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Nestlé Nutrition Workshop Series
Pediatric Program Volume 62

Personalized Nutrition for the Diverse Needs of Infants and Children



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Nestlé Nutrition Workshop Series
Pediatric Program Volume 62

Personalized Nutrition for the Diverse Needs of Infants and Children

Helsinki, September 2–6, 2007

Editors

Dennis M. Bier

Bruce J. German

Bo Lönnerdal



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Foreword

With the completion of the human genome sequence just a few years ago, it is most interesting to note that 99.9% of the genetic information is similar in all humans; it is the remaining 0.1% that varies and which makes each of us individual. Epigenetic studies have demonstrated that variation in nutrient requirements depends upon individual variations in genes which can affect nutrient metabolism. It was in this context, that the 62nd Nestlé Nutrition Workshop was dedicated to 'Personalized Nutrition for the Diverse Needs of Infants and Children' and took place in Helsinki, Finland, on September 2–6, 2007.

This was the first workshop within the 27-year history of the Nestlé Nutrition Workshops – Pediatric Program that addressed personalized nutrition in infants and young children. Individuality was discussed at the genetic, biochemical, environmental, metabolic and nutritional levels. The first food in life, breast milk, has been reported to dynamically vary between mothers, between feeds and during the lactation period. This natural individualized nutritional concept can explain in part the differences of growth pattern between breastfed and formula-fed infants. By gradually changing the composition of infant formula in a manner similar to that of breast milk, it may be possible to come closer to the goal of achieving similar growth and development of formula-fed infants relative to those which are breastfed. Bioactive factors, such as prebiotics and probiotics were discussed in the context of mimicking nature's example, breast milk. Additionally, factors that distort 'healthy' development, such as gene defects leading to inherited diseases, or epigenetic factors that can influence individual susceptibility to obesity and type-2 diabetes/insulin resistance were emphasized. Key questions during the workshop were during which time window modification of the effects can be possible, and to which extent nutrition and its personalization can contribute to optimal growth and development.

We wish to warmly thank the three chairpersons of this workshop, *Dennis M. Bier*, *J. Bruce German* and *Bo Lönnerdal* for establishing an exciting scientific workshop program. Many thanks also to *Annette Järvi* and her team from Nestlé Nutrition Nordics for the excellent

logistic support and for enabling the workshop participants to enjoy the charm of Finnish culture.

Our special thanks go to *Denis Barclay* who has coordinated the last five Nestlé Nutrition Workshops. He will move as scientific advisor for adult nutrition and enrich the Nestlé Nutrition Institute activities.

Prof. Ferdinand Haschke, MD, PhD

Chairman

Nestlé Nutrition Institute

Vevey, Switzerland

Dr. Petra Klassen, PhD

Scientific Advisor

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Developmental Perspectives on Individual Variation: Implications for Understanding Nutritional Needs

*Peter D. Gluckman, Alan S. Beedle,
Mark A. Hanson and Eric P. Yap*

In comparative biology, the principles of how developmental plasticity generates biological diversity from one genotype are well understood. A single genotype can in some species be the source of distinct morphs in which early environmental signals have induced a coordinated set of changes across systems – for example, in the female honey bee different feeding of the early larva can induce either the worker bee or queen bee phenotype. However, for most traits in most species, developmental plasticity generates a more continuous variation in response to environmental cues acting on the developing organism – the varying size of monozygous twins illustrates this. In an era of genetic enthusiasm and determinism, the major focus of molecular research has been on finding linkages between polymorphisms and phenotypic traits in the belief that variations in complex biologies can be explained by such associations. However, in general, the magnitude of these linkages has not been particularly high and much variation is left unexplained.

In the decade following the discovery of the biochemical basis of epigenetic change, namely that gene expression can be altered through methylation of CpG islands or by modifications to histones, interest has emerged in the clinical significance of these findings. While there has been considerable focus on parental imprinting, which affects a small subset of genes, a different set of genes may be influenced by environmentally induced epigenesis. The environmental factors may be internal, as in the case of cellular differentiation, or external such as altered nutrition acting in early development. Further complexity is added by the increasing evidence for cross-generational transfer of epigenetic marks, creating a transient form of inheritance [1]. The mechanisms remain speculative.

Environmental cues in early development may be gross and disrupt development, but more often are physiological and induce adaptive

responses [2]. These may serve to allow the fetus/infant to survive immediate challenges, even if there are later costs, or may induce plastic responses which evolved to provide adaptive advantage later in life. This depends on the ability of the fetus/neonate to use environmental signals to appropriately forecast its environmental future. Life history and physiological changes mediated through epigenetic processes then follow in response to this prediction [3]. The fidelity of the prediction need not be high for such mechanisms to be selected. Thus, in humans, environmental cues in early life may act to induce epigenetic change with consequences for metabolic, behavioral and reproductive phenotypes throughout life. There is growing evidence that such adaptive epigenetic changes generate much variation in how an organism can later respond to a given nutritional load [4]. Developmental mismatch can occur for many reasons, but the basic concept is that whereas the post-natal nutritional environment can change drastically between generations, the fetal environment cannot. Recent evidence suggests that maternal overnutrition can also impact inappropriately on fetal development, but it is not clear that analogous mechanisms are involved [5].

Obesity and the metabolic syndrome complex represent the net outcome of an individual living in an environment that is energetically inappropriate. Experimental and clinical evidence suggests that this variation in capacity to live in a given energetic environment is influenced by developmental factors acting through epigenetic mechanisms. In turn, this suggests that epigenetic biomarkers may provide a route for identifying who is most at risk of developmental mismatch and thus offer the opportunity for selective nutritional or other intervention.

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Factors Influencing the Establishment of the Intestinal Microbiota in Infancy

Ingegerd Adlerberth

The establishment of the intestinal microbiota commences at birth and precedes in a sequential manner during the first years of life until an adult-type, highly complex microbiota, consisting of hundreds of different bacterial species, has developed.

The first bacteria to establish in the neonatal gut are usually aerobic or facultatively anaerobic bacteria, like staphylococci, enterococci and *Escherichia coli* and other enterobacteria. During their growth they consume oxygen and change the intestinal milieu making it suitable for the proliferation of anaerobic bacteria, which successively colonize the gut. Bifidobacteria are the most common anaerobes in the infantile microbiota. *Clostridium* and *Bacteroides* are also among the first anaerobes to be established, followed by anaerobes belonging to *Lactobacillus*, *Veillonella*, *Ruminococcus*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus* and other genera. Many of the anaerobic bacteria colonizing the gut are non-culturable and are only detected using DNA-based molecular methods.

As the complexity of the anaerobic microbiota increases, the population sizes of aerobic and facultative bacteria decline. This phenomenon is thought to result from oxygen depletion, substrate competition and the accumulation of toxic metabolites.

