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Feeding during Late Infancy and Early Childhood: Impact on Health

Olle Hernell
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Preface

Three years ago, in May 2001, the World Health Assembly came to the conclusion that it was safe for the majority of infants to be exclusively breastfed for the first 6 months of life, and extending the period of exclusive breastfeeding to that age would also be beneficial. This conclusion was translated into a recommendation of exclusive breastfeeding for 6 months, and while complementary feeding of high-quality should be introduced at that age, breastfeeding should preferentially be continued beyond the second year of life. Although some beneficial effects, particularly the prevention of infections, will be more pronounced in low-income than in high-income societies, other impacts on mental development and immune functions, for example, are likely to be important also in high-income societies. It is therefore clear that the new recommendations will affect the infant feeding mode on a global level. Moreover, during the last decades it has become evident that infant nutrition has developmental and health effects which may last beyond infancy, even into adulthood.

Although, there is a consensus that breastfeeding is the optimal nutrition for most infants during the first 6 months of life, there is less agreement on the optimal feeding practices during the second half of infancy and early childhood. For these reasons it was felt timely to review current practices in complementary feeding, particularly with respect to similarities and differences within countries in Europe. What are these practices based on? Is there any evidence that different practices are related to differences in long-term outcome? More importantly, what is the scientific foundation on which current recommendations are based? Has recent understanding of the possible long-term consequences of early nutrition had an impact on the recommendations? Those were questions that were brought up prior to the workshop. The organizers strongly felt that let alone in early infancy there are still many more 'beliefs' than 'knowns', or simply tradition, rather than physiological or other scientific reasons behind current feeding practices.

It was therefore thought that an alternative and more realistic way to approach this problem and offer guidance to pediatricians was to review what do we know about the major health problems related to the introduction of

new solid foods in a child between 6 months and 3 years of age, and what could be done to avoid these problems.

A few major health problems were pinpointed. It was estimated that food allergy and celiac disease, constipation, chronic diarrhea, and the long-term consequences of weaning habits were relatively frequent and often serious enough reasons for parents to seek professional advice, and that each problem warranted special attention. Hence, the workshop was devoted to these topics, and each topic was covered by three or four invited speakers. Typically the first lecture set the physiological or psychosocial ground for the two following lectures dealing with important clinical aspects of the particular thematic topic. Recognized specialists were gathered and shared their up-to-date knowledge with a concerned audience.

The last session was meant to extract from the current knowledge, as presented by the various lecturers, possible guidelines for an 'optimal' nutrition for children from 6 months to 3 years. In the course of this difficult exercise it appeared clearly that such a valid goal was difficult to reach because of important obstacles, some of which are: (1) the poor interest in general of pediatricians in preventive health care and particularly in nutrition; (2) the often inappropriate way claims and guidelines are expressed by professionals which explains why they are not understood by parents and thus of low efficiency, and (3) the difficulty of the industry to substantiate claims on strong scientific evidence.

In concluding the Workshop, the participants unanimously expressed their wish that these difficulties be overcome by in-depth actions to promote nutrition in the pediatric curriculum, in favor of improving communication between professionals and parents, and finally by gathering the scientific knowledge that in many aspects is still missing on the impact on health of the mode of feeding normal young children.

O. Hernell and J. Schmitz

Foreword

For this 56th Nestlé Pediatric Nutrition Workshop, which took place in November 2004 in Noordwijk, The Netherlands, the topic 'Feeding during Late Infancy and Early Childhood: Impact on Health' was chosen. Moreover, it is the first time that a Nestlé Nutrition Workshop has been organized in the Netherlands, a market that recently opened to the infant nutrition business.

In its resolution WHA54.2 (2001) the WHO recommends the promotion and support of exclusive breastfeeding for 6 months, and then the provision of safe and appropriate complementary foods whilst continuing breastfeeding until 2 years of age or beyond.

This resolution naturally challenges most of the prior feeding recommendations and probably also parental habits; moreover, all recently published studies demonstrate that most infants receive their first complementary solid food before 6 months of age. On the other hand, until recently it was strongly recommended to not introduce complementary food containing gluten before the age of 6 months; this has also been challenged by data showing that a too late introduction may be problematic in terms of gluten tolerance.

It must be acknowledged that this topic of feeding during late infancy has not been investigated as systematically as that concerning feeding during the 6 first months of life; therefore, a lot of work remains to be done.

Since 1984 (Nestlé Nutrition Workshop 10: Infant Nutrition), these aspects relating to the introduction of weaning foods (baby food, cereals, etc.) have not been reviewed systematically during a Nestlé Nutrition Workshop.

This 56th Nestlé Nutrition Workshop has been specifically developed to review the medical and scientific aspects of these topics and to sustain the Nestlé Development Nutrition Program (NDNP).

I would like to thank the two chairmen, Prof. *Olle Hernell* and Prof. *Jacques Schmitz,* who are recognized experts in this field, for putting the program together and inviting the opinion leaders in these fields as speakers.

I would also like to thank Mrs. *Marjan Skotnicki-Hoogland,* Mrs. *Mieke Beemsterboer* and their team from Nestlé Nederland, who provided all logistic support, enabling the participants to enjoy the Dutch hospitality.

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The Role of Immune Tolerance in Allergy Prevention

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Introduction

Immune tolerance is an essential mechanism which maintains a state of unresponsiveness to autoantigens and food while generating protective immunity against pathogens. This phenomenon was discovered by the fact that exposure to an antigen before the development of an immune response specifically abrogates the capacity to respond to that antigen in later life [1, 2]. Tolerance-inducing strategies have been demonstrated in animal models of autoimmunity, allergy and transplant graft rejection and therefore have opened the way for testing such approaches in human diseases. Immune tolerance can be established by respiratory or oral exposure to the allergen.

Processes that regulate peripheral tolerance involve clonal anergy, clonal deletion or active suppression by regulatory cells (fig. 1). Inhibition of T-cell costimulatory molecules at the cell surface has been reported to play an important role in T-cell tolerance [3–5]. The T cells could be anergized in experimental models that bypass costimulatory signals [6–8]. The interaction between B7 and CD28 may determine whether a T-cell response develops. For example, blocking antibodies to B7–2 inhibit the development of specific IgE and allergic symptoms in mice [9].

Regulatory T Cells

Over the last years, a great deal of interest has focused on regulatory T (Treg) cells that appear to control the development of autoimmune disease, transplant rejection and play a central role in controlling the expression of asthma and allergy.

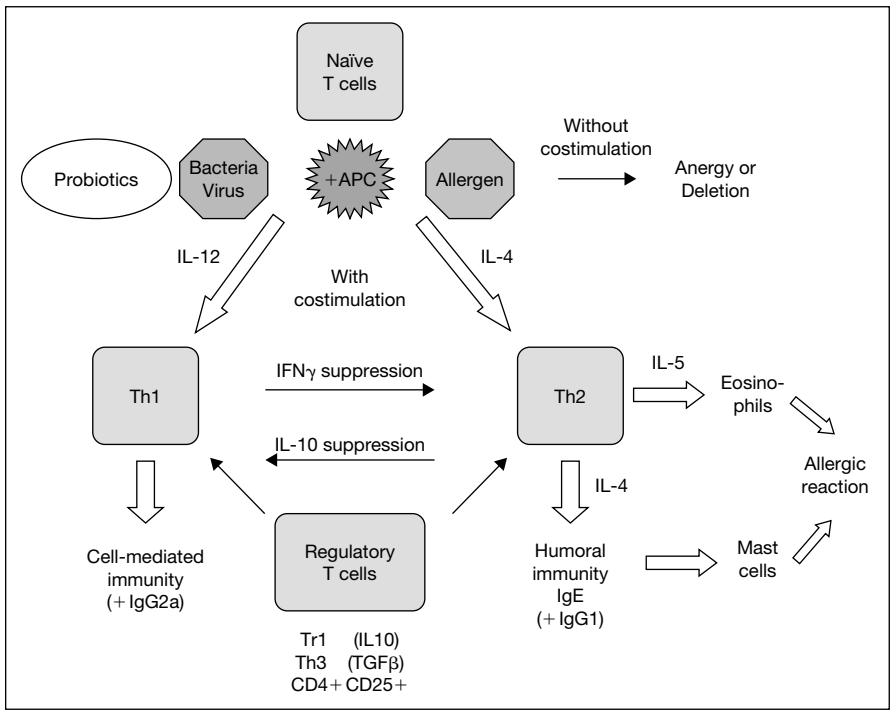

Fig. 1. Induction of tolerance through either lack of costimulation (leading to anergy or deletion) or by the suppressive action of T-regulatory cells.

Animal experiments have shown that Treg cells can suppress both Th1 and Th2 responses in vivo. In vitro engineered allergen-specific Treg cell lines protected mice from developing allergen-induced Th2 responses [10]. In humans it has been speculated that Treg cells secreting IL-10 are involved in the suppression of allergic Th2 responses. Several clinical studies supported this hypothesis [11–13] and others not [14].

Different types of Treg have been described which suppress immune responses via cell-to-cell interactions and/or the production of suppressor cytokines.

Tr1 cells were described [15] following in vitro activation of naïve CD4+ T cells in the presence of IL-10 which gave rise to CD4+ T cells with low proliferative capacity that produced high levels of IL-10, low levels of IL-2 and no IL-4. Such antigen-specific T cells suppressed the proliferation of CD4+ T cells in response to antigen.

Th3 cells were induced by oral feeding of low doses of antigen in a T-cell receptor-transgenic experimental encephalitis model [16, 17]. The CD4+ T cells isolated from mesenteric lymph nodes in such orally tolerized animals

produced high levels of transforming growth factor (TGF)-β, and variable amounts of IL-4 and IL-10 upon activation with the antigen. TGF-β and IL-10 are critical as treatment with neutralizing antibodies abrogated the disease-protective effects of these cells.

CD4+CD25+ Treg cells were described by Sakaguchi et al. [18] as a subfraction of CD4+ T cells which play a critical role in the prevention of autoimmunity, allograft rejection and maintenance of self-tolerance. Elimination of CD4+CD25+ T cells leads to spontaneous development of various autoimmune diseases, such as gastritis, or thyroiditis in genetically susceptible hosts. These regulatory cells suppress immune responses through direct cell–cell contact in a process that is dependent on signalling via CTLA-4, as well as secretion of TGF-β. Several hypotheses exist on the origin of these regulatory cells. It was proposed that thymic differentiation accounts for CD4+CD25+ T cells that are specific for self-peptides and are devoted to the control of autoimmune responses, whereas peripheral differentiation may be required for environmental antigen-specific T cells for which an undesired immune response results in pathology [19].

Other regulatory cells have been reported like CD8+CD25+, which may play a role in oral tolerance [20, 21], or γδT cells and also regulatory dendritic cells.

Oral Tolerance

Oral administration of protein antigens induces immunologic hyporesponsiveness (tolerance) to these antigens. Induction of oral tolerance has been well documented with a number of antigens in several animal models. It was first reported by Wells and Osborne [22] with guinea pigs. They noted that anaphylactic reactions to ovalbumin (OVA) could be inhibited by prior oral administration of OVA to these animals.

Immune regulation by the induction of oral tolerance to food antigens is thought to prevent food allergy [23].It has been shown that induction of oral tolerance is dependent on the age of the host [24], the dose of antigen administered, the nature of the antigen and microbial environment. This last point has been the subject of a number of hypotheses and experimental work in the last years. The interrelationship between microbes and the induction of allergy or oral tolerance involves the commensal bacteria that colonize the gastrointestinal tract. Microbial stimulation seems to provide counter-regulatory signals necessary to overcome the inherent Th2 bias of the mucosa-associated lymphoid tissue to prevent allergic disease [25]. It was reported that administration of a food allergen with a mucosal adjuvant induces allergen-specific IgE and anaphylactic symptoms in strains of mice lacking a functional receptor for bacterial lipopolysaccharide (TLR-4) but not in major histocompatibility complex-matched controls.

The importance of age has been highlighted in several studies. In neonatal rodents, oral exposure to antigen was reported to induce tolerance or priming depending on the age at the first antigen administration [26]. Starting from 7 days of age, oral administration of antigen leads to induction of oral tolerance. In a rat model we showed that oral antigen administration by the age of 14 days leads to the strongest downregulation of specific IgE responses on subsequent challenge with the antigen.

The underlying immunologic mechanisms involved in oral tolerance induction have not been fully elucidated, but recent studies suggest that antigen-presenting cells like intestinal epithelial cells and dendritic cells as well as the above-cited regulatory cells play a central role. Dendritic cells residing within the lamina propria and Peyer's patches express IL-10 and IL-4, which favor the generation of tolerance. It has been suggested that T cells primed in the local mucosal environment lead to tolerance induction. Studies in mice suggest that tolerance can be induced by one of several mechanisms: low-dose tolerance (repeated administration of protein antigens at low doses) which results from the activation of Treg cells that secrete inhibitory cytokines (e.g. TGF-β or IL-10), or high-dose tolerance (administration of a single high dose of protein antigen), which results from either clonal anergy or clonal deletion [27]. Experiments in mice have also recently shown that CD4+CD25+ T cells may play an important role in oral tolerance induction [28–31].

The phenomenon of oral tolerance has been well characterized in selected mouse strains but there are significant strain differences in terms of the ability to induce oral tolerance [32]. Oral tolerance to food antigens was also shown in several rat models [33, 34]. In humans, experimental oral tolerance induction was also attempted [35] by feeding volunteers keyhole limpet hemocyanin. It was observed that tolerance was induced at the T-cell compartment (reduction of T-cell proliferation and delayed-type hypersensitivity responses) but not at the humoral level. Recently published work [27], also using human volunteers fed keyhole limpet hemocyanin, showed that oral administration does not result in tolerance in Crohn's disease or ulcerative colitis patients on the contrary to normal controls. This may reflect an in vivo functional defect in mucosal suppression of immune responses in these patients.

Dietary Intervention for Induction of Oral Tolerance

Importance of Antigen Structure

The majority of tolerogens are soluble proteins. Larger particulate antigens, aggregated or heat-treated soluble proteins, lose their capacity to induce oral tolerance. Although oral tolerance to dietary proteins has been extensively investigated with intact antigens, few studies with antigen

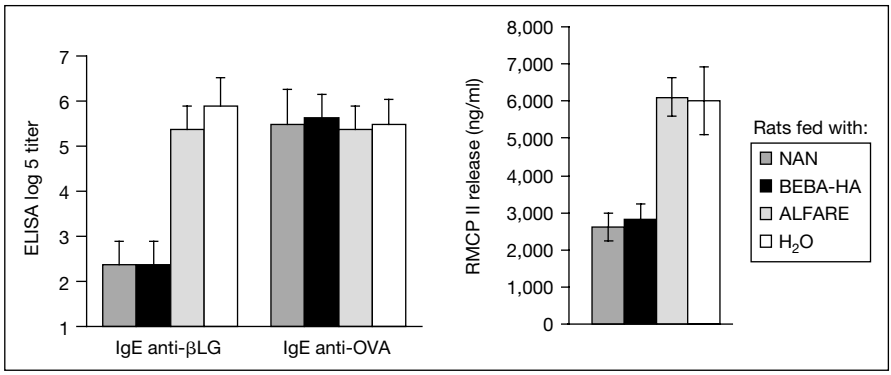

Fig. 2. Suppression of IgE and intestinal mast cell responses following oral tolerance induction with cow's milk formulas. Protocol: Groups of rats were given different experimental liquid milk formulas or water (control) ad libitum in their drinking bottles and a solid 'milk-free' pellet diet from days 1 to 19 of the experiment. Animals were given the following products: group A, NAN (standard formula containing intact cow's milk proteins); group B, BEBA-HA (moderately hydrolyzed whey-based formula); group C, ALFARE (extensively hydrolyzed whey formula); group D, water. All formulas were administered at a concentration of 120 g/l. All rats were immunized on day 5 by subcutaneous injection of 0.1 mg β-lactoglobulin (βLG) + 0.1 mg ovalbumin (OVA) in 0.05 ml 0.15 *M* NaCl mixed with 0.2 ml 3% Al(OH)3. Fourteen days later all animals were challenged orally with a whey protein concentrate (1 g/2 ml) and killed 2 h later. Blood was drawn and sera kept frozen at –80°C. Results: Specific IgE (anti-βLG) and intestinal rat mast cell protease (RMCP II) were suppressed in the sera of rats fed with NAN or BEBA HA but not in the sera of animals fed ALFARE or in control animals given water.

fragments or digests have been done. We have shown [34] that moderately hydrolyzed whey proteins are able to induce oral tolerance to intact whey proteins, whereas extensively hydrolyzed proteins are unable to achieve this (fig. 2). This was confirmed in a recent publication [36]. Up to now the mechanisms of oral tolerance induction with hydrolysates are not very clear, but medium-sized peptides appear to play a central role. So-called 'tolerogenic peptides' have been described in the literature to occur in the serum after feeding OVA to animals. A number of clinical studies have shown that for primary prevention of atopy in infants with a positive family history, partially hydrolyzed infant formulas are found useful to avoid cow's milk allergy and atopic symptoms [37, 38]. Long-term prevention has been observed in these studies, which may be due to induction of long-lasting oral tolerance with such formulas.

Intervention during Pregnancy

Animal models have shown that active induction of tolerance to dietary antigens before birth, via nutrition of the pregnant mother, is an effective

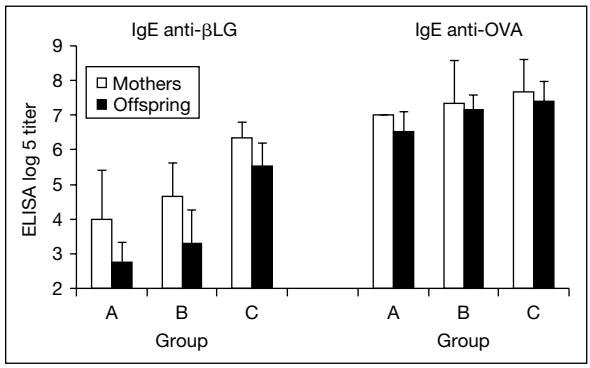

Fig. 3. In utero induction of oral tolerance in rats with intact or hydrolyzed whey proteins. Protocol: Throughout pregnancy adult Sprague-Dawley rats were given intact whey proteins (group A), trypsin hydrolyzed whey proteins (group B) or water (group C) in drinking bottles in addition to conventional chow. Four weeks after birth of the offspring, rats were immunized subcutaneously with β-lactoglobulin (βLG), ovalbumin (OVA) and Al(OH)3 as adjuvant. Fourteen days later, rats were sacrificed and serum analyzed for specific IgE. Results: The offspring of mothers fed whey (group A) or a whey hydrolysate (group B) during pregnancy had a strongly suppressed IgE anti-βLG antibody response if compared to controls (group C).

means of primary prevention in the offspring. Several authors [39–41] demonstrated tolerance induction to soya proteins, bovine serum albumin or cow's milk proteins in the offspring of rabbits and guinea pigs respectively fed these different dietary proteins. We have shown in recent experiments done in a rat model of IgE suppression that oral tolerance to cow's milk proteins can be transferred from the mother to the offspring and that this phenomenon can also be achieved with cow's milk protein hydrolysates [42]. In these experiments it was interesting to observe that the downregulation of the IgE response was antigen-specific and that cow's milk protein peptides were as efficient as intact cow's milk proteins for inducing oral tolerance in the offspring by feeding mothers during pregnancy (fig. 3).

Intervention during Breastfeeding

Breast milk contains a number of factors which may promote the development of the infant's immune system. Low levels of food allergens in breast milk, arising from the mother's diet, may also play an important role in the induction of oral tolerance of the infant. Further, recent work has shown that the quality of fatty acids ingested by the mother may have effects on the development of immunological tolerance to dietary antigens in the offspring. It was reported that Sprague-Dawley rats fed a diet deficient in essential fatty acids, rather than the one enriched with essential fatty acids, favored the induction of oral tolerance in neonatal rats via their mothers [43]. Similar

further experiments showed that the dietary ratio of n-6 to n-3 fatty acids influences the induction of tolerance to OVA in neonatal rats [44].

To date in humans, it has not been clearly demonstrated if an allergen-reduced diet by lactating mothers has a long-term protective effect on the occurrence of atopic symptoms in infants despite observations that avoidance of milk and eggs during lactation may benefit some breastfed high-risk infants with eczema.

Importance of Gut Flora

The effect of the gastrointestinal microflora on the induction and maintenance of oral tolerance to dietary antigens has been studied in several animal models with contrasting results. Oral administration of OVA was able to induce oral tolerance in axenic (germfree) mice, but the maintenance of tolerance was found to be of shorter duration than with conventional mice [45]. On the contrary, in other work [46], the intestinal bacterial flora was shown to be required for the development of an IgE-production system susceptible to oral tolerance induction. It also appears that the contribution of the microflora to oral tolerance depends on the antigen used [47]. It is further well known that bacterial endotoxins (e.g. cholera toxin) may abrogate oral tolerance to an antigen co-administered through the oral route. A recent study shows further that, in mice, infection with *Helicobacter felis* can prevent the development of oral tolerance to OVA. These results indicate that chronic infection with *Helicobacter* inhibits the establishment of oral tolerance by preventing IgE suppression, normally induced after OVA feeding [48].

In humans, it was observed that in comparison with healthy infants, babies who developed allergies were less often colonized with enterococci during the first month of life and with bifidobacteria during the first year of life. It was therefore proposed that early colonization may affect the development of mucosal tolerance, as perinatal administration of probiotics to infants at risk of allergy induced a reduction in eczematous symptoms later on [49].

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Discussion

Dr. Kaminogawa: My first question is, why does microflora increase the induction of oral tolerance? My second question is, is it useful to increase the induction of oral tolerance with probiotics or not? Thirdly, I don't think that the regulatory T cell is so important in the induction of oral tolerance; I have some evidence, but please give me your opinion.

Dr. Fritsché: To the first question, I think that the flora is really important but the proof for this is not complete because there are contradictory results from animal models. It was shown that, in germ-free animals, the flora does not appear to be important for oral tolerance induction, only for its maintenance [1]. On the contrary, other work has shown that the intestinal bacterial flora is essential for the induction of oral tolerance [2]. So the question is open here, but I believe strongly that flora might be important for at least the maintenance of oral tolerance. The other question was on the effect of probiotics?

Dr. Kaminogawa: Yes, are probiotics important for the induction of oral tolerance or not? What do you think?

Dr. Fritsché: The experiments by different groups on probiotics have shown that one might have allergy prevention by giving the mothers different probiotic strains either during late pregnancy or early infancy. This study was done by Isolauri's group, and clearly there is some downregulation of eczematic symptoms in the groups receiving probiotics. So it appears important. Is this downregulation due to the induction of oral tolerance? We don't know. It may be another phenomenon, it may be an immune deviation directly without going through anergy, deletion or T-regulatory cells. This has not been clarified to date. Please remind me of your third question.

Dr. Kaminogawa: You said that regulatory T cells are most important in the induction of oral tolerance, but I don't think so. You mentioned publications on the importance of the CD4+CD25+ T cells, but we cannot repeat these experiments.

Dr. Fritsché: Published work exists in animal models [3] where the authors depleted the CD4+CD25+ cells and they could not induce anymore tolerance. In the human situation as well, there is upregulation of CD4+CD25+ cells in infants being orally tolerized to cow's milk proteins by the age of 2–3 years [4]. So there is indirect proof that this population of cells is very important.

Dr. M. Hoekstra: I think the last study you referred to was recently published by a group from Norway in the *Journal of Experimental Medicine* [4]. They showed that the children who outgrew their cow's milk allergy had an increased number of regulatory T cells.

Dr. Heymans: In the past decades clinical evidence has shown that partial hydrolysates have a comparable preventive effect for food allergy to that of breast milk. Now we are using cow's milk as a whole protein with no physiological background. I think the only reason we do it is because we have a lot of cows, especially in this country, even more than inhabitants, and they produce enormous amounts of milk. So why shouldn't we use a partial hydrolysate in a normal formula? It has beneficial effects as we have shown; it has the same nutritional effects [5]. So why we shouldn't do it?

Dr. Fritsché: My personal view is that we should use it. There is no disadvantage using partially hydrolyzed formulas for so-called normal infants because one can never be sure that these infants really are normal, because the only history markers are the parents or siblings who are atopic or not. By restricting the administration of partially hydrolyzed formulas to at-risk groups of infants, one may end up missing a lot of indications because it has been shown that normal infants, representing only 15% at risk of atopy, represent the majority of cases who later on contract atopies and allergies.

Dr. Heymans: So let's ask Dr. Hernell his opinion about this because he is involved in these regulations.

Dr. Hernell: One first question is, what is a partial hydrolysate?

Dr. Fritsché: Unfortunately I don't have the slides to show you exactly what it is. It is a cow's milk whey-based formula, 100% whey proteins.

Dr. Hernell: I know what it is prepared from, but how can you define a partial hydrolysate in a useful way?

Dr. Fritsché: We have different definitions. In Europe one of the definitions is that its antigen-active capacity should be reduced by a factor of 100, at least according to the European Commission directives. So you have on one hand this allergenicity reduction, and on the other hand we have proven that it should also induce oral tolerance to cow's milk proteins. I think there are two parameters here which very strongly narrow the room of activity of such formulas. This is based on in vitro and animal studies, but in the human situation they should really prevent cow's milk allergy. It is clear, you have to do human studies to show their efficacy.

Dr. Hernell: There are some long-term clinical studies on allergy prevention, but I don't think they are very convincing with respect to partial hydrolysates. Again I don't think you can really use an in vitro definition based on molecular size or a reduction in the number of epitopes to classify a partial hydrolysate. It is really difficult to know what is meant when you talk about the partial hydrolysate. My second question refers to your statement that with an extensive hydrolysate oral tolerance doesn't develop. What happens after intact food protein has been introduced? I think that is what is important, rather than what is found during the experimental period.

Dr. Fritsché: This is exactly the reason why I recommend partial hydrolysates, at least something is being done to the immune system. With extensively hydrolyzed formulas nothing at all is being done; the immune system cannot be modulated with extensive hydrolyzed formulas. So when intact formulas are reintroduced later on there is a great chance that the immune system has not evolved very much, which may result in allergic sensitization to intact proteins rather than tolerization.

Dr. Hernell: I think that is an interesting hypothesis but has it actually been proven?

Dr. Fritsché: No, it has not been proven. We are presently doing a comparative study in infants between extensively and partially hydrolyzed formulas on an exclusive basis, without breastfeeding. This should give us some clues for indicating one or the other formula.

Dr. Hernell: What are the outcome variables in that study?

Dr. Fritsché: The usual ones: SCORAD, double-blind placebo-controlled challenge.

Dr. M. Hoekstra: But also parameters of the immune system?

Dr. Fritsché: Yes, absolutely, everything we can do.

Dr. M. Hoekstra: When you refer to the immune system, do you mean IgE or other immune parameters?

Dr. Fritsché: We are doing IgE, IgG1, IgG4, and we are doing cytokine of lymphocyte stimulation profiles.

Dr. M. Hoekstra: And looking at regulatory T cells as well?

Dr. Fritsché: No, not in this study. It is pretty difficult to get enough blood from babies at this young age.

Dr. Aggett: I share Dr. Hernell's reaction to the need to know what the partially hydrolyzed formula actually is. In terms of your studies, the thing that it is lacking, and is going to be lacking for many subsequent studies, is actually knowing what should be given to the babies. It would be very important to have some feeling to being able to characterize the proteins, not just grossly but also knowing what epitopes are present because what it would be fascinating to know what is inducing oral tolerance. Is it an intact antigen or is it a partial epitope? It is an epitope that is actually released so it has a greater opportunity to interact with whatever sensitizers are present. Do you have any feeling about these aspects?

Dr. Fritsché: We did a lot of work trying to identify the epitopes linked to, for example, tryptic peptides of β-lactoglobulin. We have not succeeded in this task because it is perhaps a multi-epitope here, but we have purified some candidates which could be associated with middle molecular weight peptides. We know that

intact traces of β-lactoglobulin of cow's milk proteins are not present in partially hydrolyzed formulas and, if you remember a picture I showed you, if you try to induce oral tolerance in animal models with intact proteins and partially hydrolyzed formulas, you have the same success of downregulation of the IgE response at all different doses. It means that if there were some contamination of intact proteins in partially hydrolyzed formulas then the low dose would not induce anymore tolerance compared to intact formulations, but there must be protein peptides associated with inducing tolerance. But as I said we are not at the end of this process yet.

Dr. Siafakas: It is a common practice to give corticosteroids to premature babies for lung maturity. I wonder if there are any animal or human studies with regard to the prenatal administration of corticosteroids regarding the impact that this on oral immunity postnatally?

Dr. Fritsché: I am sorry I am not aware of such studies.

Dr. Exl-Preysch: I would like to come back to the question of eHF and pHF in allergy prevention. The GINI study could perhaps give us some hints concerning the induction of oral tolerance [5, 6]. First of all, the comparison of two eHF with each other gave the result that one (eHF-casein) was effective and the other (eHF-whey) was not effective at all. Therefore the still existing dogma that 'eHF is better than pHF' has to fall, because pHF was as good as the effective eHF-casein after 3 years and the other eHF not at all.

In addition, the sensitization dates after 1 year showed clearly that the pHF had the lowest levels of sensitization against any allergens they looked into (cow's milk protein, egg allergens, 5 food allergens, 5 aero-allergens). Those results indicated for the first time what we have been finding in animal models for years: pHF induces oral tolerance at a higher level than eHF! That is what we are searching for in allergy prevention!

I would also like to draw your attention to the fact that years ago we conducted a study in Switzerland on a regular newborn population that was either fed allergen-reduced (breastfeeding and/or HE = pHF) formula or regularly fed (breastfeeding and/or regular infant formula) [7–9]. Even after 2 years the population fed the allergen-reduced formula had only half of the skin symptoms than the regularly fed infant population. Again, a reason why we should feed all non- or partially breastfed infants an allergy-reduced pHF formula!

Dr. Hernell: You mentioned that there is a critical window during which it is easier to induce oral tolerance which occurs around day 14 if I remember correctly, I believe that has also been shown in mice, but how much is really known about if there is a critical window in humans?

Dr. Fritsché: In humans I think there are no data showing this.

Dr. M. Hoekstra: It is speculated that it would be the first 18 or 24 months of life. Compared to a rat life of 2 years, the equivalent of 14 days in a rat's life would be 18 months in humans. But I think there is no more evidence than that.

Dr. Bueno: I want to come back to the question related to the role of T cells, particularly in oral tolerance, and you mentioned the change in the Th3 profile. What is your opinion about the fact that some specific cytokines like TGF-β could be interesting surrogate markers for oral tolerance?

Dr. Fritsché: Absolutely, the cytokines secreted by Th1 and Th3 are very important, TGF-β and IL-10. I think there are a number of animal studies which have shown the importance of IL-10 at least in modulating the immune system for downregulation, that is clear, they are mandatory. About the CD4+CD25+ cells, people actually think that it is a cell-to-cell contact but nobody knows if they do not also secrete cytokines which may also be very important.

Dr. Sinaasappel: In your scheme I missed the possible role of dentritic cells in the intestine. I was wondering how you imagine that the antigens are exposed to the body?

Dr. Fritsché: I think dendritic cells are in the center of the actual research activities in this domain because they have the capacity to leave the mucosa and fish the antigens through the mucosa layer. They have been categorized into different classes of dendritic cells, those inducing Th1 and Th2, so activity is strongly focused on this class of cells.

Dr. M. Hoekstra: In your rat experiments comparing partial and extensive hydrolysates, did you look at the intestinal inflammatory response in these two groups?

Dr. Fritsché: Yes, we looked at the triggering effect on intestinal mast cells because here you have sensitized mast cells lining the mucosa which are or are not covered by IgE.

Dr. M. Hoekstra: But you didn't look at antigen-presenting cells or T-cell infiltrate?

Dr. Fritsché: Not in these old experiments.

Dr. M. Hoekstra: We would really like to know something about the mechanism. Why it does work in partial but not in extensive hydrolysates?

Dr. Schmitz: I would like to ask two questions. One is related to what you said about the partial hydrolysate having a kind of tolerance-inducing ability. How would you reconcile this with the fact that it has been well shown in clinical practice that sometimes partial hydrolysates have raised acute allergic reactions when refeeding the child with normal cow's milk? The second question relates to what you said about quantity, nature and time of feeding of antigens. What makes the difference between casein, for example, or β-lactoglobulin to which allergy will decrease after 18 months or 2 years usually, and ovalbumin to which allergy will last for years and years? Have we a clue to the reason why in one case it tends to disappear and in the other case it does not?

Dr. Fritsché: I think no animal model for the moment has answered this question but it is observed in the human situation that sensitization to eggs is very early and it lasts longer; for peanuts it is very strong and it never goes away, so I don't think I can answer your question. Now on the refeeding of hydrolyzed formulas, do you mean that these were infants who were prevented with hydrolyzed formulas only or mixed with breastfeeding?

Dr. Schmitz: If I remember correctly, it was in children fed only with partially hydrolyzed formula.

Dr. Fritsché: But perhaps the formulas were used not in normal or at-risk infants but in already sensitized infants; because we always advise not to give partially hydrolyzed formulas to already sensitized infants.

Dr. Heymans: There are publications on children with proven cow's milk allergy [11–13]. It is stated that it can't be used it as treatment, it can only be used in prevention.

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Prevention of Food Allergy during Late Infancy and Early Childhood

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Introduction

The normal immunological response to food antigens is geared to the induction of tolerance, that is to say, unresponsiveness in the case of their further ingestion. Allergic sensitization may be considered as a failure or a breaking of immunological tolerance. It is becoming clearer and clearer that the development of oral tolerance is highly dependent on the intestinal microflora; indeed the intestinal bacteria have the ability to induce the formation of cytokines of Th1 immunity (particularly of INFγ) and also IL-10 and IL-12, which counteract the Th2-dependent allergic sensitization and favor the state of Th1/Th2 equilibrium that prevails later in life in normal children [1].

Since colonization of the digestive tract takes place in the first few months of life, it can be postulated that a 'window of sensitization' exists in early life [2]. In fact, infants are particularly prone to develop allergic sensitization to food antigens and this propensity usually does not extend beyond early childhood.

Epidemiological data show that the prevalence of allergic conditions continues to increase, at least in the developed world [3]. It is difficult to imagine how genetic factors, the most important risk factors for developing allergic diseases, could be responsible for such a trend. Among the environmental factors that could explain this rise in prevalence, two seem particularly worthwhile to consider because they may lead to prevention: (1) the increased exposure to (new) environmental/oral sensitizing agents that may be fought by exclusion diets, and (2) the decreased bacterial load resulting from the increased practice of cesarean delivery, the widespread use of sterile food, and the frequent prescription of oral antibiotics during infancy which may favor the Th2 allergic immunological reaction. This increased prevalence of allergic conditioning might be counteracted by the use of probiotics which would restore the normal Th1/Th2 equilibrium [4].

The present review will try to answer the following three questions: (1) is the 'sensitization window' still open during the weaning period, at the time of introduction of solid foods; (2) is it still possible to prevent/reduce food allergy by exclusion diets during the weaning period, and (3) is it possible to prevent/reduce food allergy by using probiotics in the first months of life?

Introduction of Solids as a Factor of Food Allergy

From a vast observational prospective study in New Zealand concerning 1,123 nonselected children, it was clearly apparent that, although the occurrence of atopic dermatitis at 2 years of age was mainly linked to a familial history of atopy, the second risk factor was the early introduction, before 4 months of age, of foods other than milk which increased by 50% (17.8% as opposed to 12.6%; $p < 0.05$) the risk observed in infants who received solids before the age of 4 months, despite breastfeeding, compared to the risk in children who received solids later [5]. Furthermore, in this group of children a significant correlation was observed between the frequency of atopic dermatitis and the number (but not the type or quantity) of solid foods ('beikost') introduced before the age of 4 months [5]. Ten years later, the sensitizing effect of this early introduction in the same cohort of children was confirmed: the risk of developing atopic dermatitis was 2.9 times greater in the group of children who had ingested more than 4 different solids compared to those who had taken none [6]. On the contrary the age at introduction and the number of new solid foods had no influence on the later occurrence of asthma [7].

The same effect was not observed in a smaller intervention study conducted in Sweden in 375 nonselected children. In 177 of them at 3 years of age, the avoidance of fish and citrus fruit during the first year of life did not modify the incidence of allergic signs and symptoms (skin tests, vomiting) triggered by their ingestion compared with the incidence observed in a control group of 198 children who were freely allowed to eat fish and citrus fruit during their first year of life [8]. However, in this study the age at first encounter of the allergen was not controlled and could have been rather late.

The sensitizing effect of 'beikost' was strikingly demonstrated in a group of 135 Swedish children from 'at-risk' families with a history of atopy, breastfed until the age of 6 months (or receiving an extensively hydrolyzed formula in case of failure of breastfeeding) in whom 65 of them were allowed solid foods (potatoes, carrots, meat, cereals, eggs, fish, fruits) from the age of 3 months compared to 70 infants who were exclusively breastfed until 6 months of age. At 1 year, the frequency of atopic dermatitis was half in the second group (14%) compared to the first one (35%; $p < 0.01$); all children receiving the same diet during their second semester [9]. Similarly food allergy, defined as a skin rash or vomiting after ingestion of a food item, was five times more frequent (37%) in the group of children receiving solid foods early (after the

age of 3 months), compared to those receiving solid foods after the age of 6 months (7%; $p < 0.001$). The foods most frequently responsible for sensitization were eggs, cow's milk, fish, strawberries, tomatoes and citrus fruit. Challenges with these foods 1 month after the initial reaction were still more frequent (but not significantly) in the early than in the late diversification group; 2–3 months later the difference disappeared [9]. The latter finding was interpreted as indicating a nonspecific and transient sensitization in the case of early introduction of solids in the infant regimen. Four years later the same group of children was again examined. Although atopic disease was more common in the early solid food group (40% of 62 children) compared to the late solid group (20% of 51), the difference was not significant; a similar prevalence of food allergy (6 and 4%, respectively) was not significantly different in the 2 groups. Interestingly, pollen allergy and asthma were equally or more prevalent at that age than eczema and food allergy. Furthermore pollen allergy was significantly more frequent in the early solid food group (37%) than in the late one (20%; $p = 0.04$); the same trend was noted for asthma, but did not reach significance [10]. This study suggests that solid food introduction is associated with increased risks of both precocious and later atopic manifestations.

These few studies are more than 10 years old; nevertheless no additional study focusing on this question has been made in the last years.

Prevention of Food Allergy by Exclusion Diets during the Weaning Period

Apart from the Swedish study [9, 10], no other study has tried to confirm the preventive effect of a late/restricted introduction of solid foods during the weaning period on the occurrence of allergic disease.

Conversely most of the recent studies concerned with preventing food allergy include in their design the late and ordered introduction of solids in the intervention group together with the use of breast milk, hydrolysate and exclusion diets in the mother during pregnancy or, more often, lactation. This is particularly true in the best conducted study [11] and the most frequently cited study by Zeiger et al. [12] who associated maternal cow's milk, egg, nuts and soy exclusion during the third trimester of pregnancy and lactation, and breast milk/casein extensive hydrolysate for 6 months and delayed cow's milk and solids for 6–36 months (nuts and eggs) in infants to maximize prevention. In the control group the introduction of solids was allowed after 4 months and was complete at 1 year of age. The cumulated prevalence of all allergic manifestations was significantly lower in the 'prophylactic' group (16%) than in the control group (27%; $p = 0.004$) at 1 but not at 2 years of age. This difference was linked to a significant reduction in the prevalence of eczema, urticaria, digestive symptoms ($p < 0.03$ for the combination), whereas the

prevalence of asthma and rhinitis was not modified [12]. At 3 and 4 years of age (78% of the children enrolled could be examined), the cumulated and actual prevalences of all allergic manifestations were not different between the 2 groups of children. Only the cumulative prevalence of food allergy (eczema, urticaria, diarrhea or vomiting twice after an oral challenge with eggs, cow's milk, nuts or fish) were significantly reduced in the prophylactic group ($p < 0.01$), whereas the actual prevalences were identical in both groups indicating that the cumulated effect at 3 and 4 years was obtained during the first 2 years of life [13].

In a study with a similar design (exclusion diet for the mother during lactation, exclusive breast milk or extensive protein hydrolysate until the introduction of solid foods after 9 months of age, free diet after 1 year) with the addition of anti-house dust mite treatment of mattresses and the children's rooms, not only eczema and food allergy but also asthma could be prevented in the 'treatment' compared to the control group [14].

More recently two studies have shed more light on the late introduction of solids in the preventive effect observed in the above studies. The first one aimed at comparing the allergy-prevention effect of a partially hydrolyzed formula with two extensively hydrolyzed formulas in Danish infants at high risk of developing allergic diseases [15]. The formulas were given along with breast milk until the end of the 4th month. Mothers had unrestricted diets during pregnancy and lactation. After the age of 4 months unrestricted diets and conventional cow's milk-based formula were given to the infants as needed. The outcome of the study was that the partially hydrolyzed formula was less effective than the extensively hydrolyzed formula in preventing cow's milk allergy. However, the most striking result was the globally low rate of cow's milk allergy in the 4 groups (3 formula groups and an exclusively breast-fed group) varying from 0.6 to 4.7%, even though the dietetic intervention did not include either maternal diet during lactation or dietary restriction for the exclusion of children after the age of 4 months. Food allergy controlled by a challenge was observed in only 2.5%, cow's milk allergy in 1.7% and egg allergy in 1.3% of 478 infants. This low rate of allergic manifestations was attributed to the high frequency of breastfeeding by the authors who questioned the need for dietary restrictions after the first 4 months of life [15].

In the multicenter Study on the Prevention of Allergy in Children in Europe, prevention (in 349 infants) comprised breastfeeding until 6 months of age supplemented, if necessary, by a hypoallergenic formula; gradual introduction of solid foods after 6 months; cow's milk, egg and fish being introduced after 12 months of age, and nuts after 3 years, and the use of dust-mite allergen-impermeable protection of the mattresses. In the control group ($n = 347$) exclusive breastfeeding was recommended for at least 3 months, and the introduction of solids was delayed until 6 months of age and cow's milk until 12 months of age. There was a significant reduction regarding

sensitization to any allergen test at 1 year of age (against Der p, Der f, egg, milk; 6.2% in the prophylactic group versus 10.7% in the control group, $p < 0.03$). There was only a tendency toward a reduction in definite allergy against 1 of the 4 allergens in the prophylactic (3.1%) versus the control (6%) group [16]. However, the 2 groups were not very different with regard to age at introduction of solids, and ages were rather late.

It seems, therefore, that accumulated evidence suggests that delaying the introduction of solid food might reduce the risk of clinical food allergy and eczema. However the magnitude of the effect is not well known (precise and important in one study [9, 10], and confounded with other measures in all other studies [12, 16]) and the duration of avoidance is not known at all. Although there is a consensus to avoid solid foods before 3–4 months of age, at present there are no studies enabling a decision on how long preventive measures need to be maintained. Furthermore, avoidance of oral allergens may not be appropriate: a recent study concerning peanut allergy suggests that sensitization to food allergens may also occur though the cutaneous route if the allergen comes into contact with inflamed skin and that, indeed, in this case the best prevention would be to avoid emollients containing peanut oil since in that study the risk of eczema was not associated with the ingestion of peanuts by the mother during pregnancy or lactation, or with consumption of peanuts by the infants [17].

Prevention of Atopic Disease through the Use of Probiotics

As the inverse association between infections early in life and allergic disease and the role of the commensal gastrointestinal microflora in promoting a Th1-type immunity essential in controlling Th2 allergic inflammation are being better understood, it was logical to try to prevent the occurrence of atopic disease in children by giving them probiotics in infancy [4, 18].

In a small randomized double-blind study it was possible to demonstrate that specific probiotic strains (*Bifidobacterium lactis* Bb-12 or *Lactobacillus* GG) added to an extensively hydrolyzed whey formula were able to significantly improve the skin condition (assessed by the SCORAD method) of 18 infants with atopic eczema receiving the supplemented formulas compared to 9 infants receiving the unsupplemented formula ($p = 0.002$) [18].

In a more important randomized placebo-controlled trial concerning 159 infants and their mothers in families with a history of atopic disease, capsules containing either a placebo or 10^{10} CFU of *Lactobacillus* GG were given 2–4 weeks before delivery to the mother and for 6 months to the infants. Children were examined at ages 3, 6, 12, 18 and 24 months. Atopic eczema was diagnosed in 35% of the children aged 2 years. The frequency of atopic eczema in the probiotic group was half that of the placebo group: 15/64 (23%) vs. 31/68 (46%), RR 0.51 (95% CI 0.32–0.84) [19]. Two years

later the effect was still observed: 14 of 53 children receiving lactobacillus had developed eczema compared to 25 of 54 receiving placebo (RR = 0.57, 95% CI 0.33–0.97), suggesting that the preventive effect of *Lactobacillus* GG on eczema extended beyond infancy [20]. Although these studies need to be confirmed, they open a new avenue in the prevention of atopic manifestations.

It is satisfactory to note that the recent progress in our understanding of mucosal immunity and the role of the bacterial burden on the development of tolerance in infancy make it possible to envisage food allergy prevention strategies other than exclusion diets whose efficiency is still being discussed with regard to the magnitude, the uncertain time frame, and the need for careful nutritional follow-up of the children.

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Discussion

Dr. M. Hoekstra: Thank you Dr. Schmitz for your interesting introduction and all the data you have shown us. I would like to suggest splitting the discussion in two parts, and first talk about solids. Is your question about the introduction of solids?

Dr. Gracey: My question is about the environment. There seems to be a marked difference between the prevalence of allergic diseases in children in so-called developing countries and in industrialized countries and a lot of that has been related to environmental marker levels of biological contaminations either in the home or in the environment at large. This also applies to populations that are in transition from their traditional contaminated environments going into environments where standards of hygiene are much better, and the rates of allergic disease seem to increase when children move to a more hygienic environment. I wonder whether you would care to comment on this.

Dr. Schmitz: Indeed, you are summarizing what is known as the hygiene hypothesis which states that there is an inverse relationship between the bacterial burden during the first months of life and the later occurrence of allergy. This is a tempting hypothesis, although Dr. Fritsché a moment ago did not seem to be convinced by its scientific basis. From what I have read (I am not an allergologist) I would say that there are good studies which speak in favor of it, for example those studies which were conducted a few years ago in Germany, Austria, and Denmark, mainly regarding the occurrence of eczema, or eczema and food allergy in children living on farms compared to children living outside farms, and showing less allergy in the first group. Probably this is not enough to explain all allergic diseases because there is also pollution which plays on a completely different ground. In this regard, the very often cited German study comparing the rate of allergy in western compared to eastern Germany was interesting. Contrary to what people expected, the rate of asthma was greater in western Germany compared to eastern Germany but other allergies were greater in eastern Germany mainly because of industrial pollution. So certainly the hygiene hypothesis explains part of the rise in allergic diseases and probably this is true for eczema and food allergy. For other allergic conditions, asthma for example, it is probably more difficult to say.

Dr. M. Hoekstra: Are there any other general questions about the environment?

Dr. Michaelsen: Not about the environment but about the introduction of solids. You mentioned the study by Saarinen and Kajosaari [1] that mentioned citrus fruits and fish as hyperallergenic, and I know that many countries in Europe exclude hyperallergenic foods throughout the first year of life, which I think is not based on any evidence at all. Do you agree with that?

Dr. Schmitz: I completely agree. I was amazed by the fact that we had absolutely no data to back these habits. Excluding hyperallergenic foods during the first year of life is opening the umbrella for protection, but we don't know whether this is really useful or not. In this regard it is interesting to see that Halken et al. [2], in the discussion of their article, suggest that there is no reason to postpone solids.

Dr. M. Hoekstra: Very recently there was a study published by Zutavern et al. [3] from Munich who were unable to show any relationship between the timing of the introduction of solids and the prevalence of allergic disease. I think the group was about 500 children, it was published in *Archives of Diseases in Childhood*.

Dr. Schmitz: Was it part of the SPACE study?

Dr. M. Hoekstra: It was part of the SPACE study.

Dr. Heymans: There was a meeting recently in which the investigators from the Academic Hospital in Groningen, The Netherlands, who have a unit for food allergy, presented their results on the sensitization of those children who are not exposed to certain food allergens for a prolonged period, the so-called exclusion diet. They could not show any difference after 1 and 2 years of sensitization taking IgE as a marker. So I think there is no proof yet and still we do it.

Dr. Schmitz: Yes, I agree.

Dr. Michaelsen: I just wanted to add that excluding fish for the first year of life is a serious exclusion from the diet. Fish contains n-3 fatty acids, high amounts of minerals, vitamin D and many other important nutrients. I think we have to try to advise these countries that there is no evidence supporting the exclusion of fish.

Dr. Keller: I would like to hypothesize on whether a difference is made between breastfed and non-breastfed children and the time point of introduction to solids. In analogy to celiac disease, it could be wise to introduce not multiple but a single solid food during the breastfeeding period, perhaps between 4 and 6 months or so, in contrast to non-breastfed children in whom it maybe introduced later. This is just speculation. What is your opinion?

Dr. Schmitz: Someone in the audience made the same proposition a moment ago, and it is tempting after the work of Ivarsson et al. [4]. However this depends very much on the length of the breastfeeding period, and although this is possible for example in Scandinavia where women breastfeed until at least 4–6 months of age, in France it would be very difficult to make the same proposition because the average duration of breastfeeding there is around 6 weeks.

Dr. M. Hoekstra: I have a question about these old studies by Fergusson and Saarinen. If I recall well, these groups were not randomized, meaning that it is not possible to actually compare these groups on outcome parameters, that is very difficult. So we must be very cautious with the interpretation of these studies.

Dr. Badr-Eldin: I wonder about the studies which compared the impact of giving solid foods versus just breastfeeding; whether all these studies were controlling for the mothers diet as well?

Dr. Schmitz: It depends from paper to paper. In Kajosaari's paper the mothers were not controlled, if I remember correctly. There are not so many, and for example in Zeiger's paper some foods were forbidden but the remaining food was free. So it is difficult to control the food of the mother, which might also be dangerous.

Dr. El-Din Amry: Based upon your nice presentation can I conclude that if we are obliged to introduce foods in the first 6 months of life, for example because the mother is working and so on, is yogurt the best alternative because it is very rich in probiotics?

Dr. Schmitz: Yes it is a good idea. I would not fight against adding yogurt in the 5th month of life for example. I don't know if probiotics are still important at that time because the 'sensitization' window has probably already closed by then.

Dr. M. Hoekstra: Before continuing on probiotics, can I ask you one more question about solids, the effect of the timing of the introduction of solids. Why has this never been done in a randomized control trial? I think it is easy to perform and is the type of study to investigate a question like this.

Dr. Schmitz: I agree but the timing of the introduction of solids is so much linked to local habits that randomization is difficult to envisage. We could decide now that we

should start something like that because in fact, you are right, it has not been done, but I think we are at the border between science and use, and it is probably difficult to randomize use.

Dr. Caroli: I would like to make a comment. You said that there is a delayed introduction of solid food at 4–6 months. In my opinion we shouldn't say delayed because according to the WHO this is a proper introduction of food around 6 months of age, because if we keep saying that it is delayed we allow pediatricians and mothers to give food other than breast milk or formula before that age. Then my question is, you spoke about solid foods but solid food is a very wide range of foods in terms of proteins, also in terms of fats. Do you have any data on n-3 and n-6 intake in introducing solid food earlier than 6 months of age because both are involved in the development of immunity, so I think it could be an interesting area of work.

Dr. Schmitz: Yes, that is a good point and it was just raised by Dr. Michaelsen a moment ago that excluding fish may be dangerous in this regard. I am not aware of any study having been interested in such a topic. With regard to your first comment, of course I agree but I said delayed because in many countries there is still a tendency to give solids before 4 months of age.

Dr. Badr-Eldin: I just wanted to add to what my colleague just said; giving yogurt is almost a national habit in Egypt, and mothers are used to introduce it right after 40 days or 2 months of life. We keep telling them not to give it but they do. So we telling them to use the yogurt from powder milk or dry milk because this might decrease the possibility of inducing atopy. I am not sure whether this is right or not because, after all, the incidence of atopy, eczema in particular, is not that much in Egypt, but perhaps asthma is very frequent. So I am not sure if we should continue advising the mothers not to give this yogurt as early as they do.

Dr. Schmitz: Probably Dr. Michaelsen might like to comment. If you want to have the effect of probiotics, the bacteria in the yogurt need to be living, which is not the case in many industrialized yogurts, and this must be taken into consideration when feeding yogurt early, e.g. at 6 weeks of age. Furthermore you must consider that if you give yogurt you give proteins and allergens at the same time as bacteria and probiotics, and I don't know which component of yogurt is most important with regard to your question, but Dr. Michaelsen will answer.

Dr. Michaelsen: Starting that early is certainly not recommended. There are several problems. One is that the early introduction of cow's milk can provoke microscopic bleeding from the intestine which is especially the case before the age of 6 months. Another important problem is that starting before the age of 4 months will result in a high renal solid load. The kidneys are still immature and might not be able to excrete the high amounts of protein and minerals present in human milk. So it is better not to introduce yogurt before the age of 6 months.

Dr. Heymans: May I remark on that because I think the introduction of beikosts is also based on the nutritional content of the beikosts given, iron, manganese, all kind of vitamins. I don't think that a yogurt, unless it is made of a follow-on formula, is wise to start because you don't provide the right micronutrients that actually have to come from beikosts, so that is something that should be taken into account [5].

Dr. Salminen: I completely agree with the previous speakers, and one major thing with yogurt is also that the strains in yogurt are based on technological properties, not on physiological properties, so most of the yogurts I would not call probiotic. Probiotic needs to be defined and some health-promoting activity should be shown in scientific studies before something can be called probiotic.

Dr. Sinaasappel: Can you explain the second last slide you showed us? You made the conclusion that lactobacillus GG was helpful for preventive measurements for allergic diseases, but as far as I could see from that slide, it was just the opposite.

Dr. Schmitz: Which slide?

Dr. Sinaasappel: The second last one.

Dr. M. Hoekstra: The one about the primary prevention and the follow-up study at the age of 4 years with respect to the incidence of rhinitis.

Dr. Sinaasappel: As far as I can see in the last row there is an increase in eczema, that has to be turned around, the rest is all right.

Dr. Schmitz: Yes, you are right.

Dr. M. Hoekstra: The problem is that the rest is all right, so there is a tendency for rhinitis and asthma to increase in the group who received lactobacillus.

Dr. Schmitz: You are completely right; there is no comment in the paper on this fact, there is only a comment on eczema which weakens the study.

Dr. M. Hoekstra: Any more comments on this study?

Dr. Paerregaard: Another thing that was puzzling about that study was that among the children who actually developed atopic eczema the severity was comparable in the placebo group and in the intervention group evaluated by SCORAD. That was a bit puzzling because it would be expected that the children who actually developed eczema would have less severity if treated by probiotics.

Dr. Schmitz: I agree with you, that was pointed out already.

Dr. M. Hoekstra: I do have some methodological problems with this study which was characterized by a large number of dropouts at the 1st year evaluation and at the 4th year. The second problem I have with this study is that there was no difference in sensitization between the 2 groups, so it is hard to say that the use of probiotics in this study resulted in a decrease in atopic eczema because with respect to sensitization nothing was seen. The last problem I have is that almost all the studies performed on probiotics were performed by one group, and I think it is a very good scientific principle that research should be repeated by other groups.

Dr. Schmitz: Sure, but we are waiting for the other groups.

