle fed OR bottle feed) AND (lactation). After initial removal of duplicates and not-relevant papers, sixty six papers were scrutinised by three authors before final evaluation of the evidence. Papers were considered for information on sample collection, avoidance of contamination procedures, specified bacterial analysis methods and kits used, and if they discussed maternal and infant events that may influence colonisation including mastitis and antibiotic use.

Results

Many studies considered the breastmilk microbiome and a small proportion of these considered the transfer of the bacteria to the infant and measured populations in infant faeces. Some studies followed transfer of probiotic organisms in interventional trials. Collection of breastmilk samples varied in timing (from colostrum to samples collected up to 6 months after birth) and in the method of collection (manual expression vs pump). Breasts were cleaned before sampling in many different ways including with water only, soap, saline, iodine, or chlorhexidine or some rejected foremilk before main sampling. The numbers of samples collected per study ranged from 5 to 220 and some studies looked at the whole bacterial profile whereas other concentrated on individual species. The sample storage conditions and times varied between studies along with the methods and kits used for DNA extraction and bacterial identification. In many studies there was little information on maternal health and antibiotic use.

Conclusions

Many studies reported a range of different bacteria in breast milk, however, the variation in methodology may have influenced the results. Standardisation of techniques would enhance the reliability of breastmilk microbiome research. More research is required to establish the factors determining the composition and impact of the microbiome of human milk on the colonisation of the infant gastrointestinal tract.

8. Role of placental, amniotic and cord blood microbiome in the colonisation of the infant gut: a systematic review

C.A. Edwards¹, A. Gil², E. M. van der Beek^{3,4}, M.H. Schoemaker⁵, S. Ozanne⁶, C. Belzer⁷, C. Stanton⁸, C. van Loo-Bouwman⁹, V. Marinello¹, R. Rueda¹⁰

¹University of Glasgow, United Kingdom; ²University of Granada, Spain; ³Nutricia Research, Utrecht, The Netherlands; ⁴Dept Pediatrics, University Medical Centre Groningen, The Netherlands; ⁵Mead Johnson Pediatric Nutrition Institute, Nijmegen, The Netherlands; ⁶University of Cambridge, United Kingdom; ⁷University of Wageningen, The Netherlands; ⁸APC Microbiome Institute, Teagasc, Ireland; ⁹Yili Innovation Center Europe, The Netherlands; ¹⁰Abbott Nutrition, Spain.

Background

There is increasing evidence for the potential role of the gut microbiome in human health with decreased diversity recognised as a key characteristic of a range of diseases. It is important to understand how the microbiome develops and possible strategies to stimulate a health promoting microbiota. The bacterial colonisation of the infant gastrointestinal tract is a key stage in the process, and maternal and infant events may be important determinants of the diversity and composition of the microbiome. In addition to maternal faecal and vaginal transfer at birth, it has been suggested that there is a placental microbiome which provides the first bacterial exposure to the developing infant prior to parturition. However, it is unclear how robust the evidence is for colonisation of the placenta and transfer to the infant gut, e.g. possible contamination during sample collection/analysis and pathological infection.

A systematic review of the evidence for the placental microbiome in healthy mothers and possible transfer to the infant was carried out.

Methods

Searches using PUBMED, OVID, LILACS and PROQUEST included: (“Microbiota”[Mesh] OR “Metagenome”[Mesh] OR “Dysbiosis”[Mesh]) AND (“Anti-Bacterial Agents”[Mesh]) AND (“Infant”[Mesh] OR “Infant, Extremely Premature”[Mesh] OR “Infant, Extremely Low Birth Weight”[Mesh] OR “Infant, Low Birth Weight”[Mesh] OR “Infant, Very Low Birth Weight”[Mesh] OR “Infant, Small for Gestational Age”[Mesh] OR “Infant, Premature”[Mesh] OR “Infant, Postmature”[Mesh] OR “Infant, Newborn”[Mesh] OR “Infant, Premature, Diseases”[Mesh]) ((Microbiota OR Microbiome OR Bacteria OR Microflora OR (Gut Flora)) AND (Meconium OR Amnion OR (Amniotic Fluid) OR (Gestational Sac) OR (Amniotic Sac) OR Placenta OR (Cord Blood)) AND ((In utero) OR Uterus OR Fetus OR Foetus)) AND (Umbilical cord) OR (Blood transfer) OR (Placental circulation) OR (placental transfer) OR (placental transmission) OR (Maternal-fetal exchange). Papers were checked for duplicates and relevance by three authors and then assessed for details of where bacteria were detected (cord blood, amniotic fluid or placental tissue), when infant faeces were assessed, number of samples and mothers, delivery methods, maternal health status, pregnancy history, contamination prevention during collection, sample storage and kits/processes used for DNA/RNA extraction and bacterial identification.