Many of the bacteria colonizing the neonate may be acquired from the mother's fecal microbiota during a vaginal delivery. Infants born by cesarean section are not exposed to these bacteria, which results in delayed acquisition of, e.g., *E. coli*, *Bacteroides* and bifidobacteria. However, bacteria may also be acquired from other persons and from environmental sources. Staphylococci, which are the first colonizers of the gut of both vaginally and sectio-delivered infants, are commonly acquired from the parental skin microbiota. Clostridia, enterococci and enterobacteria other than *E. coli* are easily picked up from the environment.

Many, but far from all studies find differences in the intestinal microbiota between breastfed and formula-fed neonates. Reported differences include less enterococci, clostridia, enterobacteria and *Bacteroides*, but more staphylococci in the microbiota of breastfed infants. High counts of bifidobacteria are common in both groups.

The degree of exposure to bacteria from environmental sources is an important determinant of the intestinal colonization pattern. Infants born in developing countries, whether delivered vaginally or by cesarean section, are colonized earlier by fecal bacteria such as *E. coli* and other enterobacteria, enterococci and lactobacilli and have a more complex microbiota early in life than infants in Western societies. Instead, infants in Western countries are more frequently and persistently colonized by bacteria which may be regarded as 'opportunistic' colonizers, i.e. bacteria that proliferate in the gut in the absence of competition from a complex microbiota. This includes, e.g., skin bacteria like staphylococci, and *Clostridium difficile*, an anaerobic spore-former which is common also in highly hygienic environments.

Intestinal bacteria are a major stimulus for the gut immune system, and a late acquisition of typical fecal bacteria or a delay in the establishment of a complex and diverse intestinal microbiota might have effects on the maturation of immune functions after birth.

Genetically Determined Variation in Polyunsaturated Fatty Acid Metabolism May Result in Different Dietary Requirements

Berthold Koletzko, Hans Demmelmair, Linda Schaeffer, Thomas Illig and Joachim Heinrich

The metabolic availability of polyunsaturated fatty acid acids (PUFAs) has a major impact on human health and has been related, among other outcomes, to early visual, cognitive and motor development, mental health and psychiatric disorders, cardiovascular disease mortality, immunological and inflammatory responses as well as related diseases such as allergies [1]. These and other biological effects of PUFA appear to be mediated largely by long-chain PUFAs (LC-PUFAs) with ≥ 20 carbon atoms and ≥ 3 carbon atoms, such as arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). The dietary LC-PUFA supply (e.g. AA with meats and eggs; EPA and DHA with marine foods) has a marked effect on blood and tissue contents [2]. LC-PUFAs can also be derived in human metabolism from the precursor essential fatty acids, linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3).

We hypothesized that in addition to dietary effects, variations in human genotype affect PUFA metabolism and availability, and hence related biological and health outcomes (fig. 1). In previous studies, we found a close correlation of n-6 and n-3 LC-PUFA contents in mature human milk [3], even though the main dietary sources of the two LC-PUFA families are very different, which seems to suggest that some women have a higher ability to synthesize and secrete LC-PUFAs of both families than others. Similarly, we observed considerable inter-individual differences in endogenous PUFA conversion in stable isotope studies [4, 5].

The hypothesis of genetic determination of LC-PUFA formation was tested in 727 mainly Caucasian subjects aged between 20 and 64 years who had participated in the European Community Respiratory

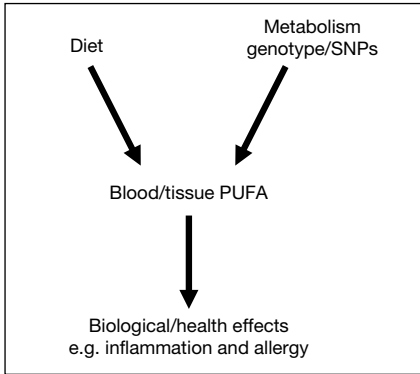


Fig. 1. Both dietary intake and metabolic handling, which may be affected by genotype and single nucleotide polymorphisms (SNPs), modulate blood and tissue contents of polyunsaturated fatty acids (PUFAs), which have an impact on biological and health effects.

Health Survey I (ECRHS I) [6]. We analyzed 18 single nucleotide polymorphisms (SNPs) of the *FADS1 FADS2* gene cluster encoding for $\Delta 5$ -desaturase and $\Delta 6$ -desaturase, the rate-limiting enzyme-mediated steps in the conversion of PUFAs to LC-PUFAs (fig. 2). We found strong associations of the less common polymorphisms and reconstructed haplotypes of *FADS1* and the upstream region of *FADS2* with higher levels of the PUFA precursors, and with lower LC-PUFA levels (fig. 2). The effect sizes were large, with a reduction in mean LC-PUFA values with 2 less common SNPs by up to about 25% of baseline values. The carriers of the less common polymorphisms and their respective haplotypes showed no differences in total or specific IgE levels, but carriers of the minor alleles of several SNPs had significantly reduced odds ratios for allergic rhinitis and atopic eczema. With the 5-locus haplotype consisting only of minor alleles, there was only half the likelihood for allergic rhinitis (OR 0.46; 95% CI 0.26, 0.83) and atopic eczema (OR 0.46; 95% CI 0.22, 0.94).

Our findings highlight the contribution of the desaturation pathways on n-6 and n-3 PUFA and LC-PUFA levels in serum lipids, and the major importance of its genetic control, and they demonstrate for the first time that the fatty acid composition of serum phospholipids is genetically controlled by the *FADS1 FADS2* gene cluster. Blood levels both of PUFAs with 18 carbon atoms, conventionally referred to as the essential fatty acids, as well as their biologically active LC-PUFA