Dr. Gracey: In the Indian subcontinent, apart from what happens in Egypt, fermented milks, usually in the form of curds, are given traditionally and very widely to young children. I am not aware of any scientifically documented studies that show that these are beneficial or not, and I am unaware of microbiological investigations that would support the use in this way. I know that there are people from the subcontinent in this room, and I wonder if anyone from Bangladesh or India or anywhere else for that matter would care to comment on what is clearly a very important subject for many millions of children in that part of the world.

Dr. Verloove: Perhaps we should broaden the question to more people in this room because these habits concerning early nutritional habits are so widely different all around the world. Moreover I was a bit amazed about the easy way you suggested doing a randomized control trial, on nutrition, in infants, looking at well baby clinics and what you hear there, that is impossible. I mean people just give their children to eat what they eat themselves or whatever. So my question to the audience would be are there studies in other countries with other cultures regarding nutrition in the early months of life, and haven't they been compared yet. I mean there must be other ways, observational studies that you can get out of these difficulties.

Dr. Schmitz: One of the first Nestlé Nutrition Workshops I attended, at least 20 years ago, was on weaning habits, and at that time the whole workshop showed that there were great differences from country to country inside Europe, as you just said, and it was impossible to find a scientific basis for these uses and whether they were detrimental or not; I am not sure that we know much more now than 20 years ago.

Dr. M. Hoekstra: In my opinion it is very difficult to compare different populations only on their varying behaviors with regard to the introduction of foods. It is the same with the yogurt question in Egypt, if you compare populations you also compare differences in environment, differences in genetic makeup. So if you really want to know

the effect of yogurt in Egypt, you have to randomize it, and that is the same difficulty when you compare populations.

Dr. Heymans: Things are difficult, but sometimes although they are difficult they are not impossible. We performed a study on the early introduction of cow's milk in newborn infants in the Netherlands, a randomized, double-blind, placebo-controlled prospective trial. We were able to include more than 1,500 children, and we were even able to follow them for 6 years. We could not show that early exposure to cow's milk in a normal population has any effect on sensitization or allergy later in life [6]. If the population and study design are chosen carefully and I think especially in Europe that it is possible to perform studies like this. The only thing is the right motivation and some money are needed for it, and sometimes the industry will to help to overcome these barriers.

Dr. Schmitz: Certainly you are right but then the question is whether it is worthwhile to enroll 1,500 children, well babies, to answer a question such as whether it is good or not to introduce citrus or orange at 6 or 3 months. It is such hard work to answer such a seemingly not so important question that eventually it is not done.

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Celiac Disease: Effect of Weaning on Disease Risk

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Introduction

From the weaning period and onwards the intestinal mucosa is exposed to an increasing number of antigens, e.g. food components and micro-organisms. Of all the antigens that reach the systemic circulation from the gut lumen, only a minority are potentially harmful to humans and need to be defended against. The majority of intestinal antigens do not require a protective immune response, but may even be beneficial for the individual. Thus, the mucosal immune system must have the capacity to discriminate between when an appropriate protective immune response to harmful foreign antigens is required and when a muted or non-response is preferable. One important component of the latter is oral tolerance, which may be defined as a systemic hypo-responsiveness or non-responsiveness of mature T and B lymphocytes to antigen challenge after prior oral exposure to the antigen [1, 2].

Celiac disease (CD), or permanent gluten-sensitive enteropathy, develops because tolerance to ingested wheat gluten (gliadins and glutenins), and related proteins from rye and barley never develops, or is broken after it has developed. The disease is characterized by inflammation of the small intestine resulting in crypt hyperplasia, villous atrophy and flattening of the mucosa. Other characteristics are an increased number of intraepithelial lymphocytes (IELs) and lamina propria lymphocytes, increased serum concentrations of IgA antibodies towards gliadin and the autoantigen tissue transglutaminase. When gluten is withdrawn from the diet the mucosal

morphology is restored, the specific antibody levels become normal, and symptoms of the disease disappear [3].

CD is an acquired disorder, which can be diagnosed in early childhood with classical symptoms such as diarrhea, malabsorption and failure to thrive, but also later in life, even in adults who show a wider and more diffuse spectrum of symptoms. Poor diet compliance and undiagnosed disease, which is frequent in adult populations [4], are associated with increased morbidity and mortality [5].

A Multifactorial Etiology

CD is a chronic inflammatory disease. Collectively, these diseases have multifactorial etiologies. The genetic component is strong in CD with a high sibling relative risk and high concordance between monozygotic twins (75%) [3, 6]. The recent Swedish epidemic of CD with classical symptoms in children below 2 years of age strongly supports an etiological role of environmental factors, such as early infant feeding practices (fig. 1) [7, 8]. Thus, while it is likely that environmental components trigger the disease in genetically predisposed individuals, the complex interplay between genetic and environmental factors makes it difficult to delineate the complex multifactorial pattern. Nonetheless, CD is the best understood HLA-linked disease.

CD is a particularly useful model for increasing our understanding of the complex immunological diseases [9]. Exposure to gluten, or certain known peptides thereof, is a prerequisite for CD development, and once established the disease can be turned on and shut off by introducing or withdrawing gluten from the diet. CD is a polygenic disease, and most of the genes involved are still unknown [6]. However, over 90% of the patients express the MHC class II molecule HLA-DQ2 and the remainder usually HLA-DQ8, both of which predispose for the disease [10].

Finally, simple access to the affected organ – the intestinal mucosa – by endoscopic or capsule biopsies allows detailed ex vivo studies such as isolation and characterization of disease-relevant cell populations. Such studies have shown that CD is associated with an abnormal T-cell-initiated immune response to gluten, but the detailed pathogenesis still remains to be elucidated. The link between environmental factors besides gluten and the development of CD also needs to be explored further.

In the mid 1980s CD became the most common chronic disease in Swedish children after IgE-mediated allergy (fig. 1) [7, 8]. The epidemic pattern is unique for a chronic disease, strongly suggesting that environmental factors contribute substantially to precipitation of the disease and, thus, prevention should be possible. A recent incident case-referent study contributed to the identification of some of these causal environmental risk factors [11], which are discussed below (fig. 2).

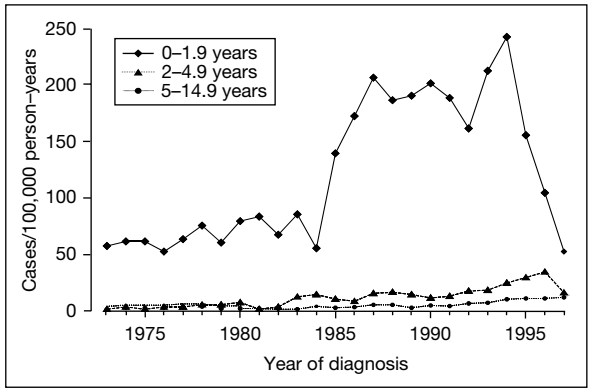


Fig. 1. The Swedish epidemic of CD. From Ivarsson [8] with permission.

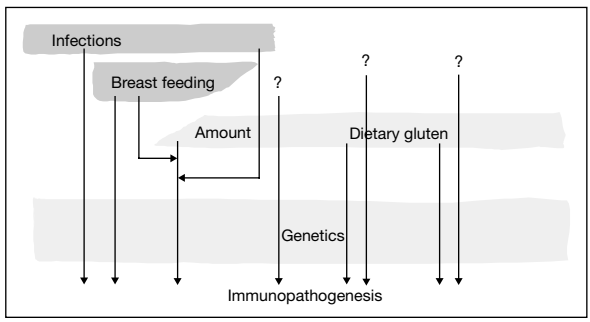


Fig. 2. A crude model on the effect of weaning on CD risk.

Amount of Dietary Gluten: An Important Causal Factor

The dose of dietary antigen ingested may influence whether or not oral tolerance develops [12]. This is easily demonstrated in experimental animals, and is possibly true for humans as well. However, whether this dose effect also applies to gluten in relation to the risk of developing CD is not known.

Healthy Swedish and Italian infants were reported to have a larger consumption of wheat gluten than infants from Finland and Denmark [13, 14] and, interestingly, the former countries also reported a higher occurrence of CD. The study design did not enable adjustment for differences in other potentially causal exposures. Moreover, a Swedish case-referent study [15] reported that CD cases, more often than referents, were introduced to gluten by means of gluten-containing milk cereal drinks (MCDs), which, by Swedish tradition, are used from 6 months of age rather than follow-on formulas.

Although the design did not allow estimation of the amount of gluten consumed by the infants, this study still suggests that the amount of gluten is a causal factor, as bottle-feeding more readily contributes a larger amount of food as compared to feeding by cup or spoon.

We recently reported results from an incident case-referent study of 491 cases and 781 referents, which was population-based and had a high participation rate [11]. Hence, the results should be representative for Sweden at large and also valid for children in general. As only incident cases, i.e. newly diagnosed ones, were included, the recall period was comparatively short which reduced the risk of recall bias. A comprehensive questionnaire, which did not reveal our focus on CD, concerning the children's diet and health in general was mailed to the families. We used a semi-quantitative food frequency questionnaire to assess the consumption of gluten-containing cereals. Multivariate analyses were used to adjust risk estimates for confounding and to suggest causal relationships. For the first time the design of the study allowed assessment of the consumption of gluten-containing cereals on an individual level [11]. The amount of gluten-containing flour consumed during introduction was assessed by the single food item which contributed the largest amount during the first 2 weeks of consumption, while the amount at 7 months of age was based on all food items consumed at that age. Introduction of gluten-containing foods in large amounts, as compared to small or medium amounts, was an independent risk factor for CD development (adjusted odds ratio (OR) = 1.5: 95% confidence interval (CI) 1.1–2.1). By use of multivariate analyses, differences in breastfeeding practices and the age of the infant when first introduced to gluten-containing foods could be adjusted for. Moreover, the type of food used as the source of gluten, i.e. solid foods or MCDs, was not a significant independent risk factor. Thus, there are reasons to argue that our observations are applicable not only to Sweden, with its traditional use of MCDs. These results strongly suggest that the introduction of gluten in larger amounts increases the risk of CD [11].

Our ecological study of the Swedish epidemic, in which we used aggregated data to explore any temporal relationship between changes in the incidence rate and in infant dietary patterns, supports the theory that the quantity of gluten consumed during infancy is a risk factor for CD. The rise in incidence was preceded by a twofold increase in the average daily consumption of gluten estimated by the use of MCDs, and later the decline in incidence coincided with a consumption decrease by one third [7].

Gluten-sensitized individuals do respond in a time-related and dose-dependent fashion to gliadin [16]. Recently an explanation for the HLA-DQ2 gene dose effect for the development of CD was proposed. Individuals homozygous for the HLA-DQ2.5 molecule on their antigen-presenting cells present a broader range of gliadin peptides to T cells than HLA-DQ 2.5/2.2 heterozygous individuals, while HLA-DQ 2.5/non-DQ2 heterozygous individuals are poor presenters and have only a slightly increased risk of developing CD

[17]. These results and others are indicative of a quantitative model for disease development. However, it is still not settled whether gluten as a risk factor for CD acts in a dose-dependent manner or whether there is a threshold effect. If the latter is true it is likely that the amount of gluten required to pass the threshold is lower in HLA-DQ2.5 homozygous compared to heterozygous individuals.

Thus, there is evidence to suggest that during infancy consumption of a large amount of gluten-containing flour, which increases the antigen dose, increases the risk of CD (fig. 2). However, the amount of gluten tolerated may be modulated not only by the genetic predisposition of the individual but also by environmental exposures besides gluten.

Does Age at Introduction Matter?

It is possible that there is an age interval during which humans have decreased ability to develop oral tolerance to a newly introduced dietary antigen [12]. Hypothetically, the age of the infant at introduction of gluten into the diet might influence the risk for CD.

Although, previous comparisons of English CD patients suggested that earlier introduction of dietary gluten resulted in earlier presentation of the disease, no such relationship was found in later studies taking differences in breastfeeding duration into account [18]. On the other hand, a delayed introduction of gluten into the diet of infants was suggested to contribute to the decline in CD incidence in the United Kingdom in the 1970s [19, 20]. In contrast, comparable dietary changes occurred in Sweden without any observed change in incidence. Furthermore, the increased incidence in Swedish children in the middle of the 1980s was preceded by a postponed introduction of dietary gluten from 4 to 6 months of age [7]. Obviously, these ecological observations are contradictory. However, a study design based on aggregated data cannot by itself provide conclusive evidence.

Case-referent studies based on individual data enable adjustments for differences in other potentially causal exposures. After having adjusted for differences in breastfeeding duration, such studies suggested that the age of the infant at introduction of dietary gluten was not a causal factor with respect to CD risk [15, 21, 22]. In our incident case-referent study we found a bivariate association indicating an increased risk for CD when dietary gluten was introduced within the age interval of 5–6 months. However, this association no longer remained statistically significant when adjusting for differences in dose of gluten given during introduction and breastfeeding variables [11].

CD and diabetes mellitus type 1 are interlinked, i.e. suffering from one of them increases the risk for the other. Recently, two cohort studies on children with increased risk for diabetes mellitus type 1 investigated the

association between the age at introduction of dietary gluten and indicators of autoimmunity. One of them suggested that it is beneficial to introduce gluten within an interval of 4–6 months of age as compared to both earlier and later introduction [23], while the other study did not find that age at introduction influenced the risk of autoimmunity [24]. Both studies considered differences in breastfeeding duration in the analyses, but not the amount of dietary gluten given during the introduction which consequently remains a potential confounder.

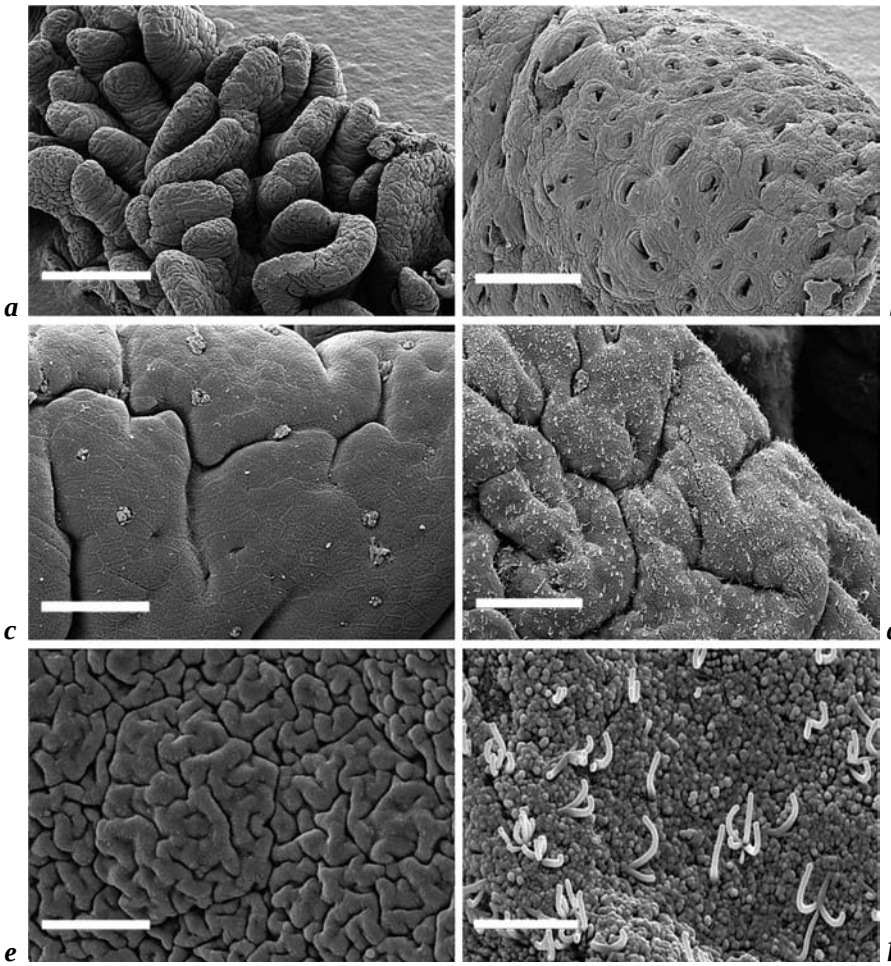
Thus, whether or not the infant's age at the time of gluten introduction is a risk factor for CD has not been settled. Most studies refute it as an independent risk factor, but it should remain on the agenda for further exploration.

Infections and Innate Immunity

In the 1980s Kagnoff et al. [25] suggested that gastrointestinal infection with adenovirus type 12 could initiate CD because of significant sequence similarities between the E1b protein produced by this virus and A-gliadin of gluten, a hypothesis later questioned but not yet excluded as a possibility. Furthermore, gastrointestinal infections cause a disruption in the barrier function of the small intestinal mucosa, which theoretically could result in an increased antigen penetration and unfavorable immune response. We found that, compared to referents, CD cases were more often born in the summer [26], and therefore more often introduced to gluten during the winter when infections are more frequent. Thus, it is conceivable that the Swedish epidemic was partly caused by a change in the infectious panorama, or an interaction between infant feeding and infections.

Interestingly, we recently discovered that rod-shaped bacteria are frequently associated with the intestinal mucosa of CD patients, both with

Fig. 3. Presence of rod-shaped bacteria in jejunal biopsies from a CD patient with active disease. Scanning electron micrographs of jejunal biopsies from 1 control patient (***a***, ***c***, ***e***) and 1 CD patient with active disease (***b***, ***d***, ***f***). ***a*** Normal villus architecture with leaf-shaped villi. ***b*** Totally flat mucosa corresponding to subtotal villous atrophy. ***c*** No bacteria and normal cell appearance with uniform enterocytes, showing regularity both in shape and size. ***d*** Presence of large numbers of bacteria and disturbed cell structure with cobblestone appearance, irregularity in shape and size of the enterocytes. ***e*** Normal ultrastructure of the apical surfaces of enterocytes with thick glycocalyx, completely covering the microvilli. ***f*** Presence of bacteria in bouquet-like groups and severe distortion of ultrastructure of the apical surfaces of the enterocytes showing prominent decrease in glycocalyx thickness and irregularly oriented and barely visible microvilli. ***g*** Frequency of jejunal biopsies from CD patients and controls with adherent bacteria. n = Number of biopsies analyzed. Statistically significant differences as determined by the χ^2 method are indicated. ***a***, ***b*** Bars correspond to 300 μm. ***c***, ***d*** Bars correspond to 30 μm. ***e***, ***f*** Bars correspond to 3 μm. From Forsberg et al. [27] with permission.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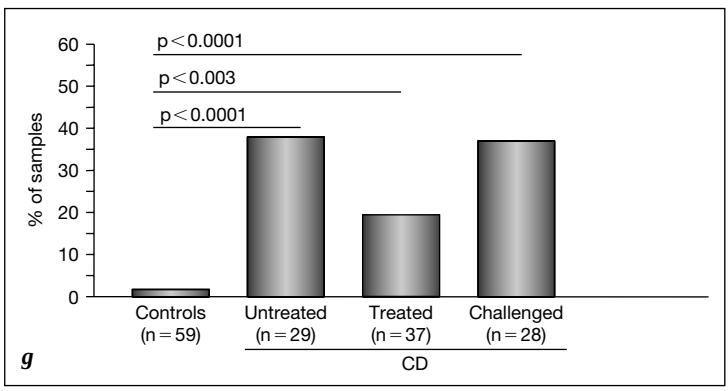
60
50
40
30
20
10
0
% of samples
p < 0.0001
p < 0.003
p < 0.0001
Controls (n = 59)
Untreated (n = 29)
Treated (n = 37)
Challenged (n = 28)
CD
g

active and inactive disease, but not with that of controls as revealed by scanning electron microscopy (fig. 3) [27]. Moreover, the presence of bacteria is associated with a particular lectin-staining pattern of the intestinal mucosa. It seems therefore that unique carbohydrate structures of the glycocalyx/mucous layer are likely discriminating features of CD patients. These glycosylation differences could facilitate bacterial adhesion. Adhesion/infection by these yet undefined bacteria could precipitate disease in genetically susceptible individuals. Alternatively, bacterial adhesion may precipitate a change in the glycosylation pattern. We have also shown that there is a strong IEL response in CD with a highly significant increased expression of the cytokines interferon (IFN)-γ and interleukin (IL)-10, without a concomitant increase in the expression of tumor necrosis factor-α or transforming growth factor-β, and with marked shift of the IFN-γ and IL-10 production from the lamina propria to the epithelium. This may cause both recruitment of IELs and a leaky epithelium. Hence, the epithelial reaction may be critical for disease development [28]. This view was recently supported by the observation that enterocyte expression of the MICA protein, a non-conventional HLA class-I molecule, is upregulated in patients with active CD [29, 30]. The expression can be induced by stress [31], but as now shown also by gliadin, or peptides thereof [29], and by IL-15 [29, 30]. MICA serves as ligand for the activating NKG2D receptor expressed at the surface of NK cells, and some CD8 α/β T and γ/δ T cells. Hüe et al. [29] also showed that NKG2D expressing lymphocytes can lyse epithelial cells, which may explain the villous atrophy typical for CD. It seems that IL-15 is a key player in these events by upregulating both MICA and NKG2D [29, 30]. The latter has been shown to play a key role in other immune-mediated disorders. It may exert beneficial functions during infections but could also serve as an immune activator that can tip the balance in favor of autoimmunity and chronic inflammation [32]. Whether the rod-shaped bacteria associated to the mucosa in CD patients stress the epithelial cell to increased expression of MICA is not yet known.

We have also found that the expression of mucin-2, α-defensins HD5 and HD6, and lysozyme is increased in active CD but returns to normal in treated CD. Their expression levels correlated to the IFN-γ mRNA levels of IELs, suggesting that this cytokine upregulates the expression of these molecules. Metaplastic Paneth cells were seen in the small intestine of children with active CD. These cells may at least partly be responsible for the increased levels of α-defensins and lysozyme [27]. Recently, Maiuri et al. [33], incubating small intestinal biopsies in vitro, gave further evidence of the involvement of innate immunity in CD. They showed that the gliadin fragment can activate this immune system, affecting the in situ T-cell recognition of dominant gliadin epitopes. IL-15 also seemed to be involved in this activation [33].

In our incident case-referent study [11] we found that children who experienced three or more infectious episodes before 6 months of age had an

increased risk of CD before 2 years of age (adjusted OR = 1.4, 95% CI 1.0–1.9; Ivarsson et al., to be published). This was true even when episodes of gastroenteritis were excluded, and after adjustments for differences in infant feeding patterns. Interestingly the risk of CD increased considerably if, in addition to having many infections, the child was also introduced to gluten in large amounts as compared to small and medium amounts.

Thus, it is possible that common infections, both gastroenteritis and other types of infections, play a causal role in the development of CD (fig. 2). This can be due to the fact that infectious episodes might increase gut permeability followed by increased antigen penetration and activation of the immune system. If so, a dose effect of gluten would be reasonable. Furthermore, infections drive the immune system towards a Th1-type response, which is also typical for CD. However, the present evidence is not sufficient to allow firm conclusions regarding the underlying mechanisms.

Breastfeeding Plays a Preventive Role

The immune defense is not fully developed at birth. In the breastfed infant this is compensated for by immunity transferred from the mother to the infant via the milk [34, 35]. It therefore seems reasonable that the introduction of a dietary antigen while the child is still being breastfed might increase the likelihood of developing oral tolerance to that antigen. However, the role of breastfeeding in the prevention of IgE-mediated allergies is controversial.

Already in the 1950s, based on case series, it was suggested that breastfeeding delays the onset of CD. Increasing breastfeeding rate was also suggested as a possible factor contributing to the declining incidence of CD in the early 1970s in the United Kingdom [19, 20]. However, the study designs used did not enable adjustment of other potentially causal factors.

In the 1980s, Italian case-referent studies [21, 22] revealed that CD cases were breastfed for a shorter duration than referents. This was confirmed in Swedish [15] and German [36] studies. However, a shortcoming of these studies is that they did not clarify whether breastfeeding had a direct causal effect, or if the protective effect was indirect resulting from the postponed introduction of infant formula (i.e. cow's milk protein), or if it resulted from a reduction in the amount of dietary gluten ingested at an early age, i.e. a dose effect.

In our incident case-referent study the above-mentioned constraints were eliminated [11]. The main finding was that the risk of CD was reduced if the child was being breastfed during the time period when gluten-containing foods were introduced (OR = 0.59, 95% CI 0.42–0.83). This protective effect was even more pronounced if the child continued to be breastfed beyond the period of gluten introduction (OR = 0.36, 95% CI 0.26–0.51), with an increasing effect for every month of breastfeeding. These risk estimates are adjusted for the age of the infant when gluten was introduced into the diet

and the amount of gluten given. Moreover, a protective effect of breastfeeding was also supported by our ecological study using aggregated data to explore any temporal relationship between the changes over time in incidence rate and changes in infant dietary patterns [7]. Both the rise and later fall in the incidence of CD were temporally related to a change in the proportion of infants introduced to gluten while still being breastfed.

It is important to note that at the time of these studies the majority of Swedish infants were being breastfed for 6 months or longer, i.e. most of the infants were introduced to cow's milk products and other foods while still being breastfed. Also, for most infants the termination of breastfeeding did not coincide with the introduction of infant formula, but rather with increased ingestion of complementary foods. Thus, these findings strongly support breastfeeding as directly reducing the risk of CD, and not merely influencing the risk indirectly through changes in other exposures (fig. 2).

Conclusions

Breastfeeding during the dietary introduction of gluten is protective against CD, as supported by several epidemiological studies of different design. Moreover, this protective effect is biologically plausible taking into account our present knowledge of breast milk composition and the impact of breastfeeding on immune responses, along with current knowledge concerning the pathogenesis of CD. A gradual introduction of gluten also seems to be beneficial. The exact mechanism behind the protective effect of breastfeeding is not yet known. However, it is tempting to speculate that this could be mediated either by reducing the number of infections or by the immune-modulating effect of breast milk, for instance by providing downregulatory transforming growth factor-β1. Our observation that CD patients often have rod-shaped bacteria adhering to the mucosa might suggest that the effect could be mediated via an effect on the bacterial colonization of the gut.

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Discussion

Dr. Branski: I have two remarks to make. One, I don't think that you are speaking about prevention of celiac disease, it is mainly postponing or delaying its presentation, and this is according to the studies from Tampere, Finland. The presentation of celiac disease in Finland was much later mainly with extraintestinal manifestations due to secondary phenomena like diabetes, arthritis, etc. [1]. The other remark I would like to make is that environmental factors are very important for the timing of the presentation and some of these environmental factors are infectious agents like viruses, not necessarily bacteria. I would like to remind the audience of the paper by Kagnoff et al. [2] many years ago about the enteric type of adenovirus; it was supposed to be one of the causes affecting the timing of presentation of celiac disease.

Dr. Hernell: Of course you may be correct. That was what I tried to stress. We really don't know if there has been a true change in the incidence, or if after 1997 we have more cases with silent disease or more cases which contract the disease after 2 years of age. That is why the screening study I was mentioning is so important. However, I don't think that you can conclude yet that there is no change in the incidence. Although, there is evidence to suggest from the screening studies that have been done around the world that 1% of the population have celiac disease, few studies have been large enough with confidence intervals allowing a firm conclusion of the true prevalence of the disease in different populations. So I think we don't really know. It may be that you are correct but it could also be that we actually have a chance for primary prevention depending on how we introduce gluten. And your second question?

Dr. Branski: It was a comment regarding the infectious etiology for the timing of presentation.

Dr. Hernell: This observation by Kagnoff with adenovirus 12 has never really been proven or refuted. We have now found that there is a strong association between repeated infections and increased disease risk. I don't think this is surprising. It could be that infections cause a decreased barrier function with increased penetration of antigens, and if that is combined with an increased antigen load, then it is not unreasonable that an enhanced risk for disease would be found. It could also be that infections shift the immune response from a Th2-type to a Th1-type response. That could also contribute to the development of celiac disease. However, it would be interesting to know whether there are certain infections that are more important than others.

Dr. Sinaasappel: Thank you very much for this provocative presentation. The question I have regards the chicken over the egg question for this bacteria, which is also the case for the cytokines. The last point, and for me you are a little bit provocative, regards starting tolerance induction by introducing gluten during breastfeeding. Is that not what was done during your epidemic?

Dr. Hernell: Your first question was whether the cytokine profile is a secondary phenomenon. I don't think so. Our studies are based on three biopsies (the old ESPGHAN criteria) and we find the same principle cytokine pattern in patients with treated disease (gluten-free diet), which is clearly different from the pattern in controls.

Dr. Sinaasappel: The kind of bacteria, what types of bacteria did you find?

Dr. Hernell: We are working on that. We are screening all bacteria with 16S rRNA. At this point we believe that it is not just any bacteria. It seems that some bacteria are more common than others. Whether there are just a few bacteria that are adhering is too early to say.

Dr. Sinaasappel: My main point is the introduction of gluten during breastfeeding. You have probably seen a number of cases during your epidemic in whom gluten was introduced at that time because the number being breastfed was decreasing.

Dr. Hernell: I think the beauty of the study is that it is a case-control study and we had the chance to control for confounders. Doing so the strongest preventive factor is to introduce gluten during breastfeeding. We could even see that when controlling for the amount of gluten, every month of breastfeeding after the first introduction of gluten added to the preventive effect. The problem is that too few mothers breastfeed up to 8–9 months to allow sufficient statistical power to the analyses, but there is a clear tendency that each month after the introduction adds to the preventive effect.

Dr. Paerregaard: You certainly convinced us that it is recommendable to proceed with breastfeeding after the introduction of gluten to the diet, but the question of timing and the amount of gluten remains to be settled. You recommend that small to medium amounts of gluten be introduced instead of large amounts. When I look at the comparative studies that have been performed between infants in southern Sweden and Denmark, it was certainly found that the amount of gluten was of importance. However, it was also demonstrated that the quantities the Swedes considered to be small to medium amounts, the Danes considered to be huge. Have you any data to recommend specific amounts of gluten with regard to formula and complementary feeding?

Dr. Hernell: No we don't have that. The only data we have are from this case-referent study and the way that we chose to define large as compared to small and medium was as I told you. We used the distribution of intake among the controls and set an arbitrary upper limit at their upper one third of intake, which was defined as the large amount. I can't give you an exact figure of what would be the ideal amount of gluten for the introduction.

Dr. Taminiau: You didn't mention the viruses. Dr. Heyman has shown that rotavirus enhances the endocytotic uptake of proteins during rotavirus infection, and also shown that during the active phase of celiac disease whole proteins are taken up by endocytosis, much more than in the treated phase. I think what you showed is very interesting, but I still think the viruses might be important and the timing of the rotavirus infection, the protection of the breastfeeding, etc.

Dr. Hernell: I completely agree. What I showed was that the number of infections matters, and our first thought was that these infections were gastrointestinal, in most cases rotavirus. However, it turned out that it was any kind of infection that mattered. Infections probably affect the mucous membranes so it could be rotavirus, it could be other viruses.

Dr. Taminiau: Because the rotavirus also influences the blood cells during the infection.

Dr. Hernell: With rotavirus infections there is a fairly long period after the acute infection of increased uptake of large protein molecules.

Dr. H. Hoekstra: I have a question regarding infections. Often clinicians make the diagnosis of celiac disease in a situation with continuous diarrhea following an acute

infection. Clinical awareness at this time will be very high. My question is: at what time of year, in what month, was the diagnosis of celiac disease made? Could it be related to more clinical awareness?

Dr. Hernell: This is a good question. Because we were aware of that we chose to use the number of infections during the first 6 months, which was before introduction of gluten. Thus, the cases were diagnosed long after the infections that were used in the calculation.

Dr. Vaarala: I found your theory about infections very interesting. There are some studies showing that the MHC genotype has an effect on intestinal colonization, at least in animal models. My question is whether you found any relation between this occurrence of rod-shaped bacteria and the HLA genotype in your patients?

Dr. Hernell: We haven't looked at that; but it is a very good suggestion.

Dr. Benninga: You showed us that the gluten-free diet changed something in the interferon-γ (IFN-γ) cells, etc. We also looked at our treated celiac disease patients, but still found a rise in intraepithelial cells. The question is, how are you sure that all these people did take a gluten-free diet?

Dr. Hernell: The only answer to that is that we had antibody responses and as I showed you those who had their first biopsy had elevated levels of both anti-endomysium and anti-gliadin antibodies and those who had their second biopsy after examination had normal antibody levels and normal mucosal morphology. But of course you can never be 100% sure that they really adhere to the diet suggested, that is correct.

Dr. Schmitz: You gave your advice in the context of the Scandinavian way of feeding infants. What would you advise in countries with a much shorter breastfeeding duration, for example if breastfeeding stops at around 3 months of age, would you say that gluten should be given at 2 months of age or at 6 months of age or later? Is it possible to give general advice?

Dr. Hernell: I have heard this question several times but not exactly as you put it. I am afraid that I do not have an answer. However. the common question is whether or not there is a conflict between our national recommendation to introduce gluten between 4 and 6 months of age and the WHO recommendation of exclusive breastfeeding for 6 months. I don't think that anyone has a final answer. If one follows the WHO recommendation it should not be a problem to postpone the introduction to 6 months as long as the mother continues to breastfeed beyond 6 months. We didn't find that there was a specific age of introduction at which the risk was increased. Others have discussed whether there is an immunological window during which the chance to develop oral tolerance is greater than before or after. I think there are no convincing data in humans. Based on the evidence that we have, and I am speaking only for celiac disease, it seems that if you want to reduce the risk you should introduce gluten while the mother is still breastfeeding, and if she chooses or is forced to stop breastfeeding before 6 months you should really consider whether to advise her to introduce gluten before she stops breastfeeding.

Dr. M. Hoekstra: I think it is very difficult to give a clear advice on this although many people try to ask for this. Is one of the reasons for this difficulty that your data are derived from observational studies and not from intervention studies? So it is not yet exactly possible to indicate what choices should be made here.

Dr. Hernell: That is correct. I think however that it would be very difficult to do a proper intervention study although that would be the ideal situation. You would need to consider genetics and the amount of gluten during the introduction and many other things. It would be a very difficult study to carry out.

Dr. Bueno: You mentioned that IFN-γ plays an important role after previous infection. Since IFN-γ increases gut paracellular permeability and subsequently may affect the uptake of gluten, it appears of paramount importance to take into account previous infectious experience or background of infection when introducing gluten to food.

Dr. Hernell: Most people do not become intolerant to gluten, so something is obviously wrong with those who develop celiac disease. I think that is a normal function for IFN-γ to actually survey the epithelial surface against the food antigens. But it is correct, if there is increased IFN-γ production there will be a leaky epithelium. That is probably one part of this in which an escalation occurs with more antigens penetrating, and then possibly the problem is that there is no upregulation of TGF-β1 to balance the IFN-γ increase.

Dr. Keller: I have a question on the differences in clinical presentation of celiac disease patients. All of us have met with less classical cases and more so-called atypical cases like osteoporosis and so on. Is the availability of celiac antibody serology the only explanation for that, and do you think there is a difference between these atypical presentations in comparison to the classical ones?

Dr. Hernell: I don't know. If we go back and look at what happened for instance when the recommendations were changed in the UK, I think it was at the middle 1970s, it seemed as if celiac disease was actually disappearing. The same change in recommendations occurred simultaneously in Sweden and there was no change in the number of cases diagnosed. So I think there are many factors contributing to the change in the expression of the disease; it is not a single factor.

Dr. Taminiau: On the genetic manipulation prevalence, it was shown that in barley, in wheat and also in oats, there are so many epitopes stimulating T cells that it will be almost impossible to manipulate the proteins in the future to get rid of celiac disease.

Dr. Hernell: That was something that I put on the slide. But I think that it will be a long time before we have a chance to actually eliminate all the epitopes. It also seems possible that not all patients are reacting to the same epitopes.

Dr. M. Hoekstra: What I still do not understand is that if you look at the revised hygiene hypothesis then this hypothesis teaches us that we should no longer speak about Th1- or Th2-mediated disease but in general of a group of immune-mediated diseases, and the chance in a lifetime that you develop such a disease depends on whether you succeeded in developing enough regulatory T cells in early life. But what puzzles me is that if this is correct and if IL-10 is one of the most important indicators of regulatory T cells, that IL-10 decreases if you treat celiac disease. I would expect exactly the opposite. I mean if you treat the inflammation, if that is mediated by regulatory T cells, then you would expect an increase in IL-10-producing cells in biopsies, but you see just the opposite.

Dr. Hernell: Actually in active disease there is an increase in IL-10 but not of TGF-β, and there are probably different populations of regulatory cells that express IL-10 and TGF-β. So I think it is more interesting why the regulatory cells expressing TGF-β are not increased, or are they increased but not functioning the way we would expect? I don't know, I am not an immunologist.

Dr. M. Hoekstra: I would like to thank Dr. Hernell for his challenging presentation. We have heard a lot about the developing immune system and about intervention during infancy and the period of weaning. But in my opinion it is not yet possible to draw firm conclusions on the timing of solids and on the administration of probiotics and which probiotics, but we will hear more about that in one of the next sessions. From the last presentation, the relationship between the introduction of gluten during or after breastfeeding has not yet been elucidated.

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Gut Microbiota in Infants between 6 and 24 Months of Age

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Introduction

The indigenous microbiota of an infant's gastrointestinal tract is created through complicated contact and interaction with the microbiota of the parents and the infant's immediate environment. Nature-induced initial colonization is enhanced by galacto-oligosaccharides in breast milk and the microbiota of the mother. This process directs the later microbiota succession and health of the infant throughout the rest of his/her life [1, 2]. Thus, understanding and positive guidance of the process through dietary means is an important target when facilitating the mother–infant relationship through birth, breastfeeding, weaning and the first years of life. This process forms the platform for healthy gut microbiota throughout the entire life and is described in figure 1 [3, 4].

Stepwise Establishment of Microbiota

Source of Original Microbiota

The basis of healthy gut microbiota is created by the mother during pregnancy and microbiota transfer at birth. The microbiota of a newborn develops rapidly after birth and it is initially strongly dependent on the mother's microbiota, mode of delivery and birth environment [1, 2]. The microbiota of the mother is determined by genetic and environmental factors. Recently, it has been suggested that stress and dietary habits during late pregnancy, prior to birth and at birth may have a significant impact on the microbiota at the time of delivery thus influencing the quality and quantity of first colonizers of the newborn. Subsequently, feeding practices including

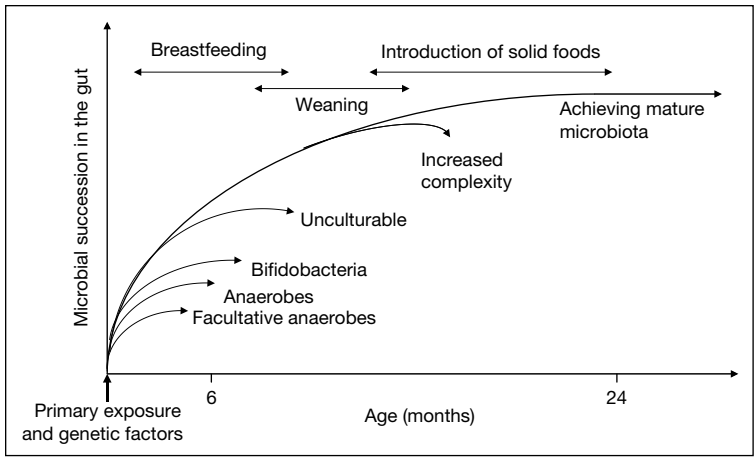

Fig. 1. Schematic description of the succession of gut microbiota during early life.

formula feeding and breastfeeding and the home environment of the infant influence the microbiota, both at the level of species composition and numbers of bacteria [1].

Succession of Microbial Communities

The stepwise process of establishing indigenous microbiota begins with facultative anaerobes such as the enterobacteria, coliforms, lactobacilli and streptococci first colonizing the intestine. These are rapidly succeeded by bifidobacteria and lactic acid bacteria [2, 4].

The establishment of the gut microbiota is usually characterized by the following steps: early colonization at birth with facultative anaerobes depending on the mode of delivery with rapid succession by anaerobic genera such as *Bifidobacterium, Bacteroides, Clostridium* and *Eubacterium* [4]. New molecular methods indicate that lactic acid-producing bacteria may account for less than 1% of the total microbiota while bifidobacteria can range from 60 up to 90% of the total fecal microbiota in breastfed infants. In formula-fed infants the microbiota is more complex, but depends on the composition of formula. New techniques indicate that the greatest difference in the microbiota of breastfed and formula-fed infant lies both in the bifidobacterial numbers and species composition within the intestinal microbiota, while the lactic acid bacteria composition appears to be rather similar. *Bifidobacterium breve, Bifidobacterium infantis* and *Bifidobacterium longum* are frequently found in fecal samples of breastfed infants, whereas the most common lactobacilli in both breastfed and formula-fed infant feces constitute *Lactobacillus acidophilus* group microorganisms such as *L. acidophilus*, *L. gasseri* and *L. johnsonii* [5]. In general, the differences between the

breastfed and formula-fed infants have decreased along with the development of improved composition infant formulas.

Characterization of the composition and function of the intestinal microbiota has been faced with considerable methodological difficulties and thus our understanding has improved stage by stage [3, 4]. As the disturbed succession during early infancy has been linked to the risk of developing infectious, inflammatory and allergic diseases later in life, it is still of great interest to further characterize both the composition and succession of microbiota during infancy [4–6].

Weaning and Gut Microbiota: The Second Stage

The practice of breastfeeding for 4–6 months has been considered to assist in the development of healthy gut microbiota. Major changes in the composition that occur during breastfeeding are related to breast milk components, especially galacto-oligosaccharides. Breastfeeding also provides an optimal environment for exchange of microbes between the mother and infant, including the contact with the mother's skin and exposure to microbiota present in the immediate environment. As a result, every individual has unique characteristic microbiota during later phases of breastfeeding [1, 2]. Thus the intestinal microbiota as a defined entity does not exist, but this population comprises a dynamic mixture of microbes typical to each individual. At the moment, there are conflicting data on the microbiota of breast milk and this form of exposure needs to be reassessed [7].

Weaning and the introduction of solid foods as well as antimicrobial drug treatment periods will break the contact and constant supply of oligosaccharides and microbes from the mother. When characterizing the establishment of bacterial communities in 2 healthy babies for the first 10 months of life by several molecular methods, the following was reported. After delivery, the sterile gastrointestinal tract of an infant was rapidly colonized. During the first few days of life the colonization profiles were simple, but they became more complex as the bacterial diversity increased with time. Clone libraries of amplified 16S rDNA fragments allowed identification of the bacterial types by comparative DNA sequence analysis; the bacteria identified included members of the genera *Bifidobacterium, Ruminococcus, Enterococcus, Clostridium,* and *Enterobacter*. The species most closely related to the genera *Bifidobacterium* and *Ruminococcus* in particular dominated the intestinal microbiota based on stability over time and the numbers [8]. Deviations in intestinal microbiota during early life may predispose the infant to diseases later [9–12].

Thus, the basic target remains in a complex microbial community that provides the barrier against foreign microbes [1, 2, 4]. Additionally, this process creates the basis for the establishment of a 'non-inflammatory' status

of the gut [4]. Such an environment in infants is distinguished by a large gram-positive bacterial population with a significant number of bifidobacteria in a species composition typical to the healthy infant (mainly *B. longum, B. breve* and *B. infantis*). Lactic acid bacteria may play a role in providing the right conditions for bifidobacteria to dominate. The collective composition of the colonizing strains in infancy also provides the basis for healthy gut microbiota later in life as the development of the disease-free state of the gut lies in the host–microbe interaction in infancy.

Gut Microbiota in Infants from 6 to 24 Months

Microbiota

Following the first 6 months of life the microbiota succession diverts towards a more diverse community [2, 8]. After weaning the differences observed between breastfed and formula-fed infants disappear due to the increase in the numbers of enterococci, *Bacteroides, Clostridium* and anaerobic cocci in the former group [6]. Increases in *Escherichia coli*, and enterococci have been reported after weaning. The levels of bacteroides and anaerobic gram-positive cocci [13] also appear to increase gradually during and following weaning, whereas enterobacteria decreased [8]. A recent pilot study by Rinne et al. [14] on 6-month-old infants and their fecal microbiota indicates that breastfed infants have high bifidobacterium levels and lower clostridial numbers than infants receiving either formula or formula with prebiotics. Adding probiotics to breast milk appears to reduce bacteroides and raise clostridia.

A small study reports the microbiota follow-up of 2 infants for a period of 2 years using molecular methods. At 6 months the T-RFLP profiles were dominated by *Bacteroides* and *Clostridium.* Between 6 and 12 months more species appeared in the feces of the infants, this increase in bacterial diversity has been reported by different studies. At 1 year there was a new shift in the microbiota and it became more diverse with *Bacteroides*, *Vellionella* and *Fusobacterium prausnitzii* increasing. The microbiota begins to resemble that of adults and there is a decrease in facultative anaerobes although these microorganisms still remain at higher levels than in adults [8]. Latter there may be a decrease in the levels of clostridia with a concomitant increase in a more diverse anaerobic microbiota including microorganisms such as fusobacteria and eubacteria [15]. Contrary to that, other studies [16] reported that in 10- to 18-month-old infants bifidobacteria predominates followed by *Bacteroides*, enterobacteria and enterococci.

At 2 years the microbiota resembles that of adults [13]. However, it has been reported that children (16 months to 7 years) still harbor higher levels of bifidobacteria and enterobacteria than adults [15, 17]. A compilation of data in figure 2 describes some of the microbiota changes between 6 months and 2 years of age.

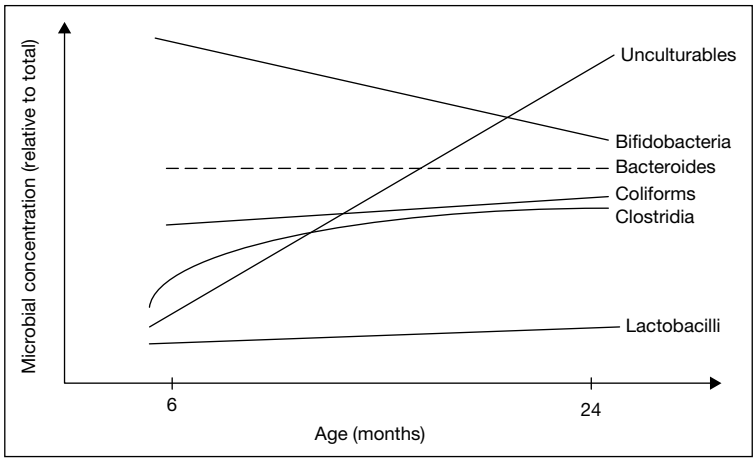

Fig. 2. Relative changes in gut microbiota composition suggested by culture-dependent and culture-independent studies.

Characteristics of the Microbiota

The succession and development of microbiota may also influence other parameters related to health. Before weaning there are differences between breastfed and formula-fed infants in the ability to ferment complex carbohydrates, being higher in formula-fed infants probably due to the presence of a more complex microbiota. Following weaning these differences disappear due to an increase in the ability of microbiota to ferment such carbohydrates in the breastfed [18]. In addition, in breastfed infants the establishment of a mucin-degrading microbiota starts later, but in both groups there is an increase in such activity between 6 and 9 months [19]. Also the conversion of cholesterol to coprostanol is initiated during the second half of the first year and it is likely to be dependent on the development of microbiota [20].

Ammonia and phenol concentrations in feces as well as β-glucosidase and β-glucuronidase activities increase after weaning, and even when higher ammonia content and β-glucuronidase activity were found in formula-fed infants, these differences disappear [21].

Creating Mature Microbiota

Following weaning the healthy microbiota, identified as the normal microbiota of an individual that both preserves and promotes well-being and absence of disease especially in the gastrointestinal tract, will gradually be created. Small differences can be reflected in later life and health as shown by studies on microbiota deviations [9, 12, 22].

In the gastrointestinal tract there is a constant challenge by diverse antigens such as microbial antigens, foods and allergens. Such priming of gut-associated lymphoid tissue is important for two opposing functions: mounting a response to pathogens, and maintaining hyporesponsiveness to innocuous antigens. An important question is how the inflammation is kept under control during weaning and how the microbiota is altered during the adaptive process. The strains of the healthy gut microbiota are likely to provide the host with an anti-inflammatory stimulus directing the host–microbe interaction towards a healthy gut [4, 5, 23].

Importantly, the host–microbe cross-talk during and after breastfeeding seems optimal for this target. At this stage the bifidobacteria-dominated environment may provide the child more anti-inflammatory stimuli than bacteria from adults which have been shown to be more proinflammatory [23].

Components of the human intestinal microbiota or organisms entering the intestine may have harmful or beneficial effects on human health, but a complex and diverse community is required for the individual balance. Abundant evidence exists to document that specific strains of the healthy gut microbiota exhibit powerful anti-pathogenic and anti-inflammatory capabilities, and are consequently involved in enhanced colonization resistance in the intestine [24, 25].

Maintenance of the Individually Optimized Healthy Microbiota

Creating a healthy gut microbiota during early life must be followed by proper maintenance and enhancement of the individual balance. During times of disease or following detectable deviations in the initial microbiota development, later maintenance can be achieved by directing the gut microbiota into healthy balance by dietary means, for instance by using probiotics or prebiotics. Probiotics are defined as viable microbes which, through oral administration, produce health benefits to the host [24]. Probiotics are members of the healthy gut microbiota and assist in mimicking the healthy microbiota of both a breastfed infant and healthy infant. Prebiotics act through promotion of specific microbes with the potential to maintain health. The prerequisite of this activity is that such strains are already available for the stimulation in the gut. Each bacterial strain and prebiotic substance has its specific effects which have to be evaluated prior to application.

In a like manner, prebiotic oligosaccharides have different microbiota-modifying properties [24]. Probiotics introduce new microbes to the gastrointestinal tract to enhance microbiota maintenance and modification while most prebiotic components have been shown to enhance the *Bifidobacterium* microbiota, which should be defined more clearly. First, the bifidogenic change alone is not a prebiotic effect. Second, the desired *Bifidobacterium* strains should be present in the infant gut for the prebiotic

Table 1. Characteristics of infant microbiota: deviations related to atopic diseases

Subject group	Before weaning (age 5–6 months)	After weaning (age 6–9 months)
Highly sensitized infants, no supplementation	High concentration of *Bacteroides* and *Lactobacillus/ Enterococcus*	Increased *Bacteroides* and *E coli* numbers
Sensitized infants	Lower levels of *Lactobacillu/ Enterococcus*	Lower levels of *Lactobacillus/ Enterococcus*
Highly sensitized infants with bifidobacterial supplementation	High concentration of *Bacteroides* and *Lactobacillus/ Enterococcus*	Decreased *Bacteroides* and *E. coli* levels, stabilization of microbiota
	Microbiota at 12 months	Microbiota at 24 months
Wheezing infants	High *Clostridium*	No data
Allergic infants	At 6 months lower bifidobacteria and higher clostridia	Less lactobacilli, high numbers of aerobic bacteria, high coliforms, higher *Staphylococcus aureus* counts
Normal infants	At 6 months high bifidobacteria, low clostridia	High numbers of aerobic bacteria

effect. Third, a clinical benefit has to be documented before a prebiotic effect can be verified.

It has been reported that some fructo-oligosaccharides enhance the levels of unknown microbes in human gut thus potentially facilitating untoward effects. Galacto-oligosaccharides in general are found in breast milk in a great variety, and they have bifidogenic effects and a health-promoting impact on the infant gut. Other than breast milk oligosaccharides, more specific prebiotics with know microbiota and health effects should be developed. Perhaps, as indicated by the mother–infant relationship of offering both microbes and oligosaccharides for the newborn infant, carefully designed combinations of probiotics and prebiotics may offer optimal means for creating and maintaining a healthy microbiota [25].

Conclusion

The healthy human microbiota is metabolically active and acts as a defense mechanism for our body. Deviations in its composition are related to multiple disease states within the intestine but also beyond the gastrointestinal tract. Examples of such deviations are given in table 1. Components of the human

intestinal microbiota or organisms entering the intestine may have both harmful or beneficial effects on human 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 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 and after weaning and during the first years of life. Another target is to understand the use of specific probiotics and prebiotics to influence microbiota development and maintenance as well as dietary management of reported health-related microbiota deviations.

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Discussion

Dr. H. Hoekstra: Thank you for this wonderful presentation. You talked about windows of opportunity for colonization. I suggest that we split up the discussion on development of the normal microbiota in the first place and then talk about possible interventions. Recently Martin et al. [1] published an article on lactic acid bacteria with probiotic properties present in human milk and on the mammary areola. Could you comment on that study?

Dr. Salminen: I have actually tried to look at that question, and when reading the article itself and looking at the isolation procedures, my first thought is that it is probably skin bacteria. However, there is a Spanish group that has hypothesized that some bacteria might be translocating from the gut to be excreted in breast milk, but I don't have any convincing evidence on that myself. So until I am proven wrong in this, I still consider them as bacteria originating from the skin. I have great difficulties in seeing how the bacteria would be excreted by the mammary gland itself.

Dr. Kleinman: You mentioned how important it is to have a balance of bacteria in the intestine and most of the focus has been on the beneficial effects of the bifidobacteria, but are there any beneficial effects from the so-called detrimental bacteria. Why are they present?

Dr. Salminen: I find it very difficult to attribute the beneficial effect to specific bacteria because even if we introduce a probiotic into the gastrointestinal tract we change the community in the intestinal contents, or perhaps not the community but the metabolic activity of the community. We may not be changing the community at the mucosal level. The other factor that I consider really difficult in research today is that most of our data are based on fecal studies, fecal recovery of probiotic bacteria, fecal concentrations of different bacteria, we really don't know what exactly is happening in the upper parts of the intestine. So I don't find it possible yet to attribute the

health effects or the harmful effects to certain groups of bacteria. Rather we could perhaps identify some bacterial groups or members of the microbiota as biomarkers of changes.

Dr. Kleinman: I am thinking about some of the work, which may have now been discredited, in which there is cross-reactivity for potential pathogens like influenza, for example, and *Escherichia coli*. Has that progressed, do we understand that a bit better now?

Dr. Salminen: Yes, that is a good point. It will certainly provide us more information also from the upper intestinal tract.

Dr. Rijntjes: I have a question concerning colonization in the atopic mother. Is there any difference in colonization if the mother is or is not atopic, or if she has a food allergy or atopic dermatitis?

Dr. Salminen: That is an excellent question that I hope to be able to answer in a year's time. Together with our Japanese colleagues we are actually analyzing that type of set up, how the microbiota is transferred, but I don't have any information available yet unfortunately. Certainly this is an intriguing question since there are genetic and environmental factors together.

Dr. El-Din Amry: Based on the available data, can we define the character of the infant with ideal microflora, the character of the mother, her feeding, mode of delivery, and so on?

Dr. Salminen: That is also a very good question. A couple of years ago, some of you may have even been involved, the European Union funded a 3-year program trying to identify what is average healthy European gut microbiota. I think an interesting result was that there is no such thing as healthy European gut microbiota because it varies so much and we have to look at the individual circumstances. If you really consider nature, the mother has already somehow adapted to the local conditions and is probably trying to transfer protection (against the threats that are in the immediate surroundings) by bacteria transferred at birth, and also by perhaps the specific antibodies in breast milk and the composition of the breast milk. So I don't think we can really identify a sort of common healthy gut microbiota for infants, not to speak about adults, it depends so much on the local conditions.

Dr. M. Hoekstra: When we study colonization of the intestine, we often take stool samples. To what extent does a stool sample reflect what is happening in different parts of the intestine?