Results

Twenty two papers were fully assessed and included placental tissue, cord blood or amniotic fluid with 2 to 165 samples each. Methods used to extract DNA/RNA varied and were not always stated. Characterisation of the microbiome differed from PCR to whole shotgun sequencing. Details of sample storage were not always clear. Few studies considered bacterial transfer to the infant.

Conclusion

Current data are limited and more research is needed to confirm the role and importance of the placental microbiome.

9. Increased risk of overweight during school age in children born to mothers with GDM

S. Shafaeizadeh¹, L. Muhandi¹, E.M. van der Beek^{2,3}

¹Nutricia Research, Singapore; ²Nutricia Research, Utrecht, The Netherlands;

³Dept of Pediatrics, University Medical Centre Groningen, The Netherlands

Background and aims

Globally, gestational diabetes mellitus (GDM) affects approximately one out of seven pregnancies amounting to approximately 8 million live births yearly. Children of GDM mothers (GDM-F1) are at increased risk of becoming overweight and developing metabolic disease in later life. We conducted a narrative review of the literature aiming to understand the growth characteristics of children born from GDM pregnancies with focus on growth after the adiposity rebound period to provide insights relevant across childhood.

Methods

Medline was searched for articles published from 1995 to Feb 2016 with selected search terms related to growth of GDM offspring. We identified 877 articles of which 12 studies were included for review using a priori chosen inclusion and exclusion criteria for eligibility based on longitudinal assessment of growth as well as data reporting in children older than 7 years of age.

Results

Three studies were conducted among GDM-F1 only, 4 studies compared GDM-F1 with non-GDM offspring and the remaining papers (n=5) compared GDM-F1 with other reference populations which included healthy, T1DM or T2DM pregnancy offspring (Table 1). Studies among GDM-F1 only (n=3) showed an increase in BMI z-score at 7 years of age compared to reference data. When compared between GDM and non-GDM offspring, 2 out of 5 the studies reported an increase in weight, height and BMI at 7 years of age among GDM-F1, while the rest reported no significant differences. Compared to other reference populations, only GDM-F1 boys were reported to have larger waist circumference at 7 and 9.5 years of age and BMI was increased in GDM-F1 at 18 years. Increased BMI and waist circumference are considered as a proxy of increased risk of overweight/obesity.

Conclusions

Although information on childhood growth characteristics appeared to be limited, GDM-F1 appears to have an increased risk of overweight, with higher BMI and larger waist circumferences at 7 years of age, which could persist into adulthood.

10. The sex-specific risk on body composition and metabolic outcomes in term born children with extreme birth weights: a narrative review

G.C. Tanner^{1,2}, K.L. Hanna¹, E.M Van Der Beek^{3,4}, L. Muhandi²

¹School of Exercise and Nutrition Sciences, Queensland University of Technology, Victoria Park Road, Kelvin Grove, Queensland, 4059 Australia; ²Nutricia Research, Singapore; ³Department of Pediatrics, University Medical Centre Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.; ⁴Nutricia Research, Utrecht, The Netherlands

Background

The influence of sex on later risk of adverse metabolic and other health outcomes is quite established, and may be related to early life growth and body composition development. It is not well understood, however, whether these outcomes are also affected by sex in children born with extreme birth weights (term small for gestational age (tSGA), term low birth weight (tLBW); large for gestational age (LGA) and high birth weight (HBW)). This narrative review aims to evaluate sex differences in development of body composition and metabolic outcomes in infants in these populations.