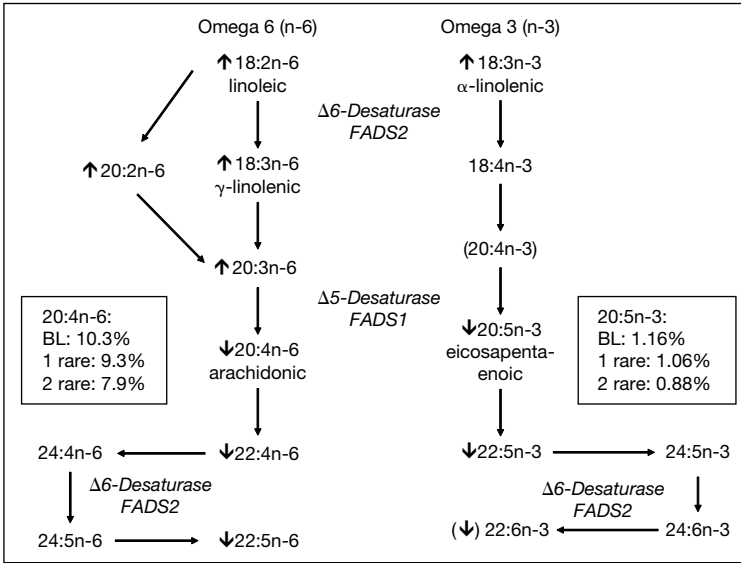


Fig. 2. Effects of single nucleotide polymorphisms (SNPs) of the enzymes $\Delta 6$ -desaturase (fatty acid desaturase 2, FADS2) and $\Delta 5$ -desaturase (fatty acid desaturase 1, FADS1) on the plasma phospholipid contents of n-6 and n-3 polyunsaturated fatty acids (PUFAs). Rare alleles are associated with significantly (in most cases $p < 0.001$) increased levels ($\uparrow$) of precursor fatty acids such as linoleic (n-6) and α -linolenic (n-3) acids, and significantly reduced levels of long-chain PUFAs (LC-PUFAs) such as arachidonic acid (n-6) and eicosapentaenoic acid (n-3), while docosapentaenoic acid (n-3) shows a nonsignificant trend to lower levels ($\downarrow$). The observed effect sizes are large: mean arachidonic acid levels (20:4n-6) with two baseline (BL) SNPs are 10.3%, with one rare mutation 9.3% and with two rare mutations 7.9%. For eicosapentaenoic acid the respective values are 1.16, 1.06 and 0.885.

derivatives depend not only on dietary intake but to a large degree also on genetic variants commonly found in a European population. The investigated SNPs explain 28% of the variance of AA and up to 12% of its precursor acids. Based on this genetic variation, individuals may require different amounts of dietary PUFAs or LC-PUFAs to achieve comparable biological effects. We strongly recommend including analyses of FADS1 and FADS2 polymorphism in future cohort and intervention studies addressing the biological effects of PUFAs and LC-PUFAs, which should enhance the sensitivity and precision of such studies.

Acknowledgements

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(Molecular) Imaging: New Developments Enabling Evidence-Based Medicine

J. W. Hans Hofstraat

Breakthroughs and continued progress in medical technologies are driving new approaches to healthcare. The increasing quality of data provided by diagnostic equipment, both in vitro and in vivo, combined with the rapidly accumulating insight into the molecular characteristics of health and disease, greatly contribute to 'evidence-based' medicine [1]. In addition, preventive and personalized medicine will first add to and, in the future, increasingly replace the present practice of symptom-based diagnosis and treatment. Early identification of individual (risk) profiles will not only have an impact on therapy, but may also lead to pro-active approaches. Examples of preventive medicine may be in 'personalized' nutrition and even in more general lifestyle advice.

(Molecular) imaging in particular offers increasing opportunities. Developments in medical imaging systems, increasingly integrating advanced high-resolution instruments with sophisticated data and image-processing tools to provide an ever-increasing quality of information (rather than data) to the medical professional, go hand in hand with developments of sophisticated functional and targeted contrast agents that provide functional information and even insight into biochemical processes at the molecular level. Since data are obtained directly from the patient, the information can be directly applied for personalized diagnosis and therapy, as well as rapid assessment of the response to therapy. The progress in 'quantitative' imaging, paving the way to '4D' imaging, significantly enhances the use of image data in tailoring and monitoring therapy, or to assess the impact of lifestyle changes.

Due to advances in nuclear imaging technologies, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), extremely low concentrations of targets can be localized and quantified. These techniques can be utilized to visualize

nanomolar and even picomolar concentrations of (radioactively labelled) molecules. The application of tailored radioactive tracers may provide direct information on the presence of biomarkers of disease, such as membrane-bound proteins, through targeting approaches. In addition, molecular tracers have been developed, which provide functional monitoring on biochemical processes (e.g., measuring increased metabolic rates, related to tumor growth, or tissue oxygenation) by dynamic imaging in conjunction with knowledge-based software, e.g. for pharmacokinetic modeling. Combination of the sensitive, but not very highly resolved, nuclear imaging techniques with other imaging modalities which provide high-resolution morphological data, such as computed tomography (CT), or complementary functional information, such as magnetic resonance imaging (MRI) and spectroscopy (MRS), leads to very powerful molecular imaging tools.

By application of advanced data acquisition and data processing tools, MRI is in itself a very powerful and versatile technique with the unique option of using the imaging instrument directly without the application of contrast agents and at the same time obtaining high-resolution morphology information *and* functional and molecular data. Examples are functional MRI, e.g. used to measure local brain activity or tumor perfusion, and MRI spectroscopy benefiting from the possibility of obtaining structural information through the measurement of molecular signatures. Furthermore, MRI can image other nuclei besides protons (such as ^{13}C , ^{19}F , or ^{23}Na), and can also be utilized for the determination of other parameters, e.g. the distribution of pH value, elasticity or temperature. These approaches have the advantage that truly noninvasive characterization can be done. In the context of the present workshop a highly relevant opportunity is the use of MRI to determine the presence and extent of potentially harmful intra-abdominal or ‘deep’ fat tissue [2, 3]. The diagnosis of deep fat is an important parameter in the assessment of cardiovascular risks (fig. 1). Significant visceral fat deposits have been linked to several conditions, including hypertension and coronary heart disease, and diabetes. Since MRI does not involve ionizing radiation, nor requires the use of contrast agents, the technique may be applied for routine screening. In conjunction with therapeutic action, MRI can also be used to determine the effect of, for instance, a particular diet plan on the amount and distribution of intra-abdominal fat by application of quantitative imaging approaches, e.g. by chemical-shift selective (‘fat only’) imaging.

The introduction of molecular imaging approaches to medical practice requires both instrumental and (bio)chemical advances. Hence, to accelerate progress in this new application area, close collaboration is required between medical technology companies on the