Dr. Salminen: Another excellent question to look at the real problem as I see it. Microbiologists have studied stool samples, as discussed earlier; the fecal recovery of different microbes that perhaps reflects the very last part of the colon but not much else. There are recent studies from probiotic interventions but if we look at the oral intake of probiotics in infants, let me say more so in adults, fecal recovery usually disappears within 1–2 weeks. However, if we take biopsies, which is unfortunately most often not possible in infants, from adult volunteers, we can see material in the colon, even in the upper part of the colon. The mucosal biopsies contain large amounts of probiotic bacteria up to 3 months after any signs of fecal recovery. So fecal counts or fecal recovery do not really reflect what is important. But that is a personal opinion and some of you might challenge me on that. I am certain that we need new ways of sampling the upper part of the intestine.

Dr. M. Hoekstra: But I can imagine that you can do animal experiments and catch the stools, and on the other hand take samples from the intestine and see whether they match or not?

Dr. Salminen: You can do animal experiments, yes I agree, especially the very elegant one that was presented by the St Louis group. Genetically defined germ-free animals were chosen for the study. It is also very challenging because the animals were

given human microbiota, and then questions were asked such as which humans, from where. We could probably learn the mechanism, but it is a great challenge for us to presently understand the meaning of the study on microbiota actually providing a reservoir assimilating quick storage of fat, but how it varies in different geographic areas, perhaps different nutritional environments, is still completely unknown.

Dr. Aggett: It strikes me though that this model from St Louis really is describing what we understand by colonic salvage. The fat that is being deposited, is that being deposited systemically?

Dr. Salminen: Actually from the study we learned that the microbes that act in the normal microbiota are the ones that rapidly make the carbohydrate part of the diet utilizable, and it was seen as histological differences in the liver and also accumulating fat storage.

Dr. Aggett: But did they look for fatty acid production?

Dr. Salminen: This was the first published experiment describing such a setting and I assume that they did.

Dr. Aggett: And going on from that, would you say something about the proteolytic activity of the colonic bacteria and the relative metabolic growth in that case, and how they may contribute perhaps to colonic salvage and other metabolic phenomena? Additionally do you think that it is possible that such microbial activity could have adverse effects as well as beneficial effects as a result of the systemic absorption?

Dr. Salminen: If I start from the last one, yes I think, as I tried to show in the last slide, we need to know more about the succession of microbes, especially after weaning, because that is where most of us have stopped at the moment. We need to know more about what happens in the upper intestinal tract and by understanding the situation a little bit more carefully it will be possible. I am quite sure there is a possibility of adverse effects and so on. Nature has taken a long time in creating the microbiota as it was perhaps before the war, and we have very rapidly introduced new ways of changing the microbiota by food processing, by sterilizing most of the foods or pasteurizing, or by UHT treatment and by diminishing general microbial exposure. Thus if we had the possibility of comparing intestinal samples from let's say 100 years ago and now, the diversity would be greater in the old samples. We are doing our best to influence the diversity and to kill all bacteria, and that probably has the greatest influence. Why do we do this? I think there is an excellent example from an American study. It is not comparable but the idea is that if you have cheese made from very clean pasteurized sterilized milk and compare it to the same cheese made from natural milk, the one made from the clean ingredients, sterile ingredients, spoils much faster and probably it is the competition of the microbes in the cheese. Again the diversity is smaller whereas in natural cheese it is greater, and somehow that creates an environment that is more stable. If you have seen the study, similar things could be considered also when we look at intestinal microbiota.

Dr. Waterland: In your list of factors influencing the gut microbiota in infants, I don't think you mentioned antibiotics.

Dr. Salminen: That is one way of trying to diminish the diversity. That is certainly one of the great impact phases, especially during early childhood, if we look at the use of antibiotics. Even looking at fecal recovery it takes 2–3 months to get back to the normal situation in the microbiota and probably longer, and we may be reintroducing the next antibiotic before the changes have returned to normal.

Dr. Michaelsen: I think that the data on the difference in microbiota and the risk of allergy between infants born by cesarean delivery and vaginal delivery are very fascinating. Don't you think we know enough to try to do something to help the infant that is born by cesarean section? Shouldn't we think about a transplant of the maternal microbiota?

Dr. Salminen: I think there are far too few studies available, but as we also have to look at the long-term effects of whatever intervention is done, we can probably point in the right direction. But I don't know what would be a good way.

Dr. Michaelsen: There are a few practical ways to do it.

Dr. Salminen: It would be interesting if there are ways of introducing the cesarean born to the mother's microbiota. It might not be a bad idea, as a matter of fact that is the approach used for chickens; commercial chickens are introduced to their parents.

Dr. Michaelsen: I think it would be reasonable to do it to all infants delivered by cesarean section as well.

Dr. Salminen: Certainly in chickens it has proven to be an effective way of reducing the risk of infections.

Dr. Hardiono Djoened: Is it better to give probiotics as a food supplement or to give it as an infant formula? How do we know whether the dose is right or the type is right?

Dr. Salminen: That is also a good question. Do we know that the dose is right if we use probiotics for instance in infant formula? It is very difficult to estimate the right dose. Today what we use for those estimates is fecal recovery. How much do you need to actually introduce enough into the intestinal tract to provide reasonable fecal recovery which would, in terms of bifidobacteria, be comparable to something that you would see in the normally born infant during the same time? Based on those estimations and very few dose-response studies, I think those have set the basis for the doses used in infant formula today. Of course at the moment we have certainly selected the strains that are the safest possible ones, which have the longest history of use also in other types of foods, trying to be sure that no long-term harmful effects are there. There are a lot of candidate probiotics which have not been introduced because we don't know enough about the long-term effects.

Dr. Bee Wah Lee: If we believe that balance and diversity is important, how does giving a single species of probiotic help in the prevention and treatment of allergic disease or infection?

Dr. Salminen: That is a very important thing to consider. I think there are two totally different options. If we think about all of us here in the room, it would be very difficult indeed to have a single probiotic that would actually change anything in us. Our diversity and complexity is already so well established that something more would probably be needed. But when one considers an infant of less than 1 year of age, maybe less than 6 months old, today we are actually giving them either lactic acid bacteria, which have been shown to specifically promote bifidobacterial microbiota in the intestine, or we give them the same types of bifidobacteria that are part of a normal microbiota. So if the early phase of life actually indicates that we have 60–90% of bifidobacteria, we are certainly promoting that part, but for later purposes we would probably need a combination or different probiotics.

Dr. H. Hoekstra: We haven't talked about the end products of fermentation. Most of the bacteria are involved in fermentation processes and produce different short-chain fatty acids. Do you have data on how to influence the type of short-chain fatty acids that will be formed? For instance, the newborn has a lactate-producing flora while the microbiota of an older child will produce butyrate. Can we influence these processes and would that be important?

Dr. Salminen: This is one of the hot topics of research today. We know that we can influence it to some degree, but rather than influencing that single component I think the issue has been whether we can change the metabolic activity in such a way that the total microbiota handles, let's say, proteins and carbohydrates in a different way. For instance there are ways of reducing the types of metabolic activity which are related to specific clostridia by giving probiotics, but again a community that could change the whole metabolic activity or the effect is very minor.

Dr. Paerregaard: I wanted to comment on the possibility of adverse effects of the probiotics. We were discussing that before, when dealing with inflammatory bowel disease, there are data to indicate that probiotics might be beneficial in mild ulcerative colitis and in pouchitis, but also that probiotics may not be efficacious in Crohn's disease. Actually in a large prophylactic study of lactobacillus GG the treatment group became worse than the placebo-treated group. Do you think this would most likely be due to the wrong strain being chosen; could other probiotic strains have been beneficial, or are there specific diseases that definitely should not be treated by probiotic intervention?

Dr. Salminen: It is a very complicated question to answer but I will try to tackle it the other way around. We have always tried to consider that if you can identify a deviation in the microbiota, this would relate to whatever disease state or aberrance you are talking about, then you can actually try to find the right strains, even the right species, for correcting that deviation. I would say that specific probiotics could be used for future purposes when we know that there is a problem in the microbiota, if the problem has been identified as much as possible, then try to find the strain or the species that counteracts the problem. Many of the clinical trials have been done that way by picking up something and trying, but without any further basis on the mechanistic side or on the gut microbiota side. I am sure that in this way we will get a lot of negative results, not necessarily due to the probiotic itself or the strain itself but rather by applying it to the wrong purpose at the wrong time perhaps. If we learn more about the microbiota we can also facilitate clinical trials in which a purpose is targeted in the microbiota and then we have the right selection of strains, because I have not seen a wonder strain. Every producer of probiotics likes to say that their strain is good for everything and that is certainly not true as most of you know.

Dr. Badr-Eldin: Would it be reasonable to use prebiotics rather than probiotics to try for example to enhance or increase the availability or bioavailability of certain probiotics? I mean using the prebiotics rather than the probiotics.

Dr. Salminen: There is a lot of debate today about probiotics and prebiotics and I am sure they have to be handled on a case-to-case basis. My gut feeling is that prebiotics worry me a little bit. We have clearly identified some of the problems in those children who later seem to get atopic diseases and have found biomarkers for them. If we do add a prebiotic to that population, we are not adding anything new, no new microbes, rather we are trying to enhance the microbes that are already there. Then I ask myself the question, are we actually enhancing the problem? Of course prebiotics can change the metabolic activity so that it does not necessarily do what I just described, but I think by introducing something which is familiar for the gastrointestinal tract but not existing at the moment and metabolically active, I would be more prone to look at the probiotic side but certainly on a case-to-case basis. In adults especially it is a totally different game because we might need them both to be able to change something that is established and stable.

Dr. Schmitz: Your lecture was planned at the start of this session because, in the idea of the organizers, there is some kind of link between the normal bacterial colonization of the gut and the diversification of foods, and the possibility that toddler's diarrhea might, in some cases, be due to an inadequacy between the food given and the way bacteria were handling it. In one of your graphs where the populations of bacteria are listed from 6 months to 2 years, the curves are very flat as if the big changes in the food ingested and, particularly, the introduction of vegetables and fibers didn't make any change here. How do you see the reaction of the colonic flora to this high input of fibers which are very special for the colon?

Dr. Salminen: Unfortunately I have to answer that most of the curves were flat but there was one that was growing and that was the unculturables, the unknowns, and

I am sure they do have an effect. There are two factors that should be taken into account. In studies by Dr. Isolauri on rotavirus diarrhea in daycare children, we actually identified that again there is a species composition difference in bifidobacteria. So there may be biomarkers for some subjects who are more prone to diarrheal diseases. But then we have to take into account the rapidly growing unculturable microbiota at that age.

Dr. H. Hoekstra: I think the last question of Dr. Schmitz is a very nice start for the next two presentations.

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Malabsorption of Carbohydrates

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Introduction

Carbohydrates are responsible for 25–50% of daily energy intake. The carbohydrate composition of the diet changes with age. In breast milk and standard infant formulas, lactose is the only or predominant carbohydrate; starches and other sugars follow with the introduction of 'beikost'. In the healthy, balanced diet of children and adults starches (and fiber) should prevail. The small bowel only absorbs monosaccharides, so dietary carbohydrates have to be hydrolyzed into their constituent monosaccharides glucose, galactose and fructose. Glucose and galactose are actively transported, while fructose absorption takes place through facilitated diffusion. Not all dietary carbohydrates can be hydrolyzed by human digestive enzymes. Unabsorbed carbohydrate enters the colon as a fuel for the gut microflora. While this is a physiologic process essential for normal colonic function, especially short-chain soluble carbohydrates may surpass the fermentative capacity of the colonic flora. Whether this leads to averse reactions depends on several factors connected to both the carbohydrates involved and local variables. There is no absolute separation between normal and decreased carbohydrate absorption and malabsorption does not necessarily imply intolerance.

Here we present the present insights into carbohydrate malabsorption and intolerance, and discuss the clinical consequences, with emphasis on the consequences for the feeding of infants and young children. Whenever relevant, we refer to the Online Mendelian Inheritance in Man (OMIM) nomenclature and the 6-digit denominators used in this database.

Digestion and Absorption of Carbohydrates

Polysaccharides

Starches consist of variable combinations of amylose and amylopectin chains and, depending on their origin, have variable physicochemical properties. Amylose molecules are straight chains of glucose units with α-1,4 bonds; amylopectin molecules have amylose backbones with additional α-1,6-linked side chains. In native form, starches are more or less inaccessible to amylase. Heating (cooking), acidification and physical degradation loosen the knots of starch granules and render them more accessible for enzymatic cleavage. Amylose digestion is primarily cared for by α-amylase. This acts only on interior α-1,4 bonds, releasing maltose (2 glucose units) and maltotriose (3 glucose units). Amylase only partly degrades amylopectin, leaving remnants of up to 6 glucose units with at least one α-1,6 bond: α-limit dextrins. Further degradation is provided by the brush border enzymes maltase-glucoamylase (short glucose polymers and starch α-1,4 bonds) and sucrase-isomaltase (oligosaccharide α-1,4 and α-1,6 bonds). Starches need processing (milling, cooking) to be digestible and part of the starch content of any given meal will resist digestion and enter the colon. This may have greater impact on colon function in infants and small children than in adults [1].

Oligosaccharides and Disaccharides

All carbohydrates have to be split into monosaccharides before they can be absorbed by the gut epithelium. At the brush border level, three enzyme complexes are available: the α-galactosidases, sucrase-isomaltase and maltase-glucoamylase; and the β-galactosidase, lactase (also called lactase-phlorizin hydrolase). Sucrase-isomaltase hydrolyzes sucrose as well as maltose, maltotriose and α-limit dextrins, while lactose is exclusively degraded by lactase. Some dietary disaccharides and oligosaccharides are not digested by any of these enzymes. These include fructans from the *Allium* species, raffinose and stachyose from beans, as well as the laxative lactulose. Interestingly, breast milk contains high concentrations (3–15 g/l) of non-absorbable carbohydrates. Over 100 different oligosaccharides are identified and, apart from favorably affecting fecal consistence, their main role is to establish a healthy colonic microflora by the suppression of potential pathogens. Recently, infant formula has been marketed containing indigestible fructo- and galacto-oligosaccharides, which should mimic the 'fiber' function of human oligosaccharides.

Lactase (OMIM 603202) hydrolyzes lactose and many other β-glycosidic molecules. It is a 1927-amino acid protein which is present in the brush border as a dimer [2]. Different from the α-glycosidases, its activity is low up to 30 weeks of gestation and peaks at term. Lactase activity remains high for the first 3–4 years of life and thereafter normally decreases: adults are left with 5–10% of newborn activity. This loss of lactase activity is caused by genetic

imprint: the lactase gene turns to low activity, and less lactase makes it to the brush border ('adult-type hypolactasia'). In this respect, mankind does not renounce its origin: in all mammals, lactase activity declines after weaning. In contrast, most Caucasians keep high (but slowly declining) lactase activities throughout their lives [1, 3]. The ability to digest lactose as an adult ('lactase persistence') is thought to be caused by a mutation which may stem back from about 10,000 years ago, at the dawn of dairying. Recent research suggests that the present distribution of lactase persistence in Europe is a reflection of gene-culture co-evolution between milk-producing cattle and humans [4]. Especially Asian and African populations have high (80–100%) percentages of adult-type hypolactasia; in Western Europe, hypolactasia is more prevalent in the Mediterranean area (~40%) than in Scandinavia (~5–10%) [5].

Monosaccharides

The absorption of glucose, galactose and fructose across the brush border and subsequently across the basolateral membrane into the blood requires three transporters. The aldohexoses, glucose and galactose, are co-transported with Na^+ from the intestinal lumen into the cytosol by SGLT1 (OMIM 182380), while GLUT5 (OMIM 601843) is responsible for the facilitated, Na^+-independent transport of the ketohexose fructose. Passive transport out of the enterocytes through the basolateral membrane is provided for by GLUT2 (OMIM 138160). Extensive discussion of the properties of these transporters can be found elsewhere [3, 6]. Fructose absorption is often incomplete and this is also the case with other monosaccharides (such as xylose) and polyols (such as xylitol and sorbitol) present in certain foods.

Indigestible Carbohydrates

'Dietary fiber' includes both carbohydrates and other plant-derived substances, such as lignin. For the purpose of this review, we concentrate on the nonstarch polysaccharide fibers, including soluble (cellulose and hemicellulose B) and insoluble (hemicellulose A, pectins, gums, and mucilages) fibers [7]. Their common feature is that they cannot be degraded by human enzymes, but they are partially or completely fermented by bacterial enzymes. Cellulose, for instance, consists of chains of glucose units coupled with β-1, 6 bonds, resistant to human glycolytic enzymes. Dietary fibers are joined in the colon by resistant starch, indigestible oligo- and polysaccharides, and the undigested and unabsorbed fraction of mono- and disaccharides.

Fate of Unabsorbed Carbohydrates

Colonic Microflora

The colon is inhabited by a bacterial mass that by far outnumbers the total cell count of the human body. Carbohydrates are their main fuel source.

About 30% of the colonic content consists of bacteria; feces contain between 10^{11} and 10^{12} bacteria/g, mostly anaerobes. Bacterial glycosidases degrade the available carbohydrates, resulting in the production of various products, including monosaccharides that are metabolized anaerobically to pyruvate, and lactate, to end up as gases (mainly carbon dioxide, methane and hydrogen) and short-chain fatty acids (SCFAs: acetate (52%), propionate (20%), butyrate (20%), and some branched-chain fatty acids) [8]. Under normal circumstances, about 90% of all SCFAs are absorbed. The bulk of the gases produced enter the circulation; carbon dioxide adds to the acid-base equilibrium and hydrogen and methane are expelled through the lungs. In short-bowel syndrome, bacterial shifts may lead to overproduction of *D*-lactate, which may result in encephalopathy and metabolic acidosis [9]. Presently, it is unclear if probiotics could play a role in improving carbohydrate tolerance by improving fermentation.

Colonic Salvage

The colonic mucosa absorbs SCFAs, which drag along water and electrolytes; monosaccharides stay behind in the fecal stream. SCFAs are subsequently utilized by mucosal cells (butyrate), liver (propionate and acetate) and other tissues (acetate). About 70% of the energy contained in unabsorbed carbohydrate is recovered through SCFAs. Butyrate accounts for 80% of the energy consumption of the colon; acetate may contribute up to 10% of the total energy expended in adults [10] and possibly considerably more in preterm infants [11]. This combined effort of microflora and colonocytes to save water and energy, reducing energy loss and fecal mass, has been dubbed colonic salvage [12]. Principally, this process is also active with carbohydrate malabsorption. Short-chain, soluble carbohydrates, such as lactose, however, increase small-intestinal transit time and therefore will more easily surpass the fermenting capacity of the microflora, thwarting the colonic salvage mechanism. On the other hand, the microflora as a whole is capable of adapting its metabolism to the types of carbohydrate supplied, increasing the salvage capacity [13, 14]. Consequently, the regular consumption of lactose in lactose malabsorption does not result in diarrhea, but rather in an increase in fecal (bacterial) mass [15]. Colonic salvage requires an established fecal microflora and a healthy colon of sufficient length. It may be less efficient in young infants and in toddler diarrhea, and it is impaired in antibiotic-associated diarrhea.

Role of Fecal Solids

Despite significant variation in the composition of the ileal contents that enter the colon and independent of total stool output, the water fraction of the feces of healthy individuals is kept within the narrow range of 70–75% [16]. The looseness of the feces in diarrhea is a function of fecal solid composition, the ratio of fecal water to water-holding capacity of insoluble

solids being increased [16]. Consequently, fat will increase the looseness of the stools, while fiber improves consistency. This may explain why the secondary lactose malabsorption found in enteropathy-associated diarrhea does not simply resolve with lactose exclusion from the diet. This also is relevant to toddler diarrhea: a low-starch, low-fiber diet reduces fecal water-holding capacity.

Role of Gut Motility

Rapid small intestinal transit results in less efficient absorption, perhaps the best example being the monosaccharide malabsorption that accompanies dumping syndrome [17]. In hypolactasia, the lactose digestion index (fraction of lactose absorbed) correlates with the orocecal transit time, but not with clinical symptoms [18]. A similar correlation exists in fructose absorption [19]. Conversely, carbohydrate malabsorption influences transit time in two ways. On the one hand, soluble carbohydrates that escape absorption will increase the osmolarity of the gut contents and thus speed up transit. On the other hand, SCFAs decrease gastric emptying through a hormonal pathway (the 'colonic break'), which may improve carbohydrate absorption [20].

Malabsorption versus Intolerance

'Carbohydrate malabsorption' and 'carbohydrate intolerance' should not be used interchangeably. Malabsorption only indicates the amount or the fraction that escapes absorption, intolerance points at the clinical symptoms that may result. (Although the EAACI recently introduced a new nomenclature for adverse reactions to foods, in which there is no place anymore for the term 'intolerance' [21], we prefer the established term 'lactose intolerance' to the proposed 'non-allergic hypersensitivity to lactose'.) When the supply of unabsorbed carbohydrates exceeds the fermentative capacity of the microflora, fermentation is incomplete, and not SCFAs but lactate and monosaccharides will prevail in the colon. This is a staged process and the end result depends not only on colonic salvage, bacterial adaptation and the type of solids present, but also on the type, the properties, the load and the rate of delivery of the carbohydrates involved. Mono-, di-, and oligosaccharides are rapidly but incompletely fermented, resulting in rapid accumulation of lactate and other small molecules and of gases. The osmotic load of these carbohydrates as well as the fermentation products is far greater than that of unabsorbed starches or fibers [22]. While fibers mainly increase fecal mass, oligosaccharides tend to increase gas production and water retention, and lactate and other breakdown products may irritate the bowel wall. This results in true carbohydrate intolerance, either as a combination of abdominal pain, distended abdomen, borborygmi and flatulence or as osmotic diarrhea, depending on the contributions of the individual factors. Figure 1 summarizes the events that accompany carbohydrate tolerance and intolerance.

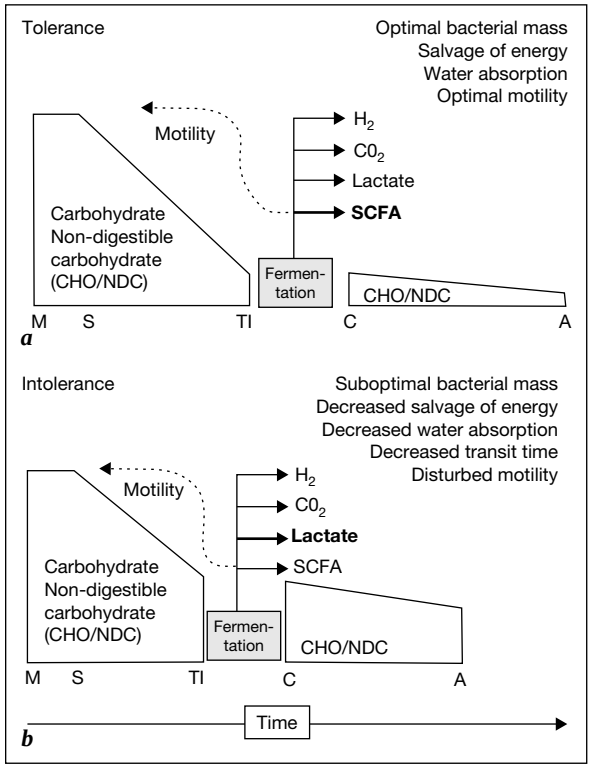

Fig. 1. Carbohydrate tolerance and intolerance. ***a*** Normally, digestible carbohydrates are absorbed in the small intestine; non-digestible carbohydrates entering the colon are efficiently fermented, with only small amounts remaining intraluminally. Efficient fermentation results in high formation of short-chain fatty acids with beneficial effects on small intestinal and colonic transit times. ***b*** In intolerant individuals, less efficient fermentation of malabsorbed carbohydrate results in shorter transit times and greater fecal loss of digestible and non-digestible carbohydrates. SCFA = Short-chain fatty acids; M = mouth; S = stomach; TI = terminal ileum; C = cecum; A = anus.

Diagnosis of Carbohydrate Malabsorption

Several techniques have been developed to study carbohydrate malabsorption in clinical practice, including assessment of small bowel disaccharidase activities and tests in which various effects of the administration of carbohydrate loads are monitored, including the breath hydrogen test and the $^{13}CO_2$ breath test. For research purposes, the lactose digestion index can be estimated accurately with a test combining ^{13}C-lactose and ^{2}H-glucose [23]. For clinical use, however, we would advocate simpler means of assessing malabsorption. In infants, because of the short colonic transit time,

determination of fecal-reducing substances and pH gives the most reliable insight into the presence of carbohydrate malabsorption. In older children evaluated for hypolactasia or sucrase malabsorption, breath hydrogen tests are simple to perform and sufficiently reliable. In contrast, the breath hydrogen test is not sufficiently discriminatory for use in the evaluation of secondary lactose absorption in suspected enteropathy, as the test outcome depends on too many variables [24], or in fructose absorption, as it is also positive in healthy children [25]. In any case, clinical symptoms following the sugar load should be evaluated as well, for breath hydrogen increase denominates malabsorption and not intolerance and thus may be irrelevant to the clinical condition of the child. Often, therefore, clinical evaluation using a test period with a lactose-reduced diet is adequate for the diagnosis of lactose intolerance in hypolactasia. When awareness of the presence or absence of lactose in the diet could produce bias, lactose provocation might be evaluated in a double-blind fashion.

Malabsorption of Glucose and Galactose

Glucose and galactose are absorbed very efficiently and clinically relevant malabsorption of these monosaccharides is uncommon. Even in acute viral gastroenteritis, SGLT1-mediated glucose transport is sufficiently preserved to enable successful oral rehydration with glucose-electrolyte mixtures. In young infants, pancreatic α-amylase and maltase-glucoamylase (OMIM 154360) activities are too low to allow the use of starches in infant formula, although generally straight-chain glucose polymers are tolerated well. Low glucoamylase activity, both as a primary and a secondary condition, was found in 15 of 511 children with chronic diarrhea [26] and in 12 of 44 children with dyspepsia [27]. The implications of these findings have yet to be established.

Glucose-Galactose Malabsorption (OMIM 606824)

This condition is caused by mutations in the sodium-glucose transporter SGLT1 and presents itself with severe diarrhea from the first week of life, resulting in dehydration and growth failure. It may be fatal if glucose and galactose are not removed from the diet. Intolerance to these sugars persists throughout life. Fructose absorption is normal. About 300 cases have been identified [28].

Secondary Monosaccharide Malabsorption

Glucose malabsorption secondary to acquired enteropathy is rare since modern insights enable adequate parenteral and enteral nutritional rehabilitation in all patients regardless of the underlying disorder. It may accompany what was called ‘intractable diarrhea of infancy’, a vicious circle of malabsorption and malnutrition, in most cases probably initiated by infection

or food-sensitive enteropathy. 'Intractable' diarrhea with monosaccharide malabsorption is nowadays mainly found in short bowel syndrome, disorders of enterocyte architecture, such as congenital microvillus atrophy and intestinal epithelial dysplasia, and immune disorders including autoimmune enteropathy [29].

Malabsorption of Fructose

Since the first demonstration of incomplete fructose absorption in children [25], this phenomenon has gained much attention. It has become clear that GLUT5 has limited capacity for fructose transport [6]. Glucose improves fructose absorption (explaining why sucrose malabsorption is rare), which has been shown to be the effect of solvent drag, as amino acids have the same ability [30]. There is much debate on the possible health consequences of fructose malabsorption, including increased energy losses in young children [31] and metabolic disturbances [32]; from a practical point of view, these seem to be limited to aggravation of toddlers' diarrhea, in which dietary imbalance is the central problem [33]. This extremely common phenomenon of incomplete absorption of fructose should be distinguished from a very rare condition, isolated fructose malabsorption, which is neither well defined nor caused by a mutation in GLUT5 [34].

Malabsorption of Sucrose

Disaccharide intolerance I (sucrase-isomaltase deficiency; OMIM 222900) results from mutations in the sucrase-isomaltase gene that result in five separate phenotypes [2]. Osmotic diarrhea starts the moment sucrose is introduced in the diet. On occasion even the presence of glucose polymers in infant feeding may cause diarrhea. Treatment consists of life-long exclusion of sucrose from the diet, if necessary combined with starch reduction. Recently, promising results have been booked with addition to the diet of sacrosidase, a liquid sucrase derived from the yeast *Saccharomyces cerevisiae* [35]. Secondary sucrose malabsorption, although less common than secondary lactose malabsorption, may be found in similar situations, especially in serious enteropathy and short bowel syndrome.

Malabsorption of Lactose in Infants

Lactose is the main carbohydrate source in the milk of virtually all mammals, and milk is the only dietary source of lactose. Human milk and humanized infant formula contain about 7 g/l of lactose; cow's milk about 5 g/l.

The average 3-month-old infant therefore consumes some 10 g lactose/kg/day; the dairy consumption recommendations for young children would imply the consumption of 15–20 g lactose/day. Healthy infants have enough lactase to ensure adequate digestion of the lactose present in breast milk or formula. Lactase activity is, however, dependent on the integrity of the small bowel mucosa and enteropathy is invariably associated with low lactase levels. In The Netherlands, until a few years ago, a lactose-reduced formula was available for infants with 'sensitive bowels'. The premise was that following acute gastroenteritis, the infant gut needed several weeks to recover and regain normal lactase levels. Nowadays, post-gastroenteritis enteropathy is considered the result of prolonged starvation due to too cautious refeeding; breastfeeding or standard formula should be continued during acute gastroenteritis.

Term and Preterm Infants

At birth, term neonates have higher lactase activities than at any moment before and after. They are optimally capable of digesting lactose. Preterm infants have low lactase levels, and seem to be not fully equipped for the consumption of human milk. Lactase activity increases rapidly after birth, however, and infants of less than 32 weeks gestation still digest at least 90% of the lactose consumed, while the lactose reaching the colon is efficiently salvaged and contributes to the energy accretion of the child [36]. Lactose intolerance is rare in this group and preterm infants should receive their mother's milk or humanized preterm milk as soon as they can tolerate enteral feeding.

Congenital Lactase Deficiency (OMIM 223000)

This is a very rare disorder, the vast majority of patients being identified in Finland. The symptoms are indistinguishable from those of glucose-galactose malabsorption [5]. Very recently a case of combined deficiency of lactase, maltase-glucoamylase and sucrase in an infant was published, possibly due to malfunction of some common regulatory factor [37]. Congenital lactase deficiency should be distinguished from congenital lactose intolerance (OMIM 150220), an even more unusual disorder (last reported in 1980) with vomiting, failure to thrive, dehydration, lactosuria, renal tubular acidosis, aminoaciduria, liver damage and cataract [38]. It is thought to result from increased gastric absorption of lactose. Although the disorder may be fatal if unrecognized, a lactose-free diet results in rapid recovery and after 6 months of age lactose is well tolerated.

Secondary Lactose Malabsorption

Secondary lactose malabsorption due to impaired lactase activity has been linked to poor growth in underprivileged children, possibly due to prolonged gastroenteritis-associated villus atrophy. It has also been suggested in several

studies (and refuted in others) that infantile colic is associated with transient lactose intolerance [39]. The etiology underlying this phenomenon is unclear. There is more proof for cow's milk protein intolerance as a cause of colic [40], but the results of these studies may have been biased by the fact that cow's milk protein-free formula is reduced in lactose as well (although oral therapy with lactase is not effective) [40]. Conversely, there seems to be no reason to exclude lactose from the feeding of cow's milk-allergic children, even though lactose might theoretically be contaminated with cow's milk proteins [41]. Lactose-reduced or lactose-free oligomeric or monomeric formula may be sporadically indicated in infants with 'intractable' diarrhea due to food protein-induced enterocolitis syndrome.

Malabsorption of Lactose in Older Children and Adults

Adult-Type Hypolactasia (OMIM 223100)

Although hypolactasia is actually the normal situation, it has certain disadvantages. The mutation resulting in lactase persistence enabled man to utilize cattle milk as an extra source of energy, increasing the chance of survival in times of famine. At present, cow's milk has become a regular constituent of the Western diet; it is considered pivotal in the supply of calcium and vitamin B_2 and therefore plays an essential role in the prevention of osteoporosis. Numerous studies have focused on the health consequences of hypolactasia.

Not only the lactose digestion index, but also lactose tolerance is shown to vary considerably in between malabsorbers and also between adults and children. Small amounts of lactose are invariably tolerated by all adult malabsorbers [39], many of them tolerating normal amounts, while children in general seem to be more tolerant than adults [41]. Although lactase activity may start declining significantly in 3- to 4-year-olds, adverse effects will not reveal themselves for several years.

Clinical Consequences of Hypolactasia

The transition from lactose tolerance to intolerance is determined by several factors. The amount of ingested lactose that reaches the colon is influenced by the type of lactose-containing food; whether or not it is consumed with a meal; the remaining lactase activity; inter-current small bowel disease, and small bowel transit time. The efficacy of colonic salvage determines whether lactose is completely fermented or partly escapes fermentation. Only in the latter case, can symptoms of lactose intolerance ensue.

All too often, the diagnosis of hypolactasia is followed by the recommendation of considerably lactose-reduced or even lactose-free diets. Most individuals will tolerate considerable amounts of lactose. In adults, a single 6-gram dose of lactose is not followed by symptoms. Also, a microflora that is

exposed to a steady supply of lactose will adapt and increase its lactose-fermenting capacity, increasing tolerance [42]. Because of its health implications, lactose restriction should be applied with reticence, especially in children. Several simple measures may reduce the chance of symptoms after dairy consumption, including preferably using full-fat milk, in smaller portions and combined with a meal, partly replacing it by yogurt, and pre-incubating lactose-containing beverages with microbial β-galactosidases [42, 43].

Secondary Lactose Malabsorption

Although also in older children, the vulnerability of the brush border and especially lactase causes a rapid decline in lactase activity when the small bowel wall is damaged due to gastroenteritis, the extent of the damage is seldom large enough to give rise to clinically relevant lactose malabsorption. Dietary lactose restriction, therefore, is not indicated in toddlers and older children with acute diarrhea. To a lesser extent, the same holds true for enteropathy. Dietary restrictions, therefore, have to be dictated first and foremost by the underlying cause (e.g., food allergy or celiac disease) and lactose reduction is seldom indicated.

Conclusions

There is a tendency to overrate the impact of carbohydrate malabsorption on physical health. The main issue is that malabsorption does not necessarily imply intolerance. Colonic salvage enables the body to retain most of the energy contained in unabsorbed carbohydrates and prevents diarrhea. Only a few conditions, mostly presenting early in life, require targeted dietary measures. Secondary lactose malabsorption is generally a transitory problem and seldom necessitates dietary lactose reduction. Adult-type hypolactasia is not a problem until school age and can often be managed by simple measures. Further research should focus on the mechanisms regulating colonic salvage and the possible role of probiotics and prebiotics in this field [44].

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Discussion

Dr. Gracey: I was interested that you didn't actually mention sucrase isomaltase deficiency by a name; I suppose you meant that it was covered by the rare syndromes in your last slide. The comment I would like to make is that, in ethnic groups with high rates of hypolactasia and very high rates of malnutrition and gastrointestinal disease, clinical lactose intolerance is a very significant problem.

Dr. Kneepkens: I fully agree as far as sucrase-isomaltase deficiency is concerned. As I was asked to concentrate on lactose and fructose, however, I left that out. As far as lactose malabsorption in underprivileged groups is concerned, there are a few studies addressing this problem in detail. On the one hand there is a study from The Gambia showing that breastfed infants have more lactose absorption problems due to infections and undernourishment [1]. On the other hand there is a study in older Tswana children from South Africa showing that all children with lactose malabsorption by nature can still tolerate lactose quite well [2]. They have more fecal production, but no symptoms other than that. So I guess it depends on age and on health whether or not lactose malabsorption is a problem.

Dr. H. Hoekstra: What would the problem of lactose intolerance in your population be: is it the lactase, is it the enteropathy, or is it a failure of normal physiological processes at the level of the colon?

Dr. Gracey: It is probably a combination of all. I am thinking particularly of a lot of studies done in Australia on aboriginal infants and children who have very high rates of malnutrition, who have environmental or tropical enteropathy and very high rates

of gastrointestinal infections and parasitic infestations. So you cannot simply disentangle one from the other. But this is a common problem in many parts of the tropics.

Dr. Kleinman: A number of gene polymorphisms are now associated with persistent lactasia and others with hypolactasia. Do you see any role for polymerase chain reaction, for example, in establishing lactose intolerance rather than using the breath test in children who are otherwise healthy?

Dr. Kneepkens: I really don't know. One slide I didn't show you presented the number of breath tests performed in our hospital, with a peak in the late 1980s of about 200/year, and it is now down to some 10/year. So we don't use the breath test anymore in clinical practice because we don't think we learn a lot from it. Most of the time the problem can be defined by just having a good history and physical; give advice to the children and wait how it turns out.

Dr. Schmitz: I was very much interested by the beginning of your talk regarding the bacterial metabolism in the colon and when you said that at the beginning the infant is more a lactate producer than an older child, which means that there is some kind of metabolic adaptation during development. Do you think, then, that the beikost or the solid foods, which are introduced during the normal feeding process between 6 and 18 months for example, always meet a competent microflora so that these additional foods are always very well metabolized or fermented, or do you think that sometimes there is incompetence of this colonic flora which makes the diversification of food problematic?

Dr. Kneepkens: Probably the flora has to learn to ferment the carbohydrates that are offered. That may be the reason why we have to be careful with the introduction of new foods to the children, giving the flora time to adapt. It is a matter of selection of the strains that are already present. While one strain is capable of fermenting a certain carbohydrate, another is not; so when you give this fermenting strain the time it will grow and do its work.

Dr. Schmitz: The question is whether it is one strain or a metabolic adaptation of a given strain. I don't think there are much data on this point.

Dr. H. Hoekstra: It could be that motility is another important factor for the establishment of a certain flora and, even more interesting, whether a specific microbiota can influence motility.

Dr. Benninga: I have a question regarding motility. You showed that carbohydrates can cause diarrhea; but last year a paper came out suggesting that carbohydrate intolerance and constipation do play a role [3]. Could you speculate on how this works and if it exists?

Dr. Kneepkens: It is very difficult to envisage but the most important thing to realize in this respect is that carbohydrate malabsorption does not imply diarrhea. Of course it may lead to diarrhea, but it may as well lead to better fermentation and that may be the explanation, I couldn't tell.

Dr. Benninga: In your practice have you seen many patients with cow's milk allergy and constipation?

Dr. Kneepkens: Not one. But to come back to your previous question. At the time we were doing a lot of lactose breath tests, I remember that, while testing a child for abdominal pain, we found both constipation and lactose malabsorption. In those cases it is always difficult to choose between the therapies: on the one hand you would suggest decreasing lactose consumption, and on the other hand lactulose was the treatment for constipation, but it just doesn't seem logical to do both at the same time. Perhaps a reduction in lactose intake would have been enough.

Dr. H. Hoekstra: May I add one comment on how to improve fermentation and the production of short-chain fatty acids in order to treat diarrhea. There was a study on cholera patients by Ramakrishna et al. [4] showing some effect with amylase-resistant

starch. With the European Working Group on Intestinal Infections we tried to repeat this kind of study using a prebiotic mixture of non-digestible carbohydrates with oral rehydration solution in non-cholera diarrhea, but it was ineffective [5].

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Chronic Nonspecific Diarrhea of Childhood

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Introduction

Over the past half century, the entity known as chronic nonspecific diarrhea of childhood or toddler's diarrhea, has followed a path from case descriptions to disease and finally, within the past 10 years, to a defined functional disorder. Chronic nonspecific diarrhea of childhood was originally thought to be part of the 'celiac syndrome'. As Davidson and Wasserman [1] noted in their seminal paper published in the *Journal of Pediatrics* in December of 1966, the pioneering pediatric gastroenterologists of the time, had defined a number of specific disorders within what was then called the 'celiac syndrome', including gluten-induced enteropathy, disaccharidase deficiencies, lympangiectasia and abetalipoproteinemia. Chronic nonspecific diarrhea was the general term within that syndrome given to those children with persistent diarrhea where no cause could be identified. Anderson [2] felt that chronic nonspecific diarrhea was a result of starch intolerance and advocated a high-protein, low-fat, low-starch diet. Davidson and Bauer [3] and Prugh and Shwachman [4] showed that starch ingestion was not the culprit and in 1956 Cohlan [5] first used the term chronic nonspecific diarrhea.

Ten years later Davidson and Wasserman [1], in a retrospective examination of the case histories of 186 children, provided a description of this entity, which remains accepted even today, 50 years later. While a strict definition of diarrhea was not provided in this paper, these children were selected because of persistent or recurring episodes of loose stools without an identifiable cause, including cystic fibrosis, identifiable pathogens, celiac disease and other enteropathies. In more than 75% of the children diarrhea began between 6 and 20 months of age, although in 12% the onset was between

birth and 5 months of age. Eighty-eight percent had cleared the diarrhea by 39 months of age, with another 10% clearing it by 48 months of age. The character of the stool was fairly consistent for the majority of children in that the first stool of the day was large and partly formed. Later bowel movements during the day were smaller and looser. Mucous in the stool was reported in 87% of the patients. When blood was present it was always associated with fissures or excoriation of the perianal skin. Every patient was growing and gaining weight normally according to reference standards of the time and functional bowel disorders, i.e. 'irritable bowel syndrome', were very common among the parents and families of these patients. Five to forty-seven percent of these patients who were treated with specific agents such as kaolin/pectin, bismuth and various antibiotics had a clear, positive response to these interventions. Perhaps most importantly, 80% improved when a full and normal diet for age was instituted.

In 1999 a working team published a set of definitions for childhood functional gastrointestinal disorders following on the Rome criteria published for adults [6]. Functional diarrhea, which the team recognized was also known as toddler's diarrhea, chronic nonspecific diarrhea and irritable colon of childhood, was defined by daily painless passage of 3 or more large unformed stools for more than 4 weeks, with an onset of between 6 and 36 months of age, and passage of stools during waking hours in children who were thriving on an adequate calorie intake. The working team emphasized the importance of avoiding restrictive diets, the fact that children recover spontaneously and that the most effective treatment is reassurance for the parents.

Etiology

Dietary fat intake was shown to play a role in a significant number of children with chronic nonspecific diarrhea. In 1979 Cohen et al. [7] reported 5 patients with the onset of chronic nonspecific diarrhea that coincided with efforts to restrict fat in the children's diets in an attempt to protect against the occurrence of coronary vascular disease in later life. By and large these patients had been placed on skim milk and the diets contained ≤27% of total calories derived from fat. In addition, several of these children were ingesting large amounts of fruit juice in the diet. When the fat in the diet was increased to between 35 and 50% of total calories, the diarrhea symptoms resolved in all 5 of these children. Cohen et al. [8] extended these observations in a subsequent report later in that same year in which they showed that increasing the fat content of the diet resulted in the resolution of diarrhea in 82% of another group of children with chronic nonspecific diarrhea. They could not account for the lack of response to this dietary change in the remaining 18% of patients in this retrospective study but felt that the carbohydrate, fiber and calorie content of the diet did not play as important a role as fat intake.

Stool analysis has not been particularly helpful in determining the etiology of chronic nonspecific diarrhea of childhood. Davidson and Wasserman [1] observed the presence of vegetable fibers, starch and fat droplets upon microscopic stool examination in a high percentage of their reported patients. Subsequently Jonas and Diver-Haber [9] suggested that the extractable water phase of stools was appreciably increased in patients with chronic nonspecific diarrhea and that this water phase contained 50% of the total stool bile acids. While their 7 study subjects had normal stool weights when compared to controls (between 5 and 10 g/kg/24 h), stool electrolyte and bile acid concentrations were moderately increased. These findings were similar to a third group of patients studied with bacterial overgrowth syndrome. The authors suggested that children with chronic nonspecific diarrhea have an induced secretory state in the large bowel as a result of bile acids entering the colon, leading to the production of loose watery stools. The validity of these findings has not been confirmed. It should be noted that those patients in the group with chronic nonspecific diarrhea were between 18 months and 12 years of age and, of the 7 children, 5 had persistent abdominal pain. Furthermore the control subjects were between 8 and 15 years of age. Neither of these groups, therefore, may truly represent the typical infant or child with or without chronic nonspecific diarrhea.

Disordered intestinal motility has also been suggested as the basis of toddler's diarrhea. Intraduodenal dextrose infusion failed to disrupt the migrating motor complex in any of 8 children studied with toddler's diarrhea [10]. However children with chronic nonspecific diarrhea do not malabsorb, as is often the case for those with severe motility disorders, and the ages of the children studied were between 3.7 and 11.5 years, an age range much more typical for irritable colon than for toddler's diarrhea.

A number of authors have suggested that the excessive consumption of beverages containing high concentrations of various sugars contributes to or causes chronic nonspecific diarrhea. By the early 1990s it was reported that children younger than 12 years of age consume 28% of all juice and juice drinks in the United States, in spite of constituting only 18% of the population. National surveys in the United States have shown that almost 90% of infants consume juice and 10% of children 2–3 years old drink more than 355 ml/day [11]. The principle carbohydrates of fruit juices include sucrose, fructose, glucose and sorbitol. Maximum absorption of the sugars, glucose and fructose, occurs when they are present in equimolar concentrations [12]. When the fructose concentration in a beverage exceeds the concentration of glucose, as it does in apple and pear juice compared to white grape juice, then more malabsorption occurs [13]. Most importantly, however, the sugars from all of these juices are equally well absorbed if an excessive amount of juice is not consumed (i.e. $\leq$10 ml/kg body weight) [14]. The consequence of carbohydrate malabsorption is the production of hydrogen, carbon dioxide, methane and the short-chain fatty acids, acetic,

propionic and butyric acid in the colon. Salvage of these gasses and fatty acids occurs in the colon thereby scavenging a portion of the malabsorbed carbohydrate. The carbohydrates and fatty acids that remain can induce fluid secretion as well as present an osmotic load to the colon leading to chronic diarrhea.

Breath hydrogen testing has been used to further examine the roles of the various carbohydrates contained in fruit juice in chronic nonspecific diarrhea. Kneepkens et al. [15] demonstrated a significant increase in breath hydrogen into the abnormal range following the ingestion of 250 ml of apple juice in both children with chronic nonspecific diarrhea as well as a group without gastrointestinal symptoms. Daily apple juice consumption did not differ significantly between the 2 groups (between 236.5 and 443.6 ml juice/day on average). When glucose was added to the apple juice the breath hydrogen concentrations decreased significantly. Thus it was felt that fructose was accounting for approximately 80% of the incomplete carbohydrate absorption and sorbitol for 20%. When apple juice was eliminated from the diets of those with chronic nonspecific diarrhea, normalization of the frequency and consistency of the stools occurred.

Hoekstra et al. [16] extended these observations and showed that other substances in fruit juice in addition to fructose and sorbitol may be implicated in causing the diarrheal stools. They compared processed apple juice, which had been treated enzymatically to produce a clear fluid with freshly pressed and unprocessed juice. When both types of juice were provided at 10 ml/kg, breath hydrogen increased to ≥20 ppm in 8 of the 10 individuals consuming clear juice compared to 5 of 10 consuming cloudy unprocessed juice. The mean breath hydrogen concentration was higher in those who consumed clear apple juice. These juices were then provided in a 4-week crossover clinical trial to 12 children. Clear apple juice significantly promoted diarrhea, and the authors suggested that nonabsorbable mono- and disaccharides present as a result of the enzymatic processing of apple pulp were an important factor in apple juice-induced chronic nonspecific diarrhea.

More recently Lebenthal-Bendor et al. [17] showed that acetylated distarch phosphate, a modified starch used in some baby foods, led to elevated breath hydrogen in 2 of 21 toddlers who consumed a formula containing 8% of the modified starch. None of these toddlers had loose stools unless they consumed a formula that contained 2% sorbitol and 5% fructose in addition to the modified starch. However, sorbitol added to the formula containing native starch, which was used as the control formula, also led to loose stools in 2 of the study subjects. Thus it is not clear that modified starch by itself plays any significant role in chronic nonspecific diarrhea of infancy in the absence of other known factors such as consumption of sorbitol and fructose.

Greene and Ghishan [18] documented that almost one fifth of the 85 patients they reported on with chronic nonspecific diarrhea consumed more

than 2.5 times their daily fluid requirement in addition to their usual diet. Most of the fluids consumed by their patients with chronic nonspecific diarrhea were hypertonic because of high concentrations of carbohydrate, although 3 of these patients were consuming large volumes of water alone. Thus it is clear that fluid intake in excess of the capacity of the intestinal tract to absorb it, and in many cases combined with a high osmotic load, is an important factor in the development of chronic nonspecific diarrhea in many children.

Treatment

In the past 50 years many agents have been used to treat chronic nonspecific diarrhea. Davidson and Wasserman [1] reported significant success with a number of different agents and approaches. Cohlan [5], who, as noted, coined the term chronic nonspecific diarrhea, reported on the successful use of Diodoquin in his description of this entity. Hamdi and Dodge [19] suggested that aspirin and loperamide are effective in the treatment of chronic nonspecific diarrhea, in part as a result of an increase in plasma prostaglandins, particularly $PGF_{2\alpha}$, that they observed in their study subjects with chronic nonspecific diarrhea [20]. The validity of the observation regarding plasma prostaglandins and its relationship to chronic nonspecific diarrhea remains to be established.

Nevertheless, treatment with both aspirin and loperamide carries significant risks, particularly when used for a condition that by and large can be treated by dietary modifications and in any event has no significant health implications. In one report, 3 children between the ages of 23 and 34 months with toddler's diarrhea unresponsive to dietary changes were treated with loperamide. All 3 became drowsy with irritability and personality changes within 3–5 days of starting treatment [21]. Loperamide has opiate-like toxic effects and may clearly cause severe central nervous system depression along with its effects on the gastrointestinal tract. Aspirin has been linked with Reyes syndrome and when used indiscriminately may result in acute or chronic salicylism. Thus both loperamide and aspirin are unacceptable treatments for toddler's diarrhea. The use of antibiotics is also inappropriate for the treatment of toddler's diarrhea. Diodoquin, for example (used by Cohlan [5]), can cause an irreversible neuropathy. Microbial resistance to antibiotics is a growing public health issue and there is no place for antibiotic use in the treatment of chronic nonspecific diarrhea.

In addition to normalizing and optimizing the diet of children with chronic nonspecific diarrhea, added dietary fiber may be of some benefit. Smalley et al. [22] reported that 7 of 23 children with chronic nonspecific diarrhea responded to an unrestricted diet alone. When psyllium was added to the diets of these 23 children, 87% responded to the combination of unrestricted

diet and bulking agent. As the authors point out, this was not a controlled study and they emphasize that a normal unrestricted diet should be the first approach to the treatment of this condition.

Conclusion

Approximately 50 years of observation and investigation have defined chronic nonspecific diarrhea of childhood or toddler's diarrhea as a functional bowel disorder with no consequences for growth, development or long-term health. The incidence and prevalence of this functional disorder is unknown, although it is clearly a common condition seen frequently by primary care physicians and pediatric gastroenterologists. Laboratory investigations are of no use or benefit when the criteria for chronic nonspecific diarrhea in infancy are met. Breath hydrogen testing in infants with chronic nonspecific diarrhea, including the fructose breath hydrogen test, is of no use [23].

The most useful approach to this disorder is to reassure the parents, to normalize the diet within current guidelines for carbohydrate, protein, fat and fluid intake, and to observe the child. It has also been noted that the symptoms of chronic nonspecific diarrhea may decrease significantly simply by asking parents to create a diet diary and record stool frequency and consistency for a 1-week period before any specific dietary interventions are initiated [24]. Thus raising the amount of fat in the diet above that recommended in current guidelines is not required. This disorder inevitably resolves by the time the child starts school. There is overlap between this disorder and irritable colon of childhood and some of those children with a diagnosis of chronic nonspecific diarrhea who have intermittent periods of diarrhea after starting school may in fact have irritable bowel syndrome.

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Discussion

Dr. H. Hoekstra: The symposium will end with educational recommendations. Do you have more recommendations in this group of children?

Dr. Kleinman: I think education is the best approach to this disorder. Primary care providers, as well as gastroenterologists need to be educated that this is a problem and not a disease. There are two target audiences, the health care providers and the parents. This can be accomplished with printed materials, or electronic formats, materials that are handed out in the physicians office and informational articles in lay magazines, which is where parents today get most of their medical information. I think that is the effective way of dealing with this issue.

Dr. H. Hoekstra: I think it is important to make a recommendation to reinstall the meal in-between. We should advise against the practice of toddlers walking with a bottle between the 3 main courses, and we should recommend the restoration of the coffee and tea break with sitting down and eating an apple instead of drinking apple juice.

Dr. Kleinman: Parents equate fruit juice with fruit and feel that if they can provide the juice then they are providing the nutritional benefit of the fruit. Thus some part of that reeducation has to be to get parents to understand that fruit is a very appropriate part of the child's diet and the juice really at the very least needs to be quite limited and could probably be eliminated from the diet with no harmful effect at all.

Dr. Hernell: I have the impression that, at least in Sweden, 10–15 years ago nonspecific diarrhea was a rather common diagnosis and then it just disappeared. Now inflammatory bowel syndrome in childhood has become a much more common diagnosis. Do you think that it is just semantics or what the explanation?

Dr. Kleinman: Anecdotally we have had the same experience. I think there is enough overlap between chronic nonspecific diarrhea (CNSD) and irritable colon, so that if the prevalence of irritable colon is increasing, as I think it is, in our own minds we are probably blending the two right now and diagnosing irritable colon more readily than we did before. But I do have the overall impression that I don't see as much CNSD as I did 20 or 30 years ago or even 10 years ago.

Dr. Sinaasapel: I remember that we had the same situation in the 1980s. We had a large number of children with this disorder and when we wanted to start a study with this group it just disappeared, and thereafter we hardly ever saw it again. I can't remember exactly, but I have the impression that at that time we did not have as many problems with constipation as we have now. So I have the impression that we changed the problem from diarrhea to constipation at that time, but I am probably wrong. One small comment, it is not only the juice in boxes but sometimes the habits of the parents that must be questioned. When they say that they just give 1 orange/day or that they give one orange juice/day, they must also be asked how many oranges they use. Sometimes in my practice I hear that the parents use 8–10 oranges/day for a small child. So when simply asking about fruit, it is also important to ask what fruit is given and how much.

Dr. Kleinman: In terms of fruit, unfortunately in the United States we almost never have to ask how many oranges parents feed their child each day because the answer generally is zero. Fruits and vegetables are really under-consumed, certainly in the United States and in other developed countries.

Dr. Branski: I would like to ask you to comment on anecdotal papers on the role of prostaglandins in causing CNSD. Also reported in these articles was that inhibition of prostaglandin synthesis via the cyclooxygenase pathway by aspirin and indomethacin (non-steroidal anti-inflammatory drugs) was helpful [1, 2].

Dr. Kleinman: There have been several papers written on prostaglandin use for CNSD. I didn't discuss them in my presentation because they are so anecdotal and the study populations so poorly defined that I didn't think it was worthwhile. As you said indomethacin was used in the past for some of these children and the high risk of an adverse effect of that or any other prostaglandin-inhibiting agent make it almost incomprehensible that anyone would use these drugs for CNDS today.

Mr. Benyacoub: Is gut permeability altered in these children?

Dr. Kleinman: I don't know of any studies that examined gut permeability in children with CNSD. I would imagine, because these are otherwise completely healthy children with healthy guts, that if one examines gut permeability it will be no different from an age-matched healthy child.

Dr. El-Din Amry: I would like to ask about the concept of a diarrheagenic diet and constipating diet. In every culture it is well known that some foods cause constipation and other foods can cause mild diarrhea, and pediatricians sometimes use this concept to help to treat their patients: with simple gastroenteritis they give them a diet that causes constipation and in the case of constipation they give them a diet that causes looser stools. What do you think of this concept?