Methods

Two search strategies were conducted for the groups from 4 electronic databases and publication dates between 2010 and April 2016.

Results

Sixteen studies were included based on a priori inclusion and exclusion criteria. Only 4 reported the influence of sex (tSGA and tLBW n=3; LGA and HBW subjects n=1). Among those infants born tSGA and tLBW, males had a higher fat free mass at 4 and 6 months, and had less odds of obesity at 4-5 years of age and fat mass at 6.7 years. No differences in metabolic markers such as impaired glucose, insulin and HbA1C during the first few months of life were reported. Among neonates born macrosomia, no sex differences were reported in the odds of overweight at 4 years of age. Other studies were sex-stratified, thus no sex-specific information can be inferred.

Conclusion

These results suggest that reported sex-specific risks on anthropometric, body composition, risk of overweight and obesity and metabolic outcomes were not altered by extreme birth weights. Further research is needed to confirm these outcomes. Future data analysis should focus on sex differences, and not only in sex-stratified analysis.

11. Prospective associations of age at weaning and exclusive breastfeeding duration with relative weight at 5-6 years within different risk groups

O. Sirkka^{1,2}, T.G.M. Vrijkotte³, J. Halberstadt¹, M. Abrahamse-Berkeveld²,
T. Hoekstra¹, J.C. Seidell¹, M.R. Olthof¹

¹Department of Health Sciences, Faculty of Earth & Life Sciences, Vrije Universiteit Amsterdam, Amsterdam Public Health Research Institute, Amsterdam, The Netherlands. ²Nutricia Research, Utrecht, The Netherlands. ³Department of Public Health, Academic Medical Centre, Amsterdam Public Health Research Institute, University of Amsterdam, Amsterdam, The Netherlands

Background

Certain maternal characteristics, such as ethnicity, education, BMI (body mass index) or neighbourhood are determinants for their child's overweight risk. There are large variations in infant feeding practices of mothers differing in these characteristics. Therefore, associations between infant feeding practices and child overweight may differ in these groups, which is important for overweight prevention strategies.

Objectives

Study the associations of age at weaning and exclusive breastfeeding duration with relative weight (BMI-SDS; standard deviation scores) at 5-6 years within different risk groups.

Methods

Using data from a population based birth cohort (ABCD) (n=4495), we formed groups of children at varying risk of overweight based on maternal characteristics: ethnicity, education, pre-pregnancy BMI and neighbourhood. Linear and logistic regression analyses were conducted.

Results

Weaning after 5 months of age was associated with lower BMI-SDS in childhood in groups of: Dutch ethnicity (regression coefficient B: -0.133; 95% confidence interval CI: -0.215,-0.050), medium-level education (-0.191; -0.308,-0.075), and high-risk neighbourhood (-0.160; -0.301,-0.019). Compared to exclusive breastfeeding for 0-2.9 months, exclusive breastfeeding for ≥ 6 months was associated with lower BMI-SDS in groups of medium-level education (-0.285; 0.455,-0.115), normal BMI (-0.184; -0.288,-0.079) and medium-risk neighbourhood (-0.189; -0.338,-0.040). Logistic regressions were in line with these results.

Conclusions

Associations between infant feeding practices and childhood relative weight differ between risk groups, implying that overweight prevention strategies should be group-specific.

12. Growth in early life and the risk of obesity among children in Aruba: a retrospective population-wide cohort study

A.J. Oudshoorn¹, D.E. Bakker², J. van Heijningen³, J.H. van der Lee⁴, R.M. van Elburg^{4,5}

¹Dr. Horacio E. Oduber Hospital, Department of Pediatrics, Aruba, ²VUmc, Faculty of Medicine, Amsterdam, the Netherlands, ³JGZ (Youth Health Care), Department of Public Health, Aruba, ⁴AMC-EKZ, Department of Pediatrics, Amsterdam, The Netherlands, ⁵Nutricia Research, Utrecht, The Netherlands

Background

Childhood obesity is an increasing health problem worldwide, causing a higher incidence of metabolic syndrome in early life. In Aruba, in 2010, the prevalence of overweight and obesity in boys at age 6 was 17.8% and 8.4% respectively, increasing to 32.4% and 14.9% respectively at age 11. The prevalence of overweight and obesity in girls was 27.6% and 11.7% respectively at age 6 increasing to 33% and 16.4% at age 11. The aim of this study was to identify if obesity was already present in the first 1000 days of life. In addition, early risk factors predicting obesity at the age of 6 and 11 years: rapid weight gain in the first 6 months of life, prematurity, being small (SGA) or large (LGA) for gestational age at birth, were investigated.