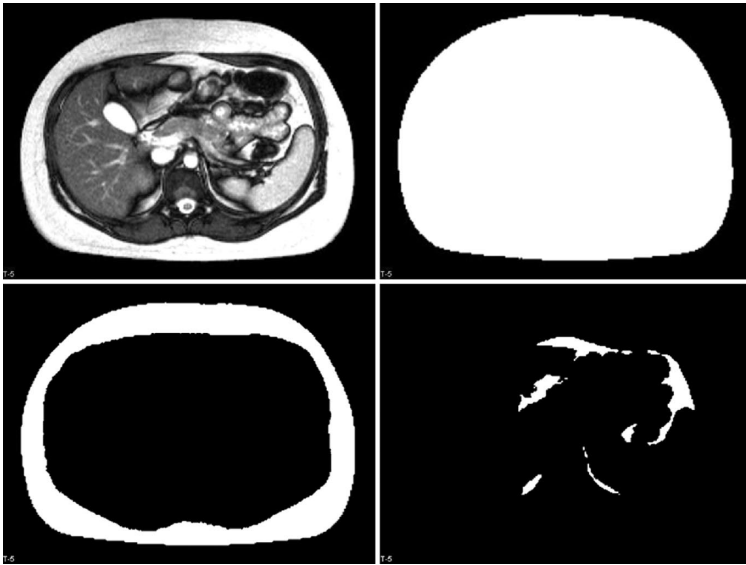


Fig. 1. MR images in 10-year-old overweight girl. Upper left: Transverse true FISP (fast imaging with steady state processing) abdominal MRI acquired as part of standard MR examination. Lower left: Subcutaneous fat region. Lower right: Visceral fat region. Fat regions were segmented separately with the region-based thresholding segmentation method, and areas can be determined. Upper right: Total abdominal fat was calculated as the sum of the measured subcutaneous fat and the measured intra-abdominal fat, where the entire abdomen has been taken as the denominator for the computation of percentage of abdominal fat. Reproduced with permission from Siegel et al. [3].

one hand, and pharmaceutical, nutritional, or contrast agent companies on the other. It is therefore Philips ambition to forge partnerships in healthcare with public and private parties with complementary knowledge and interests in a setting of ‘open innovation’.

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Metabolic Profiling

Gerard T. Berry

The concept of chemical individuality was introduced by Garrod in 1908. Inheritance of Mendelian traits including disease states has finally reached a new level of understanding based on the modern principles of gene expression coupled with new insights into the metabolism of RNA species and protein. Over 300 different perturbations in metabolite profiles with their identifying alterations in protein and/or gene structure and/or function have been identified in the past 100 years. With the realization in 1953 that the sentinel disease, phenylketonuria, can be effectively treated by nutritional manipulation tailored to the needs of each individual, we essentially entered a new phase in metabolic medicine, namely, that of nutritional therapeutics. The infant or child destined for a lifetime of debilitating cognitive and motoric handicaps may be rescued by the implementation of a unique nutritional prescription in early development. Ideally, treatment is begun shortly after birth, the direct consequence of universal newborn screening for genetic diseases. The last concept that is beginning to take hold in medicine is that of complex genetic disease, perhaps the final frontier in genetic medicine. We need look no further than phenylketonuria to realize that identical genotypes do not necessarily determine identical disease states or outcomes, even in the absence of a strong environmental pressure. Human beings are complex and the expression of disease is complex, even those that are governed by simple Mendelian factors. Patients with inherited defects that impact on intermediary metabolism need to receive nutritional therapy on an individualized basis. Metabolic profiling, i.e., the array of small molecules or analytes, as well as large macromolecules, measured with precision in body fluids or tissues, can be used to devise a nutritional therapeutic plan, as well as serve as an endpoint to evaluate the biochemical efficacy of intervention.

Table 1. Conditions under active investigation toward the development and validation of a high throughput method targeting population screening (listed in alphabetical order)

-
- Creatine metabolism (disorders of)
 - Duchenne muscular dystrophy
 - Familial hypercholesterolemia
 - Fragile X syndrome
 - Glucose-6-phosphate dehydrogenase (G6PD) deficiency
 - Infectious diseases
 - HIV
 - Toxoplasmosis
 - Cytomegalovirus (CMV)
 - Lysosomal storage diseases (partial list)
 - Fabry disease
 - Gaucher disease
 - Krabbe disease
 - Metachromatic leukodystrophy (MLD)
 - MPS I, II, IV
 - Niemann-Pick disease type A, B
 - Pompe disease
 - Severe combined immunodeficiency (SCID)
 - Smith-Lemli-Opitz syndrome (and possibly other disorders of sterol metabolism)
 - Spinal muscular atrophy (SMA)
 - Wilson disease
 - X-linked adrenoleukodystrophy (X-ALD)
-

adopted as the standard of care in the vast majority of US states. As of July 2007, screening by MS/MS is provided by 48 of 51 US states, a proportion that translates into approximately 98% of the total number of births per year. However, the extent of implementation of the full panel remains variable (fig. 1), ranging between 5 and 100% (5 states) with an overall average of 75%. Several programs are reluctant to include in their panel the whole set of secondary targets despite the unavoidable need to perform a differential diagnosis for most amino acid and acyl-carnitine markers. If conditions were to be removed from the list of secondary targets on the sole basis of not requiring a differential diagnosis from a primary condition, only argininemia and 2,4-dienoyl-CoA reductase deficiency would be candidates for exclusion from the panel [2, 3]. Limited appreciation of this reality may lead to unfortunate yet fully avoidable situations, for example the reporting of concurrent diagnoses in a patient with a complex biochemical phenotype, or the assumption that a nominal mass represents only one of several possible isobaric

compounds. Furthermore, it has become increasingly apparent that there are additional conditions potentially detectable by analysis of the same amino acid and acylcarnitine markers [4].

The evolution of newborn screening is far from being idle, as a large number of infectious, genetic, and metabolic conditions are currently under investigation at variable stages of test development and clinical validation. Table 1 is a representative, but likely incomplete, list of conditions currently being considered. Analytical development of screening tests and clinical validation through prospective pilot studies are in progress for many of them. In the US, a formal process with oversight by the Advisory Committee on Heritable Disorders and Genetic Diseases in Newborn and Children has been established for nomination and evidence-based review of new candidate conditions. If approved, these conditions could be added to the uniform panel and consequently pave the way to large-scale implementation.

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The Scope of the Problem: The Phenotype of Human Obesity

Dennis M. Bier

Personalized nutrition to prevent the development of obesity or personalized nutrition intervention to treat those who are already obese should, in theory, be a simple matter. Excess body weight reflects an imbalance between only two terms, energy intake and energy expenditure. Further, the existence of energy imbalance is readily measured in the field using a simple, highly precise instrument, a scale. Additional discriminatory power is conveyed by two other field instruments, a tape measure and a mirror. For any individual, inappropriate weight gain indicates that, regardless of their actual individual values, energy intake is increased or physical activity is inadequate for the specific person in question. Theoretically, then, a personalized corrective response can be undertaken by altering one or both of the terms in the energy balance equation. In practice, however, personalized nutrition for obesity is far more difficult since the number of variables contributing to the two terms is very large and each contributes not only a small, but also a different, fraction of the variance observed in each of the terms.

Body weight is highly heritable, but the number of genes contributing to body weight is large. Further, the distribution of the ‘weight genes’ one receives from obese parents can be skewed by assortative, non-random mating (i.e. people of like body weights are more likely to mate with each other than with people of different body weights). In addition, epigenetic effects on gene expression during fetal development due to maternal obesity and/or dietary habits during pregnancy may have permanent effects on gene expression in the adult lives of the offspring.