Dr. Kleinman: I guess I would take issue with the concept that there are foods that are likely to cause diarrhea and other foods that are likely to cause constipation. I think that most of the literature studying the question shows in fact that the stool character depends upon the amount of a particular food, the age at which it is consumed, the concentration of nutrients in that food and other factors. So the idea that

any particular food is constipating or likely to cause diarrhea I think is a notion that we probably need to move away from and instead consider the entire diet of the child and whether it is appropriate for age.

Dr. H. Hoekstra: Perhaps I can comment on this point as well. Another way to look at the stool problems in toddler's diarrhea is by looking at the water-holding properties of the stool. This aspect has been intensively studied by Wenzl et al. [3]. Diarrhea can be defined as a failure of the water-holding properties, and this may well be the case in CNSD.

Dr. Bueno: You mainly emphasized the possibility that food is involved in such a disease which is close to irritable bowel syndrome in children. Do we have any data suggesting that stressful events may also play a role? Do we now have retrospective studies suggesting that children suffering from CNSD may have a prevalence for irritable bowel syndrome in adult life?

Dr. Kleinman: In terms of stress, anecdotally virtually all of these are healthy happy little toddlers, so probably stress does not play a huge role. But as Davidson pointed out, there is often a strong family history of irritable bowel syndrome. It is very difficult in a 2-year-old to tell whether they have an irritable bowel syndrome or CNSD. Most of the time we end up making the diagnosis of irritable bowel syndrome when the condition persists beyond 4 or 5 years.

Dr. Schmitz: You said that this is a condition that occurs between 6 months and 3–4 years of age, which I think is our experience, meaning that it occurs during a window in the life of the children. As you also said, it can be corrected by a modification of the diet or induced by the diet. So my question is, what correlation do you make between the fact that it occurs at a given age and the fact that this is a time of diversification and introduction of new foods?

Dr. Kleinman: That is an excellent point. It is a period of time when the diet of the young child actually tends to be quite monotonous, and while they are adding new foods, neophobia, fear of new foods, is also very strong at that age, and so there are lots of opportunities for these young children to be on relatively restricted diets. So I think it is a very unique opportunity for us to provide not only nutritional counseling but behavioral counseling to parents, and we ought to capitalize on that and help parents to understand how to change behavior in a positive way in the young child.

Dr. Schmitz: That is a good interpretation, but there is another one which finds that this period of age is the one in which the child begins to eat fibers because he/she has never eaten fibers before that. So it could be said that chronic diarrhea is also a kind of situation of non-adaptation to a new food, which would be fibers. Then since fibers are not digested by the gut, by the small bowel, it could be viewed as a phase of adaptation of the colonic motility or flora or both, I don't know, to this new food. This would explain why CNSD is typically a situation of weaning and of food diversification. What is your opinion on that?

Dr. Kleinman: I think it is a good idea. In the US the consumption of fiber is low, particularly during those years, and I am not sure how many children that would pertain to. I think it is an excellent idea and certainly one that we could look at pretty easily.

Dr. H. Hoekstra: So it fits well with the concept of Dr. Kneepkens of inadequate fermentation.

Dr. Schmitz: Exactly, and it would be nice to have Dr. Salminen's opinion about the possibilities of bacteria in this situation. What happens to the fibers? What do you know about that?

Dr. Salminen: One thing we know is that the bacteria wonderfully adapt to different situations, so I am actually trying to reflect back to the chronic. I rather see problems in short-term diarrhea caused by bacteria because the adaptation is not always

immediate, but if you work on the basis of days and weeks, they adapt to utilizing most carbohydrate substrates. So there is perhaps an added extra to what you said.

Dr. Hernell: I think it is quite clear that this can be only part of the explanation for this type of diarrhea because we saw children with a well defined diet and just made them increase the amount of fat in the diet and many of them were cured. So this seems to be a symptom and the reasons behind it are most likely multiple.

Dr. Kleinman: And don't forget, in all of the studies anywhere from 15 to 20% of the subjects don't respond to those dietary manipulations. So either it is a heterogeneous group of children, some of whom have irritable bowel syndrome and perhaps other issues, and in addition there may be some other mechanisms that remain to be understood like the fiber issue.

Dr. H. Hoekstra: I have a question for Dr. Salminen. In a situation of very fast intestinal transit, is it possible to temporally flush out a normal flora?

Dr. Salminen: I think it is extremely difficult to flush it out totally. It will come back on the mucosal level in the long run; it can be disturbed for a long while but I would say in most of the cases it will return. You can of course disturb this by different means but it is very difficult to, unless it is in the very young infant.

Dr. Sinaasappel: With regard to your recommendations, and I think this is a disorder or a complaint from developed countries. But developing countries are imitating our habits and always walk a little bit behind us, so I think that although the problem can be solved quite easily, it might be a problem for developing countries in particular. They also have a load of infections in those areas which might increase the problem or might harm the child, although it is just a preventable cause. Although we agree on the cause and how to treat it or to prevent it, I think for developing countries it is a different matter.

Dr. Kleinman: Developing countries are going to be very busy dealing with obesity and at the same time trying to figure out how to treat hunger and starvation, so they may not have time to pay attention to this issue. But you are absolutely right, the kind of things that we have seen in the industrialized world tend to appear in the developing world 20 years afterwards. So it is something to keep in an eye on.

Dr. Keller: I have a question regarding improving toddler's diarrhea with fats. This is true in part for this nonspecific diarrhea of childhood. You told us about some overlap to irritable bowel syndrome, but there are some, at least adolescents or schoolchildren or adults, with irritable bowel syndrome whose symptoms will worsen by adding fat.

Dr. Kleinman: Yes, and in fact when those papers were published in the late 1970s it seemed intuitive. It is very clear that many of the adults with irritable bowel syndrome have symptoms that are much worse if they are eating a high-fat diet. So one of the objections to increasing the fat in the diet of children with CNSD was that it was going to make it worse, but the fact is it doesn't. For those children who have CNSD and not irritable bowel syndrome, the fat does seem to improve it. Nobody today advocates a very high-fat diet for these children. What most recommend is a diet that has about 30% of total calories from fat.

Dr. Leathwood: There is a complex communication problem to be solved. The parents are worried and upset. They are getting advice, sometimes contradictory, from many sources and they no longer know who to believe. To them, your recommendations even seem to go against what they thought was 'healthy eating'. So it should not be surprising that you have difficulties to convince them.

Dr. Kleinman: Yes, it goes well beyond what we can discuss here in the next few minutes, but clearly part of this is establishing credibility with the parents, and that generally doesn't happen the first time we meet them. The second is that they have to be convinced that what you are recommending is not going to harm their child, and that

generally doesn't happen the first time that you meet them. Perhaps, at least in part, this helps explain why many of us still do tests on these children. We were talking about the breath test and the fact that the number of breath test that we do has decreased significantly but we still do breath test and one of the times that I recommend the breath test is in the parents who are so anxious that my words aren't going to get through to them, they require some objective measure of their child's health, and if you can offer them a relatively non invasive, not terribly expensive test then I think most of us go ahead and do that, but it does require significant efforts and it is not simply a matter of saying you child is well, go home and call me in 6 months. That is particularly true with the behavioral issue, you have to give them specific recommendations on how to modify behavior and that is not easy to do for many parents. I think that is a major difference between the 1950s and 60s and today. Many parents in the United States are very reluctant to provide significant guidance for their young children in the sense that in some way by limiting freedom you harm the child's development and that is a very complex concept to work through. So I agree exactly with what you said and perhaps we will hear later in this conference some specific educational recommendations that will help us.

Dr. Steenhout: When you recommend stopping the consumption of fruit juice and installing treatment with increasing fat, how long does it take to see an improvement? My second question is, what sort of fat? Just an increased percentage of fat in the diet or have you some specific fat profile to recommend, balanced between n-3 and n-6?

Dr. Kleinman: The response happens very quickly. Within a day the character of the stool changes so it is one of those gratifying experiences where you make a recommendation and they actually see a cure in a sense, and it is a very good way to establish credibility with the patient. In terms of the fat in the diet, for most of the children who are on a diet that is severely restricted in fat, the parents have achieved that by either taking all dairy products out of the diet or shifting to non-fat dairy products. So the simplest thing to do is just to return the child to a full-fat diet or a diet containing a 1% fat dairy product. Most parents have little understanding of n-3 fats.

Dr. Steenhout: If the effect is practically immediate, in one day, by which mechanism can it be explained? Is it just by retrieving the sugar or the contaminant coming from the process of the fruit juice, or do you have an explanation by which mechanism the fat could act?

Dr. Kleinman: No one has experimentally defined the mechanism of fat in CNSD but the effect of fat on gut motility is well explored and it is the principle dietary nutrient that is responsible for the so-called duodenal break. The fat clearly slows motility. Most of these children who have a very low-fat diet are also consuming a lot of carbohydrates, a lot of juice, so it is almost always a mixed pattern. Thus the combination of adding some fat which slows motility and decreasing the amount of fluid and the amount of carbohydrate leads to a very rapid response. I don't mean to make light of the importance of having children on an optimal blend of fats in the diet. The best way we can do that is by recommending a pattern of foods that the children should be eating and not so much getting into the science of different kinds of fat.

Dr. Moreno Villares: Going back to the idea of Dr. Schmitz concerning adaptation. In the last years, as more children from other countries come to our country in this range of age, we have seen that chronic diarrhea improves with time with no infectious disease. When they go back to their own country and then return to our country they again have these episodes of chronic diarrhea as if microflora could change because of the meal pattern in the different cultures. So we do not see classic toddler's diarrhea, but this kind of episodic diarrhea in these people from foreign countries and traveling from one country to another.

Dr. Kleinman: I would ask our colleague from Finland to comment on changes in stool flora or microbiota. When moving from country to country do you think that is the principal issue that is operating here or is it just that they change diet when they go from one country to another?

Dr. Salminen: I think it is probably both and we all know from adult situations that going from country to country can change our microbiota and have some consequences. But I think it is a combination of both because the diet can be totally different and the meal patterns are different, combined with the microbial changes.

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Development of Motility

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Introduction

Advances in neonatology over the past 2 decades have resulted in the survival of very preterm infants. However, the major limiting factor to survival of such infants is the ability to initiate and maintain adequate nutrition.

Multiple maturational events are necessary for successful enteral nutrition of the infant: coordination of sucking and swallowing; effective gastric emptying; forward propagation of small intestinal contents, and finally, colonic elimination. Since normal gastrointestinal function relies on the integrated maturation of absorptive, secretory and motor function, a delay in any one of these processes will result in disturbed gastrointestinal function. Immature gastrointestinal motility manifested by vomiting, abdominal distention, delay in stooling and constipation commonly postpone the time of full enteral feeding in premature infants.

Recent advances in biomedical engineering have enabled the study of gastrointestinal motility even in very premature infants. Using miniaturized feeding catheters with an outer diameter of <2 mm, multiple recording sites and sleeve sensors and with rates of water infusion ranging between 0.005 and 0.04 ml/min, we have learned a great deal about the functional ontogeny of esophageal and antroduodenal motility in humans. In contrast, due to the difficulty in studying the human colon under physiologic conditions, very little is known about the development of colonic motility. Placement of manometric or barostat catheters in the colon requires endoscopy and cannot be justified in healthy infants, while noninvasive techniques such as scintigraphic transit studies or ultrasonographic evaluations have not yet been standardized for children.

Development of Myogenic Control

The fetal development of the structure and function of the gastrointestinal tract is a complex process. Throughout the intestine, three layers of muscle contract in a coordinated fashion: the muscularis mucosa, a thin layer that lies beneath the villi; the circular muscle, which lies outside of the muscularis mucosa and serves as the pacemaker for gut muscle contraction, and the longitudinal muscle, the outer most layer of the three muscles. These muscles have oscillatory membrane potentials and their contraction rate is reflective of the electrical slow waves. The slow wave has different frequencies at each level of the gut (i.e., 3–5 times/min in the stomach, 9–11 times/min in the duodenum, 8–10 times/min in the jejunum, and so forth). Thus, at each level of the gut, there is an intrinsic phasic contraction rate.

The muscular layers derive from the mesenchymal tissue in the gut by the 4th to 6th week of gestation in a rostrocaudal fashion [1]. The circular muscle layer appears first, followed after 2–3 weeks by the longitudinal muscle coat, while the muscularis mucosa is formed later by 22–23 weeks of gestation. Similarly, the contractile proteins of smooth muscle cells in animal models appear in a hierarchic manner; however, no such information is available in humans [2]. As the developmental changes in the contractile proteins occur, the frequency of the slow waves or electric control activity of the smooth muscle cells also changes. The frequency of electric control activity increases with the increase in post-conceptional age, reflecting developmental changes in the activity of membrane iron pumps or their modulation [3].

Until recently, some investigators suggested that groups of muscle cells located in the circular layer differentiated to form the interstitial cells of Cajal (ICCs), specialized cells provided with multiple processes that project in an ascending and descending manner throughout the length of the circular muscle and the longitudinal muscle. These cells act as pacemakers by driving the slow wave frequency and coordinate neural input to gut smooth muscle [4]. The ICCs are distinct from neurons and smooth muscle cells, and they play important roles in the regulation of gastrointestinal motility.

Anatomic studies characterizing the distribution of ICCs measure immunoreactivity to c-kit, a proto-oncogene coding for a receptor tyrosine kinase. Six distinct ICC populations were identified in the gut, including intramuscular ICCs, ICCs within the myenteric plexus, submucosal ICCs in the colon, and ICCs in the deep muscular plexus of the small intestine. A recent study reported the regional variability in colonic ICC density with the highest numbers observed in the transverse colon [5].

ICCs are present from an early stage of human gut development. Intrauterine maturation of ICCs correlates with the initiation of electrical rhythmicity, in fact in mutant mice lacking ICCs, no spontaneous pacemaker

activity is seen [6]. Such loss of pacemaker function leads to disruption of organized luminal propagation.

Recent studies have reported that a delayed maturation of ICCs could be involved in the pathophysiology of gastrointestinal dismotility seen in some neonates and children [7, 8], and abnormalities in the density and distribution of ICCs have been described in human Hirschsprung's disease and infantile hypertophic pyloric stenosis [9, 10]. However, since ICC development continues well into postnatal life, interpretation of apparent abnormalities in their distribution as being of pathological significance should be tempered.

The finding that c-kit-positive ICCs are present from 9.5 weeks, when neural crest colonization of the gut approaches completion, is consistent with a modulating effect of the fetal enteric nervous system (ENS) on ICC development.

Development of Neurogenic Control

Initiation and coordination of muscle contraction is regulated by neural and hormonal input. Extrinsic neural regulation refers to all nerves that have a cell body located outside the intestinal tract. Extrinsic neural input to the gastrointestinal tract comes from the central nervous system (CNS); the sympathetic and the parasympathetic systems. Intrinsic neural regulation refers to all nerves whose cell bodies reside in the intestine. The ENS, or gut brain, provides most of this regulation. It is capable of functioning independent of the extrinsic nervous system in animals when connections to the extrinsic nerves have been severed [1].

Components of the ENS are formed in a temporal sequence that parallels the maturation of the muscle layers. Neural crest cells migrate to the intestine via the vagal and sacral portion of the spinal cord. The undifferentiated cells are first detected in the stomach and duodenum at 7 weeks and then in the rectum at 12 weeks. They quickly differentiate along a rostral caudal axis and establish the myenteric and submucosal plexuses by weeks 12–14. Contacts between the enteric nerves and the circular and longitudinal muscle cells develop between 10 and 26 weeks [11]. It appears that there is intimate cross-talk between the developing muscles and nerves, and if either of the two fail to develop properly, maturation of the other is arrested.

Several observations suggest that development of the ENS continues after birth and through at least the first 12–18 months of life. Study of the argyrophilia of neurons in the sigmoid colon of human neonates shows that, prior to term, the nerves are unable to take up silver and that, during the first 6 months of life, neurons in the myenteric plexus gradually assume argyrophilia [12]. Thus evidence suggests that just as the majority of CNS development takes place throughout fetal life and continues through the first 18 months of life, a similar pattern occurs in ENS.

Neurotransmitters are elaborated by the end of first trimester as are almost all of the hormones and peptides. N-Methyl-*D*-aspartate (excitatory) and nitric oxide (inhibitory) have been shown to be neurotransmitters in animal studies and may be the most potent agents in modulating bowel motility [13].

Recent studies have indicated that nitric oxide is involved in the nonadrenergic-noncholinergic (NANC) innervation of the gut, mediating its relaxation. Brandt et al. [14] reported that the onset and place of development of nitrergic innervation are similar to adrenergic and cholinergic innervation and occur before peptidergic innervation. Bowel segments from the esophagus, pylorus, ileocecal and rectosigmoid regions of 14 fetuses (gestational age range from 12 to 23 weeks) were studied with NADPH diaphorase histochemistry. By 12 weeks of gestation, nitrergic neurons had appeared in the myenteric ganglia, at all levels of the gut, and had begun plexus formation. Nitrergic innervation of the submucous plexus became evident after 14 weeks. By 23 weeks of gestation, the complete nitrergic pattern had matured, as observed in the postnatal gut.

These NANC nerves mediate the reflex opening of sphincters in the alimentary tract and the descending inhibition during intestinal peristalsis. Defects of nitrergic innervation have recently been found in congenital gut anomalies such as pyloric stenosis and Hirschsprung's disease, which suggests that a lack of nitric oxide-mediated NANC inhibitory control may be responsible for the failure of relaxation of the pylorus and hindgut, respectively [15].

The combined maturation of the ENS and CNS, together with their interconnections, is likely to be responsible for many of the mayor ontogenetic changes observed in intestinal motor activity before and after birth.

Characterization of Motor Activity

Gastric Motility

Many aspects of gastrointestinal motility appear to be less mature in the preterm infant than in the term infant, and those of the term infant less mature than those seen in the child and adult.

Although fetuses in utero are able to swallow amniotic fluid from as early as 20 weeks of gestation, the sucking mechanism does not appear until 32–34 weeks of gestation [16]. Gastric emptying of swallowed amniotic fluid into the intestine may be demonstrated in the human fetus at 30 weeks of gestation [17]. Between 28 and 38 weeks of gestational age, the gastric antral contraction amplitude increases from 10 to 40 mm Hg. Emptying half-time doubles when newborns of 28–34 weeks are compared with full-term neonates independent of feeding.

Contractions may occur singly, but occasionally phasic contractions may be sustained for 3–5 min. However, preterm infants had fewer antral clusters coordinated with duodenal clusters than term infants [18].

Small Intestinal Motility

Although complete interdigestive cycles can be observed occasionally in term infants, they are very rarely seen in preterm infants. Approximately 75% of the recordings obtained from neonates are occupied by a motor pattern that is not typically seen in adults: the nonpropagating cluster of contraction. This pattern consists of contraction bursts of 11–13/min lasting 1–3 min that do not migrate from the proximal gut to the distal gut [1]. With increasing gestational age, motor contractions become more organized, the duration of a single cluster becomes longer as does the duration of the motor quiescence separating the clusters. As a result this dominant pattern still occupies 75% of the recordings of term infants but clusters are longer (3–4 min) and their occurrence is lower (6–8 times/min). The migrating motor complexes (MMCs) appear between 32 and 35 weeks post-conception, as the overall occurrence of clusters decreases [19]. Some of these MMCs are poorly organized with slower propagation velocities.

In spite of an apparent immaturity of fasting activity, the intestinal motor activity pattern in preterm and term infants changes in response to feeding. However the appearance of a fed pattern is different at different gestational ages. Term neonates shown a fed pattern similar to that seen in adults. In contrast to term infants, only 25% of preterm infants display a mature type of fed pattern while about 75% display a prompt cessation of motor contraction after feeding. This pattern, associated with a delay in gastric emptying, is probably due to the immaturity of vagal regulation.

Feeding and Development of Motility

There is convincing evidence that an acute response of motor activity and peptide release are present with the first enteral feeding and that the provision of early enteral feedings facilitates functional maturation of the human intestine. Babies can respond to enteral nutrition as early as 25 weeks of gestational age [20]. This evidence suggests that the small intestinal fed response is a more primitive form of motor activity than is the fasting motor activity. For this reason the practice of delaying the use of enteral nutrition in the very low birth weight infant may not coincide with the preterm intestinal physiology of motor function.

Several studies have shown that gut function and subsequent milk tolerance is improved by trophic feeding. Trophic feeding (minimal enteral feeding, gut priming, early hypocaloric feeding) is a practice that involves feeding small volumes of milk, nutritionally insignificant but beneficial to the developing gut. Recent studies have reported that this practice accelerates the whole gut transit probably by enhancing the MMCs. The mechanism by

which trophic feeding exerts its influence is unknown. It is responsible for surges in the plasma concentration of several enteric hormones and peptides which alter gut motility (motilin, gastrin, neurotensin and peptide YY) and may cause stimulation of the ENS [21].

The manner in which babies are fed may also trigger differences in motor responses. Maturation of motor function requires that nutrients be fed to the neonates because feeding sterile water does not produce this effect [22]. Preterm infants fed by a 2-hour infusion display a brisk increase in motor contraction that is associated with faster gastric emptying compared to infants fed by a 15-min bolus. Feeding volumes that provide as little as 10% of the daily fluid intake significantly induce the premature appearance of MMCs in comparison to those that provide 30 or 100% [23].

In conclusion, minimal feeding volumes can be used to trigger maturation of motor function, at the same time avoiding the risk of enterocolitis that larger feeding volumes include. However, since cluster represents 60–75% of the motor activity in term infants who have completed the interdigestive cycle, the motor activity in these neonates is still very dissimilar from that seen in adults, suggesting that further changes occur throughout infancy.

Colonic Motility

The role of trigger that enteral nutrition occupies in the development of gastrointestinal function also represents a major factor in the ontogeny of colonic motility. It seems that colonic motility matures late in gestation and has different characteristics in infants compared to older children and adults.

Meconium can be found in the fetal rectum after 21 weeks of gestation, and as much as 10–20% of total amniotic fluid proteins are derived from the fetal gut. These data suggest that defecation in utero occurs physiologically during the late stages of pregnancy, and it is now believed that the detection of meconium in the amniotic fluid might reflect impaired clearance of meconium rather than excessive or inappropriate elimination in the amniotic fluid.

The correlation among early enteral feeding, passage of the first stool, stool frequency and consistency has largely been discussed in the pediatric literature.

Coordinated sucking and swallowing, required for the independent utilization of milk feeds, is not achieved until 32–34 weeks of gestation, after which time most preterm infants are capable of taking feeds by mouth. This gestational age coincides with a significant increase in the defecation rate and a surge in circulating concentrations of intestinal regulatory polypeptides (gastrin, motilin and neurotensin) in response to milk feeds.

In newborn infants, who do not have voluntary control, evacuation probably occurs in response to an increasing volume of stool in the rectum. In a large study observing bowel habits in 844 preterm infants, a direct relation between the volume of milk ingested and stool frequency throughout the first 8 weeks after birth was reported [24]. Infants who received no milk had a modal

frequency of 1 stool/day whereas those receiving >150 ml/kg/day passed between 3 and 4 stools/day. Infants receiving human milk had a consistently higher defecation rate and passed softer stools than those receiving formula milk, regardless of the gestational age and feed volume. The finding of a modal frequency of 1 stool/day in the unfed neonate suggests that there is an intrinsic pattern of large bowel motor activity present as early as 25 weeks of gestation. This daily passage of stool may perform the 'housekeeping' function of clearing the colon of intestinal secretions and other unwanted material. Probably, milk feeds override the intrinsic fasting motor activity of the colon and induce regular defecation at a frequency determined directly by the volume of the products of digestion that reach the rectum: the more feeds, the more stools.

In full-term and preterm infants, the peak stool frequency occurs during the first week after birth, after which there is a decrease in spite of the increasing milk intake, indicating a maturation of the water-conserving ability of the gut. It is not known, however, whether this is due to the increasing efficiency of small intestinal absorption or colonic water retention.

Term newborn infants average 4 bowel movements/day for the first week of life. The frequency of defecation decreases with age, so that 85% of children 1–4 years old defecate once or twice daily. High-amplitude (>60 mm Hg) propagating contractions (HAPCs) are the manometric correlate of the radiologic 'mass movements' and are responsible for the rapid movement of feces. The presence of HAPCs together with an increase in colonic motility after a meal are markers of the neuromuscular integrity of the colon in toddlers and children [17]. HAPCs decrease in frequency from several per hour after a meal in awake toddlers to just a few per day in adults [25]. The gastrocolonic response also seems more prominent in younger compared to older children. Nevertheless the colon in toddlers seems to have fewer tonic and phasic non-HAPCs compared to the colon of older subjects. Information about age-related changes in colonic tone is absent.

The ongoing developmental maturation of bowel function results in intestinal hypomotility with consequent postponement of meconium passage. The first studies to measure intestinal transit in humans used amniography; aboral transport of contrast did not occur in the intestinal tract of fetuses younger than 30 weeks of gestation. Using amniography, McLain [16] observed that gastrointestinal motility increased with advancing gestational age; progression of contrast material from the oral cavity to the colon took as long as 9 h at 32 weeks of gestation, but only half of that time by the time of labor. Intestinal transit is approximately three times slower in preterm infants compared with that seen in adults.

It has been noted previously that more than 90% of full-term infants and 100% of post-term infants passed meconium within 24 h. There has been agreement on the general principle that defecation should be avoided in utero and that lack of defecation after birth is a sign of disease. In fact it is generally believed that the passage of meconium into the amniotic fluid is an indicator

of fetal distress. Nevertheless meconium-stained amniotic fluid is found in up to 30% of all deliveries, and no cause of fetal distress is found in up to 25% of all occurrences of meconium-stained amniotic fluid [26].

In premature infants with a birth weight of 1,000 g or less the first stool is passed at a median age of 3 days and 90% have their first stool by 12 days after birth [27]. Meetze et al. [28] found a median age of 43 h for passage of the first stool in 47 patients with birth weights 1,259 g or less. One forth of these infants had not passed stool by 10 days of age. Weaver and Lucas [24] reported a 32% delay in passing meconium at >48 h, with an inverse relation between gestational age and the time of first bowel action. Extreme prematurity and delayed enteral feeding were significantly associated with delayed passage of the first stool in more than one study [29, 30].

Therefore delayed passage of meconium and constipation could be induced by a delayed intestinal transit which is evident at the level of the colonic segments in particular. Naturally, normal development of the upper gastrointestinal tract (stomach; small intestine) is essential to warrant correct maturation of the colonic motility, too.

In conclusion, we have stressed that the ontogenesis of gastrointestinal motor activity is influenced by several factors such as smooth muscle activity, the CNS, the ENS and the neurohumoral system.

We have also seen that early enteral feeding plays a main role in the promotion of the development of small intestinal functions and colonic motility.

Further understanding about the timing of specific motor patterns in humans and their control mechanisms may enable neonatologists to reach optimal feeding strategies to induce better gastrointestinal function and to obtain optimal feeding tolerance.

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Discussion

Dr. Taminiau: You were talking about prematures with stomach emptying, but how does that relate to the retention? If you feed premature infants they usually have retention in the stomach and passage problems, delaying this emptying of the stomach. How is this related to the things you said about development? You said osmotic things are not important, but perhaps different foods are important.

Dr. Staiano: Dr. Benninga's studies show that in prematures, as young as 30 weeks of gestation, gastric emptying is normal as well as the antropyloric motility [1]. Perhaps

the delayed emptying is strictly related to the volume of the feed. In these last studies it was shown that with a larger volume there is inhibition of the propagated antral contractions and the appearance of isolated pyloric pressure waves that slow gastric emptying. So, the function of gastric emptying is strictly related to different aspects during gestational age. It is mainly due to volume, to the caloric density and to the composition of the meal.

Dr. Taminiau: You said that continuous infusion is an advantage. Is that in the stomach also or just duodenal?

Dr. Staiano: Also in the stomach. Berseth et al. [2] in their study explained that perhaps the continuum infusion is better in the stomach because nutrients will stimulate the G cells in the antrum and therefore there is a larger secretion of gastrin which is an important motility hormone. The way in which nutrients are instilled determines faster gastric emptying or more mature duodenal motility. It always has to be instilled in 2 h, not by a bolus in 15 min, but the result is the same even if instilled just in the stomach [3].

Dr. Taminiau: So when these measures don't work and you still have retention, do you then recommend a change of formula, going into more medium-chain triglycerides or polymers? What do you do in your unit?

Dr. Staiano: It depends if there is normal or abnormal gastric emptying. I don't advise the use of this kind of feed in all prematures just to improve gastric emptying that is perhaps normal already. It is different if we talk about a premature infant with delayed gastric emptying.

Dr. Benninga: In the last slide you showed the mechanisms of constipation and you mentioned that difficulties in defecation might be one of them, but I think this counts more for toddlers. Can you speculate on why in 60% of all constipated children, constipation already starts in the first 6 months of life? Do you have any explanation for this?

Dr. Staiano: I don't know if you are talking about infant dyschezia, due only to the lack of coordination between the increasing abdominal pressure and the relaxation of the pelvic floor during defecation. For sure in the first 6 months of life we don't just have infant dyschezia, we can also have constipated infants, and the change from human milk to a formula may worsen the bowel habit. The latter group represents the only set of children in which dietary manipulation can improve the bowel habit. It has been reported in many studies that the hardness of the stools, which is one of the most important events in establishing chronic constipation, may be improved by infant formulas containing a prevalence of palmitic acid in the S2 position of the triglycerides, with a better absorption of the fat as monopalmitin instead of free fatty acid [4].

Dr. Benninga: With regard to the diet, do you believe in the new concepts of adding oligosaccharides to the feed in an early phase to prevent constipation?

Dr. Staiano: I was expecting this question from you. I don't have any experience and I believe that in the literature there is still little information about that. But probably if the concept is that oligosaccharides have better fermentation in the colon, they can probably work similar to the disaccharides that we use to treat constipation.

Dr. Bueno: The migrating motor complex has been described as a housekeeper and a lot of work has been done on this pattern because it has two major functions, the first is to propel digesta within the small intestine and the second to prevent bacterial overgrowth. In preterm children there is a cluster-type motility pattern called the 'fetal pattern'. Do you have any clinical data suggesting that we have more or less bacterial overgrowth with this pattern compared to the migrating motor complex, or do we see a change in the frequency of intestinal infection at the time of changing from the fetal pattern to the migrating motor complex?

Dr. Staiano: To my knowledge there are no studies on the frequency of bacterial overgrowth in premature infants. One of the main reasons for speeding up the maturation of motor activity in the small bowel is just to have a migrating motor complex earlier to avoid a superimposed bacterial overgrowth.

Dr. Schmitz: Would it be possible that water fluxes or variation in the capacity of water and sodium reabsorption in the colon play a role in the very early occurrence of constipation in some children?

Dr. Staiano: There is a study, I don't know at which age but probably in the first year of life, where they tried to improve the bowel habits by increasing the fluid given to the child, and the only effect of increasing the amount of the fluid given to the infant was to have a larger volume of urine but it didn't change the stool factor [5, 6].

Dr. Caroli: You said that only 5% of children have organic constipation due to motility alterations. Can you tell us which are the most common alterations and if there is some special symptom or sign besides constipation that can be used for early detection of the special problems.

Dr. Staiano: The most frequent cause of organic constipation in children is Hirschsprung's disease with a frequency of 1 in 5,000 births, and then there are anorectal malformations and other organic causes such as endocrinal disorders or disorders of the central nervous system. Anyway, you are right that in these patients with organic constipation there are perhaps other associated symptoms different from functional constipation. The history and clinical examination will definitely help to differentiate between functional and organic constipation. For example, in patients with Hirschsprung's disease we have a delayed passage of meconium, or there are much more common obstructive symptoms that are not frequent in children with functional constipation. On the other hand encopresis or soiling, which are very common in children with functional constipation, are almost never seen in children with Hirschsprung's disease.

Dr. Benninga: I would like to make a comment on that because we have just finished a study evaluating the best diagnostic test in 130 babies suspected of having Hirschsprung's disease [7]. In 25% of the patients we found Hirschsprung's disease and it was striking that more than 50% of the healthy babies with functional constipation also had a delay in meconium production. We don't have a good explanation for the latter finding.

Dr. Staiano: Were they premature?

Dr. Benninga: No, but you are right in saying that if we have a combination of delayed meconium production with signs of obstruction then Hirschsprung's disease must be considered.

Dr. Staiano: It is interesting because very often neonatologists ask us to perform anorectal manometry and sometimes also rectal suction biopsy in children with delayed passage of meconium, but very rarely do we find Hirschsprung's disease in these infants. How do you explain this delayed passage of meconium in non-Hirschsprung's disease? Do you have any explanation?

Dr. Taminiau: Is there any system that is delayed in development? You said that one system takes about 12–15 months.

Dr. Staiano: There is a strict relation between the passage of the first stools and the first feed. The first feed definitely improves motility in premature infants. The composition of meconium is also sometimes involved in delayed elimination. Premature infants have a hard meconium in comparison to full-term infants with an increased amount of mineral and calcium and less production of intestinal secretions which allow elimination of the first stools. But I don't have an explanation in normal children.

Dr. Waterland: Why is gastric emptying quicker with breast milk versus formula feeding?

Dr. Staiano: There are different studies but no one has the answer. One study suggests that probably breast milk has a prokinetic substance that may accelerate gastric emptying. There is no reason, no explanation until now.

Dr. Taminiau: Why do you ask?

Dr. Waterland: I think it is of direct relevance to the question of how infant feeding practice affects motility and constipation and all these things. I thought there might be studies correlating duodenal motility with, for example, hormones in breast milk; anything like that might explain it.

Dr. Staiano: While I was preparing this talk I was looking for some explanation to answer this question and in that last study I found an interesting explanation which says that human milk probably contains something that will accelerate it, but it is not known yet.

Dr. Schmitz: I come back the discussion between you and Dr. Benninga about normal babies who have no Hirschsprung's disease. According to what you know about motility, what would be the physiological explanation for such a big range of stool elimination in breastfed babies? On one hand breastfed babies are said to normally have 5 stools/day, but on the other hand one can see normal breastfed babies who are producing a stool once a week or even once every 10 days without any symptoms. So what makes the difference here?

Dr. Staiano: The main reason is better fat digestion of human milk because human milk contains a specific lipolytic enzyme, the bile salt, which stimulates lipase, and because human milk has a higher prevalent proportion of palmitic acid in the S2 position. So these are the main reasons.

Dr. Schmitz: But this does not explain the range from 1 stool every 10 days to 5/day with the same breast milk.

Dr. Staiano: There are studies trying to see if improving the fat composition of milk could also improve the number of stools per day in infants fed infant formula, but the number is not improved. These special infant formulas that are much more similar to human milk improve the softness of the stools but they don't improve the amount. One conclusion is that perhaps this is better because the use of diapers is smaller and it costs less [4]. This is mostly related to the maturation of the water-conserving ability of the gut.

Dr. Schmitz: This is what I wanted you to say. At some point water reabsorption is something important in constipation.

Dr. Lafeber: What interests me is the concept of minimal enteral feeding which is very popular amongst us neonatologists and is applied now in most countries. But if you look at the Cochrane meta-analysis of this practice it is not yet evidence-based [8]. Trials, like the one performed in the US by Berseth et al. [9], were stopped because after the introduction of larger amounts of feeding there was more necrotizing enterocolitis. But what intrigues me is what is behind this concept, I mean the amounts you showed in your slide, 4 ml/kg; if a baby is 700 g it is only 3 ml so that is not so much, so what is really happening here? If you give this small amount of food, is it stimulating motility? I cannot really believe that it is doing much to the trophic function of the gut because it is such a small amount of food. If you look at animal experiments what happens if you give food: you get a trophic effect of food on the gut mucosa if you give more than 40 or 50% of the needed amount of nutrition, so I cannot imagine that minimal enteral feeding really has a substantial effect on gut function [10]. So do you think it is motility?

Dr. Staiano: I think it is not just motility, it is also hormonal secretion. In fact this minimal volume stimulates the secretion of gastrin and neurotensin and such a small volume has an effect on hormonal stimulation and inhibits the polypeptides that are against the maturation of motility. I believe that both effects may improve maturation.

Berseth et al. [11, 12] did a study comparing the 4- and 10-ml volumes against 50% of the daily fluid required, and the effect they obtained with 4 ml was better than with larger volumes, which may precipitate necrotizing enterocolitis. So I believe even if the meta-analysis does not support this finding, there is definitely an effect.

Dr. Exl-Preysch: I just wanted to add something to the discussion about gastric emptying and hydrolyzed formulas. Just have a look at the studies done by Billeaud et al. [13] and Tolia et al. [14]. They were able to demonstrate that only pHF (Nestlé NAN HA) matched the gastric emptying time of mother's milk. Therefore, as discussed before, it just cannot be the fat that is determining the gastric emptying time because those formulas have the same fat content as the other formulas based on unaltered cow's milk protein, whey or casein. The casein-dominant formulas were always those with the slowest gastric emptying time. Therefore it seems much more to be the protein source and how it has been treated (hydrolyzed or not) that determines the gastric emptying time. In addition studies conducted by Mihatsch et al. [15] and also Sievers et al. [16] showed clearly that formulas with hydrolyzed proteins had a much quicker gastrointestinal passage than non-hydrolyzed formulas, a reason why, for instance, in Germany, in preterm nutrition, hydrolyzed formulas are highly preferred.

Dr. Staiano: I agree. There are still a lot of conflicting data on this issue and probably we need further studies to clarify this aspect better.

Dr. Keller: You briefly mentioned the physiological appearance of hyperplastic ganglia in the hindgut. We were talking about these young infants having no Hirschsprung's disease, but defecation difficulty. You know the German data that intestinal neuronal dysplasia had a sort of wrong definition or a change in definition over the time [17, 18]. Do you think intestinal neuronal dysplasia still exists? Is it a disease, or is it a wrong interpretation?

Dr. Staiano: I believe that intestinal neuronal dysplasia exists, depending on the definition. If we are talking about intestinal neuronal dysplasia of the myenteric plexus then the diagnosis exists and it is a serious disease which manifests clinically as severe intestinal pseudo-obstruction. If we refer to the German definition of intestinal neuronal dysplasia, which is based only on findings from rectal suction biopsy, I don't believe that the diagnosis exists anymore. There are two well-conducted studies, one from Cord-Udy et al. [19] and one from Koletzko et al. [17], in which they showed that there is a very high inter-observational variation in analyzing suction rectal biopsy samples. Cord-Udy et al. [19] showed that infants who in the first months of life received a diagnosis of intestinal neuronal dysplasia according to the German criteria, were all healthy children at the 4- to 5-year follow-up. Most probably there is a defect of maturation in the first year of life.

Dr. Keller: That means that we need a full-thickness biopsy to rule out intestinal neuronal dysplasia.

Dr. Staiano: Yes, but we need severe clinical manifestations to think about the presence of intestinal neuronal dysplasia, not just chronic constipation.

Dr. Sinaasappel: Regarding your comment on malabsorption of fat in babies drinking cow's milk or being bottle-fed, a product is now being developed to produce bile salt-stimulated lipase, so in the future bile salts can be added to cow's milk or to formula to stimulate lipase. Are you in favor of this? Do you think that is a possibility to increase fat absorption and also probably to prevent it.

Dr. Staiano: In association with a higher proportion of palmitic acid in the S2 position, it could perhaps work.

Dr. Taminiau: In addition to what was said about the development of constipation and neuronal dysplasia, the cells of Cajal are diminished in many obstructive diseases and this seems only to be a reaction to obstruction. They are diminished whatever artificial obstruction is produced in animals, or it seems to be a secondary thing. So if

there is any obstruction the development might be delayed; this very sensitive system has not been studied as far as I know. Do you know?

Dr. Staiano: No.

Dr. Taminiau: So this may be another area that might be involved in early constipation, and it has not been studied yet.

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Motility and Allergy

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Introduction

Food allergy occurs in 6–8% of children and 1–2% of adults and is permanently increasing throughout the world [1, 2]. Most of the adverse reactions to food are immune-mediated reactions, and food antigens may cause IgE and non-IgE immune responses. Of the numerous symptoms of food allergy, at least in the early stages, gastrointestinal disorders, from food protein-induced enterocolitis to constipation, are of paramount importance and are often associated with proctitis. Celiac disease is a specific food protein-induced enteropathy, but eosinophilic gastroenteritis and esophagitis are combined IgE and T-cell-mediated disorders observed in food allergy. Most of these gut inflammatory responses are associated with diarrhea resulting from both secretory and motility disorders and constipation is only observed in IgE sensitization to cow's milk [3]. Experimental data suggest that a type-1 IgE and a mast cell-dependent hypersensitivity response, particularly for motility disorders, mediate the majority of this acute food antigen-induced allergic reaction in the gastrointestinal tract.

Colic is frequently associated with food hypersensitivity and is linked to abdominal distension, bloating and flatus resulting from gastrointestinal motor abnormalities [4]. Diarrhea and vomiting following the ingestion of food containing oral antigens are the most common symptoms of food allergy in children sensitized to various types of food antigens, suggesting the paramount importance of gastrointestinal motor disturbances in the genesis of these symptoms.

Experimental Approach

The era of experimental models for allergic enteropathy began in 1963 when Mota [5] demonstrated the ability to sensitize rats by the simultaneous

injection of antigen and *Bordetella pertussis* vaccine. Successful induction of this sensitization with dietary proteins in animals has been shown to require strict attention to the type and dose of antigen, the strain and age of animals, the need for adjuvant and route of immunization. In both rats and guinea pigs, the major antigens investigated to date have been cow's milk antigens and egg albumin. To avoid issues of tolerance, desensitization and blocking antibody, the animal is raised on a diet devoid of the antigen to be studied. In both species, immunologic responses to egg albumin are more predictable than cow's milk with regard to IgE antibody production [6]. Higher responder strains of animals are chosen, especially the Hooded-Lister rat and the Hartley guinea pig. In general, low doses of antigen favor IgE as opposed to IgG antibody production. The role of adjuvants, such as *B. pertussis* and aluminum hydroxide gel, is well established for IgE antibody elaboration in the rat and mouse. In contrast, the guinea pig achieves sensitization to egg albumin in the absence of adjuvant [7]. Immunization is generally performed by the parenteral administration of antigen to maximize the consistency of the response. Nonetheless, sensitization by oral, intratracheal and intradermal injection has been achieved with consistent gastrointestinal responses to oral challenge.

In vitro Data

Immediate hypersensitivity (type-I) reactions of the gastrointestinal tract have been characterized primarily in the small intestine. Isolated longitudinal segments of jejunal smooth muscle obtained from rats previously infected with nematode parasites contract after the addition of antigen prepared from worms, whereas issues obtained from naive rats are unresponsive [8]. This antigen-induced contraction is mediated by the release of 5-hydroxytryptamine (5-HT) from mast cells as evidenced by the ability of mast cell stabilizers, such as doxantrazole, and desensitization of the muscle to 5-HT to inhibit this response. Antigen in the form of ovalbumin administered to previously sensitized animals also contract isolated jejunal strips. However, differences exist in vitro in the response of various regions of the gastrointestinal tract. Indeed histamine, a major mediator released from mast cells, both contracts and relaxes the rat fundic strip, whereas histamine only contracts the longitudinal ileal smooth muscle. The response to 5-HT, a mediator found in the mast cells of some species, also varies among different regions of the gut. In the guinea pig ileum, 5-HT produces a contraction depending directly on the release of acetylcholine, whereas in the colon addition of 5-HT produces several responses, contraction, relaxation or both [9]. In sensitized guinea pigs, the in vitro addition of ovalbumin to isolated colonic segments of the circular layer produce a biphasic response. The initial response consists of a rapid contraction followed by a late response, which is a more sustained but smaller increase in tone and phasic activity [10]. Mepyramine inhibits the initial response

while the leukotriene antagonist, WY4852, and the mast cell stabilizer, doxantrazole, both inhibit the late response.

Motility Effects of Challenge in Sensitized Animals

The potential of food protein-induced anaphylaxis to alter gastrointestinal motility has been extensively explored in the Hooded-Lister rat and guinea pig.

In the small intestine, the anaphylactic response to challenge, whatever the route of sensitization, is characterized by IgE antibody-mediated mast cell degranulation and the release of preformed and newly generated mediators.

Numerous articles have described the changes in gastric, intestinal and colonic motility and transit following oral challenge in sensitized rats. Both gastric and intestinal slow waves are altered corresponding to a reduction in frequency; these effects being locally mediated as demonstrated by challenging isolated segments [11, 12]. In fasted rats, the intestinal motor activity is characterized by migrating motor complexes (MMCs) that are suppressed for several hours after a meal. Fargeas et al. [13] demonstrated that antigen challenge in Hooded-Lister rats sensitized to egg albumin also disrupts the MMC pattern replaced by a 'fed'-type pattern for 2–3 h (fig. 1), and these effects differ from those of two mast cell degranulators, compound 48/80 and BrX-537A, with an initial strong motility inhibition followed by a progressive recovery. Similar data were obtained by Scott et al. [12] in association with diarrhea. However, the involvement of afferent vagal fibers in the genesis of these disorders found by Fargeas et al. [13] were not confirmed by simple vagotomy, also suppressing the efferent vagal fibers and suggesting the involvement of both local and central components in the genesis of small intestine motor alterations.

Antigenic challenge also affects colonic motility in rats sensitized to ovalbumin, however these effects are biphasic corresponding to an early short inhibition that may be attributed in part to mast cell degranulation and stimulation of motility, also blocked by the mast cell stabilizer, doxantrazole [14].

Role of Mast Cell Mediators

Mast cell mediators that are released under challenge stimulate the contraction of circular and longitudinal smooth muscle activity in vitro and altered myoelectric and motor activity in vivo. Mast cell degranulation induced by two mast cell degranulators (compound 48/80 and BrX-537A) on duodenal and jejunal myoelectric activity abolishes the intestinal spiking activity of the duodeno-jejunum with progressive recovery; BrX-537A being less active. These effects are antagonized by previous administration of selective 5-HT antagonists. Indeed, methysergide (a 5-HT1 antagonist) reduces by about 80% both the duodenal and jejunal inhibition of spiking activity with early recovery of a normal pattern. Ondansetron (5-HT3 antagonist) and ICS 205–930 (5-TH3/5-HT4 antagonist) respectively shorten and suppress the

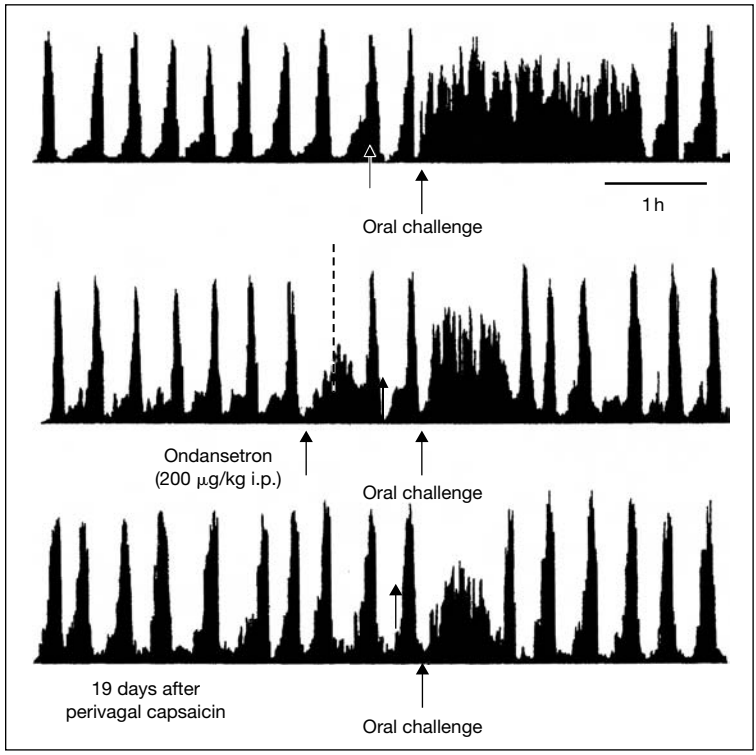

Fig. 1. Mediators and afferent nerves involved in motility disorders associated with oral challenge (ovalbumin) in sensitized rats (From Castex et al.1995).

inhibition of intestinal spiking activity with early restoration of intestinal motility in both the duodenum and jejunum. These data suggest that at least in rats: (i) the degranulation of peritoneal mast cells induces alterations in intestinal myoelectric activity through the release of 5-HT, and (ii) these effects are mainly mediated through both 5-HT1 and 5-HT3 receptors [15].

Similarly, BrX-537A inhibits colonic motility in a biphasic manner. The immediate strong inhibition of colonic motility lasting 30–40 min was inhibited by the 5-HT3 antagonist, granisetron, suggesting a local effect of 5-HT released from mast cells. In contrast the late inhibition lasting 3–4 h is partly suppressed by the 5-HT1 receptor antagonist, methysergide, and the H1 antagonist, chorpheniramine [16]. This late phase of inhibition has been shown to involve afferent nerves and particularly vagal afferent nerves since it is reduced by systemic capsaicin treatment and blocked by perivagal capsaicin [17]. More recent data also suggest that tryptase release by mast cell degranulation may participate in the inhibition of colonic motility following mast cell degranulation [18].

Table 1. Fos protein immunostaining in the brain after intraduodenal ovalbumin in sensitized rats

	Nonsensitized	Sensitized			
		vehicle	ondansetron	sham	capsaicin X
NTS	–	++++	−/+	+++	–
LPB	–	+++	+	+++	–
PVN	–	+++	+	+++	–
MMC disruption		56 ± 11	17 ± 7	48 ± 9	–

NTS = Nucleus tractus solitarius; LPB = lateral parabrachial nucleus; PVN = hypothalamic paraventricular nucleus. From Castex et al. [17].

Mediators of Challenge-Induced Motility Disturbances

The in vitro data obtained from isolated intestinal and colonic strips have suggested that mediators released by mast cell degranulation such as histamine and leukotrienes are partly responsible for the acute contractile response of ileal and colonic longitudinal layers [8, 10].

From in vivo investigations in 1988, Scott et al. [19] described the correlation between IgE titers and the intensity of diarrhea and intestinal myoelectric alterations following challenge in sensitized animals. The involvement of cholinergic motoneurons in the effects of oral antigen challenge on jejunal MMCs was established by blockade of the effects with atropine [13]. Regarding mast cell degranulation, these effects are also suppressed after non-selective destruction of afferent nerves by capsaicin. The role of substance P (SP) in these effects was identified using selective neurokinin-1 receptor antagonists that are able to block the effects of the challenge with egg albumin in sensitized rats [13]. Therefore, it was suggested that mast cell degranulation releases substances able to activate afferents fibers which in turn release SP, and SP may activate cholinergic motor neurons to produce these modifications in intestinal motility.

The role of vagal afferent fibers and 5-HT3 receptors in the effects of oral challenge was confirmed by showing that challenge activates c-Fos expression particularly in the nucleus tractus solitarius (table 1), a brain structure mainly receiving inputs from the vagus [16, 17]. In contrast, Scott et al. [12] did not observe these changes, but in these experiments the challenge was limited to isolated intestinal segments suggesting that passage through the whole gut is necessary to activate vagal afferent fibers. Previous treatment with the 5-HT3 antagonist, granisetron, just prior to challenge prevents both motility disorders and increases c-Fos expression at the nucleus tractus solitarius level suggesting that the motility disorders induced by antigenic challenge involve activation of vagal 5-HT3 receptor and are mediated through the central nervous system (fig. 1, 2). These results are in agreement with previous data

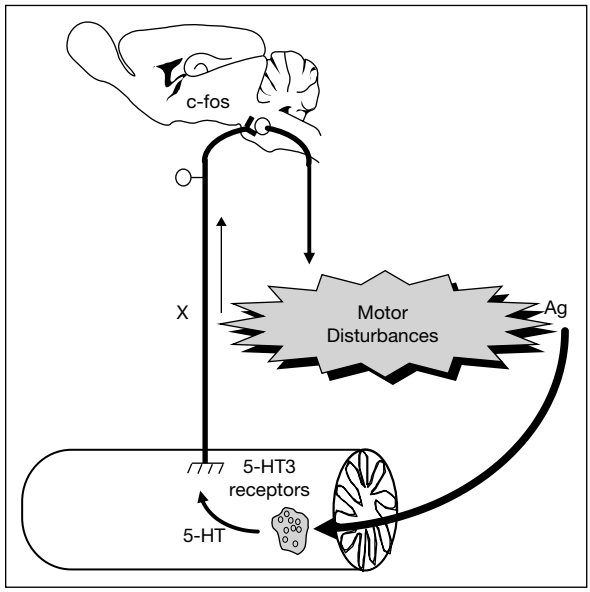

Fig. 2. Gut–brain pathways involved in anaphylaxis-induced altered intestinal motility in guinea pigs.

showing the involvement of capsaicin-sensitive fibers in jejunal secretory response to oral challenge [20]. The fact that ondansetron, a 5-HT3 antagonist, was also active in preventing the duration of MMC disruption following challenge when injected intracerebroventricularly at a 10 times lower dose than those active by the systemic route and without affecting increased brain c-Fos expression, suggests that in addition to a peripheral component, 5-HT3 receptors are also involved at the CNS level in triggering intestinal motor disorders [17].

As for the effects of mast cell degranulators, methysergide and indomethacin reduce the effects of antigenic oral challenge but not chlorpheniramine suggesting that, in addition to 5-HT3, 5-HT1 and prostaglandins are involved in the motility disorders related to challenge but not histamine directly [13].

Colonic motility and transit are also affected by oral antigenic challenge in egg albumin-sensitized rats, these effects corresponding to a colonic inhibition associated with diarrhea [13, 21]. Mast cell involvement is suggested by a significant reduction in the number of granulated mucosal mast cells in sensitized tissues after Ag challenge and in the magnitude of the Ag-induced contractile response in the presence of mast cell stabilizers. This antigen-induced response is independently inhibited by both cyclooxygenase and lipoxygenase enzyme inhibitors and by leukotriene D4 and platelet-activating factor receptor antagonists. The Ag-induced response is resistant to histamine

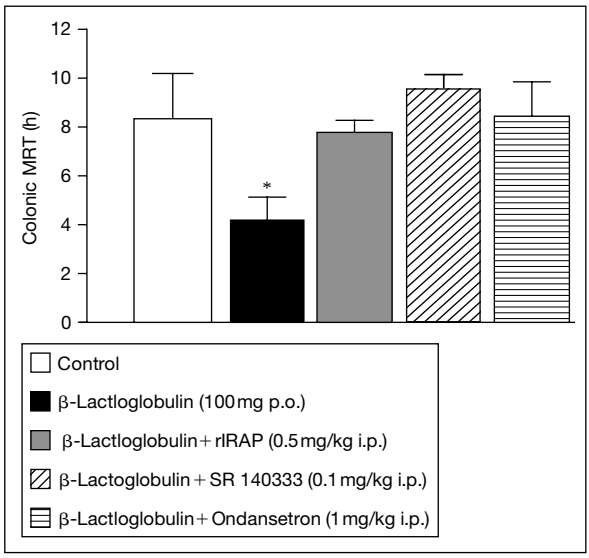

Fig. 3. IL-1β, anaphylaxis and colonic transit in β-lactoglobulin-sensitized guinea pigs. *$p < 0.05$ from control. From Theodorou et al. [22, 23].

and the 5-HT antagonists, atropine and tetrodotoxin. These results suggest that the food protein-induced contraction of colonic longitudinal smooth muscle in the sensitized rat is due to IgE-mediated mast cell activation with the subsequent production and release of membrane-derived mediators that, in vitro, act directly on the smooth muscle. However, different results were obtained in another species, the guinea pig.