Method

A retrospective population-wide cohort study of children, born in Aruba between 1 January 2001 and 31 March 2005, was conducted. Growth data obtained from White Yellow Cross and the Department of Health, section Youth Health Care, were converted to BMI-for-age z-scores (BAZs), based on the WHO growth standards, to determine the prevalence of childhood obesity, defined as BAZ ≥ 2 . Logistic regression analyses were performed to evaluate the associations between early risk factors and later obesity.

Results

In total, 41,943 measurements of 5,576 children were analyzed. In the first year of life, 34.4% of the boys and 32.4% of the girls were overweight, of which, respectively 9.3% and 7.5% were obese. The mean BAZ at birth [95% CI] was -0.13 [-0.15-0.04] and -0.07 [-0.15-0.40] for boys and girls, respectively. The mean BAZ [95% CI] for boys and girls, respectively, changed to 0.55 [0.51-0.60] and 0.47 [0.43-0.51] at the age of 2-4 months, 0.41 [0.34-0.47] and 0.32 [0.27-0.39] at the age of 5-7 years and increased to +1.03 [0.94-1.13] and +0.79 [0.71-0.88] at the age of 11 years.

The adjusted OR [95% CI] per unit increase in BAZ during the first 6 months of life for obesity at age 6 years was 1.20 [1.10-1.30]. The adjusted OR [95% CI] of being LGA at birth for obesity at age 6 and 11 years was 3.45 [2.02-5.90] and 2.78 [1.33-5.80], respectively. The associations of prematurity and being SGA with obesity at age 6 and 11 were not statistically significant.

Conclusion

The present study shows that children born in Aruba are heavier compared to WHO standards. Children whose BMI grows faster in the first 6 months are at increased risk of childhood obesity, but being large for gestational age at birth, which can be associated with maternal obesity and gestational diabetes, does give a more profound risk of childhood obesity. Prevention of obesity in Aruba should start in the first 1000 days of life, as is promoted by the WHO. Future research should focus on maternal life style and diet during pregnancy to prevent LGA born infants and early life nutrition to reduce rapid weight gain in the first 6 months of life as risk factors for childhood obesity.

13. Effects of enteral glutamine supplementation on neurodevelopmental outcomes in very preterm children at thirteen years of age

S. Twilhaar¹, J.F. de Kieviet¹, J. Oosterlaan², R.M. van Elburg^{2,3}

¹Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; ²Emma Children's Hospital AMC, Amsterdam, The Netherlands; ³Nutricia Research, Utrecht, The Netherlands

Background

Children born very preterm are highly susceptible for infection and inflammation and there is clear evidence that serious neonatal infection is associated with impaired brain development and poor neurodevelopmental outcome in this population. Enteral supplementation of the amino acid glutamine has been shown to positively affect neonatal infection rates and brain development in very preterm at school age. The objective of this follow-up study was to evaluate the long-term effects of enteral glutamine supplementation on neurodevelopmental outcomes in a cohort of very preterm children at thirteen years of age.

Methods

The current cohort was enrolled in a randomized placebo-controlled trial on glutamine-enriched enteral nutrition between day 3 and 30 after birth. Sixty-one very preterm children were assessed at a mean age of 13.30 years on measures of motor, intellectual, attentional, visuospatial working memory, academic, and behavioural functioning.

Results

No differences were found between the glutamine-supplemented and control group on motor, intellectual, academic, and behavioural functioning. Forward span visuospatial working memory performance was better in controls (crude/adjusted model: $d = 0.67/0.64$, $p = .02/.02$), whereas no difference was found on backward span. After adjustment for confounders, a difference between groups was found on parent-rated attention (crude/adjusted model: $d = 0.47/0.73$, $p = .07/.003$). However, scores of both groups were within the normal range.