In early postnatal life, we are woefully ignorant of diet-gene, diet-epigene, and diet-gut microbiota effects on the progression of developmental pathways that lead to alterations in body fat, body fat distribution, adipocyte hormone secretion, or maturation of the gut-hypothalamic appetite and satiety regulatory systems. Likewise, it is now clear from animal studies that ‘hardwiring’ of the hypothalamic

neuronal circuits responsible for regulating appetite and satiety are influenced by circulating leptin during critical periods of development, resulting in permanent changes in the way the hypothalamic-gut regulatory system functions throughout later life. Thus, simple solutions to re-balance the energy balance equation during later childhood or in adult life may not be readily achievable.

Additional personal variables that are poorly understood and, therefore, are not readily subject to individualized change are those that influence the development of eating behaviors during infancy and childhood. Similarly, virtually nothing is known about the development of physical activity behaviors in the early years of life. Moreover, the environmental variables that help determine eating and activity behaviors are almost boundless. Among the major ones are: (1) parental control of diet during the critical period of infancy when the child is fully dependent on his or her parents for nutrients; (2) parental role modeling; (3) family economics and availability of food; (4) influence of media and advertising; (5) commercial modifications to existing foods and/or introduction of new food ingredients into human diets; (6) role of education and the educational environment; (7) influence of siblings, peers, and peer group activities, and (8) effects of the local 'built environment', laws, regulations and social policies.

Given the number of variables and their permutations, developing models that will enable unique (i.e. individual) solutions appears formidable. The scope of this problem will be discussed.

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Intestinal Immune Health: A Mini Review

Michelle E. Conroy and W. Allan Walker

Fetal development and the transition from the womb imply an elegant anatomic and physiologic preparation for drastic changes in environment and exposure. The immune system of the neonate requires both instant readiness in the event of perinatal infection but also education about its new surrounds. As a result, the infant is in the unique immune circumstance of readied ignorance. This review will incorporate both well-established and recent data to present an abbreviated depiction of fetal and neonatal mucosal immune development and some of the potential molecular mechanics driving gut homeostasis.

Throughout gestation, the fetus undergoes predictably timed assembly of and protection by various immune system components and surrogates. In fact, the basic template of the mucosal immune system is established very early. This is essentially the same for the systemic immune system, the development of which occurs in parallel with the mucosal system [1]. This striking difference between fetal and adult tissue highlights the critical importance of regulatory activity in establishing peripheral tolerance in the fetus and degree of inflammatory responsiveness [2]. Thus, as the neonate readies for birth and entrance into the contaminated world, the issue of potentially excessive inflammatory responses become critical. Clearly the fetal to neonatal transition must include means through which this inflammatory default must be mitigated. Much work has been done to understand the mechanisms behind this process which leads ultimately to the enigma of oral tolerance. The amniotic environment is a sterile one that protects the developing fetus from infection. As a result, the fetus is presumably 'sterile' prior to birth. The newborn acquires a healthy bolus of bacteria while passing through the birth canal. Initial colonization via birth is also rapidly altered by the introduction of feeding. One study of 40 infants from days 3–21 of life demonstrated marked variability in colonization between breast- and bottle-fed infants. In this study, which confirms others, bifidobacter becomes the dominant

bacteria by 1 week of age in breastfed infants. Bottle-fed infants show a much more diverse flora with predominance of bacteroides [3]. Thus, breastfed babies support more 'beneficial' microbial colonization. The mechanisms by which this occurs elucidates the protective nature of human milk. In a broad sense, human milk serves to 'quiet' the hyperactive inflammatory response of the neonate. It is therefore clear that the epithelial layer of the gut interacts with microbes and milk (human or artificial) products to establish protection and immune modulation for the neonate.

As the infant is bombarded with billions of bacteria that are of variable potential pathogenicity, the epithelium must provide effective barrier protection. It has help from other mucosal cells including the antimicrobial proteins of Paneth cells and the mucous of goblet cells. It turns out that the Toll-like receptors (pattern recognition receptors) expressed on intestinal epithelia contribute to protect the intact barrier. The pattern recognition of colonized bacteria, then, likely assists the epithelium in maintaining a constitutive barrier to invasion. With one cell layer constituting such a critical separation, reparation via commensal stimulation is an efficient example of coexistence [4].

From the moment of impact, initial bacterial docking, the epithelium has devised ways of regulating the colonization of the gut. Bacteria utilize cell surface glycoconjugates as receptors for epithelial adherence. This is apparently under both regional and developmental regulation resulting in variability of terminal epithelial glycosylation by age and anatomical location. Germ animals do not appear to express these enzymes variably, regardless of age or weaning. This relationship between bacterial presence and epithelium function points again to the critical importance of proper initial and maintained colonization. A step further in logic suggests that alternative bacterial presence will result in a varied epithelial surface response. In turn, this may encourage a less symbiotic and more pathogenic bacterial effect in the gut. This bacterial and epithelial interaction is compelling. Because of its circular nature, it again reinforces the pertinence of proper initial colonization [5].

Accordingly the fetus transitions through birth to infancy with an immune system that is readied but necessarily harnessed through regulatory mechanisms. The enormous transition from sterility to non-inflammatory colonization requires intricate, adaptive responses. This is accomplished through various specific and nonspecific means, but the epithelial layer is central to the infant's ability to be colonized without harm. These interactions are critical to both the immediate need to avoid infection and the long-term goal of tolerance. Recent studies have elucidated the molecular basis of the epithelial ability to provide

barrier function, a non-inflammatory resting state, and protection against invasive organisms. The neonate is further assisted by the powerful exogenous immune influence via human milk. Not only does it enable proper colonization, but human milk clearly modulates neonatal excessive inflammation. Given the intestinal epithelial layer's open access to the environment, it seems clear that clinical intervention at this locus is inevitable. Taken in context with the widespread clinical issues of childhood allergy and inflammatory bowel disease, the gut mucosa becomes even more pertinent. The infant's acquisition of both local and systemic tolerance is complex with the reward of immunologic pearls awaiting discovery.

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Gut Microbiota and Insulin Resistance: Recent Evidence and a Lesson Learned from Antibiotic Treatments

Chieh J. Chou

Since the late 20th century obesity has become a global health problem. In the US alone, more than 65% of adults are overweight or obese [1]. Although genetic, environmental and behavioral factors are known to contribute to the evolution of obesity, specific mechanisms that could affect weight gain are yet to be identified.