Species Differences

In the guinea pig, sensitization to β-lactoglobulin is easily obtained by oral gavage or spontaneous ad libitum drinking of milk. In sensitized guinea pigs, in contrast to the effects of oral challenge in rats, oral challenge with β-lactoglobulin is associated with a strong stimulation of colonic motility giving rise to diarrhea and increased permeability to macromolecules [22]. We also established that this antigenic challenge shortens the duration of the colonic mean retention time by about 50% (fig. 3). Moreover, in this species we established that most of the digestive effects, including motility hyperkinesia and increased paracellular permeability, not only involved mast cell degranulation but also the release of cytokines such IL-1β and prostaglandins [23]. Indeed, all these effects were abolished after treatment with the

recombinant IL1 receptor antagonist, indomethacin, and a neurokinin-1receptor antagonist, (SR140333, and the 5-HT3 receptor antagonist, ondansetron (fig. 3). These last data are in agreement with the participation of the brain, cytokines and SP in these motor alterations.

Studies of intestinal motility in calves given antigenic soya protein or sucrose have shown intestinal and colonic motor disturbances linked to diarrhea. Disorders arising from feeding antigenic soya protein were distinct from abnormal motility induced by indigestible carbohydrate. These motility disorders resemble those observed in sensitized guinea pigs and are partly suppressed by chromoglycate, a mast cell stabilizer [24].

Conclusions

Motility disorders observed in food allergy affect the whole digestive tract. The experimental data suggest that they are present in sensitized animals and are exacerbated during challenge. They mostly depend upon the release of mediators from resident gut mast cells with an immediate local component involving 5-HT and histamine and cholinergic stimulation. However most of these effects involve afferent nerves; the brain-gut axis plays an important role in the vagal afferent fibers activated by the local release of mediators and the final release of neurokines such as SP. Despite some species differences in the nature of the motility disorders, their major purpose, in addition to a gut secretory response, is to eliminate the antigen from the gastrointestinal tract.

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Discussion

Dr. Benninga: Looking at your last slide in which it is proposed that mast cell degranulation leads to motility disturbances, it would be expected that most of the time infants with allergy have diarrhea. But yesterday we also discussed that a minority of these children also have constipation. With your model could you prove that it is just an observation and not a pathophysiological mechanism that causes the constipation?

Dr. Bueno: I cannot answer directly. In our model we were sensitizing animals and triggering a clear-cut anaphylactic reaction when challenging them with antigen, giving rise to inhibition of the motility by a centrally mediated mechanism, and this inhibition may promote constipation. However, in vitro, local challenge of colonic strips from sensitized animals stimulates motility. But in vivo, this effect is associated with water and ionic secretion and may initiate diarrhea. This can explain that, in terms of fecal output, both diarrhea and constipation may be observed. Moreover, depending on the degree of sensitization, we can observe more or less selective degranulation of mast cells and this factor has also to be considered.

Dr. Heymans: If mast cells are playing such an important part, is there any place for chromoglycate in trying to influence the effects on motility? We have performed studies in infants with proven food allergy and looked for gut permeability, and we have shown that if you provide them with chromoglycate before re-challenge you can influence some of the changes in permeability [1]. Would you think that you can also change motility?

Dr. Bueno: Yes, of course. Clearly the involvement of mast cells in both the secretory reaction to challenge and the motility disorder is demonstrated and mast cell stabilizers may prevent both. In animal models, we use doxantrazole but we can speculate that the same results may be obtained with chromoglycate.

Mr. Benyacoub: Can you clarify how substance P, which promotes a pro-inflammatory response, can antagonize gut motility that is in fact initiated by inflammatory signals?

Dr. Bueno: During inflammation, inflammatory mediators and also substances released by mast cells may sensitize afferent neurons which are releasing substance P responsible for the motor disorders. This was demonstrated by the fact that substance P antagonists suppressed most of the motility disorders triggered by local inflammation or mast cell degranulation. This sensitization of afferent nerves by products released from mast cells and immunocytes is responsible for gut hypersensitivity to distension as observed in inflammatory bowel syndrome (IBS). Recently, an increase in nerve terminals close to the mast cells has been described in IBS patients.

Dr. Taminiau: In pediatrics we see only a little bit of IBS, but the majority have what we call chronic abdominal pain, and everything that might be a stress disorder. So how is stress related and is it possible to investigate it in your model and separate IBS from chronic abdominal pain? Is there a pathophysiological basis which we don't have?

Dr. Bueno: A number of factors can produce sensitization or degranulation of mast cells. Stress is able to cause mast cells to degranulate under various stimuli associated with a lowered threshold, generating pain under mechanical stimuli like intestinal contractions. Moreover, in animals sensitized to food allergen, we also observe a sensitization for the development of hypersensitivity to mechanical stimuli. When mast cells are sensitized, just the same pressure applied to the wall is able to produce degranulation without any additional factors. Therefore we can speculated that chronic abdominal pain may be linked to the sensitization of mast cells to degranulate under normal mechanical or chemical stimuli occurring within the gut and then triggering pain.

Dr. Benninga: In children with chronic abdominal pain we recognize functional abdominal pain and irritable bowel syndrome. We know from our own rectal barostat studies that children with IBS have rectal hypersensitivity whereas children with functional abdominal pain do not [2]. Could you suggest that mast cell degranulation plays a role in IBS children but not in children with functional abdominal pain? Is it that easy or is it too easy to conclude?

Dr. Bueno: As I explained earlier, we can hypothesize that in viseral pain, the sensitization of mast cells is not associated with stimulation of the immune system strong enough to produce motility or secretory disorders. For example, neonatal stress is associated with long-term sensitization of mast cells to degranulate and gut hypersensitivity, but no change in intestinal and colonic transit occurs despite the presence of an increased number of lymphocytes and neutrophils.

Dr. H. Hoekstra: If this suggestion is true then it might be worthwhile to test the newly developed anti-IgE in this condition.

Dr. Bueno: Yes, you are right, but not all mast cell degranulations are linked to IgE activation. A number of neuropeptides, as well as chemical or mechanical stimuli, are able to trigger selective degranulation. Recently, it appears that mast cells may selectively deliver one or the other type of granule containing different substances. There is no continuous overflow from mast cells delivering sensitizing molecules to nerve terminals.

Dr. Baerlocher: In humans we also know what is called the pseudo-allergic situation, meaning reaction to food colorings or others. Where would you put these in your scheme?

Dr. Bueno: It has been demonstrated in rats that mast cells may be conditioned to degranulate with a simple auditory conditioning stimulus. Consequently, it is realistic to believe that this can occur in humans and that may be very important in some allergic-like reactions to food. We have to integrate these important data into the pathophysiology of allergic disease.

Dr. Taminiau: But is there any human equivalent of allergy as compared to sensitization in your animal models? Is there any allergic study in humans?

Dr. Bueno: In humans, several studies have demonstrated that in uninformed patients allergic to a specific food, only a third of them produce local allergic reactions to this food when infused intraluminally.

Dr. Schmitz: Just for the pleasure of making a comment, I congratulate you for this very elegant lecture, and particularly for the slide which shows that stress is able to degranulate mast cells through CRF. I think here you have given a physiological answer to processes that we have always seen as purely psychological, and it is interesting to understand the biological mechanism through which psychological processes can affect the gut. My question is how important is this factor compared to the others? My comment relates to what was said yesterday that allergy is a growing condition in the modern world, particularly in developed countries. Could it be that the stressing life we are all living could be a factor of increased allergy? Can we speculate on that?

Dr. Bueno: Stress, as many other factors, has an effect on epithelial cells for the lung as well as the digestive tract. Stress increases tight junction permeability and consequently the uptake of allergens. Consequently, we can speculate that stress may favor the development of asthma and food allergy.

Dr. Taminiau: What extra slides do you have to answer questions we didn't ask. Can you give us the answer and then we will guess what the question should be.

Dr. Bueno: I take this opportunity to extend the data related to the mechanisms by which stress affects gut paracellular permeability, the uptake of allergens and toxins, and subsequently hyperalgesia. Stress activates mast cells promoting the production of cytokines by T lymphocytes and particularly interferon-γ responsible for the contraction of the epithelial cell cytoskeleton through an MLCK-dependent mechanism. This contraction of the cytoskeleton opens the tight junction. Such opening of the tight junction by stress is followed by a paracellular bacterial translocation and activation of the local immune system.

Dr. Taminiau: The slides?

Dr. Bueno: This slide shows you that a number of mediators released by mast cells are able to activate or to sensitize sensory nerve terminals. Among them, serotonin and tryptase directly activate receptors located on the nerve terminal to induce nociceptive signals.

Dr. Steenhout: Would you also have some explanation to make a relation between food allergy and migraine?

Dr. Bueno: Yes. Calcitonin gene-related peptide (CGRP) is a neuropeptide contained and released from afferent nerve terminals. CGRP has vasodilatatory effects and is considered to play a role in migraine. Therefore, we can speculate that under chronic activation of the sensory nerve, there is a high level of circulating CGRP suspected of triggering migraine.

Dr. Bee Wah Lee: Can I ask whether there is systemic inflammation when events in the gut result in mast cell degranulation? We have patients who have very severe abdominal symptoms and in whom food allergy is a possible cause, but they have absolutely no signs or hematologic evidence of any systemic inflammation. That is, they have severe gut symptoms but no signs of inflammation.

Dr. Bueno: No skin or other manifestations?

Dr. Bee Wah Lee: No.

Dr. Bueno: That probably depends upon the degree and localization of the activated mast cells. We can speculate that if the sensitization is only limited to the mucosal mast cells within the gut, then the presence of allergens will only activate these mast cells because they are strongly resident mast cells. When more mucosal cells are sensitized, mast cell degranulation may concern mast cells that have migrated or are present in other organs such as the lungs.

Dr. Sinaasappel: With your talk and also the earlier talk, we didn't pay attention to the influence of the narrow endocrine system and mediators at the cellular level on electrolyte and water transport, and there is a close connection between these two. Probably at this time it is not possible to give an answer to that, but it reminds me that in allergic diseases and also in other conditions that raise in stimulants of the intestinal tract that there is also an influence on water and electrolyte transport.

Dr. Bueno: We only have some preliminary data about the chloride transporter that can be modulated by granulate cyclase C presentation of enterocyte which can be activated in some stressful situations. So chloride and water secretions are affected by stress. Many mediators released by mast cells or during stress also affect secretions by the epithelial cells. Indeed, 5-hydroxytryptamine is an important mediator of these effects, and also cytokines from the activated immune system stimulate enterocyte secretions.

Dr. Fritsché: Looking at your slides, is it possible that stress has an influence on the IgE-mediated reactivity?

Dr. Bueno: Stress is able to modulate the sensitization to mast cells, and so perhaps also the synthesis of receptors for IgE. No experiment has demonstrated that stress may directly enhance the synthesis of IgE. However, as already mentioned, stress may affect the degree of the possible uptake of allergens and consequently the production of IgE or the reactivity of mast cells to IgE by sensitizing them to degranulate.

Dr. Taminiau: Thank you very much for your excellent discussion and presentation. I can highly recommend reading the articles in *Gut* on neonatal maternal deprivation and the changes in colonic epithelial barrier and immunity changes in the long term [3], and also in *Gastroenterology* [4] in which he explains the studies he was talking about.

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The Role of Dietary Fiber in Childhood and Its Applications in Pediatric Gastroenterology

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Introduction

Since the 1970s the importance of dietary fiber for human health has been acknowledged and investigated. In the 1970s a relation was found for the first time between constipation, hemorrhoids and fiber-depleted food. The term dietary fiber is familiar to most people, although many do not fully understand the nature of dietary fiber and its role in the diet. Dietary fiber is a normal constituent of healthy food. Both in enteral and oral feeding the presence of fiber is necessary; not only in the face of problems like constipation and encopresis but also for a wide range of other disorders in adults and children such as diabetes mellitus, hypercholesterolemia, high blood pressure and colon cancer. In this chapter we will review the nomenclature, physiological properties and fate of fiber in man and its applications in pediatric gastroenterology [1]. The role of fiber in colorectal neoplasia will not be discussed here.

Definition

Since Hipsley [2] introduced the term dietary fiber in 1953, the exact definition has been controversial as scientists have studied various aspects of the impact of food supply and dietary fibers upon health. Two important questions arise when a definition for dietary fiber is sought: first which polymers should be categorized as dietary fiber? And secondly, can the term

'fiber' be correctly assigned to substances that are not metabolized, and are also not fibrous in chemical structure. Here we will adopt the definition for dietary fiber as put forward by the Dietary Fiber Definition Committee of the American Association of Cereal Chemists [3]: 'Dietary fiber is the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation and/or blood glucose attenuation.' However, this focus on digestibility has been contested in Europe [4]. There is no proof that digestibility is beneficial. Having a definition on the percentage of non-starch polysaccharide content of natural foods in food tables better serves the potential benefits of these plant cell walls (table 1).

According to the American definition, food components having the above properties can also be taken as dietary fibers such as resistant starch and non-digestible oligosaccharides. Resistant starch is the sum of starch and starch-degradation products not absorbed in the stomach and small intestine. Three types can be separated: RS1, physical non-approachable starch (lentils, beans); RS2, ungelatinized starch (bananas and potatoes), and RS3, retrograded starch (mainly amylose). These RS fibers are fermented at different rates in the colon and the amount in food is dependable on food production (heating and cooling down) [5, 6]. Legumes appear to be the single most important source of resistant starch, with as much as 35% of legume starch escaping digestion [7].

Non-digestible oligosaccharides are naturally present in food, mostly in fruits, vegetables or grains, or produced by biosynthesis from natural sugars or polysaccharides and added to food products because of their nutritional properties [8]. They consist mainly of fructo-oligosaccharides (FOS; one glucose molecule connected to as many as 60 fructose molecules or fructose molecules alone; the bond is of the $\beta_{(2-1)}$ type). In nature these are mainly found in inulin, a mixture of FOS that can be turned into a mixture of FOS of 8 units by hydrolysis. If the fructose molecule is exchanged by a galactose molecule then galacto-oligosaccharides (GOS) occur. The latter are found in soybeans. GOS can also be synthesized from lactulose. FOS and GOS can be obtained quite pure and can be added to food as functional ingredients.

Today both FOS and GOS are also recognized as prebiotics. Prebiotics beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Recent data indicated that a prebiotic mixture of FOS and GOS was able to stimulate the development of a microbial flora similar to that of breastfed infants [9]. The authors suggested that prebiotics might play a role as modulators of the postnatal development of the immune system. Furthermore the GOS/FOS mixture significantly increased the number of bifidobacteria and reduced the number of pathogens in term as

Table 1. Dietary fiber content of foods (g/serving)

Food groups	Food	Serving size	Total dietary fiber
Fruits	Apple, large with skin	1 apple	3.7
	Banana	1 banana	2.8
	Figs, dried	2 figs	4.6
	Orange	1 orange	3.1
	Peach, canned	1/2	1.3
	Pear	1 pear	4.0
	Prunes, dried	5	3.0
	Raisins	1 miniature box (14 g)	0.6
	Strawberries, raw	1 cup, sliced	3.8
Vegetables	Beans, kidney, canned	1/2 cup	4.5
	Broccoli, raw	1/2 cup	1.3
	Brussels sprouts, cooked	1/2 cup	2.0
	Carrots, raw	1/2 cup	1.8
	Celery, raw	1/2 cup	1.0
	Lentils, cooked	1/2 cup	7.8
	Lettuce, iceberg	1 cup, shredded	0.8
	Peas, green, canned	1/2 cup	3.5
	Peas, split, cooked	1/2 cup	8.1
	Potatoes, boiled	1/2 cup	1.6
	Spinach, cooked	1/2 cup	2.2
Grains	Bread, white, wheat	1 slice	0.6
	Bread, whole wheat	1 slice	1.9
	Cheerios	1 cup	2.6
	Crackers, graham	2 squares	0.4
	Cream of wheat	1 cup	2.9
	Oat bran muffin	1 muffin	2.6
	Oatmeal, cooked	3/4 cup	3.0
	Raisin bran	1 cup	7.5
	Rice, brown, cooked	1 cup	3.5
	Rye crisp bread	1 wafer	1.7
	Shredded wheat	2 biscuits	5.0
	Wheat bran flakes	3/4 cup	4.6
Other	Apple pie	1 piece	1.9
	Chocolate cake	1 slice	1.8
	Nuts, mixed, dry roast	28 g	2.6
	Yellow cake	1 slice	0.2

Source: USDA Nutrient Database for Standard Reference.

well as in preterm infants when compared with a group of infants fed a formula without supplement [10]. Stool consistency and fecal pH were also positively affected. These data were confirmed in a double-blind randomized controlled study in infants comparing a FOS-supplemented cereal (0.75 g FOS/cereal) with placebo [11]. The FOS-supplemented cereal was well tolerated and improved stool regularity and consistency.

Table 2. Function of non-fermentable dietary fibers

Non-fermentable dietary fibers are hardly digested in the colon but still have important functions such as:
• Shortening the transit time
• Fluid uptake, feces content increase and softer stools
• Positive effect on gut integrity by trophic effects on colonic mucosa (increase in cell turnover and secretion of gut hormones)

It has to be addressed that the effect of prebiotics is only temporary and strictly related to intake. More research is needed to delineate optimal fiber intake for infants and children <2 years of age, the quantity and types of fiber that would be most appropriate, and if prebiotic supplementation leads to measurable long- and short-term benefits for infants.

The Effect of Dietary Fiber on Gastrointestinal Function

The effect of dietary fiber on the gastrointestinal tract is explained by its osmotic properties, its stimulating effect on intestinal motility and the water-retaining capacity in the intestine (table 2). The water-retaining capacity of crude fibers is greater than that of fine fibers, and raw fibers have better laxative effects than cooked ones. Insoluble fibers such as cellulose and lignin are minimally degraded by colonic bacteria and thereby retain water, increase fecal bulk and decrease the intestinal transit time [12]. Soluble fibers such as hemicellulose and pectin are largely broken down by the colonic microflora. They have little effect on fecal weight, but they increase fecal volume and soften the stool by increasing the bacterial mass [13].

Dietary fibers are also able to bind bile salts and fatty acids in the small intestine. They are liberated in the colon after fermentation of fiber and thereby have a laxative effect. Moreover, during fermentation of polysaccharides, gas and short-chain fatty acids (SCFAs) are produced. The predominant acids include acetate, propionate and butyrate. The production of SCFAs through fermentation of oligosaccharides by colonic flora is important because the SCFAs have well-described effects in the intestinal tract. For example, it is largely accepted that butyrate has an essential role in maintaining the metabolism, proliferation and differentiation of the different epithelial cell types [14]. Although, it has to be admitted that, despite its prominent role, the taxonomy, population structure, and dynamics of predominant butyrate-producing bacteria in the human intestinal tract are poorly understood [15]. Current research is focussed on developing new

probes such as the 16S rRNA-targeted oligonucleotide probe to investigate the quantitative and qualitative distribution of bacteria in the gastrointestinal tract [15]. However, even experience with this new probe could not detect bacteria in all fecal samples further emphasizing the diversity of the colonic microbiota at the strain level. Future research probably will find inter-individual differences possibly due to diet, genetic constitution or geographic location.

Fiber Intake Recommendations

The amount of fiber needed by children varies by the age and weight of the child. The first recommendations about fiber intake were given by the American Academy of Pediatrics published in 1981. The revised recommendations were published in 1994 and 1995 and were based on the age of the child, health benefits such as controlling or preventing obesity, hyperlipidemia, diabetes and colon carcinoma and safety concerns [16]. In both European and American studies children consume amounts of fiber that are inadequate for health promotion and disease prevention [17–19]. Therefore, the American Health Foundation and the American Academy of Pediatrics recommends a minimal intake for children and adolescents 3–20 years of age to be equivalent to the age of the child plus 5 g of dietary fiber/day (age + 5). The age + 5 g level of fiber intake for children is similar to the American Academy of Pediatrics recommendation (0.5 g/kg/day) up to the age of 10 years, but lower for older adolescents. Furthermore, this recommendation is consistent with current guidelines for adult dietary fiber intake (25–35 g/day).

The current concern about recommending a high-fiber diet is that it has the potential for reduced energy density, reduced calorie intake, and poor growth, especially in very young infants. Secondary to these factors is the concern that such diets reduce the bioavailability of iron, calcium, magnesium and zinc. However, most investigators nowadays state that when dietary fiber intake is according to the recommendations given above and the dietary fiber is consumed within a proper balanced diet, mineral deficiencies will be of no real concern [16].

Despite the availability of fiber supplements it is sometimes difficult to achieve the recommended fiber intake. Especially constipated children are often trapped in a vicious circle of poor appetite resulting in poor intake. Moreover, side effects such as intolerance, ineffectiveness and tastelessness of the fiber product may lead to poor compliance of ingesting adequate fiber.

Despite the good intentions of the parents and advice by their primary care physicians, only half of the children receive the recommended amounts of dietary fiber [17]. Further public education with regard to fiber intake is warranted.

Constipation

When healthy volunteers add fiber to their diet, such as cereal brans, psyllium seed husk, methylcellulose or a mixed high-fiber cereal, stool weight increased and gastrointestinal transit time decreased. The increase in stool weight is caused by the presence of the fiber, by the water content of the fiber and by partial fermentation of the fiber which increases the amount of bacteria in stool. Already in 1927 a publication in the *American Journal of Physiology* suggested the laxative action of wheat bran [20]. Since then many papers have emerged in which a possible association is suggested between fiber intake and motility disorders. However, the association between fiber intake and constipation is still controversial [21]. To date, there are no large randomized clinical trials that have addressed the role of fiber in the treatment of constipation in otherwise healthy children.

Two case-control studies in children showed a lower fiber intake in constipated children compared to healthy controls [22, 23]. Discriminant analysis showed that only fiber intake was independently correlated with constipation [22]. On the other hand, it has been demonstrated that constipated children do generally not consume less fiber than healthy persons and treatment with increased fiber intake did not result in large clinical effects [18, 24–27]. Side effects such as intolerance and tastelessness of the fiber product may lead to poor compliance. Moreover, in the studies by Guimaraes et al. [26] and Mooren et al. [18], no correlation was found between dietary fiber intake and transit time in each of the colonic segments studied. Those children with prolonged colonic transit time did not differ in fiber intake compared with the group of children with normal colonic transit time. Surprisingly, patients with a fiber intake below the recommended levels had a shorter right, left and total colonic transit time (although not reaching statistically significant levels) than those with adequate fiber intakes.

Recently, two small double-blind placebo-controlled trials in 20 neurologically impaired constipated children and in 31 otherwise healthy constipated children showed the beneficial effects of glucomannan (a fiber gel polysaccharide from the tubers of the Japanese Konjac plant that has no unpleasant taste or smell) 100 mg/kg body weight (maximum 5 g/day) on defecation frequency, stool consistency, soiling episodes, suppository use and side effects [25, 27]. Although the defecation frequency significantly increased after glucomannan intake no correlation between fiber intake and transit time was shown. Tse et al. [28] documented a very low fiber intake of 2 g/day in children (3–17 years) with severe developmental disabilities living in residential institutions. By increasing fiber intake to 17 g/day relief of constipation and a significant reduction in the use of laxatives was achieved. A further increase in fiber intake to 21 g/day showed a further reduction in the use of laxatives. Although the authors suggest continuing to recommend increasing the fiber intake in children with constipation, larger clinical trials are needed to confirm

the outcome of these studies. In contrast to the studies by Staiano et al. [27] and Loening Baucke et al. [25], in a small randomized double-blind clinical trial (n = 30) Motta et al. [29] in Brazil showed no positive effect on treatment outcome and gastrointestinal transit time of soya polysaccharide fiber (10–20 g/day) in children with chronic constipation.

Diarrhea

Diarrheal disease is one of the two main causes of death in children in developing countries, claiming the lives of more than 3 million children every year [30]. Although standard glucose-based oral rehydration therapy corrects the dehydration caused by cholera, it does not reduce the diarrhea. SCFAs, which are produced in the colon from non-absorbed carbohydrates, enhance sodium absorption. In a beautiful randomized controlled trial Ramakrishna et al. [31] showed that 50 g of high-amylose maize starch, an amylase-resistant starch, per liter of oral rehydration solution significantly lowered diarrheal output compared to the standard oral rehydration therapy in 48 adolescents and adults with cholera. Furthermore, the mean duration of diarrhea was significantly shorter in the amylase-resistant starch group than in the conventional treatment group.

Recently, a significant clinical improvement in diarrhea was described in an 11-year-old patient affected by congenital chloride diarrhea after oral butyrate intake at a dose of 100 mg/kg/day [32]. As already discussed above, SCFAs have a great capacity for stimulating ion and water absorption; they provide energy and induce a trophic effect on both colonic and small bowel mucosa. Moreover, it has been shown that SCFAs, particularly butyrate, are avidly absorbed by the intestinal mucosa and that this process is responsible for the transport of Na^+ and Cl^- through different mechanisms, primarily by the stimulation of an electro-neutral NaCl absorptive mechanism activated by parallel Cl^-/butyrate and Na^+/H^+ exchanger and secondarily by upregulation of the Na^+/H^+ and Cl^-/HCO_3^- exchangers [33]. Finally, butyrate is able to limit Cl^- secretion, inhibiting the Na^+-K^+-$2Cl^-$ cotransporter activity.

Cystic Fibrosis

Patients with cystic fibrosis (CF) often have gastrointestinal complaints. Atypical abdominal pain, constipation, and obstruction from inspissated intestinal contents in the terminal ileum (distal intestinal obstruction syndrome, DIOS) are frequent complications. Slowing of intestinal transit secondary to persistent steatorrhea is believed to play a role. Gavin et al. [34] compared the mean daily intake of fibers in 28 children with CF and compared their data with 28 age-matched controls. The mean daily fiber intake in CF

children was significantly lower compared to healthy controls. Furthermore, they found that the mean fiber intake in children with moderate or severe abdominal pain was significantly lower than children with occasional but mild symptoms. The authors suggested that abdominal complaints and DIOS might be secondary to the low dietary fiber content in the diet of patients with CF. In contrast, in Belgian children with CF no relation was found between fiber intake and gastrointestinal complaints or DIOS [35]. The overall intake of fiber was adequate in this group of CF children. Further studies are needed to evaluate the need of dietary fiber in this specific group of patients.

Appendicitis in Children

It has been postulated that acute appendicitis is a serious disease to emerge with the adoption of fiber-depleted diets. In order to investigate the possible role of fiber in the etiology of acute appendicitis, Adamidis et al. [36] studied 203 consecutive appendectomized children with histologically proved appendicitis and 1,922 controls using the diet history method. This Greek group of researchers found that appendectomized children had a statistically significant lower mean daily intake of fiber (17.4 vs. 20.4 g, $p < 0.001$) including all fiber fractions: cellulose, pentose, exose and lignin. No statistical significant difference was found for energy, protein, carbohydrate and fat intake. Discriminant analysis proved that only cellulose and exose were independently correlated to appendicitis and lower fiber intake was thought to be the cause in 70% of the cases. Their results suggest that low fiber intake might play an important role in the pathogenesis of appendicitis. In contrast, Naaeder and Archampong [37] in their (much smaller) study of 173 children and adults did not find a correlation between dietary fiber intake and appendicitis. It is clear that more studies are needed to clarify the exact role of fibers and its relation with acute appendicitis, but it exemplifies the importance of sufficient fiber intake in children.

Irritable Bowel Syndrome

The main aim of dietary intervention in irritable bowel syndrome (IBS) is to manipulate colonic fermentation. High-fiber diets have long been used in adults with IBS but no studies exist in children with IBS. As fibers decrease the whole gut transit time, fiber-enriched diets may be more useful in the subgroup of children with IBS and constipation. Hammonds and Whorwell [38] examined the outcome of 13 trials in which fiber was used to supplement the diet of IBS patients. Only one 1 of 6 studies using bran reported an improvement in symptoms. The outcome of their survey was that the role of fibers is limited to those patients whose problem is predominantly constipation.

In patients with IBS and symptoms such as bloating, diarrhea and flatulence, low fiber or exclusion diets are the treatment of choice. Response rates of between 50 and 70% have been reported [39].

Conclusion

Fiber likely plays a valuable role both in the prevention and treatment of several gastrointestinal disorders. However, there is an obvious need for large clinical trials to test the efficacy and safety of fiber as a therapeutic agent in the clinical treatment of children with constipation, diarrhea, IBS and acute appendicitis.

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Discussion

Dr. Aggett: Can I ask you to comment because I think one of the biggest problems concerning fibers is that no one knows what they are. You gave us a definition, but when it comes to labeling food, planning diets, giving recommendations, anticipating what the outcomes may be of manipulating so-called fiber intakes, etc., there is very little awareness of the sensitivity of what the components of fiber are actually doing or how one can actually measure them. As far as I know there are something like 3 or 4 different accepted ways to measure fiber for the sake of labeling foods. There is now a standardized approach within the European Union even though it is not necessarily accepted with enthusiasm amongst the constituent members. You gave reference to the ESPGAN Committee on Nutrition commentary on fibers, and one of the main points behind getting that report drafted was the comments and concerns that I have just to

expressed. So I was wondering if you would like to comment on the difficulty of defining it and whether or not we should stop using the term fiber and start to be much more discriminatory about the components of fiber and what we think their specific effects might be. That would be better for our development of products and also our practice.

Dr. Benninga: If, as you, the experts in the field find it very difficult to give a clear answer to this question at this time, I do have not a better suggestion.

Dr. Aggett: I didn't want you to worry about the definition. What I am implying is that perhaps we should forget about the definition; perhaps we should start thinking about the various independent components of this thing we call fiber, the same way we are starting to mature our thoughts about fat. Now fat is totally meaningless to me in many ways, and similarly I think fiber is as well, because as you said one would be far more concerned about resistant starch or α-amylase-resistant starch. In that case is it a native resistant starch or is it a natural state starch that has been cooled and has gone into a glass state and is therefore α-amylase-resistant? Are we talking about some of the sources of gums, all of which have different effects, and really I think understanding these effects and how they arise is going to take us forward far more effectively than just being concerned about fiber. I don't think it helps us characterize the benefits and the problems.

Dr. Benninga: I am not aware at this moment if there is a diagnosis test or a laboratory procedure which gives you insights into which fiber you deal with. I am not aware of this, I don't know if the audience has some suggestions about this.

Dr. Taminiau: But if you wish to separate it, then the goal might be to say I want this fiber separate because there is evidence, or we as pediatricians should study it in a certain context. Is that what you mean?

Dr. Aggett: Yes, we already have one simple demarcation between soluble and insoluble fibers, and we envisage that insoluble fibers work by water retention perhaps. Let's face it, there is some degradation and fermentation in the colon on some of the insoluble fibers, and then one comes to the soluble fibers which might have different effects. Of course it is in the soluble fibers that many people are looking for due product development, and one of the big discussions recently has been in the area of probiotics. Whether or not one could actually accept, not fiber but inulin for example, as a fiber, that was the first grade discussion. The decision is whether or not one would like to accept inulin as a non-digestible carbohydrate fructo-oligosaccharide in the diet for a specific effect, and it is this functionality that I am really asking about, I am not really looking for a description of the state of the art. There is strategy for organizing our current knowledge to take it forward so we can then think in terms of the intraluminal fate of these various components and then in turn their impact on gastrointestinal and systemic function.

Dr. H. Hoekstra: Perhaps I can help a little bit in the discussion. We have defined fermentable and non-fermentable fibers. In a previous discussion we talked about the water-holding properties of the feces [1]. It seems that water-holding properties in non-diarrheal stools are very constant, and normal and hard stools may not differ so much in this respect. If the non-fermentable fibers are responsible for the water-holding properties the net difference in the situation of constipation might be the aspects of the fermentable fibers. So if there is good fermentation that leads to good colonic function, we can explain the studies you presented. In a situation with adequate amounts of non-fermentable fibers more of these sorts of fibers will not be beneficial, but more fermentable fibers such as glucomannan could be helpful. So I would suggest having studies addressing both components, the fermentable and the non-fermentable, in constipation.

Dr. Benninga: I agree with this opinion. However, if you look at the diet of children then all kinds of fibers will be included. It will therefore be very difficult to strictly

separate the soluble and the insoluble, or the fermented or the non-fermented fibers, and truly know which effect of fibers is beneficial in children with constipation.

Dr. Hernell: Isn't that one of the problems, because most of the studies that you showed discussed only dietary fibers. With respect to functional outcomes you don't really know exactly what people have been comparing because, as Dr. Aggett says, dietary fiber is not well defined. I think we need to agree on some kind of definition. If we want to compare functional outcomes we must really know what type of fiber we are comparing.

Dr. Benninga: I agree. But the same is true if you look at the studies in adults with a lower risk of developing colonic carcinoma. It is not known if this is caused by the effect of fibers or that other supplements are important in decreasing the risk of colorectal cancer.

Dr. Leathwood: Once you have defined fiber to your own satisfaction and identified the effects, the next problem is to communicate this information to consumers. We must not forget that many consumers attribute all sorts of benefits(?) to fiber, and these do not necessarily bear much relation to expert opinions about the benefits of fiber.

Dr. Benninga: Yes, but it gives rise to the same discussion. As we really don't know how to define fibers and how to divide them, it makes it difficult to explain.

Dr. Hernell: When you give a recommendation as you did, age plus 5 g, one may wonder if the same type of fibers is applicable to all ages, or if different types of dietary fibers should be recommended for different age groups?

Dr. Benninga: I haven't really thought about it. If you look at children's diets, 75% of the fiber intake is non-soluble whereas only 25% is soluble, so perhaps we have to make this recommendation.

Dr. Hernell: I was thinking about breast milk. 20 or 15 years ago, we used to say that infants should not have too much fiber in their diets because they were not used to it, there is no dietary fiber in breast milk. Then we changed the definition of dietary fiber to non-digestible carbohydrates and all of a sudden there are a substantial amount of dietary fiber (oligosaccharides) in breast milk. So I mean it is perhaps time to question what type of dietary fiber should be recommended for what age group? May be we shouldn't recommend dietary fiber, we should recommend how much fruits and vegetables children in various age groups should eat.

Dr. Taminiau: Is there any concern about micronutrients, with regard to age or risk?

Dr. Aggett: I don't think there is. As Dr. Benninga pointed out, the opinion is that if one eats fiber at a reasonable level then there will not be a negative impact on nutrition in general and particularly on the minerals. Now clearly some of the issues arising from mineral availability relates to perceptions that there may be ionic binding between cations and fibers that would limit their availability. But interestingly I don't think there are really any good studies over an extended period to substantiate if there is a negative impact of so-called high-fiber intakes. This has mainly been done in vegetarians, there is clearly a lot of adaptive capacity to acquire the calcium, magnesium, iron and zinc that is necessary. Perhaps when there is so much non-digestible carbohydrate that it displaces other items from the diet then there may be a negative impact, but that would apply to all nutrients and not just minerals.

Dr. Schmitz: Is it a question of definition to explain the contrast between two of the results you presented, the first one being the nice slide in which the increase in the amount of ingested fiber increased stool weight, and the following slide in which you showed that in the pediatric age there is nearly no difference between the ingestion of fibers in constipated and non-constipated children? Otherwise this contrast is difficult to understand.

Dr. Benninga: Adding more fibers to the diet is the first-line treatment in adults with constipation. More importantly it works in these patients. However in children with constipation, we don't find beneficial effects of fibers on defecation frequency and stool consistency. Children don't often take the fiber supplements because of the nasty taste. A solution might be the use of glucomannan.

Dr. Taminiau: You presented 14 g in Brazil, 11 g in Greece, and Holland 7 g. Is there a difference in fiber intake in the world?

Dr. Benninga: Although there are not many papers describing the amount of fiber ingestion, I think that there will not be a large difference between the Western world and South America. Even in higher socioeconomic class families, the same intake of fiber was found.

Dr. Taminiau: So is there any epidemiology in fiber content in the world you didn't mention?

Dr. Benninga: There are not very many papers talking about fiber ingestion. But if you estimate there is not a big difference between the Western world and South America; in all countries there is a decreased intake of fibers, even when looking at higher socioeconomic class families, and it didn't make any difference when they looked at fiber intake. So I think Holland is not very different from the rest of the world.

Dr. M. Hoekstra: I would like to confront you with one of your statements with respect to the effect of fibers on constipation. You said that there is a need for larger studies, but in my opinion a large study is not always better than a small study. So I would like to ask you whether the negative studies were underpowered? My second question is: if you try to make a conclusion from several studies, you almost always end up with inconsistencies. The solution to that is that the studies are compared with respect to the patients, whether they are the patients that are being treated as well, or looking at the methodology. Are the pro studies better than the con studies?

Dr. Benninga: You pointed out the difficulties in studying constipation. I think that you have to ask Dr. Staiano if she thinks that her study was underpowered. Of course you are correct that we don't always need higher power studies, but in the majority of studies we did we always needed to have only a small beneficial effect, at least 150 patients, so I think 20 is perhaps not enough, but we will hear it from Dr. Staiano in a few moments. Another very difficult point in studying children with constipation is that there is not one definition for constipation. As I showed you in the Brazilian and Greek studies, I really think that the definition of constipation was not good. Therefore I think it is important that in a few weeks new criteria will be made and if we all stick to these criteria we might get the same population and more insight into the pathophysiology and how to treat these patients. I think that is the main weakness of our studies.

Dr. Kleinman: Do you think that there is some value in separating prevention from treatment when talking about constipation, given that for the most part when we treat constipation now most recommend increased dietary fibers? The compliance is so poor, however, that most of us now turn to a synthetic polyethylene glycol mixture that can be used very effectively often without additional stimulant laxatives. In discussing this, clearly if you are talking about a population-based approached, changing the diet makes a lot of sense, and yet if you are talking about treatment so many other things impact on successful resolution of constipation, particularly when it has been in place for months or years, that increased dietary fiber alone is likely to have less benefit there.

Dr. Benninga: I think it is a good point to talk about prevention in these children. Future studies will hopefully answer your question if early adjustments of prebiotics, such as FOS and GOS, will cause less constipation.

Dr. Sinaasappel: To continue this point: is it possible to identify risk groups that are prone to constipation? When prophylactic measures are needed, I think it is wiser to concentrate on these risk groups and not on the whole population.

Dr. Benninga: That is a very good question too. It will be difficult however to identify risk groups. We know now that 30% of the children with constipation have a first- or second-degree relative who has constipation too. It might be useful to follow the children of parents who had childhood constipation themselves.

Dr. Verloove: Can I come back to this prevention issue? Most of you from the Netherlands are aware that a nutritional analysis of Dutch children was done. From that, as I remember it, it was clear that the consumption of fiber-containing foods by children in the Netherlands is tremendously low. So if you could change the dietary habits, as you showed last week during the pediatric conference in the Netherlands, by letting them eat full-fiber pasta, if you get children to eat that kind of pasta and brown bread and fruits and vegetables, I think 50% of the problem would be solved, and you don't need to identify risk groups and give them additional fibers whatsoever. But that should be our first concern in my opinion.

Dr. Benninga: I think this is wishful thinking. As I stated before, in 1995 a conference was held in the US on adding fibers to supplements for children in the US. Disappointingly the outcome of this conference was that the fiber and vitamin intake didn't change despite an enormous advertising campaign and information to the public.

Dr. Verloove: You are probably right. Tomorrow morning we are going to talk about junk food, so I won't say anything more but I will come back to it tomorrow.

Dr. M. Hoekstra: Not looking at your slide, it must not be too difficult for industry to make a fiber-enriched product that tastes good to children.

Dr. Benninga: You mean that it won't be difficult because there are of course already fiber-enriched supplements, but are you talking about healthy or are you talking about constipated patients?

Dr. M. Hoekstra: We can talk about all categories, but if you talk about constipated patients then it must be possible. I mean children don't always like fruits, they want other things that taste better in their opinion, and it has to be possible to make something that tastes sweet and contains fibers. Why can't we make that? It is not that difficult.

Dr. Benninga: I will ask the people from Nestlé.

Mrs. Gailing: We are following the recommendation of age plus 5 for the toddlers after 1 year of age, but between 6 months and 1 year it is more difficult to make a precise recommendation. I was just doing the calculation in our infant cereal. For stage 1 globally we have two portions, so between 4–6 and 6–8 months, we have about 2.5 g fiber from infant cereal. So if some fruits in jars are added in which there are also fibers and vegetables, particularly carrots, the intake of fibers could be fulfilled. But we don't know if age plus 5 must also be followed between 6 and 12 months.

Dr. Benninga: Was it is not difficult to make the product?

Mrs. Gailing: No, it is not difficult to make the product, but a difference must be made between cellulose and other dietary fibers because it is probably less palatable when cellulose is increased.

Dr. M. Hoekstra: But I think you have to make something like a candy bar, something that taste like Bounty or Mars and contains fibers. You really have to adjust it to the tastes of the children.

Dr. Verloove: Let's wait until tomorrow with that discussion.

Dr. Caroli: It seems to me that we are going to medicalize our children too much, because if we are going to make a candy bar with vegetables I know many more tastier foods that can be useful in this respect. In your slides I did not see the length of the observation in the treatment of constipation with vegetables and fruits, nor the age of

the subjects. So I would like to know your opinion on the minimal time of using normal and tasty food before going on to using a laxative, because children comply differently to adults. In my opinion miracles do not appear all the time, so we probably need time to get good results using correct food.

Dr. Benninga: I can't answer, I don't know what the best time to start with laxatives is. As you know lactulose is also a non-digestible carbohydrate and we start it immediately if we think that the child needs it, and that can be already after 10 days or even earlier. I never wait in starting laxative if the child really has problems. It is also very difficult to define what constipation is because I think that is what you mean, how long can you wait until the defecation problems resolve, and I can't answer this question. If the child has pain during defecation, if he cries around the defecation and the defecation frequency is less than 3 times/week, then I think you have a good reason to start laxatives. We know now from studies by Dr. Staiano and our group that if you start treating these constipated infants early, then they tend to do better in life than children who started treatment later.

Dr. Taminiau: Dr. Staiano can you comment and then can you comment on what Dr. Benninga published on glucomannan. There is a limit of 5, why didn't you give more? Perhaps Dr. Kneepkens can also comment because he worked with glucomannan in the stomach to delay emptying.

Dr. Staiano: I want to say that we should make a difference between the efficacy of fibers in normal subjects and in constipated children. In adults, fibers have a very good efficacy on stool habit, even in constipated adults because one of the effects of the fibers is to increase stool size which determines the distention of the lumen and this evokes peristalsis. We know that in most constipated adults the problem is delayed transit in the proximal colonic segments. In contrast, in children constipation is mainly due to delayed transit in the rectum [1]. The effect of fibers in rectal constipation is different than in patients with a delay in the more proximal segments of the colon. In fact, it has been reported that an increased amount of fibers in adults with rectal dyschexia, i.e. a rectal delay in the transit time just at the level of the rectum, may worsen the constipation due to the difficulty in the elimination of stools with an increased size. So far, in children with functional constipation, if we increase the amount of fibers too much we could create a further problem in the elimination of this larger stool. In the past, we evaluated the efficacy of glucomannan, a soluble fiber, as a treatment for chronic constipation in children with severe brain damage [2]. The study demonstrated that glucomannan has a beneficial effect only on bowel habits but not on gastrointestinal transit time. The increased bowel frequency despite the prolonged transit time, could be explained by the frequent passage of small amounts of less consistent feces, without improvement in the progression of the intestinal contents. So, in these patients, severe damage to central structures could be responsible for the disregulation of normal content progression through the large bowel. Differently, in the last study done by Loening-Baucke et al. [3], the effect of glucomannan and placebo was evaluated in 31 children with chronic functional constipation with and without encopresis, recruited from the Pediatric Clinics of the University of Iowa and the University of Naples. We used glucomannan at a dose of 100 mg/kg body weight daily, maximal 5 g/day, just to be sure not to give too much fiber so as to have an opposite effect. Also in these children we found fiber to be beneficial in the treatment of constipation with and without encopresis, with an improvement in bowel habits. Symptomatic children already on laxatives still benefit from the addition of fibers. In conclusion an adequate amount of fiber in the diet is certainly very important for the treatment of constipated children, however I believe that we have to be careful in advising large amounts of fibers because in children there is a delay in the rectum and sometimes fibers could worsen the condition.

Dr. Kneepkens: I don't have much to add to that, but we have to realize that glucomannan is not very much different from galactomannan, present in carob gum, that we use in the treatment of regurgitation in infants, and we know that it also influences the stools of the children. Both galactomannan and glucomannan are fermented completely in the proximal colon, but at a rate which is a lot lower than, for instance, lactulose. It may act as something in between lactulose and non-fermentable fiber and have an influence especially on bacterial growth, bacterial mass, and fecal mass. So there may be a possibility for galactomannan and glucomannan to be used in constipation, but I don't think they are better than what we use at the moment, microgal, which also increases fecal weight.

Dr. Benninga: I totally agree.

Dr. Taminiau: I would like to reemphasize what Dr. Benninga showed about the digestible fibers and that digestion is not solid and water absorption but also the energy absorption. Adults can absorb about 80–100 g, it is 400 kcal in the colon and also medium-chain triglycerides, if they arrive in the colon, they are digested and about 100 g of medium-chain triglycerides can be put into short chains and absorbed, so there is about 400–800 cal. Then there are the beautiful studies by Diamond on maximal absorption in animals. He used a python as a model and let him eat a sheep to study the upregulation of absorption, what the maximum is. He showed that our nutrient-absorbed carriers such as the glucose sodium carrier are not upregulated in the human because we probably have so much reserve capacity in the colon, also in the newborn and the premature, up to 800 cal. So it is really the digestive organ that is very important because we don't upregulate in the small bowel. I would like to thank you all for participating. I would like to thank Dr. Staiano for talking about motility, Dr. Bueno for going from motility to transport and pathophysiology, Dr. Benninga for addressing fiber with all its problems, and Dr. Aggett for defining the problems we have with the definition.

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Early Influences on Taste Preferences

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Introduction

In this review, the 'early influences' considered include genetic, prenatal, early postnatal and childhood influences on perception and taste preferences. 'Taste' is used in its broad sense, including taste, flavor, and texture. 'Preferences' include responses to tastes and flavors, as well as to foods and beverages.

People differ in taste and smell sensitivity [1, 2], and in behavioral traits important in food choice [2]. At least some of these differences are of genetic origin. Intrauterine influences include experiences early in pregnancy, such as the effects of the mother's morning sickness on the salt preference of her offspring [3] and, later in pregnancy, where flavors experienced in the mother's amniotic fluid may influence subsequent preferences [4, 5]. Flavors experienced during the first 2–3 months of life can influence food preferences later in childhood [6] and perhaps into adulthood [7]. Experience with a variety of foods during weaning can ease acceptance of new food flavors [8]. Lastly, longitudinal studies suggest that food preferences and choices at 2–3 years old have a predictive value for preferences later in childhood [9] and, for some foods, into adolescence [10, 11].

We first describe some of the findings mentioned above and then explore their theoretical and practical implications, suggesting ways to help mothers successfully introduce new foods during weaning and beyond.

Development of the Senses

Anatomically complete taste buds can be identified in the human fetus by the 15th week of gestation, and olfactory neurons, apparently functional, are present by about the 25th week. Although it is difficult to establish at what

stage in gestation the fetus actually begins to experience tastes and flavors, it has been known for more than 100 years that infants born 1 or 2 months prematurely respond to some tastes. In 1859, Kussmaul [12] reported that premature infants sucked with apparent satisfaction on sweet substances and rejected bitter solutions. This was confirmed by later studies [for review see 13, 14]. In the last months of gestation the fetus 'inhales' amniotic fluid and so may be exposed to any aromas present therein [14]. In addition, newborn infants respond to aromas that could only have been experienced prenatally [5] (see 'Effects of prenatal sensory experience on later reactions to foods', below) so it is reasonable to conclude that during the last weeks of a normal pregnancy the fetus is able to detect food aromas reaching the mother's amniotic fluid.

Kussmaul [12] was also the first to report that newborn infants clearly detect and respond to different tastes. His observations that they lick and smack their lips when offered a sweet solution, purse their lips when tasting strong acids and spit out strong bitter tastes have since been confirmed by many others. Lipsett [13] provides a good historical review of this work, also noting, as do Beauchamp and Mennella [14], that neonates show no distinctive response to weak salt solutions and do not refuse weak acid or bitter tastes. Many different cultures provide infants with sweetened 'pre-lacteal' feeds [14, 15], often consisting of honey or sugared water, sometimes supplemented with oils or herbs. Although the rationales for pre-lacteal feeding vary, they are certainly considered pleasing for the infant [14], thus confirming that a 'liking for sweet tastes' by newborn infants is easily detected.

Similarly, newborn infants detect and respond to some odors. Steiner [16] reported that about 50% of newborn infants responded positively to butter, banana and vanilla aromas, and speculated that these reactions may be innate. More recently, Schaal et al. [4] provided an alternative explanation, showing that responses to food aromas can be learned in utero.

Genetic Influences on Perception and Food Preference

The earliest influences on development of perception are (a) genetic predispositions to like some tastes and dislike others, and (b) differences in sensitivity to some tastes and flavors inherited from parents. Genetic predispositions are not fixed and can change according to experience. This was nicely demonstrated by Moskowitz et al. [17] who reported that an Indian community, used to eating tamarind, liked strong acid and bitter tastes. Inherited differences in sensitivity to particular tastes and flavors can influence preferences and food choices [18, 19], but so far the effects observed are not large. The most completely studied are the inherited differences in sensitivity to 6-*n*-propyl thiouracil (PROP). Some people perceive 0.001*M* PROP as

extremely bitter, while for others it is not bitter at all [18]. These differences can be linked (although usually with poor predictive power) to differences in food preferences. Thus, Turnbull and Matisoo-Smith [20] showed that 3- to 6-year-old children who were PROP tasters disliked raw spinach (but not cooked spinach or raw or cooked broccoli) more than did non-tasters.

Children who are PROP tasters also seem to like less intense sweet tastes than do non-tasters [21]. Following up on this, Lin [22] recently reported an inverse correlation ($r = -0.51$, $p < 0.0001$) between sensitivity to PROP and the frequency of dental caries in 6- to 12-year-old children. This possible health consequence of a genetic difference in taste sensitivity merits further study.

Behavioral traits can also be genetically influenced, so it is probable that neophobia and variety-seeking are to some extent inherited. At present it is not possible to evaluate the relative contributions of genetics and environment to these behaviors but they need more exploration.

Effects of Prenatal Sensory Experience on Later Reactions to Foods

Aromas of at least some of the foods eaten by the pregnant woman find their way into her amniotic fluid, so if she eats garlic then her amniotic fluid smells of garlic [23], if she eats curry it smells of curry [24], etc. Two recent studies [4, 5] show that aromas experienced in utero appear to influence the infant's responses to aromas and foods. Schaal et al. [4] gave women an anise-flavored drink daily during the last weeks of pregnancy. In the first hours after birth, the infants showed positive facial responses to anise aroma if the mother had consumed anise during the last weeks of pregnancy. This observation offers a possible explanation for the results of Steiner [16]. As noted above, he found that about half the newborn infants he tested appeared to like banana aroma. It is possible that their mothers ate bananas in the last weeks of pregnancy whereas the mothers of non-responding infants did not.

Mennella et al. [25] found that, if mothers consumed carrot juice for several days during the last weeks of pregnancy, at weaning the infant showed more enthusiasm for a carrot-flavored cereal than an unflavored one. An evident limitation of this study was that the mother could not be 'blind' to the treatment, so may have influenced the behavior of her infant by pathways other than flavor experience.

For the moment, we can conclude that these first results are fascinating and promising, but that much more needs to be done before we fully understand prenatal influences on subsequent food choice.

Crystal and Bernstein [3] identified another prenatal influence, specifically on salt taste preference. They showed that the offspring of mothers who had

experienced moderate or severe morning sickness during pregnancy had greater salt preference and greater salt use. As women with different levels of morning sickness did not differ in reported salt use prior to pregnancy or as a function of their pregnancy symptoms, this effect seems to be specifically linked to vomiting early in pregnancy. The mechanism could involve a change in expression of (or response to) angiotensin II. Whatever the mechanism, the long-term increases in salt use could have long-term health consequences in salt-sensitive hypertensive people.

Effects of Early Postnatal Flavor Experiences on Subsequent Food Preferences

Flavors experienced in the first 2–3 months of life can influence subsequent food preferences.

The flavors of at least some foods eaten by lactating mothers quickly find their way into her milk. If the mother eats garlic, her milk smells of garlic [26] and the baby responds, often by taking more milk (at least, during the first garlic-flavored feed). Similarly, if the mother consumes vanilla, her milk tastes of vanilla [27]. Thus, in contrast to infant formula, mother's milk provides a potentially rich and complex sensory experience for the infant, reflecting in part the mother's eating habits and food culture [28].

Sullivan and Birch [29] showed that, at weaning, breastfed infants adapted more rapidly to new foods than did bottle-fed infants, suggesting perhaps that their richer sensory experience facilitated acceptance (or that they had already come across aspects of the new flavors in their mother's milk).

For evident ethical and practical reasons it is not easy to study the long-term effects of flavors in the mother's milk or bottle formula. However, in a series of studies using hydrolyzed infant formula, Mennella et al. [6, 30–32] cleverly used a 'natural experiment' to show that flavor experiences in the first few weeks of life can indeed influence later preferences. Hydrolyzed formula is appropriate for children with severe allergies to milk proteins and has a distinctive (and to adults, very unpleasant) acid, bitter, 'burnt' taste due to the free amino acids and small peptides it contains. As some children are obliged to consume them from a very early age, their use provides useful information on taste acceptance in the first months of life, and about the influence of early sensory experiences on later food selection. The key findings from this work were as follows:

(1) Infants up to 2–3 months old readily accepted hydrolyzed formula [31, 32].
(2) At 7.5 months, when presented with hydrolyzed formula for the first time, they strongly rejected it [31].
(3) If they regularly consumed hydrolyzed formula during the first 7 months, they readily accepted it at 7.5 months. If a different brand of hydrolysate

was offered to these infants, it was less well accepted [32]. (Note: for adults, the two hydrolyzed formulae had different tastes and flavors but were equally unpleasant).

(4) If hydrolyzed formula was given during 4 of the first 7 months, it was accepted at 7.5 months, but with less enthusiasm than if it had been experienced for the full 7 months [31].

(5) Children who had experienced (the bitter and acid) hydrolyzed formula for several months early in life more readily accepted acid tasting (but not bitter tasting) drinks at 4–5 years of age. This effect on acceptance of acid taste was no longer evident at 7 years. Children who had been fed soya formula, which is bitter and astringent, more readily accepted bitter tasting foods at 4–5 years old [6, 30]. In contrast, children's preferred levels of sweetness at 4–5 years old correlated best with the sugar content of their current breakfast cereal, and whether or not the mother routinely added sugar to their foods [30].

Mennella et al. [5] also demonstrated that if lactating mothers consumed carrot juice for several days shortly after giving birth, the flavor was transferred to their milk and, at weaning, the infants showed more enthusiasm for cereal prepared in carrot juice than for an unflavored cereal, although they did not eat significantly more. Once again, as the mother could not be 'blind' to the treatment, pathways other than flavor experience may have influenced the infant's behavior.

A very different study, carried out by Haller et al. [7], illustrates how flavor experiences in the first months of life may well have an impact on preference lasting well into adulthood. In Germany, for many years, infant formula has been flavored with vanilla. Haller et al. [7] asked over 130 German adults if they had been breast- or bottle-fed, and then asked them to taste two samples of tomato ketchup and note, which one they preferred. One ketchup was flavored with vanilla, the other not. The result was striking. Two thirds of the respondents who had been breast-fed liked the normal ketchup best. In contrast, two thirds of those who had been bottle-fed preferred the vanilla-flavored ketchup.