Conclusions

This is the first study on the long-term effects of enteral glutamine supplementation on neurodevelopmental outcomes. Our study provides no evidence for beneficial or adverse effects of enteral glutamine supplementation in very preterm children on motor, neurocognitive, academic, and behavioural outcomes at thirteen years of age. The positive effects of enteral glutamine supplementation on neonatal infections, time to full enteral feeding, and brain volume as shown at earlier ages are nevertheless promising. Future studies may therefore clarify under which conditions enteral glutamine supplementation is most beneficial.

Satellite symposium

Sponsored by Nutricia Research, Utrecht, The Netherlands

Early-life programming is becoming an established concept that states that the environment during early development affects health and disease in adulthood. Accumulating evidence suggests that nutrition during pregnancy and early postnatal life is one of the most important environmental cues that programs microbiological, metabolic, and immunologic development. The neonatal period is crucial for the early microbial colonization of the gastrointestinal tract of the new born infant. Exposure to environmental microbial components is key in the maturation process of the immune system, in turn the immune system shapes the microbiota composition. Communication between the gut microbiota, immune system and brain is driven by hormonal and neural regulation, as well as immune and metabolic pathways. Disruptions in the evolving gut microbiota during this critical developmental period can eventually impact brain development.

The speakers of this symposium will zoom in on the development of the immune system. More specifically on the concept and capacity of training innate immunity, which underlies the responsiveness of the immune system to later challenges throughout life. The protective effects following specific vaccinations in infants are dependent on the trained immunity. Inappropriate activation of trained immunity can lead to excessive inflammation in autoimmune and autoinflammatory diseases. Therefore, its putative role as well as the long-term consequences of disruptions in early life will be discussed. For instance, early life events (antibiotic use and maternal inflammation) and possibly food allergy play an important role in the development of psychiatric disease. The relevance of gut-microbiome-immune-brain axis in neurodevelopmental disorders such as autism will be elaborated on based on (pre)clinical data. Collectively both talks will shed some light on the importance of immune development in early life.

17.40 Short introduction

Prof Dr Eline van der Beek
Nutricia Research, Utrecht / UMCG Groningen,
The Netherlands

17.45 Innate immune adaptation and disease

Prof Dr Mihai Netea
Radboud University Nijmegen, The Netherlands

18:30 When things go wrong

Prof Dr Aletta Kraneveld
Utrecht University, Utrecht, The Netherlands



Trained immunity: innate immune adaptation and disease

Prof. Dr. Mihai G. Netea

Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed *trained immunity* characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, *trained immunity* has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on *trained immunity* have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce *trained immunity*, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. Inappropriate activation of *trained immunity* can also lead to excessive inflammation in autoimmune and autoinflammatory diseases. The concept of *trained immunity* represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

Food for thought: the gut-immune-brain axis in neurodevelopmental disorders

Prof. Dr. Aletta D. Kraneveld

Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science; Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Many neurodevelopmental disorders are associated with gastrointestinal deficits characterized by motility problems, low-grade inflammation and leaky gut, pain, alteration of microbiome composition and activity. The recently reported leaky gut, intestinal inflammation and changes in the composition of the microbiome in patients point to the relevance of gut-microbiome-immune-brain axis in neurodevelopmental disorders such as autism. Based on (pre)clinical data the talk will shed some light on the possible mechanism of the crosstalk between gut and brain in CNS disorders with a focus on immunological mechanisms. The talk will present (pre)clinical data on gut-immune-brain axis & immune programming in autism focusing on the role of the microbiome. Early life events (antibiotic use and maternal inflammation) and possibly food allergy play an important role in the development of autism. A poor gut function leads to a poor brain development and function; therefore targeting the microbiome/gut with nutritional interventions or pharmaceutical compounds modulating immune processes could be a new approach for the therapy of neurodevelopmental disorders.

**NUTRICIA
RESEARCH**



Nutricia Research
Utrecht Science Park
Uppsalalaan 12
3584 CT Utrecht
The Netherlands

Nutricia Research
Singapore
30 Biopolis Street
Matrix Building
#05/01B Singapore 138671

www.nutriciaresearch.com