Recent data suggest that gut microbiota may be involved in obesity and fat accumulation. Comparative metagenomic analyses examining the gut microbiome of ob/ob mice have shown that the amplitude of dominant gut bacterial divisions, Bacteroidetes and Firmicutes, changes in obese animals compared to their lean counterparts [2]. Associated changes in the gut microbiome of ob/ob mice affect their ability to harvest energy from dietary fibers [3]. When inoculating germ-free mice with the ob/ob gut microbiota, the recipients accumulated more body fat than the mice that received cecal contents from a wild-type control donor, suggesting that obesity is possibly a transmissible trait [3]. In a human study, obese patients on different weight loss diets experienced a shift in their fecal bacterial profile, comprising increased Bacteroidetes and less Firmicutes; an analogous result to the prediction based on animal data [4].

At the Nestlé Research Center, we have been examining whether gut microbiota is involved in the pathophysiology of insulin resistance and type-2 diabetes. We attempted to answer this question by giving antibiotics to genetic obese and insulin-resistant ob/ob mice. To rule out the potential side effect of antibiotic treatments on food intake of ob/ob mice, we also included a pair-feeding control group. Our results demonstrated that a 2-week intervention with a combination of nor-floxacin and ampicillin in drinking water (1 g/l each) significantly

suppressed the number of total bacteria and enterobacteria in cecal samples of ob/ob mice. The excursion of blood glucose and plasma insulin during oral glucose tolerance tests markedly reduced in the treated mice. The improved insulin sensitivity was independent of food intake or adiposity because the pair-fed ob/ob mice were at least as glucose intolerant as the mice in the control group. Downregulation of hepatic G6P and PGC-1 α mRNA supported the normalization of fasting glycemia in the antibiotic-treated group. In addition, hepatic steatosis of ob/ob mice was also reduced by the same treatment. Reduced expression of lipogenic genes (ACC1 and FAS) and increased expression of fatty acid oxidation genes (ACO and Cyp4a10) in the liver positively correlated with the reduced amount of liver triglycerides, suggesting that treatment significantly changed the hepatic lipid metabolism.

Existing data offer encouraging insight into the interaction between gut microbiota, obesity and type-2 diabetes. However, more evidence is needed to confirm that gut microbiota is a valid target for the treatment or prevention of obesity and type-2 diabetes. With emerging technology to measure and evaluate gut microbiota, we can further explore the diversity and complexity of the gut microbial ecosystem to understand the implications for human health.

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Individual Epigenetic Variation: When, Why, and So What?

Marcus V. Gomes and Robert A. Waterland

Epigenetics is the study of mitotically heritable changes in gene expression that occur without changing the original DNA sequence. Epigenetic mechanisms include methylation of CpG dinucleotides in DNA, autoregulatory DNA-binding proteins, and various modifications to the histone proteins that package DNA in the nucleus. DNA methylation of cytosine residues within CpG dinucleotides is one of the best characterized epigenetic modifications, and has been shown to be influenced by diet in early life.

Just as genetic differences among different people explain individual differences in disease susceptibility, so too can epigenetic differences. We currently have a very poor understanding, however, of the factors that contribute to inter-individual epigenetic variation. Inter-individual variation in methylation has been reported at specific regions in the genome, including at specific transposable elements, genomically imprinted genes and in the 'inactive' X chromosome in females. Sources of epigenetic variation among individuals include environment, genetic and epigenetic inheritance, and random (unexplained) variability. Among environmental influences, nutrition during prenatal and early postnatal development has been shown to affect the establishment of epigenetic regulation at specific gene regions.

We propose that the field of nutrigenomics, which has focused on understanding how inter-individual genetic variation affects nutrient requirements for optimal nutrition, should also consider inter-individual epigenetic variation. Future research will elucidate not only the mechanisms by which nutrition affects the establishment of an individual's epigenotype, but also the ways in which epigenetic differences among individuals might affect the personalized nutritional needs of infants and children.

Interaction of Early Infant Feeding, Heredity and Other Environmental Factors as Determinants in the Development of Allergy and Sensitization

Erkki Savilahti

The role of early infant nutrition in the development of allergic symptoms and allergic sensitization has been disputed for 70 years. How the mother, through the delicate immunomodulatory system of breast milk (BM), contributes to the maturation of her infant's immune system and its regulation and how the infant's early feeding is related to its hereditary predisposition to allergic immune response is mostly unexplored. Interactions between genes and environmental conditions for the development of allergies have been explored since the 1990s and seem to be complicated. The same genotype may lead to either an increased or decreased prevalence of asthma depending on the environmental conditions, such as the high endotoxin concentration met during infancy. The interaction between genetic factors and infant feeding has been limited to studies searching for a relation between parental heredity for allergy and the length of breastfeeding (BF), as well as a few studies on the qualities of BM.

In the 10 original studies comparing the development of allergic symptoms among children, in whom BF duration was used as a separate risk factor among those with either positive or negative parental heredity for atopy, no definite answer could be found. The effect of early feeding was even changed in both heredity-negative and positive groups when looking at symptoms at age 2 and 5 years. In the first study, long BF was a risk for the development of allergic symptoms by age 2 among infants without family history of allergy (FHA), while it did not have any significant association with atopic symptoms among FHA-negative infants [1]. Among the same children at age 5, long BF was associated with an increased risk for atopic symptoms among

those with a positive FHA, while no association was found among those with a negative FHA [2]. When the risk of allergy associated with long vs. short BF was categorized to increased, no change, or decreased, out of 9 possible combinations among children with either positive or negative FHA, 6 combinations were present in the 10 studies and none in more than 2 studies. For sensitization, long BF was a risk in 3 of 5 reports if FHA was positive and in 2 if FHA was negative.

Low IgA food antibodies in BM was a risk for the development of allergies [3]. Conflicting results have been reported with regard to the possible difference in immunologic factors in the BM of allergic and non-allergic mothers [3, 4]. However, BM cytokines were not associated with the development of allergic symptoms or sensitization [3, 4].

The complexities of genetic, environmental and epigenetic influences makes one think that it is not possible for such a simplified association, as looked for in this review, to exist, and as such the above analysis is valid. Gene environmental analysis concerning infant nutrition needs to be much more focused, both in defining the nutritional parameter and the disease endpoint to be studied, and great care must be taken to have a similar environment for the study population in all other aspects.

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Personalized Care of Pediatric Cancer Patients

Karen Rabin, Tsz-Kwong Man and Ching C. Lau

It has always been the goal in oncology to customize therapy for cancer patients in order to optimize long-term survival while minimizing the side effects of therapy. This is particularly important in the treatment of children with cancer because the potential side effects of therapy on the rest of the patient's rapidly growing body could be unacceptable or irreversible. Such personalization of treatment is usually based on an assessment of the aggressiveness of the cancer as well as the potential response of the cancer and the rest of the body to treatment. The former assessment is traditionally based on the extent of the spread of the disease at diagnosis as well as histologic subtypes within the same diagnostic group that are associated with poor outcome. The latter assessment is based on our previous observations of the response of a particular cancer type to standard therapy and the toxicity patients had experienced. However, it has been difficult to predict the response to treatment or the side effects in a particular patient before therapy is initiated.