Effects of Early Post-Weaning Experiences on Development of Food Preferences in Young Children

Sullivan and Birch [29] asked mothers to offer the same vegetable (pea purée) for 10 days to their infants at weaning. All the infants (4–6 months old) showed significant increases in acceptance and intake, suggesting that simply being exposed to a new food increases its acceptability. However, as Birch [33] pointed out, it sometimes requires 8–10 exposures to achieve clear increases in acceptance, so caregivers should be encouraged to be persistent and continue to offer (without pressure) new foods that are initially rejected [33].

Brown and Grunfeld [34] gave infants sweetened or non-sweetened baby foods during the first 3 months after weaning and then measured acceptance and intake of (new) sweetened or non-sweetened fruits. They found no differences between the groups in acceptance or intake of sweet fruits, suggesting that, while exposure does facilitate acceptance of a food, the effect of sweetness does not necessarily generalize from one food to another.

Gerrish and Mennella [8] showed that experiencing a variety of foods at weaning facilitates subsequent acceptance of new foods. For 10 days, groups of mothers fed their infants the following weaning foods: either carrot purée, potato purée, or a variety of purées (pea, potato and squash). On the 1st and 11th days all infants were offered carrot purée, and on the 12th day, chicken purée. The chicken was an entirely new flavor to these infants. In addition, at the beginning of the study, mothers were asked how often her child had consumed fruit purées (never, occasionally, or daily). The results were as follows:

(1) Infants who experienced a variety of purées more readily accepted the carrot purée than did infants who had received only potato (108 ± 11 vs. 64 ± 12 g). This result is open to several interpretations, including: (a) experience with variety facilitated acceptance of carrots (favored by the authors); (b) infants who had eaten potato purée for 9 days ate less of the carrot purée perhaps in the expectation it would have a similar caloric density to potatoes (the potato purée had a higher caloric density), or (c) the flavors and textures that became familiar during the variety condition generalized to carrot purée.
(2) The infants who had experienced a variety of flavors more readily accepted the completely novel chicken purée than those who had not (31 ± 7 vs. 13 ± 2 g). This result clearly suggests that experience with variety *did* facilitate acceptance of this new food.
(3) Regular consumption of fruit did not diminish later acceptance of vegetables. In fact, earlier regular exposure to fruit was linked to a significantly enhanced acceptance of carrots on day 1 of the study (74 ± 10 vs. 43 ± 5 g).

In a recent cross-cultural study on weaning practices, Maier et al. [35] showed that exposure rates during weaning similar to the 'control' and 'variety' conditions used by Gerrish and Mennella [8] can actually be found in different regions of Europe, confirming the practical significance of the above findings.

In a longitudinal study specifically addressing the development of preference for sweetness, Beauchamp and Moran [36] measured the relative intake of water, 0.2 and 0.6 *M* sucrose at birth, 6 months and 2 years in 63 infants. Newborn infants consumed more 0.6 *M* sucrose than 0.2 *M* and more 0.2 *M* sucrose than water. At 6 months, the infants were divided into 2 groups: those who had regularly been fed sugar water and those who had not.

Although the 2 groups did not differ in their intake at birth, those regularly fed sugar water consumed, in an intake test, more sugar water (but not more water) than those who had not. At 2 years old, children who had been fed sugar water consumed more sucrose (with no difference if exposure was <6 or >6 months). Thus early exposure to sugar water had long-lasting effects on acceptance of sucrose in water. There were no differences in consumption frequency of other sweet foods nor did the effect generalize to other sweet drinks. (The same infants were tested with 0.6*M* sucrose in Kool-Aid. All of them drank more of the sweetened than the non-sweetened Kool-Aid.) Lastly, intake of 0.6*M* sucrose at 2 years was correlated with intake at 6 months (but not at birth) suggesting that innate acceptance of sweet tastes can be modified early in life. Beauchamp and Cowart [37] proposed that a sense of what should or should not be sweet rather than a general hedonic responsiveness to sweetness itself is what may be influenced by dietary experience. Thus a familiar food, only experienced without added sugar may not be enhanced by making it sweeter, while for an unfamiliar food, a sweetened version would be more acceptable. This study is one of the few covering the period from birth to 2 years old and has important implications for the studies described below, linking tastes at 2–3 years old with later preferences.

Preferences at 2–3 Years Old and Other Factors as Predictors of Liking Later in Childhood

Skinner et al. [9] examined the evolution of 97 children's food preferences, taking measures at 2–3, 5 and 8 years old. They found that patterns of preference over this period were remarkably stable. Children who liked the most foods at 2–3 years old liked the most at 8 (correlation, r = 0.79). They also liked the same foods at each time, so that consistency (percent agreement for specific foods) was 84%. In addition, there was no significant increase in the number of foods liked. The best-liked foods were carbonated soda, popcorn, white bread rolls, salted crackers, raw apples, French fries, potato chips and chocolate chip cookies. Disliked foods were preponderantly vegetables. Mothers tended not to offer foods they themselves disliked and if mothers liked many foods, the children were less likely to be neophobic.

Nicklaus et al. [10, 11] longitudinally followed the evolution of food preferences in over 300 children beginning at 2–3 years old (in which actual food choices were measured in about 100 meals for each child); these subjects were followed up from between 4 and 22 years of age. Pre-adolescent preferences were rather well explained by choices at 2–3 years old, but evolved with age and gender thereafter, particularly for meats and vegetables, so that in adolescence preferences were only modestly related to

early choices. In contrast, current preference for cheeses was well explained by choice at 2–3 years old across all age groups.

Cashdan [38] and Fischler and Chiva [39] explored the evolution of children's food choices, reporting that at 2–4 years old children became noticeably more conservative with respect to the foods they would accept. This was sometimes interpreted by parents as becoming more 'particular' eaters.

Very recently Liem et al. [40, 41] examined later determinants of sweet preferences of children in Holland. In children aged 4–5 years whose parents restricted access to sweet foods and drinks, they showed that consumption of sugars in beverages was lower than in 'control' children whose parents did not restrict (40 ± 23 and 63 ± 37 g/day, respectively; $p < 0.01$). The restricted children also tended to eat less sweet sugars at breakfast ($p < 0.09$). They did not, however, consume significantly less sweet sugars over the whole day (110 ± 54 vs. 122 ± 54 g/day, respectively; $p = 0.41$). In addition, restricted children preferred significantly higher concentrations of sucrose in orangeade than did non-restricted children. Unfortunately with the study design used, it was not possible to identify the direction of causality, so it was not clear if restricting access to sweet foods and drinks increased sweet preferences or if restrictions tended to be imposed because children showed more marked sweet preferences [40].

In a second study with slightly older children, Liem and de Graaf [41] showed that 8 days of exposure to orangeade containing added sucrose was enough to increase preference for sweeter orangeades ($p < 0.05$), once again suggesting that children habituate to sweeter versions of foods. In contrast, exposure to an equally liked, but more acid orangeade did not increase preference for more acidic orangeades. They also checked if the increased sweet preference in orangeade (above) generalized to another food (yoghurt). The children showed a non-significant trend towards an increased preference for sweet yoghurt ($p = 0.09$).

Discussion and Conclusions

From an evolutionary psychobiology perspective, these results provide a reasonably coherent picture suggesting that early influences on later food preferences are well adapted to a hunter-gatherer existence but perhaps less so to the modern economy.

Genetics provide sensitivity to and preference for sweet tastes (useful for identifying ripe fruits) and a dislike for strong bitter tastes (useful for identifying bitter poisons). These are not fixed likes and dislikes and can change according to experience (thus acceptance for non-poisonous bitter foods can easily be learned). Inherited differences in sensitivity (particularly

to bitter tastes) can influence food preferences and choices. So far, the effects observed are not large, although the observations that PROP-insensitive infants tend to like sweeter foods [21] and are more likely to have caries [22], certainly merit further examination.

There is an evident adaptive advantage for infants to develop a mild but not overwhelming preference for food flavors experienced in their mother's milk. These flavors reflect her food choices and the food choices of her culture. The simple fact that she survived long enough and was fit enough to reproduce and suckle her child shows that her food choices must have at least have been adequate, if not positively good.

As the infant grows up, other influences on food preference and choice come into play, so these early effects cannot be expected to lead to exclusive preferences. Cashdan [38] has argued that, for early humans living out their lives in the same food environment, there would be a survival advantage for the child to be open to accept new foods during the first 2 years (i.e., early exposure to food flavors in mother's milk plus a full seasonal cycle of exposure to adult foods) followed thereafter by a gradual decrease in willingness to experiment (where the costs of experimentation would be higher as the child becomes more mobile and is less protected by parents). Taken together, the early influences on food preferences described in the earlier sections of this review fit closely to this pattern. In the first few months of life, infants do accept unusual flavors more easily and they do tend to like them years later. Similarly, foods liked at 2–3 years old are liked 6–8 years later, and few new foods are added to or subtracted from the list of liked foods. Few studies have examined the evolution of preferences in the age range 8–24 months, so influences on food acceptance during this phase are still poorly understood. It seems, however, that once a particular food becomes accepted and familiar at this stage of development, the preference can be long lasting. Thus preferred levels of sweetness or saltiness in particular foods may be established in the first 2 years. We do not know the extent to which these preferences generalize to similar foods or to other foods. This is an important unanswered question.

This reading of early influences leads to several practical conclusions (some of which certainly need to be studied further before being unequivocally recommended).

(1) Mother's milk reflects the flavors of foods she eats and these can influence her child's food preferences later in life. This suggests that the lactating mother should regularly eat the range of healthy foods that she wants her child to accept later on.
(2) Infants more readily accept new foods and flavors during the period from weaning to about 2 years of age, and surprisingly few new foods are easily accepted in the remaining years of childhood, so it seems worthwhile to make sure that the child experiences a wide range of healthy foods during this period.

(3) 'Repeated exposure' can increase acceptance of new foods. If the infant dislikes a new food on the first 2–3 occasions it is offered, many mothers give up. As Birch [33] suggests, it may be better to offer it without coercion and not give up until 8–10 tries.
(4) Preferred levels of sweetness (and saltiness) in particular foods seem to be influenced by early and current experiences, so perhaps it is better to begin with the levels of sugar and salt one considers healthy. It may be difficult to reduce preferred levels later on.
(5) For parents wishing to reduce their child's sugar intake, setting restriction rules for access to sweet foods and drinks does not seem to work very well.
(6) Lastly, even short-term exposure to a sweeter version of a food or drink may increase the preference for sweetness; so once again, it really does seem important to begin and persist with the levels one considers healthy.

As Benton [42] has pointed out, educational strategies typically involve attempts to impart basic nutritional information. An alternative strategy, worth exploring, is to teach parents more about child development in the hope that an understanding of innate tendencies, effects of early experiences and child psychology will be more successful in teaching healthy food preferences.

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Discussion

Mrs. Maier: With respect to the influence of exposure to new foods and flavours on their acceptance, I would like give an example from the work we are presently doing in Dijon. The first time babies were given puréed peas, some obviously didn't like them, but after 8–10 exposures most readily accepted them.

Dr. Leathwood: Yes, even though mothers tend to give up on an initially rejected food after 2–3 tries, it is easy to demonstrate increasing acceptance for some foods after more exposure than that, suggesting that, if an infant does not accept a new food, it is worth persisting in a relaxed, non-coercive manner on up to 8–10 occasions [1].

Dr. Verloove: I have exactly the same pictures of my own son eating his first apple sauce which was sterile, so many of us will recognize this kind of behavior.

Dr. de Nef: Is it not better to advise the parents to give natural products, fruits and vegetables, instead of industrially prepared bottles of vegetables?

Dr. Leathwood: It seems that the important point is to give a wide variety of vegetables. Parents can cook the vegetables themselves or they can use prepared jars. It is interesting to note that in some countries (France, for example), mothers tend to give one vegetable at the time apparently because they want their child to experience the tastes and flavours of a wide range of individual vegetables. In some other countries, mothers tend to offer mixed vegetables and a limited range [2]. It would be very interesting to know more about the longer term consequences, if any, of these different approaches.

Dr. Hernell: Yesterday when we discussed dietary fiber we agreed on that we would like children to eat more fruit and vegetable. From what you said it seems that to start complementary feeding with fruit and vegetable would be the right thing to do. But in reality, is it not true that fruit and vegetable are not what most children prefer when they grow older? So what is the reason, are we doing something wrong when we introduce complementary foods?

Dr. Leathwood: We have shown that experiencing a variety of vegetables increases acceptance of new foods, but we only have good evidence for relatively short term effects. Others have shown that preferences at 2–3 years old predict quite well preferences later in childhood so we do know that if children like and eat a wide variety of foods at 2–3, they are more likely to be eating a wide variety when they reach 8–10 years of age [3]. Unfortunately, there is very little information

available about predictors of fruit and vegetable preferences in 2–3 year olds. One study, on sugar water preference, showed that liking for sugar water at 6 months was a reasonably good predictor for liking for sugar water at 2 years, but the effect did not seem to generalize to an increased liking for sweetness in other beverages [4].

Since many children like and eat fruit and vegetables, perhaps we should be doing retrospective studies to check if their parents gave a greater variety of vegetables early on.

Dr. Hernell: I think we have quite large observational studies because this is a typical way to wean infants; to start with fruits and vegetables. This seems natural and I think is a rather strict weaning mode in many countries. Still you have the problem that when the children grow older they don't prefer fruits and vegetables.

Mrs. Gailing: Yes, they are different. The recipes for baby foods in jars are different in different countries; they are adapted to local tastes.

Dr. Verloove: Perhaps that explains some of the differences.

Dr. Salminen: It was really fascinating to listen to you. I was thinking along the lines of Dr. Hernell. If one considers the Nordic countries, there is still great seasonal variation in the availability of fruit and vegetables. Is there any information about how that may have influenced the eating habits of old generations?

Dr. Leathwood: I am not aware of any systematic studies on this point but it is certainly worth exploring further.

Dr. Verloove: There could be an additional advantage in that you are forced to eat different things in different months of the year, so maybe one week you would eat the same thing but the week after it would be something different.

Dr. Steenhout: I like the data showing that we should not abandon the introduction of a new food before having tried 8–10 times. If a child doesn't like something today, should we repeat it tomorrow or in a very short time? Is there a timing in the introduction or can we just give more space in between, and in terms of development, repetition is very often important or not?

Dr. Leathwood: Repeatedly offering an initially rejected food is certainly a good way to increase the probability it will be accepted. As for the frequency at which the food should be offered, we are currently studying this.

Dr. Verloove: Have there been any studies connecting the food habits of the mother during pregnancy to these kinds of things?

Dr. Leathwood: The best-known studies in this domain are the two I briefly mentioned in my presentation. Schaal et al [5] showed that, if mothers regularly consumed an aniseed flavoured drink during the last weeks of pregnancy, the newborn infants showed a significant preference for aniseed aroma. Mennella et al [6] found that, if the mother consumed carrot juice for several days during the last weeks of pregnancy, at weaning the infant tended to show more enthusiasm for a carrot-flavoured cereal than an unflavored one. As I pointed out, an evident limitation of this study was that the mother could not be 'blind' to the treatment, so may have influenced the behavior of her infant by pathways other than flavour experience.

Dr. Verloove: If the mother's habit was to eat different foods every day, this could be different situation for the child as compared to a child whose mother ate, say, pizza practically every day throughout pregnancy.

Dr. Waterland: Given that flavors found in the mother's diet are transported to breast milk, are there any good data indicating that breastfed infants are more accepting of a broad range of foods later on after weaning compared to formula-fed infants?

Dr. Leathwood: Yes there is at least one study. Sophie Nicklaus [7] recorded the number and types of food chosen from a selection of 8 different foods (and, on

average, for 110 meals per child) by 418 children aged 2–3. She reported that length of breastfeeding was significantly and positively correlated with the variety of foods selected.

Dr. Kleinman: Young children, even during infancy, are increasingly being exposed to longer durations of television viewing. There is evidence linking that to attention deficit disorder. Do you think that there is an influence on food preference at that age from television viewing?

Dr. Leathwood: Older children certainly see and hear about foods on television. This can influence the foods they want to try, but as those of us who are parents well know, it up to us to decide what they get, and to give our children robust guidance.

Dr. Kleinman: I was thinking more of 1–2 year-olds. The mother is perhaps offering a wide variety of so-called healthy foods and yet that 1-year-old is sitting in a chair watching television for 3 or 4 h/day perhaps. I wonder if that has been studied.

Dr. Leathwood: I don't know of any research on effects of television viewing on food preferences in 1-year-olds.

Dr. Hardiono Djoened: If exposure to a variety of foods is better for taste preference why do mothers always stick to one infant formula instead of changing the formula for example every week or every month? You told us that exposure to a variety of foods facilitates acceptance of new foods by the infant. Why don't we recommend that the mothers change the infant formulas, for example change to another brand every week, and then next week to another brand.

Dr. Leathwood: You raise a very interesting point. I think mothers stay with a particular formula for reasons other than the taste. With respect to providing formulas with a variety of aromas, there are in fact strict constraints and producers are not allowed to provide formula with a range of aromas. This is perhaps because people haven't fully considered the potential advantages.

Dr. Exl-Preysch: I think this question needs to be considered in its broader health context. I remember very well when, for example, it was permitted to add vegetables to infant formula. Misuse of such mixes could lead to health problems such as allergies and diarrhea. I think we are glad that putting vegetables into formulas is no longer allowed. However, I wanted to ask if are there any data or knowledge on the taste preferences of infants from vegetarian parents or even macrobiotic parents? If they are eating nothing but vegetables then the infants should prefer that when they are older as well.

Dr. Leathwood: With appropriate planning and (sometimes) supplementation, infants of vegetarian parents grow adequately and have adequate nutritional status [8]. For families eating macrobiotic diets, there is evidence for growth stagnation at the time of weaning followed by (partial?) catch up between 2 and 4 years old [9]. This study did not identify any long-term consequences of macrobiotic diets for mental status [9]. However, I am not sure if the children all preferred vegetables when they were older.

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Junk Food or 'Junk Eating'?

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Introduction

Despite the widespread epidemic of overweight and obesity, little attention has been given until recently to the potential of 'junk foods' in its causation. This essay discusses the role of 'junk food' in nutrition-related disorders and some associated factors that affect this problem. It also considers whether 'junk foods' are intrinsically unhealthy, whether their pattern of consumption is contributory, and whether the term 'junk eating' is useful.

Dietary Patterns and Obesity

Junk food or junk eating and lowered exercise patterns have been linked to the steep rise in overweight and obesity, which has become 'a public health crisis', particularly in industrialized and industrializing countries, over recent decades [1, 2]. However, there is limited objective evidence of the potential contribution of 'junk foods' or 'fast foods' and/or mechanisms by which they may promote excessive weight gain.

Because obesity is considered elsewhere in this symposium, only a brief overview will be given. For example, rates of childhood, adolescent and adult overweight increased steeply in the USA from the late 1970s to early 1990s [3]. The situation in the United States is of such concern that the US Surgeon General has said 'we've seen a generation of kids who grew up off the playground and on the PlayStation' and 'with obesity the fastest growing cause of disease and death in our nation … we need to have a consistent, uniform message that changes the culture of America as it relates to eating and physical activity' [4]. By 1999–2002, 65% of US adults were overweight or obese (30% obese, 5% extremely obese); 31% of children were at risk of overweight (body mass index, BMI, for age ≥85th percentile) and 16% were overweight (BMI for age ≥95th percentile) [5]. The situation in western Europe is similar with

prevalence rates differing from country to country [1]. Overweight and obesity in children and adolescents are also increasingly prevalent in Australia. The 1995 National Nutrition Survey of almost 3,000 subjects aged 2–18 years showed that, overall, 15% of boys and 15.8% of girls were overweight and a further 4.5% of boys and 5.3% of girls were obese [6]. Of 11,247 randomly selected persons examined in 1999–2000, the overall (25 to 75+ years) prevalence of overweight in adults by BMI was 39% and by waist circumference was 25.5%; obesity rates were 20.8% by BMI and 30.5% by waist circumference. Therefore, the prevalence of adult overweight and obesity in both sexes was almost 60%; this was 2.5 times higher than in 1980 [7].

Junk Food

'Junk food' is well recognized by the mass media and information can be obtained publicly through the Internet. Some sites urge 'junk food lovers of the world to unite' and to learn more about this subject through enormously long lists of so-called 'junk foods', which are categorized; they request readers to add items to these already vast lists. Many mention brand names which will not be used here; categories include: (1) 'fast foods'; (2) 'soft' (sweetened) drinks; (3) confectioneries and sweets, including chocolate-containing products; (4) biscuits (cookies) and cakes; (5) chips ('crisps' in some countries); (6) frozen dairy products, including ice creams and yogurts, and (7) breakfast items.

Are these all inevitably 'junk' or 'rubbish'? For example: (a) what faster, yet nutritious, food could there be than an apple, pear or banana; (b) are the now widely recognized 'fast foods' invariably 'junk' (that is, 'worthless') unless they are consumed habitually and in excessive quantities; (c) should all sweets and confectioneries be banned – surely they are not inevitably hazardous to health if consumed in moderation; (d) the same comments could be made about biscuits and cakes; (e) fried potatoes, if not fried in very thin strips or wedges in deep oil, can be an acceptable component of a balanced diet; (f) although some frozen dairy products contain substantial amounts of fat, low-fat alternatives are widely available, and (g) the nutrient and energy density contents of breakfast cereals are very variable – many have added sugar and other ingredients of which the consumer may not be aware, while others can be a good source of nutrition, fiber in particular. On balance, these groups of foods should not automatically be labeled 'junk'.

Impact of Junk Food Consumption on Body Weight

In many 'Westernized' countries 'fast foods' are recognized as items that are purchased for immediate consumption at the point of sale or elsewhere soon afterwards. This is equivalent to 'takeaways' or 'junk' foods. Until recently little

attention was given to the possible effects of consumption of such foods on nutrition or health. A study of more than 6,000 children and adolescents aged 4–19 years in the USA, who participated in the nationally representative Continuing Survey of Food Intake from 1994 to 1996 and the Supplemental Children's Survey in 1998, examined associations between 'fast food' consumption and measures of dietary intake quality. This study employed between-subject comparisons involving the entire cohort and within-subject comparisons using data from 2,080 subjects who consumed fast food on one but not both survey days [8]. On a typical day 30% of subjects reported consuming 'fast food'. This was prevalent for both genders, all racial and ethnic groups, and in all regions of the USA. After controlling for socioeconomic status and demographic variables, 'fast food' consumption was independently associated with being male, of older age, coming from higher income households, being of non-Hispanic race/ethnicity, and living in southern parts of the USA. Children who ate 'fast food', compared with those who did not, consumed more dietary energy, more energy-dense food, more total dietary fat, more total dietary carbohydrates, more added sugars, more sugar-sweetened beverages, less milk, and fewer fruits and non-starchy vegetables. The authors linked these factors to 'an adverse effect on dietary quality in ways that plausibly could increase risk for obesity' [8]. A recent study showed that adolescents over-consumed 'junk food' regardless of their body mass, although this phenomenon was more pronounced in overweight subjects and they were less likely to adjust their intakes so as not to exceed their usual intakes than were lean subjects [9].

Not all investigators agree with these findings. For example, a study of obese and non-obese adolescents found that total energy intake from high-calorie, low-nutrient-dense (HC) foods was higher in the non-obese subjects [10]. After adjusting for under-reporting, the percentage of dietary energy provided by the HC foods was similar in both groups, except for ice cream which was significantly greater in the non-obese group. Despite this study involving only 22 non-obese and 21 obese subjects and using cross-sectional methodology, the authors conclude that obese adolescent Americans do not consume more dietary energy from HC foods than do their non-obese peers. Contentiously, they suggested that their findings did not support the widespread notion that obese adolescents consume more 'junk food' than non-obese adolescents; they commented that health professionals should appreciate that excess dietary energy can come from many other sources in their foods and drinks [10]. Whatever the role of fast foods in obesity, it should be noted that their consumption has trebled in the past two decades [11].

'Junk' Drinking Patterns

More attention should be given to the contribution to dietary energy intake from drinks in the diets of infants, children and adolescents and their influence

on overweight and obesity. There has been a dramatic increase by almost 500% in per capita soft drink consumption in the USA over the past 50 years [12]. The American Academy of Pediatrics (AAP) has recognized that the prevalence of US children being overweight has doubled in the past 20 years and, according to their policy statement on 'Soft Drinks in Schools', overweight is the commonest pediatric medical problem in that country [13]. That statement associates the following with high intakes of sweetened drinks: (a) overweight/obesity; (b) displacement of milk consumption causing calcium deficiency and risks of osteoporosis and fractures, and (c) dental caries and risk of erosion of dental enamel; dental damage is exacerbated by the acidic nature of many soft drinks (pH range is often 2–3). It is not widely recognized that each 360 ml of a soft-drink contains about 10 teaspoonfuls of sugar and 628 kJ of dietary energy. In an analysis of more than 12,000 nationally representative American 11- to 18-year-olds, soft drink consumption more than doubled in females and almost trebled in males from the mid-1960s to the mid-1990s [14]. A study of more than 1,800 nationally representative 2- to 18-year-olds in the USA showed that dietary energy intake was positively associated with consumption of non-diet soft drinks. The mean adjusted energy intake was 7,659 kJ/day for children who drank an average of about 270 ml of sweetened drinks daily. Those in the highest category of soft drink consumption drank less milk and fruit juice than those in the category who drank the least amount of sweetened drinks [15]. Among more than 500 ethnically diverse children (median age 11.7 years), for each single daily serving of sugar-sweetened drink consumed, both BMI and the frequency of obesity were increased after adjustment for appropriate variables; daily consumption of a single can of soft drink may increase a child's risk of developing obesity by 60% [16].

The term 'sugar' conventionally means mono- and disaccharides. The National Dietary Guidelines usually identify 'added sugars' as those that are eaten separately at the table or used as ingredients in processed or prepared foods, such as cakes, biscuits, soft drinks or ice cream. In the USA consumption of added sugars has increased steadily from 1970 [17]. The largest source of added sugars (one third) in that country comes from non-diet soft drinks [17]. Johnson and Frary [17] reviewed evidence linking increasing sugar consumption to several disorders including dental caries, dyslipidemias, overweight/obesity, compromised bone health, and generally impaired diet quality. They remind us of the risks associated with the long-term use of baby feeding bottles containing fermentable sugars; the habitual use of pacifiers (or 'dummies') smeared with honey or jam to quieten babies or help them to sleep has reemerged as a very serious risk to pediatric dental health in some places, including Australia. Increasing sugar consumption has been identified as a threat to dental health in China, India, Vietnam, Thailand and other Southeast Asian countries, particularly because of the recent rise in the consumption of sugar-containing carbonated beverages [18].

A Possible 'Doomsday Scenario'

Type-2 (non-insulin-dependent) diabetes mellitus (NIDDM) and other related 'lifestyle diseases' such as overweight, obesity, increased cardiovascular disease risk, and chronic renal disease and failure, are epidemic in many parts of the industrialized world and cause millions of deaths annually [19]. Prevention and control of these major non-communicable diseases present a huge challenge which Zimmet [19] likens to a 'Doomsday scenario'. To develop cost- and health-effective programs will require mobilization of government agencies, lobbying of politicians, collaboration with international agencies such as the World Health Organization, the United Nations' Development Program, the United Nations' Children's Fund and the World Bank, and will require education and involvement of communities and populations at large, as well as active participation and advocacy by a range of health professionals [19].

These are becoming global health problems of immense proportions; this is exemplified by dramatic rises in the prevalence of these degenerative, chronic diseases in rapidly urbanizing populations such as in the Pacific and Indian Ocean regions and in Asia [20]. Environmental influences including rapid changes in dietary consumption patterns, the introduction of Westernized diets (including 'junk foods') and more sedentary lifestyles are important and may be compounded by a genetic predisposition in some ethnic groups. A significant accompanying factor in this process was colorfully labeled by Koestler [21] almost 30 years ago as 'Coca-colonization'. This is why NIDDM is now so prevalent in transitional populations such as indigenous North Americans, Native Canadians, New Zealand Maoris, and urbanized New Guineans and Nauruans [20, 22–24].

This situation is not confined to cities, other urban areas or country towns. In Australia, for example, changes over recent decades from the traditional, active hunter-gatherer lifestyle of Aborigines have rapidly introduced 'Westernized' dietary patterns, including 'junk foods' and fatty and salty, energy-dense foods to their community food stores and at roadhouses in isolated areas, and there have been significant declines in physical exercise, even in very remote areas and in isolated, small communities in 'the outback'. This has been termed 'surrogate urbanization'; it is associated with energy-dense, high-fat, high (refined)-carbohydrate, high-salt diets that are accompanied by very heavy consumption of sweetened soft drinks [25]. This predominantly 'junk eating and drinking' pattern contrasts with their traditional hunter-gatherer diets that were low in energy density, high in micronutrients, high in carbohydrates with a low glycemic index (such as starch-rich foods), low in highly refined carbohydrates, such as simple sugars (monosaccharides and disaccharides), extremely low in salt and low in fat but with a higher proportion of polyunsaturated fats and less saturated fat, a protective factor against the development of cardiovascular disease; they also

had plentiful natural 'bush tucker' – native fruits, nuts and vegetables, and hunted for wild animals [26]. These people now have among the world's highest rates of NIDDM and very high rates of associated 'lifestyle diseases' including overweight, obesity and cardiovascular disease; in many Aboriginal communities one third or more of the adults have diabetes [27]. Australian Aborigines are now developing diabetes and cardiovascular risk factors in childhood and adolescence [28, 29].

From my observations of food consumption patterns in the People's Republic of China over the past 25 years, particularly among children and other young people in cities and large towns, I am struck by the dramatic change from a total absence of Westernized dietary influences in the late 1970s to a galloping proliferation of international 'fast food' and 'junk food' outlets in the early 21st century. The nutritional and other health-related consequences of this change are yet to be seen. As Diamond [22] has said, the prevalence of NIDDM is 'exploding' in many populations, perhaps because of 'the genetic and evolutionary consequences of geographical differences in food history' and altering dietary and other lifestyle patterns; 'junk foods' and 'junk eating' are very significant contributory factors in this scenario.

Other Issues

Television viewing and other sedentary pursuits like computer games affect patterns of 'junk food eating' among today's youngsters. TV viewing tends to replace their fruit and vegetable consumption by foods and drinks advertised on TV [30]. It also encourages food snacking, junk food consumption and excessive consumption of foods in general, and inactivity. These are now prevalent in urbanized societies and are linked to much greater risks of being overweight [25, 31–33]. In some places TV advertising of 'fast foods' is legally restricted during children's popular viewing times, even up to 9 o'clock at night. Most 'junk foods' have extremely high energy density; among regular consumers of these products this would tend to promote weight gain and obesity [34]. Humans tend to consume a similar bulk of food regardless of its energy density because of inadequate recognition of its high-energy density and subsequent downregulation of intake to maintain energy balance [34–36]. This may help explain why frequent and habitual consumption of inappropriately excessive amounts of energy-dense foods, that is 'junk eating' habits, as well as the composition of junk foods themselves contribute to overweight and obesity [9]. Thus, the frequent consumption of palatable, energy-dense 'fast foods' of enormous portion size, with highly refined starch and with added sugars, for example in sweetened drinks [9], strengthens the proposal that 'junk eating' patterns as well as 'junk foods' contribute to overweight and/or obesity and their consequences in millions of people.

Yet the news may not all be bad. According to the Associated Press, a major international 'fast food' chain has been prompted by obesity lawsuits in the USA to launch diet-conscious meals with salads and bottled water and has started to provide information to customers about how to choose meals to decrease their fat, calorie and carbohydrate consumption. This has begun in Australia. A series of essays about food and its producers, eating patterns, obesity and other nutrition-related disorders, the food industry, food policy and the roles of governments, the media and individuals was published in *The Economist* in December 2003 [37]. There is now a documentary movie, 'Super Size Me', showing the risks of doubling energy intake by grossly excessive and continual consumption of high-energy-density junk food. These developments point to a need for better cooperation between various sectors to better understand and help reduce this very serious international health burden.

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Discussion

Dr. Steenhout: At the end of your presentation, you mentioned the new regulation that Great Britain wants to introduce to ban TV advertisement for children. We should consider that our civilization is evolving, and in fact now, at least in my personal opinion, we are relying a little bit too much on the decisions made by politics, states, law, instead of trying to educate people to have their own responsibility and to try also to develop their own ethics and their own ways. This is certainly something on which we

should work very early, tough educational programs at school. Finally industry will implement and follow what the population and their leaders would like. It is not always the industry that, as is sometimes said, tries to drive the changes in the population.

Dr. Gracey: You made some very good points and I agree with them. I think probably the most important point is the complexity of the problem and where the responsibility lies.

Dr. Caroli: I really appreciated your presentation. I gave a presentation at the European Meeting on Obesity last May in Prague about television and childhood obesity. I had to study the topic a little bit more deeply and I saw that there is a gradient of prevalence of obesity from the north of Europe to the Mediterranean countries. Sweden, Norway, the Scandinavian countries, have very strict laws on advertising directed at children during children's programs, while in Italy, Spain and Greece there is just self-regulation by the industry. I have to admit that in Italy the situation is particular, but we have a more than 30% obesity rate as compared to less than 20% in Scandinavian countries. The reason why the English Government decided to change the law is because they have a 27% obesity prevalence in childhood and so they decided to reduce the advertising to children. There was a very interesting study by Borsanowsky a few years ago on the effect of food advertising on children aged between 2 and 6 years. He saw that even 30 seconds of advertising can affect the choice of food in children of this age, and the effect is double if the same advertising is shown twice. So I think that in order to increase the fruit and vegetable intake of our children we should just learn from advertising. Fruit and vegetables must be presented to children in a positive way, and just not give up, because if they are pushed in a positive way, smiling, they don't care if you say that their food is good for their health, they don't care at all. Parents are really interested in that, and you have just to say that they are good as the advertising says that all this food is absolutely perfect, especially for getting everything in your life. But I do agree that we should have a very strong law at the European level to adjust advertising for the better heath of our children.

Dr. Gracey: You made some very good comments and perhaps I should just make one supplementary comment. Although I am not an expert on Europe for obvious reasons, I understand that there is a great diversity in the prevalence rates of obesity in children in different parts of Europe, even in different parts of the same country, even in Italy I believe, for example, so that any regulations or public health measures that are used to try to improve the situation have to be tailor-made for local conditions. Although the European Union is now one of the largest political groupings in the world, as an outsider I feel that a one size fits all approach would not work. Am I right?

Dr. Caroli: You said that the law must be adjusted for local situations?

Dr. Gracey: I think so, yes.

Dr. Caroli: You are probably right. We need a strong background, similar in all the countries, because the same advertisement that is prohibited in Greece, for example, is still allowed in Poland. We must have some general rules to follow in all Europe and then adjust for single and particular national problems.

Dr. Gracey: That was what I expected. So you need guidelines and principles to follow.

Dr. Caroli: No we don't need guidelines, we need law, because in Italy we don't care about guidelines.

Dr. Verloove: So perhaps we need sets of regulations which we can be adapted for each nation.

Dr. Sinaasappel: May I play the devil's advocate, that is we have what we ask for. In other words it is not only the industry that is offering this stuffs, but also society. The children of our community or ourselves are also in some way asking for these products, we like them, we use them. The question is how we can change that?

Dr. Gracey: I don't have an answer.

Dr. Verloove: Do you have any suggestions yourself?

Dr. Sinaasappel: I think education at home was already mentioned. But it is not only a question of the children, it is also a question of the parents. The parents are very much used to a lifestyle which is very convenient for them, and we probably have to start there to change it.

Dr. Exl-Preysch: I would like to make two comments and a suggestion regarding the previous question. Concerning the teeth, in Switzerland, for instance, we have a fantastic system for educating children on how to clean their teeth already in kindergarten. This system has been able to decrease caries in these children to zero. Those who go through this education system just clean their teeth every time they eat something, and I don't think they are eating less sweets and drinking less soda drinks than the other children. I think the most important thing is to educate children on how and when to clean their teeth. I was astonished that we haven't heard anything about portion sizes. There is fantastic literature on the development of portion sizes, especially in the United States, and I think this is very impressive. Very recently a study came out in the United States in which students received lunches of various portion sizes over several weeks. What was eaten for the rest of the day was studied and two things were realized: first of all, for the rest of the day the students ate the same amount of food regardless of how much they had eaten for lunch, and on average those who had the biggest portions were getting 400 kg cal/day more than the others. So portion size is really something everybody has to think about: restaurants, food industry, everybody. Finally regarding the education topics that were just raised, and knowing that therapy in obese children is almost useless, everybody here and everybody concerned with children, we all have to come up with educational programs for children starting in kindergarten, and there are some around, Nutrikid for instance. A change must be brought about in the eating habits of the children. It is fascinating to see that later on the children educate their parents because they go home and say 'no mother that is not for me'. So this is where I think we really should put the emphasis and not on difficult to understand recommendations.

Dr. Gracey: I would like to comment on those last two issues that you raised, it is extremely important. I did not mention portion sizes not because I was not aware of their importance, it is obviously important in terms of caloric intake and overall dietary consumption patterns, and I agree with you entirely. I would also like to mention the recently produced film called 'Super Size Me', which came from the United States and I hope that many of you in this room have seen it. The film is about an experiment that a young man in the United States did on himself. He fed himself in fast food chains in various parts of the United States, for 30 days he ate nothing but fast food, and when he was offered a super size of whatever he was ordering he accepted, and he did this with great graphic detail about what he was eating, and he had a gastroenterologist looking after him, a cardiologist and a general practitioner. In the space of 30 days this man gained 11 kg or 25 lbs in weight, he developed a firm fatty liver, his liver function tests were drastic, and his gastroenterologist said that if this was alcohol-induced he would probably die within a fairly short space of time, and he was warned off the diet, he lost his energy, he lost his sex drive, as his girl friend said anyway. It was an artificial uncontrolled experiment on an individual who was trying to make a point, but I think the point was made graphically, and the film I believe is well worth seeing. Anybody who is interested in diet, nutrition and related conditions, I would really recommend seeing it, not to make any more money for Michael Moore, I am sure he has made lots and lots of money, but perhaps not enough money to keep off the lawyers from McDonalds. Did you want to make another comment?

Dr. Exl-Preysch: I think we really should also mention that in an interview Moore himself said it was not McDonalds finally, it was the fact that he took 5,000 cal/day and he could have easily done that in a 4-star restaurant. The difference is that it would

have been quite a bit more expensive and certainly much more pleasant, but the effect would have been exactly the same. In addition, it seems that he 'organized' his weight gain in such a way that he had to succeed. Scientifically spoken, it was almost impossible that he gained so much weight in such a short time in a commercial way!

Dr. Gracey: I was going to come to the amount of calories that he was consuming and that was excessive, I agree completely, but it is just a very instructive film. Now your second point about teaching young children I believe is extremely important. I am working with Aboriginal people who live under miserable conditions, they no longer hunt and gather their food from berries, nuts and hunt for wallabies and kangaroos and snakes and goannas and so forth. Their food is brought in from hundreds or thousands of kilometers away. They have fatty fried salty foods; they consume enormous amounts of calories; they drink these 2-liter containers of a certain company's sweetened drink, I won't mention the company's name, and their children are turning into obese, hypertensive, diabetic teenagers from being underweight youngsters at the age of 8–10 years, and we are adopting the same approach with these children. I work through Aboriginal people and they have their own languages and ways of communicating, but we get the message to the family and to the community through the children. The children go back to the family and the community and say to the mothers or the fathers and the young men, you shouldn't eat that, that is dangerous, that will give you that sugar disease and then you go blind and you lose your feet or you get kidney disease, and this has a very powerful impact. So children actually can be agents of change in that sort of community setting, and that is where I believe that people who work in public health as I do, rather than in a hospital setting, have a great opportunity to get the message through the children to the rest of their community. It is very difficult, patient and hard work; it takes months and months to get these messages across.

Dr. Verloove: Yesterday when we were talking about similar subjects, someone called me a dreamer. Do you have any comment on this kind of thing Dr. Benninga? Do you think it is possible to change behavior?

Dr. Benninga: If you put in the effort then hopefully they do. But as I said yesterday they gave a lot of information which advocated adding fibers to the diets of high socioeconomic class families in the US, and they didn't have success.

Dr. Verloove: That is a nice opening to a question I have. Is it possible to change the way of thinking in such a way that you could, for example, add fibers to a hamburger? Accept the fact that people want to eat a hamburger but then change the hamburger so that it doesn't have these side effects? One aspect of it is portion size of course but another aspect is content like fluoride to water or fibers to hamburger or whatever.

Dr. Gracey: I don't see any reason why not. If the choice is there and you can convince the manufacturer or the person who is preparing the hamburger to change from a low-fiber flour to a rye flour or some other flour with more fiber or a wholewheat flour, then you can do so, but you have to work with the industry in order to achieve that objective.

Dr. Aggett: In a way what you are suggesting is something that is running through people's mind in terms of so-called functional foods; creating foods in which added value can be derived by manipulating a particular ingredient. This is very well developed in many areas and explored. It is a highly focused area. What you described is a challenge that we are actually facing in the UK as a part of an issue arising from that review in the newspaper mentioned by Dr. Gracey, and what is happening in the UK now is part of the same initiative in public health. They want to have red, green and amber labels on foods, so a red food one would eat sparingly, green plenty, and amber somewhere in-between, so sometimes it is good, sometimes it is bad. The present debate, as I picked up on the television last night, is that someone is saying well, what if we have high-fiber bun, a nice thick juicy fatty piece of meat and a lump of lettuce,

how are we going to give that a red, green or amber label. And there are going to be lots of people running around arguing, debating this. So it has been appreciated to a certain extent that there must be some real way of doing a risk-benefit analysis of these foods. Instinctively one would feel that a high-fiber bun would probably not compensate for 50% of calories coming from saturated fat as may be the case, but the initiative in terms of foods more generally is now developing to this concept of nutritional profiling where the risk-benefit analysis is being applied to products by trying to synthesize and interpolate from compositional data something about the food. It has yet to be tested whether or not that is going to work. People have actually started doing it but now appreciate that it is one of these things that is far more easily said that done.

Dr. Gracey: What you said is very sensible, and again I come back to the complexities of altering what is in the diet, what people know about what is in the diet and how to perhaps change their attitudes and their behavior. After this film 'Super Size Me' was produced and went around the world, there was a lot of pressure on McDonalds in particular to change their products and the way they marketed their products in McDonalds outlets, and McDonalds started to introduce salads as a response, saying here we are, we are now giving salads as a healthy alternative. But what happened was that they are adding so much salad dressing to the salads that the salads in fact contain more fat and more calories than what people had been consuming previously.

Dr. Aggett: I think the basic answer, and it is probably relating to some comments I think Dr. Leathwood made, that intrinsically some of us try to understand food. To teach and educate people about food rather than nutrients is a real challenge and I think what Dr. Gracey has just illustrated is a very good example of that.

Dr. Waterland: I just wanted to follow-up on your previous comment about children acting as agents of change in this whole issue. I think it is good if we can teach children to try to influence their parents about healthy eating and that sort of thing, but I really have echo Dr. Steenhout's comment that fundamentally the responsibility comes down to the parents. It made me think of a recent innovation in the United States which is diet dog food. When I think that my dog is fat I just give him less food, but the pet food industry is trying to convince us that you have to provide your dog with a proper balance of nutrients to help him lose weight. This is analogous to the idea of banning junk food advertisements for children. It seems kind of silly when really it is the parents who are buying the stuff, and if the parents aren't bringing the stuff home the kids don't have access to it. So I think that is really where we need to focus.

Dr. Leathwood: I think we must not forget the historical perspective. The vast changes in the agriculture industry that have occurred 70 years were at least partially influenced by fear of famine. Even in the 1950's, Europe was not able to produce enough food for the population, so agricultural policy was aimed at increasing production. In consequence, a few years later there was over-production, with 'beef mountains' and 'butter mountains'. In relation to income, food today is generally cheaper than it used to be and more easily available, so the time cost and the money cost of obtaining food is lower. In consequence, it is easier to eat more and value food less.

This leads directly to my next point. The expression 'junk food' expresses something about the food and can also be used to express something about the people who eat it. There is sometimes a veiled implication that people who eat 'junk' food are 'junk' people. So some criticisms of junk foods may be a form of social posturing.

Lastly, I would like to comment on the point that advertising is often presumed to be spectacularly efficient while nutritional education seems to be remarkably inefficient in changing people's behavior. Should we not treat this as an opportunity and develop programmes where advertisers and nutritionists work together?

Dr. Gracey: I do agree that the term 'junk' used in relation to food or dietary habits does have a pejorative connotation. We do have to be careful about that particularly as it tends to be the less fortunate strata in the society that are affected most by junk food, partly because it is relatively cheap and it is quick, it is convenient and it is tasty because it is salty and fatty.

Dr. Kleinman: I actually have some comments rather than a question. The first has to do with what was brought up about going to a very good restaurant in Paris and being able to eat the same amount of calories. That is absolutely true; the problem with that line of reasoning is that by and large the restaurants that serve haute cuisine are extremely expensive and if one wants to eat more food in those restaurants one spends even more money. The restaurants that most of us eat at will give you a lot of food for very little money, and that is the point of the documentary movie 'Super Size Me', that for a few more cents you can go from a 350-ml beverage to a 470-ml beverage or from a 470-ml beverage to a 710-ml beverage. That comes back to Dr. Leathwood's comment; this really is a very complex and integrated system of rewards. Food by and large is very cheap now in the developed world. Portion sizes are often enormous, and it is not difficult to eat an extra 400 or 600 cal/day beyond what is expended.

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Does Nutrition during Infancy and Early Childhood Contribute to Later Obesity via Metabolic Imprinting of Epigenetic Gene Regulatory Mechanisms?

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Introduction

An epidemic of obesity is occurring in the US and many other developed countries, and appears to be responsible for an associated increase in the prevalence of type-2 diabetes, dyslipidemia, and hypertension. Alarmingly, this trend for increasing adiposity and its comorbidities is not limited to adults, but is also threatening children at younger and younger ages. Over the last three decades, the prevalence of overweight among children age 2–19 years has nearly doubled in the US [1]. What is the cause of the dramatic increase in adiposity among our children and adults? The increasing availability of highly palatable foods combined with more 'modern' lifestyles involving less physical work and play are creating a more obesigenic environment. But why do some individuals respond to these environmental changes with increased adiposity, while others seem naturally resistant to increases in body weight?

It has long been presumed that individual genetic variation alone explains individual differences in obesity susceptibility. According to the 'thrifty genotype' hypothesis [2], individuals whose genomes evolved to maximize energy intake in times of plenty, and minimize energy expenditure, developed an evolutionary advantage allowing them to survive prolonged periods of food deprivation. In our modern society, these same 'thrifty' genes now promote excessive weight gain and type-2 diabetes. A complementary explanation for individual variation in obesity susceptibility, the 'thrifty phenotype' hypothesis [3], proposes that individual differences in satiety mechanisms,

endocrine interactions that regulate metabolism, and neurological and behavioral mechanisms affecting physical activity are determined not only by genes, but also by environmental influences during development. This chapter will review briefly evidence from human studies and animal models that during infancy and early childhood nutrition serves as an important signal for 'fine-tuning' various metabolic systems, and thereby influences obesity susceptibility throughout life. The primary focus of this article will be an evaluation of the hypothesis that nutrition during infancy and early childhood modifies obesity susceptibility by perturbing epigenetic mechanisms.

Metabolic Imprinting

Several years ago, Waterland and Garza [4] proposed the term 'metabolic imprinting' to encompass a subset of adaptive metabolic responses to early nutritional influences. Metabolic imprinting is characterized by: (1) susceptibility limited to a specific ontogenic period early in development (i.e. a 'critical window'); (2) a persistent effect lasting through adulthood; (3) a specific and measurable outcome, and (4) a dose-response or threshold relation between exposure and outcome [4]. When this definition was proposed, no phenomenon characterized in either human populations or experimental animal models met the stringent criteria of metabolic imprinting. The postulation of metabolic imprinting was thus intended as a challenge to researchers in this field. Once specific examples of metabolic imprinting are characterized we will, by definition, have gained substantial insight into the underlying developmental processes. Considering that obesity can result from dysregulation of myriad physiological systems, it is not appropriate to postulate metabolic imprinting of obesity. Obesity not a specific outcome. This point underscores the most important distinction between metabolic imprinting and 'programming' [5]. Whereas it would be reasonable to propose nutritional programming of obesity, metabolic imprinting can be postulated only in the context of a specific candidate mechanism.

Early Postnatal Nutrition and Adult Obesity Susceptibility

Several excellent reviews [3, 6–8] have considered whether adult obesity susceptibility is influenced by nutrition during critical periods of development. Of particular relevance here, retrospective studies have reported that adiposity is lower in individuals who were breastfed as infants, relative to those who were formula-fed. These data have led to the provocative hypothesis that breastfeeding protects against later obesity [9, 10]. Because socioeconomic status is associated with both obesity and breastfeeding rates, these studies

adjust for the influence of socioeconomic status. It is impossible, however, to rule out potential residual confounding. Recent findings that obesity physiologically impairs lactation [11] suggest yet another cause for an inverse relation between breastfeeding and obesity. For these reasons, and because of the discordant findings among apparently similar studies, the effect of breastfeeding on later obesity remains highly controversial [12]. Other human observational approaches suggesting that early postnatal nutrition contributes to later obesity may likewise be explained by alternative pathways. For example, several studies link rapid weight gain in infancy to overweight in adulthood [13]. Although consistent with the hypothesis that infantile overnutrition causes adult obesity, such relations can more parsimoniously be explained by an individual tendency for excessive food intake manifesting in infancy and persisting to adulthood.

By far the most compelling human data demonstrating that early postnatal diet can influence metabolic parameters relevant to obesity are from Lucas [14] who performed a longitudinal study of preterm infants randomly assigned to one of two formulas or breast milk during the first few weeks of life. At 15 years of age, the ratio of serum leptin to fat mass was 25% higher in subjects who were fed a special preterm formula in early infancy, compared to those who received either the standard infant formula or banked breast milk [15]. Leptin is secreted by adipose tissue and acts as a satiety signal in the hypothalamus. Thus, although there were no significant differences among the groups in body mass index or fat mass at age 15, the effect on serum leptin normalized to fat mass indicates that early postnatal diet caused a permanent change in adipose tissue leptin secretion and/or hypothalamic leptin sensitivity. It is likely that these physiological differences will cause group differences in adiposity as the subjects get older.

Several animal models provide strong support for the hypothesis that nutrition in the early postnatal period influences developmental pathways that affect adult obesity susceptibility. In the rodent suckling-period litter size model, the offspring from several litters of rats or mice born on the same day are randomized and redistributed to foster dams in small, normal, or large litters. These animals experience, respectively, overnutrition, normal nutrition or undernutrition during the suckling period. At weaning, small-litter pups are heavier and fatter than pups suckled in normal-sized litters, and these differences persist to adulthood [16, 17]. Animals suckled in small litters also display a persistent impairment in glucose-stimulated insulin secretion in vivo and in vitro [16, 18, 19]. Patel and co-workers [20] have for several years been developing the ‘pup in a cup’ model for early postnatal induction of obesity in the rat. Within a few days of birth, rat pups are cannulated and fed enterally until postnatal day 18. The rate of formula delivery is adjusted so that formula-fed pups gain weight at the same rate as mother-fed animals, and after day 18 all animals are provided free access to the same standard rat chow. Rats fed the high-carbohydrate formula during

the suckling period become heavier, fatter, and hyperinsulinemic as adults, relative to rats who are either enterally fed a high-fat formula (similar to rat milk) or suckled by their mothers [20]. In another model, it was recently shown that male mice whose mothers are fed a low-protein diet during the suckling period only are thereafter protected from the induction of obesity by a highly palatable diet [21].

Compared to nutritional exposures during gestation, nutrition in the early postnatal period appears to have a greater impact on metabolic imprinting of physiological parameters relevant to body weight regulation [4]. In fact, in many of the models purporting to show 'fetal programming' of obesity, such as the maternal caloric restriction model [22], it is unclear whether adult obesity results from the prenatal restriction per se or from dietary compensation (catch-up growth) in the early postnatal period.

Epigenetics, Development, and Nutrition

The vast majority of cells in the human body contain the same complement of DNA: the entire human genome. Yet hepatocytes express a very different subset of genes from, say, neurons or adipocytes. These tissue-specific patterns of gene expression are maintained by 'epigenetic' mechanisms. Epigenetics is the study of mitotically and/or meiotically heritable alterations in gene expression that are not associated with changes in DNA sequence. Epigenetic processes include DNA methylation, various modifications of the histone proteins that 'package' DNA in the nucleus (including acetylation, ubiquitination, and methylation) and feed-forward autoregulation by specific transcription factors [23]. All of these processes interact synergistically to maintain specific regions of the genome in an open, transcriptionally active state and others in a highly condensed and transcriptionally silent state. By definition, these cell-type-specific epigenetic alterations, once established during development, are maintained through successive rounds of cellular proliferation throughout life and can in some cases be transmitted through the germ line. Of the various epigenetic alterations, DNA methylation is among the best characterized. For this reason, and because of the direct influence of diet on the DNA methylation pathway [24], we have focused on nutritional influences on DNA methylation during mammalian development.

In mammals, methylation of cytosine to 5-methyl-cytosine occurs on both DNA strands within palindromic CpG dinucleotides. (The 'p' denotes the intervening phosphate group.) CpG methylation contributes to transcriptional regulation by affecting the binding of methylation-sensitive DNA-binding proteins. CpG methylation in a gene's promoter region is generally associated with transcriptional repression, but CpG methylation in discrete regulatory regions can also augment transcription. During development, the high levels of genomic CpG methylation in the sperm and oocyte are largely erased following

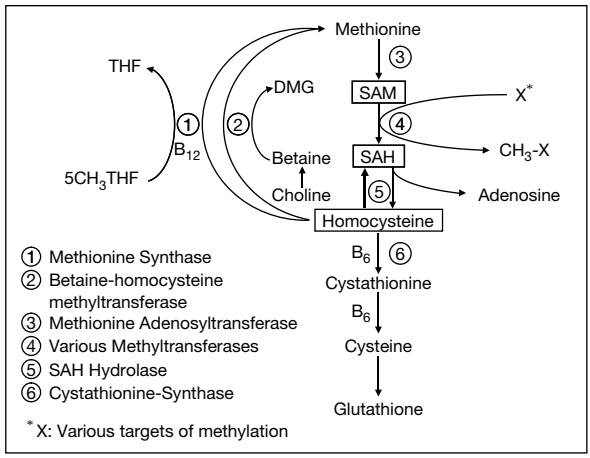

Fig. 1. Mammalian one-carbon metabolism provides the methyl groups for methylation of DNA and other substrates (indicated as 'X'). DNA methylation is directly dependent on nutrients including folate (shown here as 5-CH_3-tetrahydrofolate), cobalamin (vitamin B_{12}), choline, and methionine.

fertilization, and around the time of implantation a wave of de novo methylation occurs in the embryonic genome [25]. Tissue-specific patterns of CpG methylation are established during prenatal and early postnatal development, and are thereafter maintained during cellular replication through the action of a 'maintenance methylase' DNA methyltransferase-1 (DNMT1). The DNA methylation pathway is directly dependent on dietary methyl donors and cofactors (fig. 1). Hence, during critical ontogenic periods, a dietary excess or deficiency of key nutrients such as folic acid, methionine, and vitamin B_{12} may affect the establishment of CpG methylation in specific regulatory regions of genes [26]. These induced epigenetic alterations can be maintained to affect adult gene expression and phenotype. Importantly, just as genetic variation influences individual susceptibility to various diseases, it is becoming increasingly clear that individual differences in epigenetic regulation can affect disease susceptibility [27–29]. Unlike genetic variation, however, which is determined by parental inheritance, we currently know very little about the factors that determine individual variation in epigenotype.

Perturbation of DNA Methylation by Environmental Influences during Development

Recent studies in animal models have demonstrated early environmental influences on mammalian developmental epigenetics. Wolff et al. [30] showed that maternal dietary methyl donor supplementation affects the coat color of

viable yellow agouti (A^{vy}) mice. The *agouti* gene encodes a paracrine-signaling molecule that regulates the formation of a yellow pigment. *Agouti* is normally expressed only in hair follicles during a specific stage of hair growth, causing a yellow band on an otherwise black hair; this results in the brown (agouti) coat color of a normal mouse. In A^{vy} mice a retrotransposon (intracisternal A particle, IAP) has inserted into the *agouti* gene. The IAP insertion introduces a cryptic promoter, and destabilizes the establishment of DNA methylation at *agouti* [31]. Consequently, within a single litter of isogenic A^{vy}/a animals, some animals will have a high level of CpG methylation at A^{vy}, while others will display systemic hypomethylation at A^{vy}. Hypomethylation of A^{vy} allows ectopic agouti expression from the IAP cryptic promoter and, not surprisingly, these animals have yellow coats. Because the agouti protein binds antagonistically to the melanocortin-4 receptor that contributes to hypothalamic regulation of satiety, ectopic agouti expression also causes hyperphagia, obesity, and hyperinsulinemia [32]. In A^{vy}/a animals with hypermethylation at A^{vy}, ectopic agouti expression is silenced, recapitulating the brown, lean phenotype of an *A/A* mouse (fig. 2a).

Waterland and Jirtle [33] demonstrated that dietary methyl donor supplementation of female *a/a* mice before conception and during pregnancy shifts the coat color distribution of their A^{vy}/a offspring by increasing A^{vy} CpG methylation (fig. 2b, c). The nutritionally induced change in offspring epigenotype affected all tissues and was maintained into adulthood. Hence, a transient nutritional stimulus during a critical ontogenic period caused an epigenetic alteration that persisted to influence the adult phenotype. Because viral-derived transposable elements comprise roughly 40% of the human genome [34], it is highly likely that many human genes share the 'epigenetic metastability' [35] that renders the A^{vy} locus labile to early nutritional influences on epigenetic regulation.

In another example of early environmental shaping of the mammalian epigenotype, Weaver et al. [36] studied the 'programming' of stress response by maternal caregiving in the early postnatal period. In an inbred strain of rats, they reported substantial interindividual variation in maternal caregiving behavior; some dams spent a great deal of time licking, grooming, and nursing their pups (high-LGN) whereas others spent much less time on these nurturing behaviors (low-LGN). Weaver et al. [36] discovered that maternal caregiving behavior affects the establishment of CpG methylation in the glucorticoid receptor (GR) promoter region in the hippocampus. Methylation is completely absent at specific CpG sites within the GR promoter before birth, and increases dramatically by 1 day after birth (fig. 3). In the offspring of high-LGN dams, the GR promoter in hippocampal DNA is almost completely demethylated by day 6 postnatally. In offspring of low-LGN dams, however, it remains methylated, and this group difference persists into adulthood. Cross-fostering studies demonstrated that the GR epigenotype is determined by the LGN phenotype of the foster mother, not by the

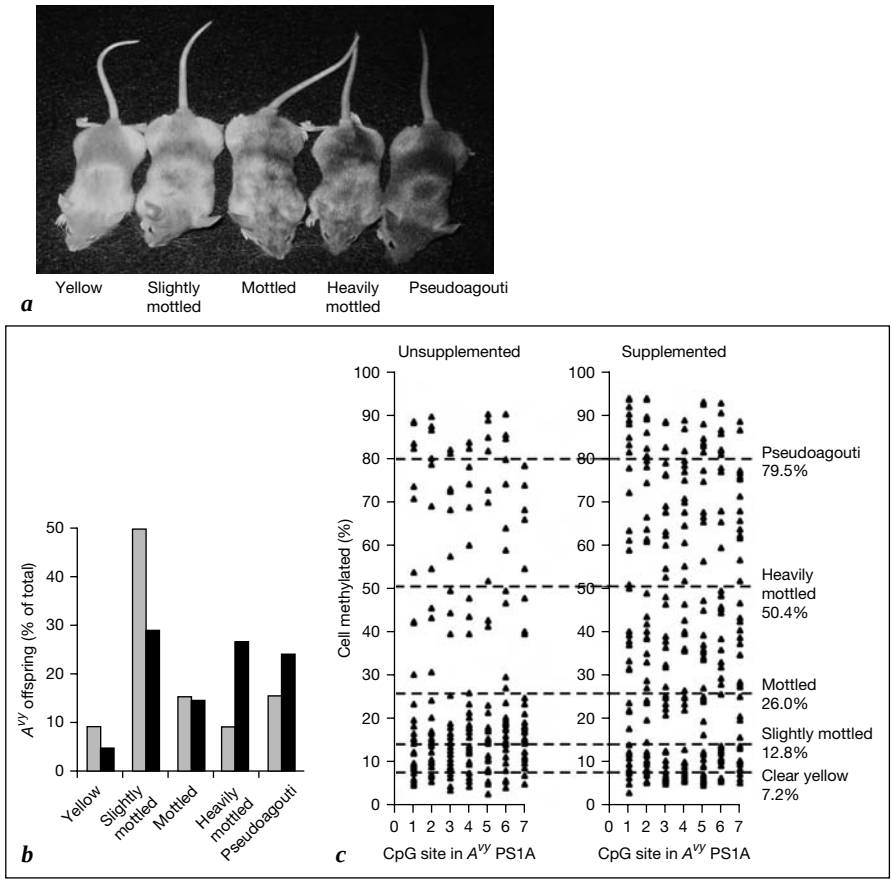

Fig. 2. ***a*** Phenotypic classes of A^{vy}/a mice. Yellow mice are hypomethylated at the A^{vy} allele, whereas pseudoagouti mice are hypermethylated at A^{vy}. ***b*** Coat color distribution of all A^{vy}/a mice born to 9 unsupplemented dams and 10 supplemented dams. The distribution of the supplemented offspring is shifted toward the pseudoagouti phenotype ($p = 0.008$). ***c*** Percentage of cells methylated at each of seven CpG sites in the A^{vy} region of all A^{vy}/a mice born to 9 unsupplemented dams and 10 supplemented dams. Average A^{vy} methylation is increased in supplemented offspring ($p = 0.005$). From Waterland and Jirtle [33].

inheritance of subtle genetic or epigenetic differences between high-LGN and low-LGN dams [36]. This elegant study provides a compelling example of a subtle environmental influence during postnatal development affecting the establishment of gene-specific epigenotype.

These two examples demonstrate that environmental influences during embryonic and early postnatal development can affect the establishment of gene-specific DNA methylation. How late into the postnatal period

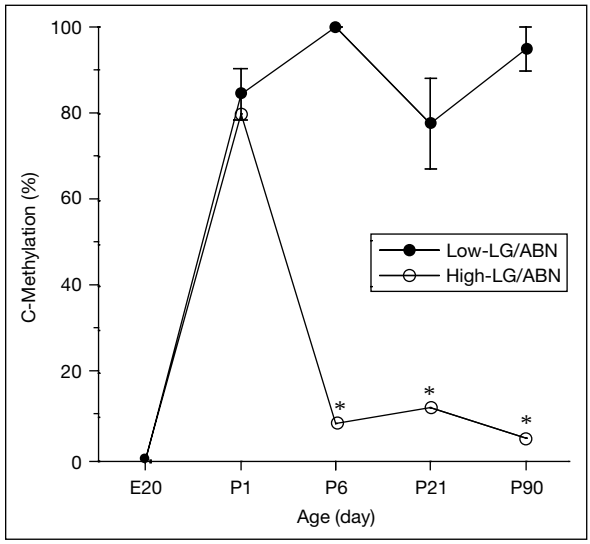

Fig. 3. Ontogeny of methylation at a critical CpG site within the glucorticoid receptor promoter region in the rat hippocampus. The site is unmethylated before birth (E20) and becomes 80% methylated by 1 day after birth. In pups suckled by mothers with high maternal caregiving (High LG-ABN), methylation at this site returns almost to zero by day 6 postnatally. In rats suckled by less attentive mothers (Low LG/ABN) this developmental demethylation does not occur. This epigenetic response to maternal caregiving persists to adulthood. From Weaver et al. [36].

are mammals susceptible to environmental influences on epigenotype? A continuous supply of methyl donors and cofactors is required to maintain established tissue-specific patterns of CpG methylation through the rapid rounds of cellular proliferation that continues into the postnatal period in many tissues. Maintenance of CpG methylation may therefore be hampered by an inadequate supply of dietary methyl donors and cofactors in the postnatal period, and such affects may be locus-specific [26]. We have been exploring this hypothesis by examining the effects of post-weaning diet on the epigenetic regulation of genomically imprinted genes. Genomic imprinting is an epigenetic phenomenon whereby certain mammalian genes are expressed preferentially from either the allele inherited from the mother or that inherited from the father [37]. The 'monoallelic' expression of genomically imprinted genes is regulated by allele-specific CpG methylation that is established differentially in the oocyte and sperm genomes of the parents and transmitted to the next generation. In this manner, CpG methylation appears to serve as the 'imprint' that allows the maternal and paternal alleles of imprinted genes to be distinguished. The gene encoding insulin-like growth factor-2 (*IGF2*) is imprinted in mice and humans, and is

expressed predominantly from the paternal allele. Waterland and Jirtle [38] weaned C57/Castaneus F1 hybrid mice onto either a normal control diet or a synthetic diet lacking methionine, folic acid, vitamin B_{12} and choline. By 60 days post-weaning, dietary methyl donor deficiency caused a dramatic increase in relative expression of *Igf2* from the maternal allele [38]. Most importantly, after this 60-day diet exposure, when the deficient animals were 'recuperated' for an additional 100 days on the control diet, the increment in maternal *Igf2* expression persisted, indicating that the post-weaning dietary deficiency induced an epigenetic alteration that was maintained despite a subsequent return to a replete diet [38].

These animal models demonstrate that epigenetic mechanisms can be affected by environmental influences during development. Clearly, the organism is most susceptible to environmental influences when epigenetic states are undergoing developmental transition. Epigenetic metastability (and nutritional lability) at A^{vy} appears to occur only in the very early embryo, and once an individual's A^{vy} epigenotype is 'set' it is relatively stable throughout life. Developmentally programmed changes in methylation at the *GR* in the hippocampus span an entirely different ontogenic window, during the first few postnatal days, during which maternal caregiving can have a profound and permanent influence on GR methylation and expression. As demonstrated by the studies of post-weaning effects on *Igf2* allelic expression, even after epigenetic states have been established, severe nutritional deficiency during late postnatal development can interfere with their maintenance and thereby cause persistent changes in the epigenetic regulation of certain genes. As nutritionists, we tend to think about inducing alterations in DNA methylation with a 'supply-side' approach, i.e. by dietarily producing either an excess or deficiency of the S-adenosylmethionine required for DNA methylation (fig. 1). Importantly, the study by Weaver et al. [36] indicates that simply inducing (or repressing) the transcriptional activity of a promoter region during a critical period of development can cause a persistent alteration in its epigenetic regulation. Early nutritional exposures unrelated to one-carbon metabolism may therefore have a profound effect on developmental epigenetics simply by affecting gene transcription during critical ontogenic periods.

Epigenetics and Obesity

There are currently no compelling data indicating that epigenetic mechanisms play a major role in the most common types of human obesity. This evidential void contrasts markedly with the convincing body of evidence that dysregulation of epigenetic mechanisms is an important cause of human cancer [28, 39]. This does not indicate that epigenetic factors do not affect human obesity susceptibility. Rather, establishing links between epigenetics and cancer is relatively straightforward. The ability to compare

tumor tissue with healthy adjacent tissue from the same individual enabled the identification of numerous epigenetic abnormalities associated with carcinogenesis [39]. In obesity, coordinate endocrine dysregulation of multiple organ systems makes it extremely difficult to identify the primary cause of chronic energy imbalance. This complexity will hamper attempts to understand the role of epigenetic dysregulation in the etiology of obesity.

Nevertheless, there are already extensive data indicating that epigenetic dysregulation can lead to obesity. Prader-Willi syndrome is a genetic syndrome that causes hyperphagia, hypogonadism, characteristic facial features, and obesity [40]. The disease results from the lack of expression of genes on a portion of 15q11-q13 that are genomically imprinted and normally expressed from only the paternal allele. Prader-Willi syndrome is most often caused by paternal deletion of 15q11-q13 or uniparental disomy for chromosome 15 [40]. In a small number of 'sporadic' cases, a very small genetic deletion in an imprinting center in the 15q11-q13 region causes the paternally inherited allele to be epigenetically silenced (in addition to the normally silenced maternal allele). Notably, this epigenetic disease initially causes feeding difficulties and failure to thrive in early infancy. The onset of rapid weight gain does not occur until 1–6 years of age [40].

Cloned mice provide another demonstration that obesity can be caused by epigenetic dysregulation [41]. When DNA from somatic cells of adult animals is used to create clones, the epigenetic 'programming' of the differentiated adult cell must be reset to allow the totipotency required for embryonic development. Our understanding of how to restore epigenetic totipotency remains fairly rudimentary, as suggested by the extremely low success rates for cloning. When mice are successfully cloned, they often have normal birth weights but develop adult-onset obesity [42]. Similar to obese humans, these mice also develop hyperinsulinemia and hyperleptinemia. While we do not yet understand the specific mechanisms that cause obesity in this model, it is clearly an epigenetic, not a genetic, phenomenon. The viable yellow agouti (A^{vy}) mouse model described above also provides an example of epigenetic dysregulation leading to obesity. In A^{vy} mice, hypomethylation in the A^{vy} genomic region enables ectopic expression of agouti protein. When expressed systemically, agouti protein interferes with melanocortin signaling in the hypothalamus, causing hyperphagia and obesity. Notably, the 'agouti-related peptide', which shares sequence similarity to the mouse agouti protein, acts on the hypothalamus to stimulate eating behavior in humans.

Developmental Epigenetics and Obesity: Potential Links

Obesity is a highly complex disease that can result from dysregulation of literally any physiological system involved in regulating energy intake or the various components of energy expenditure. How can we identify

physiological systems in which early postnatal nutrition may plausibly modify epigenetic mechanisms that will have an impact on later obesity susceptibility? The most compelling insight from the animal models is that epigenetic mechanisms are most labile to environmental influences when they are either first being established or undergoing developmental transition. In this section two physiological systems that contribute to body weight regulation (and its dysregulation in obesity) will be considered from this perspective.

Neurological Development

The hypothalamus is a master regulator of eating behavior, as it detects and integrates peripheral signals to maintain energy homeostasis. It is likely that food availability in the early postnatal period acts as a signal to 'tune' the sensitivity and responsiveness of hypothalamic regulation of energy intake. Recent data suggest that this developmental maturation occurs via epigenetic mechanisms. Bouret et al. [43] demonstrated that circulating leptin in the early postnatal period acts as a developmental signal that drives the formation of projections from the arcuate nucleus of the hypothalamus (ARH). These projections are severely deficient in leptin-deficient (*ob/ob*) mice. However, when *ob/ob* mice received leptin injections during the suckling period (days 4–12 postnatally) they developed ARH projections similar to those of wild-type animals. Unlike untreated *ob/ob* mice, which are hyperphagic, post-weaning food intake of mice treated postnatally with exogenous leptin was comparable to that of wild-type animals. Importantly, this developmental action of leptin is limited to the early postnatal period. When adult *ob/ob* mice were treated with leptin for 20 days, there was no effect on the density of ARH projections [43]. This indicates that a developmental maturation of the hypothalamus, likely epigenetic in nature, occurs in the early postnatal period in the mouse.

Several studies have documented profound changes in global [44] and gene-specific [45] CpG methylation in specific regions of mouse brain over the early postnatal period, further validating the hypothesis that early postnatal neurological development occurs via epigenetic mechanisms. The developmental progression of Rett syndrome, an epigenetic neurological disease that is among the most common causes of mental retardation in girls, indicates that postnatal neurological development in humans involves epigenetic mechanisms. Rett syndrome is caused by mutations in the X-linked gene encoding methyl-CpG-binding protein-2 (MeCP2) [46]. MeCP2 is a methylation-dependent DNA-binding protein that binds to methylated DNA regions, helping to stabilize them in a transcriptionally inactive state [46]. Girls with Rett syndrome appear to develop normally for the first 6 months of life, then head growth falters and neurological symptoms manifest. Considering that MeCP2 is normally widely expressed throughout the brain in the prenatal and early postnatal period [46], why

does MeCP2 deficiency not cause symptoms until 6 months after birth? One explanation is that MeCP2 is required for the stabilization of progressive epigenetic silencing that is necessary for normal neurological maturation in late infancy.

Maturation of the Insulin Axis

Peripheral insulin resistance, hyperinsulinemia and hyperglycemia are commonly associated with obesity. While it is widely assumed that obesity leads to secondary insulin resistance which causes compensatory hyperinsulinemia, disentangling the causal pathways linking these comorbidities has perplexed endocrinologists for decades. It therefore remains plausible that primary defects in peripheral insulin sensitivity and/or pancreatic glucose-stimulated insulin secretion cause human obesity. Glucose-stimulated insulin secretion in the endocrine pancreas is blunted in newborn rodents and humans and displays a developmental maturation during early postnatal life [47, 48]. This process can be affected by postnatal nutrition, as demonstrated in rodent models showing persistent effects of suckling-period litter size [16, 18, 19] and high-carbohydrate formula feeding [20]. Waterland and Garza [19] showed that the persistent defect in glucose-stimulated insulin secretion in rats suckled in small litters (compared to those from normal-sized litters) correlated with persistent changes in gene expression within isolated pancreatic islets. The quantitative stability with which these expression differences were maintained from weaning to adulthood suggested that early postnatal overnutrition had affected the establishment of epigenetic gene regulatory mechanisms in islet cells [19].

Although it is likely that nutrition during postnatal development influences epigenetic mechanisms involved in functional maturation of the endocrine pancreas, surprisingly little is known about the epigenetic regulation of genes that play critical roles in insulin axis function. For example, we know little about DNA methylation of the insulin gene itself, let alone whether it undergoes developmentally programmed changes during the early postnatal period. One study, however, provides precedent for early-postnatal ontogenic changes in gene-specific DNA methylation within the endocrine pancreas. Matsusue et al. [49] showed in rats that pancreatic expression of cholecystokinin type a receptor (*Cckar*) increases markedly from age 14 to 21 days postnatally. The increase in *Cckar* expression was associated with concurrent demethylation of *Cckar.* The dearth of knowledge on the ontogeny of gene-specific epigenetic changes in the endocrine pancreas extends to other tissues important in insulin axis function, including skeletal muscle and adipose tissue. Given that persistent nutritional influences on epigenetic mechanisms are most likely to occur when these mechanisms are undergoing developmental changes, it is critically important for us to gain more information on the developmental epigenetics responsible for functional maturation of the insulin axis.

Conclusions

Clearly, environmental influences during prenatal and early postnatal development can both permanently alter body weight regulation and affect the establishment and maintenance of epigenetic gene regulatory mechanisms. Epigenetic dysregulation can cause obesity, and epigenetic development of physiological systems relevant to energy homeostasis continues into the postnatal period. It is therefore likely that postnatal metabolic imprinting of epigenetic gene regulatory mechanisms plays a role in determining individual susceptibility to obesity [8]. Improving our understanding of the biologic mechanisms whereby early nutrition influences developmental epigenetics may eventually enable the formulation of early postnatal nutritional interventions aimed at decreasing individual obesity susceptibility.

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Discussion

Dr. Woltil: What is known about body composition determinants and the development of epigenetic regulation disturbances? Preterm infants and small-for-gestational age babies grow best during their hospital stay, there is evidence for that. There are now formulas available for this group of infants. The effects of these formulas have been shown, not only in weight gain but also the gain of fat-free mass, on absolute fat mass but not relative fat mass. Is it known which of these parameters affect the epigenetic regulatory mechanisms, and if not can you speculate on that?

Dr. Waterland: There is very little known about how nutrition influences epigenetic gene regulation in humans and we are just now starting to use animal models to get a better understanding of the specific genomic characteristics of gene regions that are epigenetically labile to early environmental influences. As far as speculating on what types of genes might be involved in regulating body composition in humans, there too it is almost an open question. I gave one example regarding maturation of the hypothalamus because it is clear that the nutritional transitions that occur at birth, and also at weaning, are important developmental windows. These might also be critical periods when development of epigenetic processes is occurring, but that remains to be seen.

Dr. Schmitz: I would like to ask two questions. The first one is: what is the evidence that this modification in the epigenetic mechanism can extend in the first or second year of life? The second question is: do you think that the quantitatively small variations in feeding during infancy might be able to modify these very important mechanisms and the one you are showing?

Dr. Waterland: The first question is whether these types of induced modifications of epigenetic mechanisms occur in the postnatal period in humans. There are no good data available in humans yet, but what we have to do to start with is to really understand comparative genetics and comparative genomics and determine whether these processes occur the same way in mice as in humans. Regarding your second question, I think that our study in mice is really relevant, the supplemented diet was only about 3-fold higher on average in those various nutrients relative to the unsupplemented diet, so that wasn't an extreme mega supplementation or anything. When you consider the various practices in terms of maternal vitamin intake and supplementation of children, I think that in terms of weaning foods and weaning practices there is a very broad variation in food intake during at least late infancy. So I do think that there is a potential for even these relatively subtle differences in diet to have a big impact down the road.

Dr. Verloove: I think it has been shown that the behavior patterns of the parents can reduce stress hormone levels in early childhood and infancy. There are changes in the hormonal axis. I don't know if any of you is more familiar with the literature than I am, but it has been proven in humans from the behavioral and the hormonal side.

Dr. Waterland: But still it is difficult to disentangle that completely from the potential genetic contributions to those epigenetic mechanisms.

Dr. Verloove: As far as I understood they came quite a long way in proving that.

Dr. Singhal: I would like to make a comment on the evidence for epigenetic factors in humans in terms of postnatal programming. There is huge interest in imprinted genes in which the effect of the gene depends on whether it is inherited from the mother or the father. These epigenetic phenomena are likely to influence early postnatal growth which itself is likely to influence early appetite, a key area of interest. Although we don't have much data, there is some evidence to show for example that growth in the first 2, 3 days of life (which is nutritionally dependent) affects your IgF-1 levels permanently [1]. So the critical window is very early on, and is likely to involve imprinted mechanisms. I think human data are beginning to emerge to support this.

Dr. Waterland: In terms of the relevance of the mouse studies to humans, the imprinted genes actually give a nice example because in a lot of mouse studies it has been shown that different stimuli during early embryonic development can cause persistent changes in epigenetic regulation at genomically imprinted genes. And as far as humans go, very recently we are starting to get data on various epigenetic diseases in humans born by assisted reproductive technologies and there appears to be a higher prevalence of some of these epigenetic diseases such as Angelman's syndrome and Beckwith-Wiedemann syndrome in individuals who are produced by these artificial technologies that require incubating an early embryo in vitro for a couple of days before implantation. So we can see how this provides another indication of the similarities in the way these things work between mice and humans, and it is a very scary thought when you think of how many children are being produced by these techniques now. We think we have a great understanding of everything and can control nature but now we are finding out that even though we can successfully produce an infant with these techniques, we might actually be causing some problems as well.

Dr. Singhal: How do you explain nutritional programming or metabolic imprinting in identical twins, a model in which the mothers have the same diet but you still see the same programming phenomena? This is one area in which I don't understand how the epigenetic mechanisms work.

Dr. Waterland: That is actually an excellent example because in a lot of cases of identical twins you actually have very different placentation, so it is very common to have one twin whose birth weight is significantly lower than that of the co-twin. When you think that these differences in birth weight are actually based on different nutrient supply through the placenta, then it is very easy to understand how this could cause epigenetic differences between the twins.

Dr. Heymans: A prospective study in humans is not feasible so we have to stick to things that happened. We are still investigating a population that was exposed to famine in Amsterdam in 1944. There is a huge difference between those who were exposed in the beginning of the pregnancy and those who were exposed at the end [2]. Wouldn't that be an interesting group to try and research in your project, and see what the differences in methylation are, for instance?

Dr. Waterland: I would be very interested in following up with that cohort eventually. That early paper by Ravelli et al. [3] looking at obesity as an outcome in those individuals was the very first paper that sparked my interest in this whole field. My goal is to be able to use these animal models to really refine our specific hypothesis of what we want to test in these human populations because there are several cohorts like that

where we have documented differences in early nutritional exposures, but the question is, before we try to follow up on these individuals, if we are going to be asking for blood samples and that sort of thing, I would like to have a really good idea about exactly where in the genome we expect to find epigenetic differences caused by these early exposures.

Dr. Verloove: For your information, both the Amsterdam cohort and the new cohorts from other places in Holland where we are trying to study these effects, all the blood samples are frozen. So if you have thought about what you would like to know, you can ask and we will see what we can do.

Dr. Kleinman: Have we evolved any mechanisms to protect ourselves against imprinting? For example, once methylation occurs, is it a permanent phenomenon or is there a repair mechanism that is available to protect against 'mistakes' that might work to the disadvantage of the animal or human?

Dr. Waterland: That is an interesting question. As far as evolving mechanisms to fix these things, the flip-side of that is why we might have this type of environmental susceptibility in the first place. So really if you think about it, having some kind of plasticity built into these epigenetic processes might actually have given an evolutionary advantage, allowing a newborn infant to adapt to the specific nutritional conditions which were prevalent at that time. But now the same types of developmental plasticity can be detrimental for example in developing countries where the infant might have made adjustments to one environment and now 30, 40 years later the nutritional environment is quite different. But as far as the ability for these things to change during later life, with age there is a gradual loss of epigenetic information throughout the genome. For example the overall level of genomic methylation declines with age in most tissues, suggesting that a gradual loss of this information, as some people have postulated, might be implicated in the aging process itself. But as far as repair mechanisms are concerned, I am not aware of too much work in that area.

Dr. Kleinman: If you think about this from an evolutionary perspective we have evolved a number of mechanisms to protect ourselves against our nutritional environment, excessive intake of iron for example or inadequate consumption of carbohydrate, and certainly 500 years ago there were much wider swings in nutritional intake from day to day likely, and certainly season to season. So in a sense this imprinting would seem to be counterproductive if it leads to an inability to respond to a changing environment. At least if you look at it from a long evolutionary perspective, our diets have become much more consistent over the past 50 years than they were over the past previous 900 years, so it is a little bit difficult to understand the benefit of imprinting in the context of being able to adapt to a changing environment.

Dr. Waterland: We could do a lot of hand-waving about the exact evolutionary perspective, but I think it is really unknown exactly how well nutrition in infancy provides a mechanism for the individual to sense its environment to some extent. I don't know what would be the appropriate nutrition in infancy to prepare one to be 'super-sized' later in life, so the food environment that we have in the US, and increasingly in other countries, is a problem and it will be important to understand how these processes interact with the environment.

Dr. Hernell: We have been discussing very extensively during the last decades metabolic imprinting or programming and it seems clear that early events do have lasting effects. Still there are many environmental factors that affect for instance development of obesity in adulthood. Would you even care to speculate how much could these early events, including epigenetic effects, contribute to the total risk panorama during a lifetime?

Dr. Waterland: I know that one problem a lot of people have with this whole early origins hypothesis is that people may just give up, they say, 'My birth weight was only

2.3 kg, I am doomed, so there is no sense exercising or eating a healthy diet'. Now I entirely disagree with that. I still believe that the largest part of staying in good health is taking care of yourself and eating properly and exercise and all that sort of thing, but I don't want to deny the potential importance of even a minor effect because if it is influencing the entire population it is going to have a pretty big overall effect on health. But in terms of obesity I want also to mention that some epigenetic alterations are actually meiotically as well as mitotically heritable, so there is the potential for epigenetic inheritance to occur. This might actually allow trans-generational influences of early nutritional exposures, and that is another area where imprinted genes are especially relevant because genomically imprinted genes have essentially evolved to propagate epigenetic information trans-generationally. We know that the methylation patterns at imprinted genes are established during late gestation and the early postnatal period. So if nutrition during these periods can influence methylation in these critical imprinted gene regions then those changes could actually be transferred to the next generation. One could imagine a feed-forward trans-generational effect of increasing levels of obesity, by which even a small effect could actually accumulate over successive generations.

Dr. Verloove: This reminds me of our discussion yesterday about allergy, food allergy and that the such. Do you think there might be a link there?

Dr. Waterland: I think we are going to find more and more that epigenetic processes are fundamentally involved in almost everything.

Mr. Turini: In clinical settings antibiotics together with anti-inflammatory drugs are often administered to children. Anti-inflammatory drugs can affect the methylation of histones, for instance, and it is also known that some aspects of anti-inflammatory processes could limit the development of tolerance as suggested in a paper published in *Nature Medicine.* Do you think giving aspirin to young children is a risk for allergy development, especially during the weaning period?

Dr. Waterland: It is important to consider that, even though I focused my research on nutritional influences, as I demonstrated with that example on maternal care-giving behavior, many other types of environmental influences can impact the establishment and maintenance of epigenetic processes during development. Pharmacological exposures could certainly cause epigenetic changes just as nutritional exposures.

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Long-Term Effects of Weaning Habits: Type-1 Diabetes

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Introduction

Type-1 diabetes is an autoimmune disease which attacks insulin-producing β cells in the pancreas. This autoimmune process is characterized by the appearance of circulating autoantibodies against β-cell antigens, such as insulin, glutamate decarboxylase (GAD) and tyrosine phosphatase. The infants of mothers with type-1 diabetes do not have autoimmune diabetes despite the transfer of IgG antibodies, including autoantibodies to β cells, to the child via the placenta. This indicates that autoantibodies against β-cell antigens, although being markers of β-cell inflammation, do not transfer the disease. Also in animal models of type-1 diabetes the evidence indicates that autoimmune diabetes is mediated by autoreactive T cells which infiltrate the islets and mediate the destruction of β cells.

The genes of the HLA complex, among them especially the DQ genes, are most important in type-1 diabetes susceptibility. HLA DQ2 and/or DQ8 alleles are found in about 90% of children with type-1 diabetes. On the other hand, the genotype of disease susceptibility is found in about 22% of the population, showing the impact for environmental factors in the modification of disease development. We have seen a rapid increase in the incidence of type-1 diabetes during the last decades, in particular in young children [1]. This kind of change in the incidence cannot be totally explained by genetic changes and thus it is obvious that environmental factors modify the incidence of type-1 diabetes. Dietary factors, especially during the first year of life, have been intensively studied as putative environmental risk factors for β-cell autoimmunity and type-1 diabetes.

Accumulating data suggest that the gut immune system plays a key role in the development of type-1 diabetes, supporting the view that dietary factors

are able modify the risk of type-1 diabetes. In non-obese diabetic (NOD) mice, islet-infiltrating lymphocytes express α4β7 integrin, a homing receptor of the gut mucosa, and antibodies blocking this receptor prevent diabetes [2]. Mesenteric lymphocytes from young non-diabetic NOD mice include diabetogenic lymphocytes which are able to transfer diabetes to NOD/scid mice [3]. In humans, some reports suggest that autoreactive T cells may originate from the gut immune system. T cells derived from the pancreas of a patient with type-1 diabetes adhered to the mucosal and pancreatic endothelium [4]. GAD-reactive T cells from patients with type-1 diabetes expressed gut-associated homing receptor α4β7 integrin [5]. As markers of intestinal immune activation, increased expression of HLA class-II antigen, α4β7 integrin, IL-4 and IL-1β has been found in the intestinal mucosa of children with type-1 diabetes without associated celiac disease (CD) [6]. Several studies suggest that patients with type-1 diabetes, especially if diagnosed at an early age, have failure of oral tolerance manifested with increased immunity to dietary cow's milk (CM) proteins [7] as well as to wheat gluten [8]. Aberrancies of the gut immune system in children prone to type-1 diabetes may at least partly explain the harmful effects of diet in these individuals, whereas the same dietary factors may be innocent in the majority of children.

Weaning to Hydrolyzed Cow's Milk Formula as Prevention of Type-1 Diabetes

Dietary factors, especially when modified during the weaning period, affect the development of autoimmune diabetes in animal models of type-1 diabetes: biobreeding (BB) rats and NOD mice. In these animal models, autoimmune diabetes develops spontaneously, and there is no direct evidence showing that any putative dietary factor(s) could directly cause the development of destructive insulitis and autoimmune diabetes. Weaning to a diet of hydrolyzed CM formula [9, 10] protects from autoimmune diabetes in both the above-mentioned animal models. A diet of hydrolyzed protein induces functional changes in the islets infiltrating lymphocytes [10], which indicates that an immunological link between diet and autoimmune diabetes exists. Studies in the NOD mouse model suggest that weaning to casein hydrolysate induces regulatory T cells which are responsible for the prevention of autoimmune diabetes [11]. Casein hydrolysate, when given either continuously or for 10 weeks after weaning, was highly effective in preventing autoimmune diabetes (32-week incidence 4.6 vs. 58.8%) in NOD mice. Spleen cells from protected NOD mice failed to adoptively transfer diabetes into irradiated syngeneic recipients. Furthermore, splenocytes from diet-protected mice inhibited adoptive diabetes transfer with splenocytes from diabetic donors (incidence 50 vs. 94%, $p < 0.001$). The animal studies thus suggest that weaning to a hydrolyzed

diet instead of weaning to a diet containing whole proteins is protective from type-1 diabetes, but do not directly suggest a diabetogenic role for CM.

Epidemiological studies, however, propose dietary CM proteins as a putative trigger of type-1 diabetes by showing a correlation between the consumption of CM products and the incidence of type-1 diabetes in several countries [12]. Also the exposure to CM proteins during early life has been reported to imply an about 1.5- to 2-fold risk of type-1 diabetes in children who were breastfed for <3–4 months or exposed to CM formula before the age of 3–4 months [13–16]. A Swedish study indicated that early exposure to CM proteins was a risk factor for type-1 diabetes diagnosed at an early age [16]. When the effect of the HLA risk genotype was considered, the risk of diabetes related to early CM exposure was more pronounced in those individuals who had the 'high-risk' genotype of disease [15]. An association between early CM exposure and the risk of type-1 diabetes has not been observed in all epidemiological studies performed, but according to two meta-analyses of case-control studies, the overall risk of type-1 diabetes was slightly increased being about 1.5-fold when the child was exposed to CM proteins before 3 months of age [17, 18]. Most of the studies are based on retrospectively collected infant diet data and in some studies the participation rate of controls was lower than that of the cases, factors which may have biased the results of these studies [17]. Thus, prospective studies are needed to confirm the possible link between CM exposure and type-1 diabetes. Recent reports from the DAISY study and the German BABYDIAB study did not show an association between CM exposure and β-cell autoimmunity [19, 20]. The definition of CM exposure may differ between different studies; for instance in the German study all CM-containing products (also hydrolyzed formula that has been suggested to be protective) were recorded, and thus the results are not comparable to studies in which exposure to CM formula containing whole CM proteins was indicated as a risk factor, e.g. in a Finnish birth-cohort study [21]. Since the follow-up time in all these studies was relatively short and the number of children who developed type-1 diabetes was limited, the association of CM exposure with the emergence of β-cell autoimmunity, and not with the risk of type-1 diabetes, was reported. In the Australian BABYDIAB study of children from families with type-1 diabetes, no association of CM formula exposure and the appearance of β-cell autoantibodies was demonstrated [22]. Instead, in a Finnish birth-cohort study, the Diabetes Prediction and Prevention study, exposure to CM formula before 4 months of age implied an about 5-fold risk for the development of multiple autoantibodies and especially autoantibodies against tyrosine phosphatase in children who all carried the HLA DQB1*0302 risk allele of type-1 diabetes [21]. The methodological problems in the follow-up studies of dietary exposures are obvious, and thus randomized controlled intervention studies are needed to answer the question of the protective effect of CM avoidance during the first months of life or weaning to hydrolyzed formula.

The international Trial to Reduce IDDM in Individuals at Genetic Risk study was planned to study the question whether elimination of CM proteins in the diet and the use of hydrolyzed formula during the first 6 months of life could be protective from type-1 diabetes [23]. By 24 months of age, 1/53 (1.9%) of the subjects in the casein hydrolysate group had developed autoantibodies, whereas 6/48 (12.5%) in the control group had done so ($p = 0.036$, Fisher's exact test) [23]. By the age of 5–7 years life-table analysis, showed, after adjustment for duration of study formula feeding, a significant protection by the intervention from positivity for ICA ($p = 0.02$) and at least one autoantibody ($p = 0.03$) [24].

In some epidemiological studies not only early exposure to CM proteins, but a high intake of CM proteins later in life implied a risk of type-1 diabetes [14, 25]. This was also evident in a prospective follow-up study of the healthy siblings of Finnish patients with type-1 diabetes [25]. These studies suggest that CM could contain a diabetogenic factor which contributes to the development of type-1 diabetes.

Several candidates for a diabetogenic factor in CM have been suggested, such as bovine serum albumin, β-casein and CM insulin. Bovine insulin in CM induces primary immunization to insulin in infants exposed to CM formulas [26, 27]. According to a hypothesis, weaning to CM results in immunization to bovine insulin, and if this happens during the time when gut maturation is incomplete, an aberrant immune response to insulin may be developed. Later this immune response to dietary insulin could be activated against insulin-producing β cells [7].

In our follow-up study of children at genetic risk of type-1 diabetes, the levels of bovine insulin-binding antibodies increased after the primary immunization (i.e. exposure to CM) in children who later developed β-cell autoimmunity, whereas in autoantibody-negative children the insulin-binding antibodies remained at lower levels [28]. This may indicate a dysregulation of oral tolerance in children prone to β-cell autoimmunity. Indeed, subclinical intestinal immune activation has been associated with type-1 diabetes [6, 29] and could favor the development of a harmful immune response to insulin.

The weaning to CM during early infancy may also influence the development of autoimmune diabetes by nonspecific mechanisms. The introduction of CM proteins to the infant's diet is a strong immunological stimulation manifested as the development of antigen-specific humoral and T-cell responses to foreign CM proteins [30] and induction of soluble adhesion molecules [31]. Thus, early stimulation of the gut-associated immune system by foreign proteins may prime the gut immune system and further activate β-cell autoimmunity by nonspecific bystander mechanisms. It is possible that a diet containing hydrolyzed proteins, i.e. less immunogenic peptides, instead of CM proteins prevents autoimmune diabetes due to less stimulation of the intestinal immune system during the first months of life.

Wheat Gluten as a Trigger of Type-1 Diabetes

CD is more common in patients with type-1 diabetes than in the general population; the prevalence varies between 5 and 8% in studies of patients with type-1 diabetes in different populations [32]. The increased prevalence is at least partly explained by the shared HLA-risk genotype, DQ2 and DQ8.

Studies in animal models suggest that wheat gluten could trigger autoimmune diabetes. Wheat gluten when added to the diet of BB rats causes an increased incidence of autoimmune diabetes suggesting that dietary wheat gluten could contribute to the development of autoimmune diabetes [33]. A gluten-free diet prevents autoimmune diabetes in NOD mice [34]. In NOD mice a wheat-based diet induced a Th1 response in the gut [35].

Weaning to gluten-containing food before 4 months of age has been suggested to increase the risk of β-cell autoimmunity in the German BABYDIAB study [19]. This is an interesting finding, but should be interpreted carefully since it is based on a small number of individuals who developed β-cell autoantibodies. In the North American DAISY study both early (i.e. before 3 months of age) and late introduction of gluten-containing cereals after 7 months of age implied a risk of β-cell autoimmunity [20]. The authors suggest that there may be a window of exposure to cereals in infancy outside which initial exposure increases the risk of β-cell autoimmunity in susceptible children.

Some studies suggest that dietary wheat gluten may trigger impaired β-cell function and could accordingly be a candidate to trigger autoimmune diabetes also later in life [36]. Interestingly, in a trial of 17 autoantibody-positive healthy first-degree relatives of diabetic patients, the insulin response to intravenous glucose improved after 6 months of a gluten-free diet. Autoantibody titers did not show significant changes. After returning to the normal diet the acute insulin response decreased in 10 of 13 subjects during the following 6-month period. These findings indicate that 6 months of gluten elimination does not influence humoral autoimmunity, but may have a beneficial effect on the preservation of β-cell function in subjects at risk of type-1 diabetes [36].

The possible diabetogenic mechanisms of wheat gluten are not known, and studies of the mechanisms are few. It is possible that wheat gluten triggers autoimmunity and/or inflammatory changes in individuals who carry the HLA risk genotype common to CD and type-1 diabetes. This view is supported by Italian studies reporting that in vivo and in vitro stimulation with gliadin results in an enhanced local intestinal immune response in patients with type-1 diabetes [29, 37]. This inflammatory response to challenge was found in diabetic patients with normal CD serology (anti-gliadin antibody, anti-endomysial antibody, and anti-tissue transglutaminase antibodies) and a morphologically normal jejunal mucosa. It is possible that gluten-induced inflammatory changes favor the activation of β-cell autoimmunity in the gut immune system without any specific cross-reactive autoimmunity between

wheat gluten and β-cell antigens. However, recently antibodies to wheat storage protein were demonstrated in patients with type-1 diabetes but not in control individuals [38]. The reactivity to the gluten-derived protein was associated with β-cell damage in BB rats, and the authors suggest that immunity to wheat gluten may be involved with the pathogenesis of autoimmune diabetes. It is evident that more clinical and mechanical studies are needed to clarify the possible role of wheat gluten in β-cell autoimmunity and type-1 diabetes.

Low Vitamin-D Intake and Type-1 Diabetes

A large multicenter trial covering 7 centers in Europe and comprising data on vitamin-D supplementation to 820 patients with type-1 diabetes and 2,335 controls showed a protective effect (odds ratio 0.67) of vitamin-D supplementation in infancy [39]. In a Finnish birth-cohort study including children born in 1966 in northern Finland, a low vitamin-D intake during the first year of life implied an increased risk of type-1 diabetes [40]. Children who regularly took the recommended dose of vitamin D (2,000 IU daily) had a relative risk of 0.22 compared with those who regularly received less than the recommended amount. Children suspected of having rickets during the first year of life had a relative risk of 3.0 compared with those without such a suspicion. It is difficult to estimated whether high doses of vitamin D or its analogs could be effective in the prevention of human type-1 diabetes in the general population in whom vitamin-D supplementation was followed.

The mechanisms of vitamin D in the development of autoimmune diabetes were studied in animal models, but in these studies high doses of $1,25(OH)_2D_3$ were administered and not vitamin D. The protective mechanisms of $1,25(OH)_2D_3$ or its analogs in the NOD mouse model were suggested to be mediated by increased apoptosis of Th1 cells, induction of skew from Th1 to Th2 immune response, and activation or induction of regulatory CD25 high CD4 cells [41].

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Discussion

Dr. Paerregaard: You mentioned that type-1 diabetes is a T-cell-mediated disease and also that in animal models you could transfer T cells and the disease. We know that lymphocytes are present in breast milk and that by ingestion of breast milk the infants also introduce maternal T cells into their gut and that these T cells interact with the immature immune system of the infants. We also know that in certain respects this can have consequences later, for instance if the infant needs an organ transplantation at a later stage, it may influence whether this organ is rejected or not. Could ingestion of T cells from diabetic mothers be a risk factor for the infants? I know that breast milk in general probably promotes protection against the type-2 diabetes, but in the offspring of diabetic mothers could these maternal T cells actually be a risk factor for type-1 diabetes?

Dr. Vaarala: First, I think that we have actually not studied breast milk enough in this context. We know that breast milk contains cytokines, growth factors and immune cells. We also know that the cytokines, for example breast milk transforming growth factor-β, modulate the development of at least the antibody responses in children [1], so this is definitely a very interesting area of study. If we think about breast milk from diabetic mothers, whether it is protective or not, we have some epidemiological data that actually show very clearly that the risk of developing type-1 diabetes is decreased in the offspring of mothers with diabetes when compared to the offspring of fathers with diabetes [2]. There is no explanation for this, but something occurs there during pregnancy or the first months of life, and maternal diabetes actually protects from the genetic risk load. Whether it is mediated by immune cells against β-cell antigens present in the breast milk, that is an interesting hypothesis, but the effect is the opposite to what you suggested.

Dr. Exl-Preysch: Could you comment or speculate on that huge epidemiological study published last year in Germany in which a group of children with newly diagnosed atopic eczema was compared with paired controls, and the incidence of type-1 diabetes was looked at [3]. The group with eczema had a significantly lower incidence of type-1 diabetes than the group without eczema. They speculated on the Th1/Th2 ratio, and stated that when these children have a Th2 increase they have allergy but not diabetes and also the other way round. I would like to have your opinion on that.

Dr. Vaarala: When we think about Th1/Th2 polarization, the next idea of course is to think that if children with atopic diseases have a type-2 response there is downregulation of the type-1 response that is associated with diabetes. Thus you don't see these two diseases in the same individuals. I think that this is the hypothesis behind this kind of study. Now there are several studies on the association of atopic asthma and atopic diseases with type-1 diabetes [4], and the studies are quite discrepant. There are even studies showing that atopic asthma is actually associated with type-1 diabetes [5], so I think that this issue is yet not clear enough and personally I do not believe too much in this simple type of explanation based on type-1/type-2 polarization for the epidemiology of allergic and autoimmune diseases. Th1/Th2 polarization may be the factor in the effect on antigen-specific T cells. But it is much more likely that T-regulatory cells are more important in the context of disease development. They control both type-1 and type-2 immune responses and similar kinds of environmental factors, for example microbial load that regulates the induction of regulatory cells may be a risk factor for both allergies and type-1 diabetes, the incidence of these diseases is increasing at the same time. So I would think that the answer will be found in the regulatory mechanisms, and the genetic background of the children will determine whether they get type-1 diabetes or another type of immune-mediated disease such as atopic allergy.

Mr. Benyacoub: Coming back to this aspect of a potential epigenetic effect, is anyone investigating the methylation profile of these children, particularly with regard to the 2 or 10% of them who developed type-1 diabetes?

Dr. Vaarala: When I was listening to the previous presentation I got the idea that this should be studied now, especially the effect of dietary factors on T cells and immune responses, the development of T cells.

Dr. H. Hoekstra: The clinical association between type-1 diabetes and celiac disease is mostly that first diabetes and then celiac disease are diagnosed. Is this not a point against your theory?

Dr. Vaarala: I must say that I don't understand how would it be against my theory, because I think it supports my theory. If you think that triggers of the intestinal immune system are associated with type-1 diabetes, the presence of these triggers will lead to the development of type-1 diabetes, and the same factor is actually affecting the development of celiac disease very near the diagnosis of type-1 diabetes. So you don't need to wait 10 years, they occur together just because of this association with the trigger.

Dr. H. Hoekstra: The clinical presentations of classical celiac disease and diabetes are mostly quite obvious. So if celiac disease is an important factor to de-clench diabetes, wouldn't you expect that celiac disease will present itself earlier than diabetes?

Dr. Vaarala: Actually my point is that exposure to gluten and an aberrant immune response to gluten is associated with type-1 diabetes. It could play a role in triggering type-1 diabetes, but celiac disease as a trigger of type-1 diabetes, that was not my theory. The children who get type-1 diabetes do not have or quite seldom have celiac disease. They have celiac disease because they share the same genetic background, so the incidence of celiac disease is higher in patients with type-1 diabetes because of this accumulation of the celiac disease-risk genotype. But the occurrence of celiac disease is not higher than that estimated from the HLA-risk genotype when compared to the population. But the response to gluten may be aberrant because of this binding

ability of gluten peptides by HLA molecules and triggering of T cells. But the process stops, there is sub-clinical inflammation in the intestinal immune system and this does not necessarily lead to a celiac disease.

Dr. Bee Wah Lee: Have there been any trials on probiotics preventing type-1 diabetes when given very early in life or in experiments on animal models?

Dr. Vaarala: There is one animal study from Japan in which lactobacillus GG was given to NOD mice and it resulted in the prevention of autoimmune diabetes [6]. We started a study 2 years ago in which probiotics, a combination of 4 different probiotics or bacteria, were given to children with a genetic risk of type-1 diabetes. We have seen immunological changes induced by this preparation but, of course, we have no data to show whether it has any effect on β-cell autoimmunity. But we hope to be able to bring this study to an end and then have some information.

Dr. Fritsché: You mentioned in your talk that you have identified the hydrolyzed peptide. Can you tell us a little bit more about its function in deviating to the Th2 pathway?

Dr. Vaarala: This evidence comes from animal models and I still do not have any human data on this. Evidence is based on a feeding protocol: the mice and rats that received hydrolyzed peptides at weaning have this kind of type-2 immune response phenotype in the islets when compared to animals receiving normal proteins [7]. So we have not actually identified a single peptide in hydrolyzed formula that drives this kind of development, and we still do not have evidence from human studies, but this will be studied in the TRIGR and the FINDIA studies.

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What Is known? Short-Term and Long-Term Effects of Complementary Feeding

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Introduction

Much is known about the short-term effects of complementary feeding (CF), especially how an optimal diet can prevent poor growth, malnutrition and nutrient deficiencies. The CF period has always been identified as a period during which the infant has a high risk of developing stunting, protein-energy malnutrition and specific nutrient deficiencies such as iron-deficiency anemia and rickets. Furthermore, during this period, the risk of infectious diseases increases dramatically and the mortality from infectious diseases during this age is closely associated with the nutritional status. It has been estimated that malnutrition is a contributing factor in more than half of the deaths of children below 5 years and a considerable part of these deaths happen during the CF period [1]. If resources and good hygiene are available and if the caregiver has the required knowledge, it is not difficult to provide a sufficient CF diet which will prevent poor growth and severe nutrient deficiencies.

However, we are only beginning to understand how feeding during this sensitive period characterized by high growth velocity influences the growth pattern, development and health during later childhood and adulthood. The emerging knowledge about programming or early origin of adult diseases has previously focused on the intrauterine and the early postnatal period, but there is increasing evidence that nutrition and growth velocity during the CF period also has important long-term effects. We know that CF is a determinant of growth velocity and there are some data showing that growth velocity during this period is associated with risk factors for later development of cardiovascular disease and metabolic syndrome [2, 3].

This chapter will discuss some of the existing recommendations on CF from developing and industrialized countries. Furthermore, some aspects of macronutrients (fat, protein) and the use of cow's milk will be reviewed, ending with a discussion of the research issues that need to be addressed if we want to optimize CF. This will focus on the potential long-term effects of CF.

Complementary Feeding in Low Income Countries

In low income countries the feeding period, from 6 to 18 months, is critical for the promotion of optimal growth, development and health. Global statistics have helped to identify this period as the time when malnutrition and stunting develops. In figure 1 the SD scores (Z scores) of weight-for-age and height-for-age are shown for the three regions, Africa, Asia and Latin America, including the Caribbean, compared with the current WHO reference data [4]. For weight-for-age there is a marked decrease from the age of 3–6 months until the age of about 12 months. After that there is no further deterioration indicating that children after the age of 12 months have an average weight gain very close to the reference population. For length-for-age the pattern is almost the same except that the deterioration is slower and lasts longer, until about 24–36 months. The increase at 24 months is an artifact as the reference data are constructed with the children lying down until the age of 24 months after which they are measured standing. Thus, the dramatic deterioration in nutritional status, resulting in a high prevalence of malnutrition and stunting, happens exactly during the CF period. After this period there is no further decrease and also no catch-up. These data support other studies showing that, at least at the population level, it is very difficult to reverse stunting after the age of 2 years [5, 6]. The main causes of this deterioration are an insufficient CF diet, which typically has a low energy density, no or very small amounts of animal foods and a low content of essential micronutrients together with a high prevalence of infectious diseases.

The same pattern of growth deterioration during the CF period is also seen among infants and young children from industrialized countries with failure to thrive caused by an insufficient diet. A very illustrative example is the Dutch study of infants and children who received a macrobiotic diet (fig. 2), which is a diet with no animal products, high in fiber content and low in energy density [7]. The growth pattern of these infants and young children (fig. 2) was close to the pattern seen in many countries in Africa and Asia. These children also had a high prevalence of delayed motor development, rickets, iron and B_{12} deficiencies; all conditions that are common in developing countries [8].

Because of the health problems in low income countries, especially the high mortality in infancy and early childhood caused by low rates of breastfeeding and inadequate CF, the WHO has developed a comprehensive

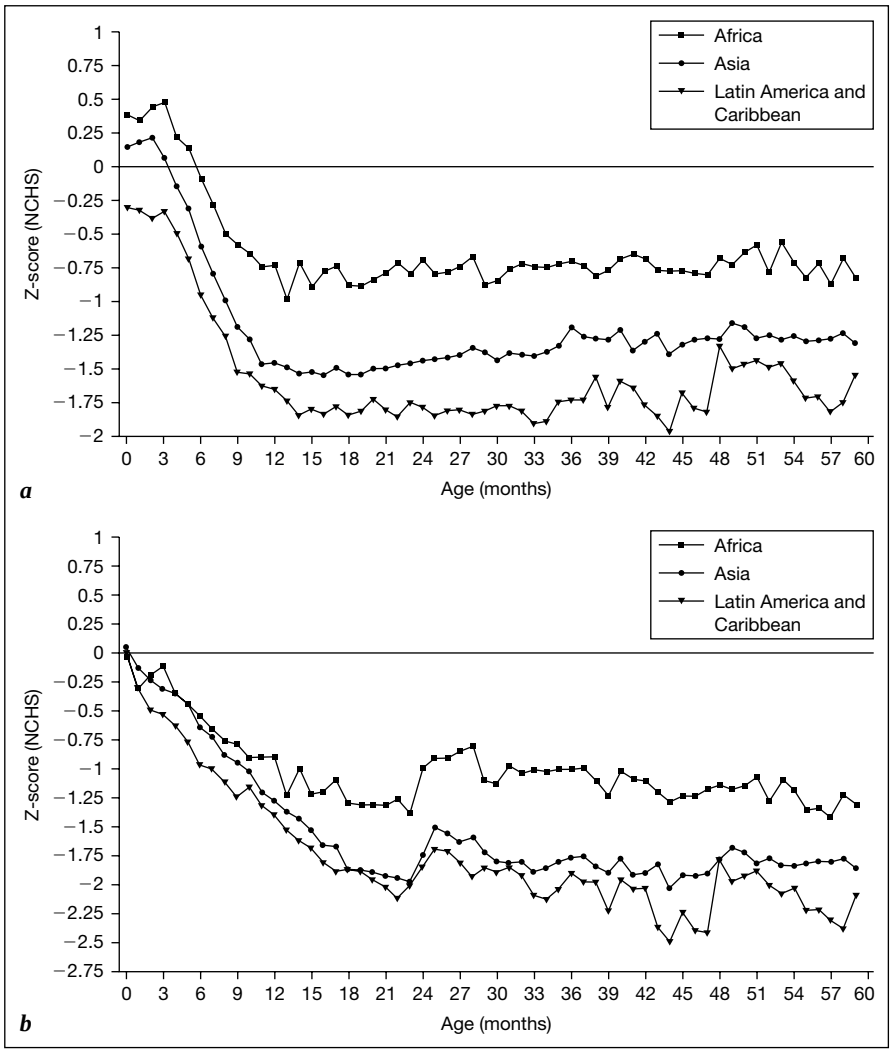

Fig. 1. Mean weight-for-age (***a***) and length-for-age (***b***) Z scores, relative to the NCHS reference, by region. From the WHO Global Database on Child Growth and Malnutrition. Reproduced with permission from Pediatrics [4]. Copyright 2001 by the AAP.

global strategy on infant and young child feeding [9]. The promotion and protection of breastfeeding have been a high priority for the WHO for many decades, but it is more recently that CF has also been given a high priority. Several expert consultations on CF have been convened. The guiding principles for CF from a WHO global consultation on CF in Geneva in 2001 are

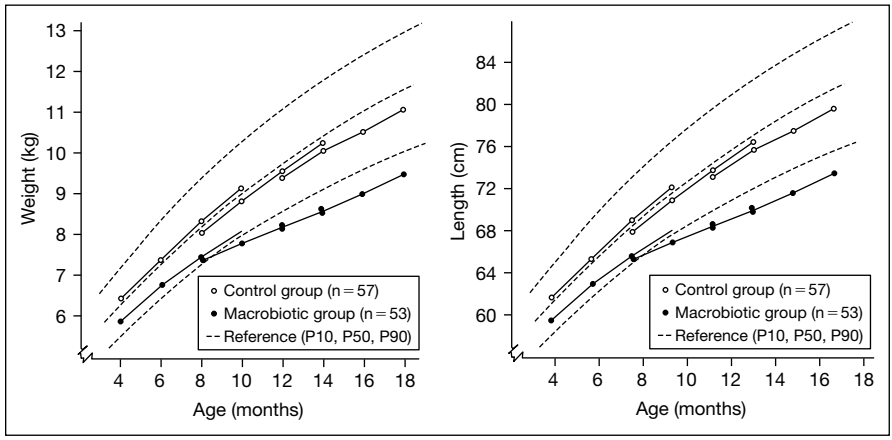

Fig. 2. Weight-for-age and length-for-age in children receiving a macrobiotic diet which is characterized by no animal products, a high fiber content and a low energy density. Reprinted by permission from European Journal of Clinical Nutrition [7], Copyright 1989 by Macmillan Publishers Ltd.

shown in table 1 [10].The scientific evidence for most of these guidelines has been summarized in a number of papers published in the same issue of the *Food and Nutrition Bulletin* [10] and in a review of the current scientific knowledge published by the WHO [11]. For some of the guidelines there is no strict scientific evidence. Instead, the guiding principles are based on the best available knowledge. Although these guidelines are mainly directed towards solving the global problems with malnutrition, poor growth and high mortality, most of the guidelines are highly relevant to industrialized countries as well. The guidelines focus on the duration of exclusive breastfeeding and age of introduction of CF, continuation of breastfeeding, amount, energy density, nutrient content and consistency of complementary food, meal frequency, the use of vitamin-mineral supplements and fortified products, safe preparation and storage, and feeding during illness. Furthermore, the guidelines emphasize responsive feeding, giving a set of principles for psychosocial care during feeding.