In this presentation, we will use pediatric acute lymphoblastic leukemia (ALL) and osteosarcoma to highlight the impact of personalized treatment in the clinical outcome of patients and to illustrate how we have begun to augment the risk assessment of cancer patients by including novel molecular markers identified by high throughput genomic technologies. One of the great success stories of clinical oncology over the past several decades is the treatment of pediatric ALL with the cure rates improving from around 10% in the 1960s to nearly 90% today [1]. The primary factor responsible for this remarkable improvement is the personalization of treatment, with stratification of patients based on both disease and host characteristics in order to optimize therapy. While age, WBC, and immunophenotype provide a rudimentary system for categorization of ALL, molecular factors are playing an increasingly important role in further individualization of ALL therapy.

We have much experience customizing therapy for leukemia patients based on risk assessment as described above. However such therapeutic strategies have not been as well developed in the treatment of solid tumors until very recently because of the lack of validated prognostic makers. In the past few years, we and others have tested the feasibility of using comprehensive molecular technologies to identify biomarkers for both diagnostic and prognostic purposes. Using osteosarcoma as an example, we will illustrate how these biomarkers have been developed and validated. One such application is the use of a multi-gene signature to predict the response to chemotherapy at the time of diagnosis prior to the initiation of therapy.

Recently we reported the analysis of 34 pediatric osteosarcoma samples by expression profiling in an attempt to identify a molecular signature that can predict chemoresistance before treatment is initiated [2]. We identified 45 genes that discriminate between good and poor responders to chemotherapy in 20 definitive surgery (post-chemotherapy) samples. A support vector machine classifier was built using these predictor genes and was tested for its ability to classify initial biopsy (pre-chemotherapy) samples. Five of six initial biopsy samples that had corresponding definitive surgery samples in the training set were classified correctly. When this classifier was used to predict eight independent initial biopsy samples, there was 100% accuracy.

In conclusion, as we continue to improve our strategies in personalized care of children with cancer, genomic profiling analysis offers an exciting possibility for refining the diagnosis, stratification and therapy of pediatric cancers. It is reasonable to imagine that in the near future, predictive individualized care based on molecular classification and targeted therapy will become a reality for children with cancer.

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Personalizing Nutrient Intakes of Formula-Fed Infants: Breast Milk as a Model

Bo Lönnerdal

The growth pattern of formula-fed infants is quite different from that of breastfed infants. There may be several reasons for this difference, ranging from different endocrine responses to feeding and the presence of growth factors in breast milk to the different control of food intake, but it is highly likely that differences in nutrient composition of the food (breast milk or formula) have a major effect on growth. Infant formula is in most countries used more or less exclusively up to 6 months of age and as part of the diet up to 12 months of age and during this period its composition remains the same (although some countries also use so-called 'follow-on' formula). In striking contrast, the nutrient composition of breast milk changes during the lactation period, most dramatically during early lactation, but with pronounced differences throughout lactation for many nutrients. The concentration of protein is very high in early lactation and exceeds that of infant formula, then rapidly decreases and becomes considerably lower than in infant formula. The protein composition also changes during this time, with a high concentration of whey proteins early on, but little casein, whereas these two protein classes approach 50:50 during mid lactation. Since whey proteins and caseins provide different bioactivities, this change in protein composition may also have functional consequences for the infant. The lactose concentration, on the other hand, is low in early milk and then increases to reach a more constant level. Oligosaccharides, however, which are believed to provide several physiological benefits to the breastfed infant, are higher in early milk and then decrease in concentration. The concentration of lipids is also low during early lactation, which together with the lower lactose concentration results in a lower caloric content (metabolizable energy) of breast milk during early lactation. Several micronutrients, such as zinc, are also very high in concentration in

early milk and then decrease significantly to be much lower than in infant formula.

It has been stated as a goal that the performance of formula-fed infants should be as similar to that of breastfed infants as possible, and attempts have been made to modify the composition of infant formula to achieve this goal. However, although the concept of 'individualizing' the nutrient intake of premature infants fed their own mothers' milk has been used, there has been no systematic attempt to gradually change the composition of infant formula in a manner similar to the changing pattern of breast milk. This represents a technical and nutritional challenge, but is now possible. Although many bioactive components are unique to breast milk, present dairy technology allows isolation of bovine milk fractions that may at least provide some of the bioactivities of breast milk components. Addition of such components at physiologically relevant concentrations at each developmental period may result in improved performance of formula-fed infants.

Human Milk Oligosaccharides: Evolution, Structures and Bioselectivity as Substrates for Intestinal Bacteria

*Bruce J. German, Samara L. Freeman,
Carlito B. Lebrilla and David A. Mills*

Human milk contains a high concentration of diverse, yet structure-specific soluble oligosaccharides. These unusual molecules are carbohydrate polymers formed with unique linkages by a stereospecific group of mammary glycosyltransferases from a relatively small number of different monosaccharides. To date, this class of molecule, found exclusively in mammalian milks and in unusually high abundance in human milk, has been poorly understood especially with respect to their unique functions in the context of the health of infants consuming breast milk. Novel methods combining liquid chromatography with high-resolution mass spectrometry have identified approximately 200 unique oligosaccharide structures varying from 3 to 22 sugars. These methods were used to develop high-throughput chip-based HPLC mass spectrometry. Now in place commercially, these methods have been applied to examine the structures of oligosaccharides from milks of various mammals and across various human milk samples.

The increasing structural complexity of oligosaccharides in different mammalian milks follows the general pattern of mammalian and primate evolution although the concentration and diversity of these structures in homo sapiens is strikingly more abundant. There is also considerable diversity among different human mothers in the structures of oligosaccharides. Milks from randomly selected mothers contain as few as 23 and as many as 130 different oligosaccharides. The genetic, nutritional or pathogenic basis of this diversity is not yet known, nor are the functional implications of this diversity described, and much less understood. It is not yet known for example whether mothers whose milk contains greater complexity or abundances of oligosaccharides provide their infants with distinct benefits.

Despite the role of milk to serve as a sole nutrient source for mammalian infants, the majority of the oligosaccharides in milk are not digestible by human infants. This apparent paradox raises the obvious questions about the functions of these oligosaccharides and how their diverse molecular structures affect their functions. The nutritional function that is most frequently attributed to milk oligosaccharides is to serve as prebiotics, a form of indigestible carbohydrate that is selectively fermented by desirable gut microflora. This function was tested by purifying human milk oligosaccharides and providing these as the sole carbon source and measuring the growth of various intestinal bacteria in isolated culture. Results confirmed remarkable selectivity for microbial growth attributable to the complex mixture of oligosaccharides pooled from dozens of human milk samples. Among a variety of Bifidobacteria tested, only *Bifidobacteria longum biovar infantis* was able to grow extensively on human milk oligosaccharides as its sole carbon source.

In order to understand the genetic basis of this organism's unusual growth characteristics, its genome was sequenced in its entirety. Analyses of the genomic sequence of this strain revealed approximately 700 genes that are unique to *B. infantis*, including a variety of co-regulated glycosidases, relative to other Bifidobacteria. These results are consistent with a co-evolution of human milk oligosaccharides and the genetic capability of select intestinal bacteria to utilize them. The goal of ongoing research is to assign specific functions to the combined oligosaccharide–bacteria–host interactions that emerged from this evolutionary pressure. The diversity of oligosaccharides in human milks may contribute to directing the diversity of organisms in each human's microbiome during the period of breast-feeding. As this new aspect of human biology is revealed it may be of considerable value to guide the development of bacteria in each individual's intestine as they proceed through weaning and the establishment of their adult and persistent microbiome.

Opportunities for Improving the Health and Nutrition of the Human Infant by Probiotics

Seppo Salminen and Erika Isolauri

The best documented benefits of specific probiotics have been demonstrated in the reduction in the risk of gastrointestinal diseases such as necrotizing enterocolitis, rotavirus diarrhea, antibiotic associated side effects, and the treatment and prevention of atopic diseases. Several intervention studies especially on atopic diseases are under evaluation and currently being published.

The practical benefits of specific probiotics and specific probiotic combinations in infant nutrition may lie in the microbiota modification.

First, modifying the microbiota of the pregnant mother is important. This approach may provide benefits to the microbiota and the wellbeing of the mother during pregnancy by influencing both the composition of intestinal microbiota and its metabolic activity. Bifidobacteria and lactic acid bacteria have been demonstrated to be transferred from the mother to the infant during delivery and breastfeeding. Thus, the balance of a mother's intestinal microbiota and vaginal microbiota may influence the outcome in the infant. Microbiota may also predispose infants to later health problems as has been reported in the case of diarrheal and atopic diseases, and recently for obesity development.

Second, specific probiotics may be important for providing stimuli to the intestinal system during early infancy to assist in development of a healthy gut microbiota and the barrier against harmful microbes and dietary components. Some beneficial effects associated with breastfeeding, such as protection against diarrheal diseases, atopic diseases and even obesity, may be facilitated by the breast milk bacteria and oligosaccharides. Thus, it may be important to correct potential deviations in infant microbiota and to offer formula-fed infants bacterial stimuli in a form of safe probiotic lactic acid bacteria and bifidobacteria. It may be that the probiotic bacteria-supplemented formulae may better mimic the effects provided by breastfeeding.

The most important focus point in probiotic research for infant nutrition is to recognize the individual properties of probiotics. Each strain is different and the properties of each strain and each strain combination are unique. Therefore, the scientific documentation behind probiotics always focuses on specific probiotic strains or specific probiotic combinations.

The healthy human microbiota is metabolically active and acts as a defense mechanisms for our body. Deviations in its composition are related to multiple disease states within the intestine but also beyond the gastrointestinal tract. Components of the human intestinal microbiota or organisms entering the intestine may have both harmful or beneficial effects on human health and clearly the genomic approach on the human infant side and the probiotic side will assist in formulating new approaches to benefit infant health.

The available information focuses mostly on the crucial role of infant microbiota and the first colonization steps to later health. Especially bifidobacteria play a key role in this process. The mother–infant contact has an important impact on initial development. The mother provides the first inoculum at birth, promotes the bifidogenic environment through prebiotic galacto-oligosaccharides in breast milk, and introduces environmental bacteria through her skin and other contact with the infant thus providing the means to promote the guidance to the development of individually optimized microbiota under the existing environmental conditions for each infant.

The future target is to further clarify both the sequelae and the succession of microbial communities especially during breastfeeding and at weaning. Another target is to characterize the use of specific probiotics and prebiotics to influence microbiota development and maintenance as well as dietary management of reported health-related microbiota deviations.

Do We Need Personalized Recommendations for Infants at Risk of Developing Disease?

Olle Hernell and Christina West

Historically, the main objective with nutrition recommendations was to prevent deficiency disorders. Today nutrition recommendations have shifted their main focus from prevention of deficiency disorders to maintaining good health and preventing major chronic diseases, e.g. coronary heart disease, obesity, diabetes, cancer and osteoporosis. Current nutrition recommendations are directed towards populations and are based on estimated nutrient requirements for these populations, to which a margin of safety has been added to generate a recommended intake for energy and each nutrient. Hence, they are intended to meet the needs of most individuals within that population, or subgroup thereof (children, pregnant and lactating women, elderly) regardless of the considerable variation in genetic makeup. For infants with specific genetic polymorphisms, i.e. some inborn errors of metabolism, adherence to current recommendations will cause disease symptoms and they do need personalized nutrition recommendations. Other known genetic polymorphisms, for instance adult lactose intolerance, may vary considerably between ethnic groups and within populations making it necessary to take them into account when recommendations are prepared, although recommendations are generally not personalized. For polygenic diseases such as type-1 diabetes, celiac disease and allergic disease, current knowledge is insufficient to suggest personalized recommendations for all high-risk infants, although it may be justified to provide such recommendations on an individual level based on heredity together with the genotyping currently available for that disease, should the parents ask for them.

Seemingly healthy individuals differ in a variety of single nucleotide polymorphisms. In fact such polymorphisms are normal, and only a minority of them causes disease or may cause symptoms only when a nutrient is consumed in excess.

In the field of gene-diet interactions, nutrigenetics and nutrigenomics are two emerging concepts. The former addresses the importance of genotype (mainly single nucleotide polymorphisms) on the risk of nutritionally related disease. Genetic polymorphisms are identified and studied to see if they modulate the relationships between nutritional exposure and risk. The aim of nutrigenetics is thus to generate recommendations on an individual basis regarding the risk and benefit of specific dietary components. Nutrigenomics addresses the inverse relationship. It focuses on the effect of food-borne components on gene transcription, proteomics and metabolism. Thus, these new technologies collectively aim to identify the genetic variation accounting for why some individuals react differently to dietary components than others. The question is if such individual differences should impact on dietary recommendations to the extent that they become individualized for each genetic makeup? The fact that nutrients may have more than one function makes personalized nutrition recommendations even more problematic. A recommendation that may be beneficial with respect to one function may be harmful with respect to another.

Be that as it may, these technical developments are promising tools with which current recommendations can possibly be refined to meet individual requirements and the potential of individualized nutrition be explored. It seems likely that in the future it will be technically possible to offer personalized recommendations to more subgroups within a population. Questions that remain to be solved are: who will pay and who will provide such recommendations.

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