Complementary Feeding in Industrialized Countries

Although the principles for a CF developed by the WHO as part of the infant and young child feeding strategy are global, they focus on preventing malnutrition, growth faltering and a high rate of infectious diseases. The guidelines followed in many industrialized Western countries, where these

Table 1. Summary of the Guiding Principles for CF of the Breastfed Child [10]

1 Duration of exclusive breastfeeding and age of introduction of complementary foods
   Practice exclusive breastfeeding from birth to 6 months of age, and introduce complementary foods at 6 months of age (180 days) while continuing to breastfeed
2 Maintenance of breastfeeding
   Continue frequent, on-demand breastfeeding until 2 years of age or beyond
3 Responsive feeding
   Practice responsive feeding, applying the principles of psycho-social care. Specifically: (a) feed infants directly and assist older children when they feed themselves, being sensitive to their hunger and satiety cues; (b) feed slowly and patiently, and encourage children to eat, but do not force them; (c) if children refuse many foods, experiment with different food combinations, tastes, textures and methods of encouragement; (e) minimize distractions during meals if the child loses interest easily, and (f) remember that feeding times are periods of learning and love – talk to children during feeding, with eye to eye contact
4 Safe preparation and storage of complementary foods
   Practice good hygiene and proper food handling by (a) washing caregivers' and children's hands before food preparation and eating; (b) storing foods safely and serving foods immediately after preparation; (c) using clean utensils to prepare and serve food; (d) using clean cups and bowls when feeding children, and (e) avoiding the use of feeding bottles which are difficult to keep clean
5 Amount of complementary food needed
   Start at 6 months of age with small amounts of food and increase the quantity as the child gets older, while maintaining frequent breastfeeding. The energy needs from complementary foods for infants with 'average' breast milk intake in developing countries are approximately 200 kcal/day at 6–8 months of age, 300 kcal/day at 9–11 months of age, and 550 kcal/day at 12–23 months of age. In industrialized countries these estimates differ somewhat (130, 310 and 580 kcal/day at 6–8, 9–11 and 12–23 months, respectively) because of differences in average breast milk intake
6 Food consistency
   Gradually increase food consistency and variety as the infant gets older, adapting to the infant's requirements and abilities. Infants can eat pureed, mashed and semisolid foods beginning at 6 months. By 8 months most infants can also eat 'finger foods' (snacks that can be eaten by children alone). By 12 months, most children can eat the same types of foods as consumed by the rest of the family (keeping in mind the need for nutrient-dense foods, as explained in item 8 below). Avoid foods that may cause choking (i.e., items that have a shape and/or consistency that may cause them to become lodged in the trachea, such as nuts, grapes, raw carrots)
7 Meal frequency and energy density
   Increase the number of times that the child is fed complementary foods as he/she gets older. The appropriate number of feedings depends on the energy density of the local foods and the usual amounts consumed at each feeding. For the average healthy breastfed infant, meals of complementary foods should be provided 2–3 times/day at 6–8 months of age and 3–4 times/day at 9–11 and 12–24 months of age. Additional nutritious snacks (such as a piece of fruit or bread or chapatti with nut paste) may be offered 1–2 times/day, as desired. Snacks are defined as foods eaten between meals – usually self-fed, convenient

Table 1 (continued)

	and easy to prepare. If the energy density or amount of food per meal is low, or the child is no longer breastfed, more frequent meals may be required
8	Nutrient content of complementary foods Feed a variety of foods to ensure that nutrient needs are met. Meat, poultry, fish or eggs should be eaten daily, or as often as possible. Vegetarian diets cannot meet nutrient needs at this age unless nutrient supplements or fortified products are used (see item 9 below). Vitamin A-rich fruits and vegetables should be eaten daily. Provide diets with adequate fat content. Avoid giving drinks with low nutrient value, such as tea, coffee and sugary drinks such as soda. Limit the amount of juice offered so as to avoid displacing more nutrient-rich foods
9	Use of vitamin-mineral supplements of fortified products for infant and mother Use fortified complementary foods or vitamin-mineral supplements for the infant, as needed. In some populations, breastfeeding mothers may also need vitamin-mineral supplements or fortified products, both for their own health and to ensure normal concentrations of certain nutrients (particularly vitamins) in their breast milk. Such products may also be beneficial for pre-pregnant and pregnant women
10	Feeding during and after illness Increase fluid intake during illness, including more frequent breastfeeding, and encourage the child to eat soft, varied, appetizing, favorite foods. After illness, give food more often than usual and encourage the child to eat more.

The guidelines were developed by the WHO and PAHO and are available in full from www.paho.org.

problems are not common, focus also on other aspects of CF. Some countries have no official guidelines, while others have guidelines given by the health authorities or by pediatric associations. These recommendations are often based more on tradition and the availability of foods than on science.

The recommendations on CF covers some main issues: (1) duration of breastfeeding – exclusive and partial; (2) when to use infant formula and cow's milk; (3) the order in which different foods should be introduced and the consistency; (4) securing a sufficient energy intake through adequate energy density, fat content, and meal frequency; (5) prevention of iron deficiency; (6) prevention of micronutrient deficiencies, and (7) prevention of allergic diseases in infants with a family history of allergic diseases.

There are a number of issues in which the recommendations differ among the countries. Many countries have followed the WHO recommendation on exclusive breastfeeding for 6 months. However, some countries like Finland have maintained the recommendation that CF should be introduced between the ages of 4 and 6 months. Although the WHO recommends that breastfeeding should continue until the age of 2 years, most countries in Europe recommend that breastfeeding should continue until the age of 12 months or longer, without stating when it should be stopped. While many countries have no recommendations on when eggs could be introduced, some

state eggs could be introduced from the age of 6 months while several countries state that egg whites should not be introduced before the age of 12 months. The reason for this recommendation is to reduce the risk of allergy to eggs, but the advice is not evidence-based [12]. Recommendations on when to introduce cow's milk are mentioned in the section on cow's milk. Most countries recommend vitamin D drops while only some countries recommend vitamin A supplementation, which might be a remnant from the time supplementation with cod-liver oil, which contains both vitamins D and A, was common.

A set of guidelines based on infant and young child feeding and aimed at the WHO European region has been developed [13]. These guidelines are aimed at a group of countries including both highly industrialized countries and low income countries. The EU European region includes, in addition to Europe, all the former Soviet republics including the Central Asian Republics. Thus, it comprises highly industrialized Western countries, low income countries with poverty and high rates of infectious diseases and many countries in transition.

Fat

During the last decade there have been considerable discussions about the timing of the reduction of the dietary fat content during the CF period. Breast milk has a high fat content, about 50 energy percent (E%) and, in most countries, the recommended fat content of the family diet is 30 E%. The concerns have been that a fast reduction might reduce energy intake and thereby impair growth, and a too slow reduction could have a negative effect on risk factors of cardiovascular disease, especially if the intake of saturated fat is high. Furthermore, there has been concern that a high fat content could increase weight gain and thereby the risk of overweight.

No population-based studies from industrialized countries have shown an association between low fat intake and growth. In the Finnish STRIP study children with the lowest fat intake, which at 13 months was 22 E% from fat (FE%) and at 5 years 26 FE%, had normal growth [14]. However, in this cohort the children were monitored closely and growth faltering is likely to have resulted in some sort of intervention. In reviews considering data from developing countries, Uauy et al. [15] concluded that it was not before the FE% was <22 that there was a risk that growth was impaired. This is in line with the review by Prentice and Paul [16] who concluded that the FE% should be a minimum of 20–25 FE% to prevent growth impairment.

A high fat content will result in high energy density and thereby a theoretical risk of excessive weight gain, which is a concern in populations with a high prevalence of childhood obesity. However, there are several

studies in early childhood showing no association between fat intake and weight gain. In cross-sectional studies of 2- to 5-year-old children there were no associations between FE% and body mass index [17, 18] or body fat percent [19]. Furthermore, there was no difference in energy intake, weight, length or body composition at the age of 2 years in children receiving milk with 2 or 3.5% fat from the age of 12–24 months [20].

If the fat content of a diet is increased by adding oil, butter or margarine, the nutrient density of minerals and most vitamins and also other nutrients will be reduced as the intake of other foods will be reduced. In extreme cases, for example if a teaspoon of oil is added to a 100-gram serving of maize porridge prepared with water, the content of protein and iron will be reduced by more than 50%, when expressed per unit of energy. Therefore, if fat is added to the CF diet to increase energy density and prevent growth faltering it is important that only moderate amounts are added. Just adding 3 teaspoons of oil (15 ml) replaces about 14% of the energy needs of a 1-year-old infant.

A working group under the Danish Nutrition Council reviewed the literature about the possible associations between fat intake during the first 3 years of life and the risk of developing cardiovascular diseases later in life [21]. The working group found that there was a very limited scientific basis for evaluating the importance of fat intake during the first 3 years of life for later development of atherosclerosis. Although young children do develop fatty streaks in the aorta, they are reversible and are probably not influenced, to a major degree, by traditional risk factors for atherosclerosis. The working group found that these risk factors might influence the vascular function in children, but its importance for later development of atherosclerosis is unknown. Furthermore, no investigations have been carried out on whether the amount or quality of fat intake in the first 3 years of life influences the risk of developing atherosclerosis later on. The working group found that there was no risk of reduced growth if the fat content is above 25 E%. To avoid a very low fat E% there is a recommendation in Denmark that a teaspoon of fat or oil, preferably vegetable fat, should be added up to the age of 12 months to each serving of homemade mash and porridge, which otherwise will have a very low fat content, which was supported by the working group. Though a high content of saturated fat and cholesterol in the young child's diet increases the cholesterol level in the blood, the cholesterol level during childhood is considerably lower than the adult level. As there are no positive effects of a high intake of saturated fat in this age group, the working group found it prudent to reduce the intake of saturated fat from the age of 12 months in order to establish healthy dietary habits. Therefore, it recommended that from the age of 12 months the intake of saturated fat should be reduced to the same level as that recommended for adults, i.e. maximum 10 E%. To reduce the intake of saturated fat it was recommended that from the age of 12 months children are preferably given semi-skimmed

milk (1.5 fat%) and from the age of 3 years preferably low fat (≤0.5%) milk. The recommendations of the working group were adopted by the National Board of Health as official recommendations.

The dietary recommendations for the Nordic countries have recently been revised [22]. According to these, the FE% should be between 30 and 45 during the 6- to 11-month period, lowered to 30–35 during the 12- to 23-month period and, thereafter be between 25 and 35 with a population goal of 30 E%. This is a reduction compared to the previous recommendation. The reason for this reduction is that it was found that the evidence for a negative effect of a low fat intake on growth is not very strong.

There are many other potential health aspects of fat quality than the effect of saturated fat on cardiovascular risk factors. The effects of long-chain polyunsaturated fatty acids and especially the effects of the relation between n-3 and n-6 fatty acids on cognitive and visual development, growth and the immune systems have been studied in detail in early infancy where the interest has been to identify the optimal fat composition of infant formula. The composition of the fat intake during the CF period could potentially have the same effects and could also be of importance for the development of allergic diseases. However, only very few studies have explored the potential health effects of fat quality during this period. Such studies should be given high priority.

Protein

The average protein intake in late infancy is high, above the physiological requirements. Typically, it is 3–4 g/kg body weight, which is 3–4 times above the physiological requirements of about 1 g/kg body weight [23, 24]. However, some infants receive a protein intake which is much higher, i.e. 5 g or more. The short- and long-term effects of such a high intake are not clear.

A high protein intake results in high levels of amino acids in the blood which can have specific metabolic effects. Branched-chain amino acids stimulate insulin secretion that have a growth-stimulating effect during infancy [25] and some amino acids have hormonal effects or effects on neurotransmission. The potential effects of protein on growth is discussed in more detail in the section on cow's milk intake below.

A high protein intake influences the size of the kidney, most likely through an effect of the glomerular filtration rate. In adults, a 6-month intervention study showed that an 18% increase in protein intake resulted in a 2.5% increase in kidney size [26]. A similar effect of protein intake is most likely the explanation that the kidneys were larger in 3-month-old formula-fed infants compared to breastfed infants [27]. At the age of 18 months when there was no major difference in diet, there was no difference, suggesting that the effect on size was reversible.

It has been suggested that a high protein intake during early life increases the risk of overweight and obesity later in life. Rolland-Cachera et al. [28] suggested that it could be caused by an increase in insulin-like growth factor-1 (IGF-1) stimulated by the high protein intake. This hypothesis is been tested in an ongoing large EU multinational project [29]. In a prospective longitudinal study of term infants we found that the protein intake at 9 months was not associated with any measure of body fat at the age of 10 years [30]. However, the group was small and there were only a few obese children at the age of 10. There was, however, an association between protein intake and weight and height at 10 years which might be explained by a growth-stimulating effect of early protein intake as explained in the following section on cow's milk.

In a cross-sectional study of 2.5-month-old children we found a significant positive association between protein intake and systolic pressure [31]. The effect was substantial as a 1 SD increase in protein intake corresponded to a decrease in systolic blood pressure of 3 mm Hg. Such associations have also been found in older children [32] and adults [33, 34]. It has been speculated that this could be caused by certain amino acids mediated through the production of nitric oxide. It is unknown if the same effect of protein intake on blood pressure is also seen during the CF period and if such an effect could have long-term effects.

Cow's Milk

In industrialized countries cow's milk is an important part of the CF diet. In the WHO guidelines for CF, cow's milk is not mentioned as it is assumed that the infant/young child continues to be breastfed up to the age of 2 years. In most industrialized countries this is far from the reality and cow's milk is an important part of the diet, as a supplement to breast milk during late infancy, or as a substitute for infant formula when the child has reached an age where it is recommended to change from infant formula. Recently, a technical background paper commissioned by the WHO on the feeding of the non-breastfed infant was published, together with guiding principles from an informal working group [35]. The need to provide a sufficient diet to non-breastfed infants from HIV-positive mothers was one of the reasons for providing these guidelines. According to these guidelines undiluted cow's milk could be introduced from the age of 6 months, provided that iron supplements or iron-fortified foods are given and the overall amount of fluid is sufficient.

Recommendations for the intake of cow's milk in industrialized countries differ considerably among countries. Many countries recommend that cow's milk is not introduced before the age of 12 months. The most important reason for this recommendation is the iron status. Cow's milk has a very low iron content and the iron in the milk is poorly absorbed, while infant formula

is iron-fortified. Some countries recommend that cow's milk be gradually introduced from the age of 9 (Denmark) [36] or 10 months (Sweden) [37]. To solve the iron problem in Denmark it is recommended that infants not getting at least 400 ml of iron-fortified formula between the ages of 6 and 12 months should have medicinal iron. In the WHO/UNICEF guidelines for CF for the European region, which also covers some low income countries, the recommendations are that cow's milk should not be given before the age of 6 months [13]. In the period from 6 to 9 months only small quantities (e.g. in mashes) should be given. Milk should not be given as a drink before the age of 9 months, after which it can be introduced gradually. Once it is introduced the amount should not be more than 500 ml to secure a diversified diet.

Cow's milk has both positive and negative effects [38]. It contains high-quality protein, is a good source of important micronutrients and is an important calcium source. Furthermore, it contains peptides and other potential bioactive sources which might have beneficial effects. On the negative side, it is a poor iron source with a low iron content and a poor bioavailability, it may cause gastrointestinal bleeding, especially during the first 6 months of life, it has a high protein and mineral content resulting in a high potential renal solute load and has a high content of saturated fat.

New data suggest that cow's milk can stimulate linear growth in young children, even in those who are already receiving an adequate diet with high protein content. In a cross-sectional study of 2.5-year-old children we found a significant positive association between milk intake and height and IGF-1 levels [39]. These data were supported by an intervention study we performed in 8-year-old boys [40]. A very high intake of skimmed milk (1.5 liters/day) for a week resulted in a 20% increase in IGF-1 levels while an intake with the same amount of protein from meat had no effect on IGF-1 levels. Surprisingly, the milk also resulted in a 100% increase in fasting insulin levels, while there were no effects on fasting glucose levels [41]. The meat intake had no effect on insulin levels. The stimulation of insulin levels by intake of cow's milk is supported by meal studies of the effect of different foods on the insulin index. It was shown that the insulin levels after intake of milk was considerably higher than after other foods, while the glycemic index of milk was lower than the glycemic index of several other foods [42]. In an evolutionary perspective it is not surprising that milk and not meat stimulates growth. Milk has evolved as a diet to support the newborn during a period of high growth velocity, which is not the case with meat. Thus, the traditional concept that growth is optimal if there are no deficiencies may be too simplistic.

It has not been shown that cow's milk given during the CF period can stimulate growth and IGF-1 levels, but we find it likely that it is also the case during this period. It is well established that infants receiving infant formula based on cow's milk have a higher linear growth velocity and a higher weight gain than breastfed infants [43], which could be caused by a growth-stimulating factor in cow's milk not present in breast milk.

Table 2. Recommendations on research issues that need to be addressed, identified by the IPA/ESPGHAN workshop in Casablanca 1999 [44]

How should 'optimal' growth and body composition be defined and appraised? Long-term studies of functional outcomes related to growth and body composition during the period of CF and to CF itself are needed
How does CF influence the development of taste and smell and appetite control?
What impact does CF have on the development of immunotolerance, enteropathies, and atopic disease?
Does CF affect metabolic imprinting or programming?
Is health in later life influenced by CF?
Does the timing of introduction or amount of complementary foods affect breast milk frequency and intake, and the duration of breastfeeding?
How does CF interact with and influence the physiologic maturation and metabolic competence of infants to digest, absorb, and metabolize non-breast milk and non-formula-based foods?
What are the accurate nutrient requirements during infancy?
What strategies would improve nutrient supply and bioavailability in complementary foods?
Should complementary foods be different for breastfed and formula-fed infants?
What are the potentially modifiable constraints to adoption of parenting practices that are geared to children's developmental and nutritional needs, and to the maintenance of food safety?

If cow's milk intake has an effect on growth velocity during the CF period it is not known if this has a lasting effect or if it will be compensated later during childhood and if it has any positive or negative effects on health.

Research Recommendations

The current interest in CF has resulted in two sets of research recommendations. The many research issues and questions that need to be addressed to improve the understanding and practice of CF were discussed in a workshop arranged by the International Paediatric Association (IPA) and the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in Casablanca 1999 [44]. The proceedings include a number of short papers, many of which have included research recommendations. The workshop also agreed on a number of more general recommendations that are given in table 2.

The global expert consultation on CF held by WHO/UNICEF in Geneva 2001 recognized that although the evidence for the guiding principles decided on (table 1) were sound, further research was needed to broaden and refine

Table 3. Research priorities identified by the WHO global expert consultation on CF in Geneva 2001

Estimating energy and nutrient requirements of children living in especially vulnerable circumstances, such as infants of HIV-positive mothers who choose to breastfeed, preterm infants, and low-birth weight infants
Assessing the effects of variations in energy density, feeding frequency, food quantity, and food variety on total energy intake, including the intake of breast milk
Identifying factors affecting children's appetite and appropriate treatment of anorexia
Determining the optimal amount and type of lipid and fiber intake by children
Examining the use of linear programming for developing context-specific CF guidelines
Identifying alternative approaches to create demand for affordable and effective processed food products
Assessing the efficacy and effectiveness of fortified complementary foods, sprinkles and spreads in addressing dietary gaps, including optimal levels of formulation and ration sizes to improve nutrient intakes
Determining the bioavailability of iron and zinc in locally available foods
Determining methods and criteria for characterizing the responsiveness of feeding styles in various settings and identifying effective methods for promoting responsive feeding
Determining the impact of improved responsive feeding on child growth and developmental outcomes
Developing and testing appropriate and effective strategies to ensure the safe storage, preparation, and feeding of complementary foods to infants and young children
Developing strategies for maintaining and sustaining breastfeeding as complementary foods are introduced and the young child progresses to the family diet
Developing and testing indicators and tools for designing, implementing, and evaluating programs promoting appropriate infant and young child feeding

the strategies and interventions [10]. The consultation identified the research priorities shown in table 3.

Future Directions

We know how to feed the infant and young child during the CF period to avoid malnutrition, growth retardation and micronutrient deficiencies. The challenge in developing countries is to implement strategies that secure the infants an optimal feeding during this critical period. Both the WHO and UNICEF have given this a high priority through the Global Strategy for Infant and Young Child Feeding. This strategy also identifies the difficult situations that require special attention. These include infants and young children that

are already malnourished, low birth weight infants, infants of HIV-infected mothers, and infants and young children that are victims of natural or human-induced emergencies.

In industrialized countries the challenge is to understand the potential long-term effects of CF. There is increasing evidence that postnatal nutrition and growth play an important role in the early origins of adult disease hypothesis [45] and that CF is likely to program long-term effects. Breastfeeding has many positive long-term effects and it is plausible that other foods given during infancy and early childhood can also have long-term effects. However, the evidence for such effects is not strong at present.

With the alarming increase in overweight, obesity and allergic diseases during childhood, it is of special importance to explore if the risk of developing these conditions could be reduced by optimizing CF. Regarding this there are a number of areas that are of special interest.

Gastrointestinal Microbiota

How is it influenced by the composition of CF?

What are the effects of pre- and probiotics?

Is there a long-lasting effect of the timing of the introduction of CF?

What is the influence of non-digestible carbohydrates?

Gastrointestinal Barrier

There seems to be a marked effect when CF is introduced to the exclusively breastfed infant and the function of the gastrointestinal barrier seems to be important for the development of allergic diseases.

What are the mechanisms?

How does the introduction of different foods influence the gastrointestinal barrier?

Maturation and Polarization of the Immune System

What is the need for n-3 fatty acid content of the CF diet? How does the n-3/n-6 balance affect immune function?

Is there a long-term effect?

How does the gastrointestinal microbiota influence the immune system?

Obesity

Is there an effect of protein intake on later development of obesity?

Does the composition of the CF diet, especially the fat quality, have an influence on body composition on the short- and the long-term?

Do the changes in body fat content during the CF period influence body composition on the long-term of the early n-3/n-6 intake?

Does the gastrointestinaal microbiota have an influence on the risk of obesity?

Food Preferences and Appetite

How does the composition of the CF diet and the diversity affect later food preferences?

How is appetite regulated during the CF period?

How is the CF diet influencing leptin levels?

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Discussion

Dr. Vaarala: You showed that drinking milk causes high production or release of insulin. Have you any evidence that insulin resistance is induced by drinking milk?

Dr. Michaelsen: Strictly speaking these children had some degree of insulin resistance because they had normal fasting glucose and increased insulin levels compared to baseline [1]. However, the intervention lasted for only 1 week and we examined the effect of a milk intake (1.5 liters/day) much above the recommended intake, so based on our data we cannot say that milk causes insulin resistance. I know that there are studies by Nilsson et al. [2] from Sweden showing that milk is especially effective in stimulating insulin secretion after a meal and it seems as though specifically whey stimulates insulin, but they only studied the acute effect after a meal and after some hours the insulin values were down to starting values.

Dr. Vaarala: I only wanted to say that there are two studies, one from Australia [3] and one from Finland [4], in which a high intake of milk during childhood has been associated with the risk of type-1 diabetes. It could be that an increase in insulin and insulin resistance enhances the presentation of insulin to the immune system, which may be the factor. This is really a fascinating study.

Dr. Michaelsen: Do you know how high the milk intake was? What were the levels?

Dr. Vaarala: It was more than 3 glasses/day, so more than 600 ml/day.

Dr. Michaelsen: I think it is important that children don't get much more than 500 ml milk as recommended in some countries in order to keep the diet diversified. I think milk is an important part of the diet, providing important micronutrients. Furthermore, it seems that milk stimulates linear growth, which is important in developing countries where stunting is common. The long-term effects of such a growth stimulation in industrialized countries is not known.

Dr. H. Hoekstra: What is the actual recommendation concerning cis and trans fatty acids for the young child?

Dr. Michaelsen: I don't know. Perhaps Dr. Hernell could answer. He participated in the EU working group on infant formula under the Scientific Committee on Food.

Dr. Hernell: In infant formula I think the most recent recommendation, which is the European Union revised infant formula directive of 2003 (5) is to keep trans fatty acids as low as possible and a maximum level was set to 3% of total fatty acids.

Dr. Michaelsen: But the discussion about trans fatty acids is whether cow's milk trans fatty acids count as much as hydrogenated.

Dr. Hernell: In that recommendation we didn't discuss the potential difference between different sources of trans fatty acids.

Dr. El-Din Amry: What do you mean by cow's milk? Is it raw milk?

Dr. Michaelsen: Here I mean pasteurized cow's milk with a normal fat content or a low fat content. It is not cow's milk-based infant formula.

Dr. El Din-Amry: But in developing countries excessive consumption of cow's milk could lead to rickets because of the calcium phosphorus ratio.

Dr. Michaelsen: I didn't know that. I know that, in developing countries where milk is not part of the diet, if there is a very low calcium intake this could cause rickets.

Dr. El Din-Amry: It is not because of the calcium intake but because of the calcium phosphorus ratio in the milk. The calcium absorption from the intestine could lead to rickets and it is advised that vitamin D supplementation is given in this case.

Dr. Michalesen: Many countries recommend universal vitamin D supplementation, and in countries where rickets is prevalent this should be considered. At this meeting we also heard that vitamin D could be important for diabetes prevention.

Dr. Steenhout: A recent article published in *Pediatrics* [6] showed that multivitamin supplementation increases the risk of atopy and asthma in certain sub-classes of the

population. The authors were speaking about a whole range of vitamins but also more specifically about the risks of vitamin D supplementation. What is your opinion on that?

Dr. Michaelsen: I don't think I have any further comments. We are faced with a lot of research data that are difficult to draw public health conclusions from. Also some of the data I gave on cow's milk are difficult to transform into simple advice. So there is still a lot to do in complementary feeding research.

Dr. Schmitz: One comment that can be made from the workshop and from what you said is that the pros and cons for every nutrient must be taken into account, and this is difficult for cow's milk for example. In your talk you did not comment on solids and the number of solids. In my talk, I stressed the fact that the introduction of too many solids too early was dangerous. In the European document you showed us, are there data concerning the number and the timing of solids?

Dr. Michaelsen: No, we didn't make a recommendation on that. I know that several countries recommend that one food at the time be introduced in order to be able to know if there are allergic reactions, but as far as I know this is not evidence-based. I think it makes it too complicated and too disease-oriented.

Dr. Schmitz: I agree with you completely.

Dr. Caroli: Thank you very much for your absolutely wonderful presentation. As you said, in Italy we have a very high intake of protein during weaning, even during the first year of life between 6 and 12 months of age, we even have an intake of 5 g protein/kg. The reason for this is that pediatricians use this to increase iron intake, which is really a silly thing because in meat baby food in Italy the iron content is not labeled. There is not enough advice given to pediatricians on the use of fortified supplemented iron or cereals. There is a WHO declaration saying that it is impossible to reach the recommended iron level if you don't use unrealistic and unbelievably high amounts of meat. I think that this should be stressed for teaching mothers how to wean because otherwise we will still have this problem of protein intake and obesity and so on. What do you suggest?

Dr. Michaelsen: I think that iron-fortified complementary foods have a place in some situations, but I don't think we should restrict meat intake because of the fear of protein intake. In any case infants and young children don't eat large beef steaks; they eat small amounts of minced meat. If we address the issue of iron supplementation, we are not addressing the zinc issue. Meat is a good source of zinc and also other nutrients. I think it is important to introduce meat as part of the complementary feeding diet. Most countries recommend its introduction at about 6 months. Last week in Milan there was a discussion on meat introduction and I was very surprised to see that there was such a lot of reluctance among Italian pediatricians to introduce meat early. They talked about the histidine content of meat; they talked about mad cow's disease; they talked about other diseases in chickens. So people are very afraid and I don't think these issues are appropriate. I think meat is an important part of the complementary feeding diet.

Dr. Moreno Villares: When you recommend less than 0.5 liters of milk, does this also apply to the dairy products or only milk?

Dr. Michaelsen: I think in some countries dairy products are included. This is not a very sharp limit but I think for the diversity of foods we shouldn't feed our children too much milk. Once in a while in Denmark we see children being admitted with severe iron-deficiency anemia. They drink 1.5 liters of milk (so-called milkaholics) because the child and the mother think that it is a very easy way to cover the needs. So I think we should be active in keeping milk intake at a reasonable level.

Dr. Wei Cai: During your talk you mentioned that a high protein intake could induce a high prevalence of obesity. A couple of papers published recently recommend 4 g amino acids/kg/day. Is there a long-term effect in children?

Dr. Michaelsen: I mentioned the hypothesis that a high protein intake could induce obesity but I don't think it has been proven. There have been a few studies

showing an association and a few studies showing no association. There is a large ongoing multinational study in Europe addressing this. So I don't think we should cut down on protein for obesity, we don't have the evidence.

Dr. Mexitalia: You mentioned the high protein intake, but in most developing countries the animal source protein intake is very low and the problem is the low protein intake. What is the recommended protein energy ratio?

Dr. Michaelsen: A protein intake at 10–15 energy percent would be adequate. In developing countries protein intake can be a problem, especially if there are no animal sources and only few vegetable protein sources. If there is a mix of vegetable protein the situation is better, and many studies have shown that if it is possible to add a little animal protein, not only because of protein quality but also because of the minerals and other micronutrients to be gained from animal food, then there might be a considerable stimulation of growth and thereby improved health. So I think if a little animal food can be given with a mixed vegetable diet, then protein problems would be very rare.

Dr. Mexitalia: If we want to increase the energy density we can add oil, but it would lower the protein energy.

Dr. Michaelsen: That is right. But again breast milk, which is the food with the highest growth velocity, contains only 5 energy percent of protein, but it is of high quality. So for a composite diet 10–15 would be appropriate.

Dr. Lafeber: During the ESPGAN meeting in Paris in July 2004, Dr. Rey told me that the European Committee, which advises on normal formulas, is no longer recommending follow-on formulas. Looking at the continuity of protein in formulas, just regarding normal infants, not special formulas, and the introduction of cow's milk, some attempts have been made to lower the amount of protein in formula milk. There is now a formula available with I think 1.8 g of protein/100 kg/cal. That is alright, but it is advised that the formula be given for 6 months and then changed over to a follow-on formula, particularly with iron in it. But if there is now a new European recommendation that no longer advises the use of follow-on formulas, then the only way out is to introduce cow's milk with a high protein content at 6 months.

Dr. Michaelsen: In the report with suggestions for a new EU directive on infant formula, it is recommended that follow-on formula has the same protein content as infant formula [7].

Dr. Lafeber: Also the continuity of a low protein formula. Of course if you have that and then at 6 months change to a protein-fortified follow-on formula, that doesn't make sense either.

Dr. Michaelsen: My personal idea is that we don't need a follow-on formula. We can stay with the starter formula throughout. With the low protein content we might then, at least for the first 6 months, get a lower growth velocity, as Dr. Singhal also stated, which might then result in a lower growth velocity with potentially beneficial effects. What the optimal growth velocity from 9 to 12 months is, and what the protein content should be, I don't think we really know.

Dr. Bee Wah Lee: Is there any negative effect of using a soy formula in place of a cow's milk-based infant formula?

Dr. Michaelsen: The report from the Scientific Committee on Foods on Infant Formula concludes that there are no nutritional advantages to using a formula with soy as the protein source [7]. Furthermore, they mentioned some potentially negative effects that have not been fully evaluated, and advocate that the effects of soy-based infant formula needs to be better evaluated.

Dr. Caroli: As far as I know, in Italy at least, the high protein intake is always associated with a very low fat intake because, when looking at some old nutrients used in the traditional way of weaning in Italy, it is found that even at 8 months of age or 11 or 12 there is only 30% of the fat calorie intake.

Dr. Michaelsen: What kind of protein is given to the infants when there is no fat in it? It is my impression that much of the protein is from milk, from cheese, and from meat. Of course some of the meats given will be pretty lean, but is it because a lot of meat is given that there is not so much fat?

Dr. Caroli: In Italy pediatricians use a meat baby food which is very low in fat. When a baby eats 80 g of baby food, he cannot eat many other things. If the nutrients in the traditional Italian way of weaning are counted, then it is found that the fat intake is very low. I am worried about brain growth in the first 2 years of life. What is your opinion? Are there any data on that?

Dr. Michaelsen: I think brain growth is not so much a question of total fat content, but fat composition might have an effect. There must be a reasonable balance between n-3 and n-6 and, if that is not the case, it might influence brain and psychomotor development. As I said, in most children a fat content of about 30% would not cause growth retardation. If a low fat content is found in combination with a mother who is not very responsive to the hunger and satiety clues of the infant, an infant who eats only a few meals a day and perhaps has many infections, a low fat content might be a limiting problem. For some years I was more worried about a low fat intake but as long as it is about 30 fat energy percent, it is not very likely that fat intake will have a negative effect on growth.

Dr. Lafeber: You showed us Dr. Singhal's slide which also provokes the question: what is optimal growth? One of the observations made by Dr. Singhal and Dr. Lucas is that there is a period of in utero growth restriction resulting in intrauterine growth retardation, and there is growth restriction shortly after birth because, in the case of low birth weight, it takes a while before proper nutrition can be given, and often there are signs of malnourishment. If you do not react to this with an adapted diet and give a low protein diet, it has been demonstrated that the brain will not develop properly, so we are very much in favor of at least giving enough protein to spare the brain. But then comes the question of how long we should continue that protein gain. Regarding the Barker hypothesis, it is always said that if there is an insufficiency before birth and an insufficiency after birth, then there is no problem, there will be no disease, there will be no heart disease, there will be no type-2 diabetes. But if you are supplying very rich formulas after birth then the problems start. I think it is the same in these preterm infants, they must be given more protein for brain development at a certain time, but then there is a risk if the diet is continued for too long a period. What are your thoughts on this?

Dr. Michaelsen: Neonatologists often ask about the optimal growth rate of preterm infants. It is a very difficult question for which I have no clear answer. There are studies showing both positive and negative effects of a high growth velocity, depending on the outcome. The long-term effects of growth velocity are likely to be different for term and preterm infants, and depend on a time window. The first 6 months could be very different from the effects of growth velocity during the last part of infancy, when complementary feeding influences growth velocity.

Dr. Lafeber: There is still a lot to be studied regarding insulin growth factor insensitivity and sensitivity.

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Educational Recommendations for Processed Foods for Infant Feeding

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Introduction

There is relatively little systematic evidence on which to base any informed advice about the introduction of foods other than breast milk or breast milk substitutes. Arguably once solids have become the major component of a young child's diet there is even less evidence about optimum feeding [1]. Typically diversification of an infant's diet has been very much influenced by parental belief and cultural practice.

It is difficult in such circumstances to give firm evidence-based guidelines on feeding practice for young children, and on the foods involved, and even less easy to conceive defensible regulatory approaches to this issue, although there are, for example, some compositional guidelines for cereal-based weaning products.

Even so, there is considerable interest in the potential for harm that can arise from inappropriate early childhood feeding and strategies to minimize this form the basis of most recognized educational needs in the issues of early childhood feeding.

Here the principal message is that there is key knowledge based on the nutritional needs of young children and practice which should be widely disseminated as part of the societal responsibilities of all concerned in the food chain of young children. Once their diet has become diversified, regardless of whether or not processed foods are used, it is not realistic to regulate for ideal feeding practices. Ideal feeding practices should be seen as a key component of the inter-professional and interagency governance of public health nutrition. This principle, in itself, indicates the nature of the educational needs and the research that in turn is needed to enable these to be met.

This topic should be viewed in the context of the WHO Global Strategy for Infant and Young Children Feeding [2]. Here key points from the World Health Assembly (WHA) agenda are abstracted from its May 2002 meeting. The 55th WHA perceived 'that complementary feeding practices are frequently ill-timed, inappropriate and unsafe'. It recognized that infant and young child mortality could be reduced by improving the nutritional status of women of reproductive age using nutritionally adequate and safe complementary feeding through the introduction of safe and adequate amounts of indigenous foodstuffs and local foods, while breastfeeding continues until the age of 2 years or beyond.

The WHA was 'also aware that inappropriate feeding practices and their consequences are major obstacles to sustainable socioeconomic development and poverty reduction'.

As a matter of urgency, the WHA urges member states:

(1) to adopt and implement the global strategy, taking into account national circumstances, while respecting positive local traditions and values, as part of their overall nutrition and child health policies and programs, in order to ensure optimal feeding for all infants and young children, and to reduce the risks associated with obesity and other forms of malnutrition;
(2) to strengthen existing, or establish new, structures for implementing the global strategy through the health and other concerned sectors, for monitoring and evaluating its effectiveness, and for guiding resource investment and management to improve infant and young child feeding;
(3) to define for this purpose, consistent with national circumstances, (a) national goals and objectives, (b) a realistic time line for their achievement and (c) measurable process and output indicators that will permit accurate monitoring and evaluation of action taken and a rapid response to identify needs;
(4) to ensure that the introduction of micronutrient interventions and the marketing of nutritional supplements are not in place of or undermine support for the sustainable practice of exclusive breastfeeding and optimal complementary feeding;
(5) to mobilize social and economic resources within society and to engage them actively in implementing the global strategy and in achieving its aims and objectives in the spirit of resolution WHA49.15.

The WHA also requests the Codex Alimentarius Commission to continue to give full consideration, within the framework of its operational mandate, to action it might take to improve the quality standards of processed foods for infants and young children and to promote their safe and proper use at an appropriate age, including adequate labeling, consistent with the policy of the WHO, in particular the International Code of Marketing of Breast Milk Substitutes, resolution WHA54.2, and other relevant resolutions of the CAP Health Assembly.

The WHA also requests the Director General:

(1) to provide support to member states, on request, in implementing the strategy, and in monitoring and evaluating its impact;
(2) to continue, in light of the scale and frequency of major emergencies worldwide, to generate specific information and develop training materials aimed at ensuring that the feeding requirements of infants and young children in exceptionally difficult circumstances are met;
(3) to strengthen international cooperation with other organizations of the United Nation's system and bilateral development agencies in promoting appropriate infant and young child feeding;
(4) to promote continued cooperation between all parties concerned with implementing the global strategy.

Processed Foods for Early Childhood

The common reference point is that processed foods should be microbiologically safe, and free of pollutants, contaminants and potential antigens. Regulatory frameworks exist to cover additives, novel foods and processes, packaging, flavorings, hygiene and microbiological safety, contaminants, milk fats, natural mineral waters and foods for particular nutritional purposes, which embrace infant formulas and follow-on formulas, and processed cereal-based foods and baby foods for infants and young children. Some elements of risk assessment in the food chain of children have been considered in an earlier Nestlé Nutrition Workshop [3].

Processed foods offer benefits for the carers and children alike. They offer extended availability of foods free of seasonality, and the opportunity for products to be designed to meet issues relating to convenience, low cost, changing lifestyles and ideals relating to the health and well-being of infants and young children.

There are some possible disadvantages arising from a dependence on commercially produced processed foods. It is conceivable that their availability might compromise existing cultural preferences and practices.

The use of processed foods designed for children responsibly meet the known and current concepts of good nutritional practice for young children but convenient access by carers to processed foods designed for the broader 'adult' market could lead to young children being given inappropriate foods.

There is no guarantee that parents and carers will provide their infants and young children with processed foods meeting current nutritional guidelines such as reduced contents of refined carbohydrates, sodium chloride, saturated and trans fatty acids, and calories. Confused messages may, however, also lead to young children having too high intakes of fiber, too low intakes of energy.

A mixed blessing of globalization, or perhaps more accurately the regulatory framework of global trade and food safety, could be that it erodes the traditional base of a locally sustainable and diverse early diet, because

such regulations by enforcing homogeneity will reduce opportunities for commercial and culturally sensitive diversity, and, perhaps paradoxically, create nutritional risk. This illustrates the need for consideration of anthropological and cultural perspectives in the educational background to the feeding of infants and young children with processed foods, particularly if one expects the processed foods to educate the palate of young children for their adult diets. Clearly, it is feasible to do this with processed foods, but it may be necessary to have marketing strategies, and to educate carers on how to use products to do this.

What Do Infants and Young Children Need?

There is a universal need for an awareness that the science base for regulation of composition of processed foods is of variable quality. This is due in no small part to the insecurity of the reference values for dietary intakes of children in general [4, 5]. There is a lack of good quality data on the nutrient requirements of children [6]. Reference values for this age group are largely extrapolated from estimates for older and younger age groups. This is a major source of uncertainty as is the need for better insight into any critical windows relating metabolic programming, growth and development, and crucial timing and duration of exposure to nutrients. Coupled with the need for more markers of adequate nutrition [5–7]. An example of these problems is the current uncertainty about the susceptibility of cognitive and psychomotor development to anemia, iron deficiency, other correlated nutritional deficiencies, social factors, or a combination of these [8].

There is an opportunity, however, to refine knowledge about the nutritional needs of young children during the systematic development of processed foods. Some of this opportunity arises from ideas concerning health and lifestyle and the development of 'functional foods'. These are based on the concept that such foods may provide beneficial effects beyond those seen as critical events from which to derive reference values and the possibility that components other than traditional nutrients might also have beneficial effects [9]. In fact this whole area is so insecure that any such new knowledge can be seen as a key to deriving better qualitative and quantitative reference values for dietary intakes [5]. An additional assumption is that it may be possible to make claims for such processed foods, and that the basis for these claims will be a sound portfolio of evidence using studies based on humans and suitable outcomes [10].

In this scenario it is possible to envisage a confluence of interests that collectively would create an innovation-implementation cycle that would educate, identify knowledge gaps and generate both basic and applied research to provide new information. This cycle is illustrated in figure 1.

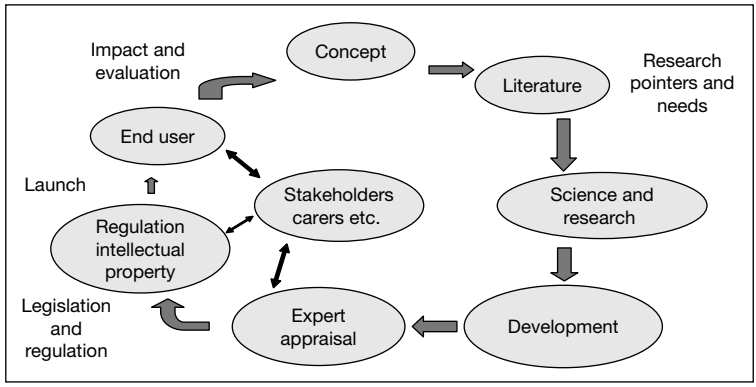

Fig. 1. The innovation-implementation cycle of nutrition and product development and improvement.

The cycle [11] involves firstly the development of a concept that is refined by a sound search of the literature, and then realized and explored by research before being applied via development and involvement of all potential stakeholders (e.g. regulators and expert appraisal, users, carers amongst others as the case may be) and, after consideration of intellectual property issues, finally being launched and appraised after use. The latter evaluation could lead to new concepts and further development. All stages of the cycle need a suitable education to prepare them for their particular role in the cycle, and in the context of this meeting to enable them to share in the effective generation and dissemination of new knowledge, information and practice, as well as new processed foods.

Processed Foods and the Market and Claims

A 'health claim' has been defined as any claim that states, suggests or implies that a relationship exists between the food category, a food or one of its constituents and health. Claims can relate to nutrient function claim; enhanced function claim, or a reduction of disease risk. Claims are seen to have potential in education in that they can achieve a high level of consumer protection and facilitate consumer choice, improve the free movement of goods within the internal market, increase legal security for economic operators, ensure fair competition in the area of foods, and promote and protect innovation in the area of foods (COM (2003) 424 final: Article 2) [5, 12].

Thus claims are seen as a means of educating consumers and encouraging processors to think about their products, for example, in the innovation model described above. It would be reassuring if one could feel confident that claims or the process involved in their derivation and justification could be translated to

Table 1. Food components and modifications of interest in processed foods for infants and young children

Nutrients
Lipids: saturated fatty acids, poly- and monounsaturated fatty acids, cholesterol
Carbohydrates: fructose, glycemic indices, complex carbohydrates
Minerals: calcium, iodine, iron, zinc
Electrolytes: sodium, potassium
Vitamins: vitamin D, vitamin C, vitamin A, folic acid
Energy and nitrogen requirements
Nucleotides
Non-nutrients with possible functional food benefits
Non-digestible carbohydrates
Phytostanols and phytosterols
Breast milk bioactive factors
Bovine milk bio-active components
Organosulfurs
Polyphenols
Cryptoxanthines
Fat replacers
Components that could be reduced in content
Phytate, allergenic epitopes, sodium, energy and total fat and trans fatty acids

Derived from Aggett [5], Koletzko et al. [7] and Diplock et al. [9].

processed foods for infants and young children and the related aspects of public health. As is evident from the elements described in the previous paragraph, claims are intended to operate in a commercial environment, but one would hope that given the WHA's exhortation mentioned initially, an ethos will emerge that will favor an evidence-based approach to their use in infant feeding.

This meeting has addressed needs relating to energy metabolism, obesity and the metabolic syndrome, disturbed intermediate metabolism, immune function and avoidance of allergy, and gastrointestinal function. Additionally there are other important endpoints relevant to monitoring both the short- and long-term impact of early childhood nutrition. For example, a recent European Union concerted action discussed functional endpoints relevant to development, growth and body composition, bone health, cognitive and psychomotor development, and responses to effects induced by environmental hazards, and oxidative damage [7, 9]. A variety of food components and modifications were considered in this exercise (table 1), one important outcome of which was the appreciation that there is a need for new markers and methodologies for assessment of exposure and of outcomes relevant to homeostasis, function and safety which will enable the determination of more accurate reference intakes and secure guidelines on feeding young children. Increasingly it is appreciated that post-genomic molecular biology will provide opportunities for such markers.

Nutritional Safety

The nutritional safety of early feeding addresses the longer term outcomes of early feeding and the possibility that, despite the best metabolic and dietary logic, there can be unexpected effects.

There may be a need to develop an approach analogous to the guidelines that have been proposed for the long-term nutritional assessment of infant formulas. These contained groups of recommendations relating to the standardization of quality control and benchmark reference data based on outcomes in breastfed children; the use of standard study designs and harmonized protocols, and the means of handling data such that they could be aggregated and pooled to form a repository of reference data and to facilitate long-term follow-up [13, 14].

Recommendations and Opportunities

This scenario demonstrates many educational needs concerning the use of processed foods for young children, and a need for an interdisciplinary or interprofessional approach to developing an appropriate ethos to deliver safe and adequate nutrition to these children regardless of whether they are fed processed or unprocessed foods. The latter is a less formal approach and not open to as easy regulation as are processed foods, but the overall feeding of young children has to be seen as an informal exercise. Within this, key competencies in nutrition and food safety as part of public health, need to be conveyed to all involved in caring directly or indirectly for children.

This includes the agriculture, food and fishery industries, farmers, small and medium enterprises and large companies, as well as regulators, legislators and economists and others who are stakeholders in a broader investment in child health.

This indicates the needs for cross-cutting competencies as well as more specific nutritional and industrial food skills; amongst these I would include risk management, communication as well as knowledge and information management, anthropology and cultural sensitivity, and consideration of economics of health and sustainability. The latter can be seen as a means of ensuring that processed products can address local issues.

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Discussion

Dr. Leathwood: I would like to raise the issue of consumer understanding of the health claims. It is easy to say that consumers ought to be able to understand claims. But normal people are not nutritionists and interpret claims in unexpected ways.

Dr. Aggett: You were telling me earlier about the example of dairy products. Do you want to explain that as an illustration?

Dr. Leathwood: The example that I gave was where the recommendation that, to ensure adequate calcium intake, children should be given 'dairy products'. This was interpreted by many mothers as meaning that the best thing to give was full cream dairy milk (the perfect 'dairy product') and the further one moved away from this, the less calcium the food would contain so that skimmed milk would have less calcium, cheese would have less calcium, and yogurt would have less calcium. This is not quite what the dieticians intended and underlines the importance of checking how the message is received.

Dr. Aggett: One of the points that has certainly been developed in the European guidelines is that these claims must be tried so that consumer understanding can really be tested, and that is not very easy.

Dr. Waterland: You stated that in your opinion optimal nutrition doesn't really exist. I am just wondering what you really mean by that?

Dr. Aggett: At the moment from my point of view, optimal nutrition is the promotion of products, it isn't strongly evidence based. The point is that many of the existing recommendations for reference intakes are based on the avoidance of deficiency, and the point about optimal nutrition is that if one recommends and provides intakes over and above reference intakes then it will almost certainly be beneficial. These benefits

are not necessarily that well defined, and then they lead up to these difficulties of getting to limits which may be super-physiological and psychological.

Dr. Waterland: But we nutritionists have moved beyond just avoiding deficiency and are looking for ranges of nutrient intakes that can benefit in many ways, such as the Mediterranean diet as an example of perhaps being protective against cancer. What term would you propose for that if not optimal nutrition?

Dr. Aggett: What I am objecting to is the fact that people call this optimal nutrition rather than calling it adequate nutrition, which in itself should be optimal.

Mrs. Gailing: I have two questions. One is about claims. You probably know that in the future, following CODEX, new guideline claims will be allowed in a lot of countries on normal food but will be banned or not authorized for food specifically designed for infants and young children. So here there is unfair competition and mothers are encouraged to use normal food for their babies. As Dr. Leathwood just said, dairy products make health claims, with high protein levels, saturated fat, and discourage the use of food specifically designed for infants and young children. How do you see the application of guidelines compared to consumer taste? A lot of experiments or trials have been made, and when the sugar level is reduced or sugar is deleted in some baby foods the consumers don't buy them because the competitors' products are sweeter or appear more tasty to the mother due to sugar or salt levels. Unfortunately here we are not much supported by pediatricians in advising products without sugar or salt in their daily practice. So when mothers buy the products for their babies they buy according to their own taste, and they don't have strong recommendations from pediatricians.

Dr. Aggett: I don't really know if I can actually say much except to sympathize. In terms of the claims and whether or not claims can be made, I think this is going to depend on whether or not there is a demonstrable base, whether we can actually say this is an evidence-based claim, and some of these points have been made. The other point about the lack of support or the unfair advantage of people producing what you call sweet products and you are not getting support from pediatricians: I think these are some of the key educational needs, whether they could be seen to be educational needs for the carers, I certainly think you can educate consumers through carers of children through some of these guidelines. I think in terms of educating professionals that is sometimes more difficult, and I think it is sad that there is probably not great engagement by many pediatricians in these sort of community issues.

Dr. Schmitz: Following the line just raised by Mrs. Gailing, there was a very nice paper in *Acta Paediatrica* a few months ago concerning the introduction of solids [1]. It was the result of an inquiry of about 500 parents and the questionnaire concerned breastfeeding, formula feeding, and the time of introduction of weaning food. It appeared that compliance to guidelines and particularly the timing of diverse foods (gluten, fish, eggs) was low (less than 50% of the parents following the recommendations). This raises questions regarding the recommendations that we can make because if the industry follows recommendations and the parents do not follow them, it is very difficult to modify the trends in alimentation.

Dr. Aggett: That study was similar to one done in north-east Scotland. It is amazing that they have strong local traditions, some of them analogous to those in Scandinavia, of adding cereal mixes to formulas at an early age, 3–4 weeks. In some cases we come across parents who add marshmallows to formulas. It is very sad that one does find these recommendations. Products are produced according to the regulations, but we then find that many of the health professionals, who are involved with the care, are themselves not that familiar with the regulations in the first place, let alone have any great sense of motivation to implement them.

Dr. Kleinman: Just a brief comment to follow up on Mrs. Gailing's observation. In the Unites States several companies that focus on complementary foods for infants and young children have in fact used recommendations about sugar and salt to gain a competitive advantage. They have pointed out that their competitor's foods contain added sugar, salt, or thickeners for example, and have used that to try to create a competitive advantage. The point that I am trying to make is that if we are raising health consciousness among parents, care providers and journalists (because they clearly have a huge influence over what parents do), then that creates a kind of counter-pressure against the advantage of adding a sweetener or a thickener. I think that the diagram Dr. Leathwood created is excellent because it shows the role we have to play there and how it can counter the pressures to yield to perhaps some inherent taste preferences or beliefs that parents have about foods.

Dr. Aggett: And does it work?

Dr. Kleinman: Yes, absolutely, there are many examples.

Dr. Exl-Preysch: I just wanted to add something to what was discussed before between Dr. Schmitz and Mrs. Gailing. We should really make a difference between products that are specifically made for small children up to the age of 3 years and fall under the EU regulation, and I think those producers are following all the recommendations quite fairly. What they have to be careful about is that the products are tasty, because if they do not taste good enough for the mothers they will not buy them. But I think what we really should be a little bit worried about is all those products that are not specifically designed for infants or small children. I don't want to give names but every body knows them, they are full of sugar, full of fat, and they are not processed specifically for these children. But if you look at their labeling, their appearance and advertising, you know exactly that they are used from the age of 1 year, and these are the products we are really concerned about, and I think that this is the most important problem we should deal with.

Dr. Aggett: I agree, and I think now that regulatory authorities, certainly food standardization and several organizations, are being set up in member countries of the European Union. There are now opportunities to discuss this and also to try to see what can be done to counteract it. But I don't know how it can be done systematically yet.

Dr. Lafeber: When you see all the legislation now in Europe, things have changed over the last 8–10 years. In America there is the Food and Drug Administration that has strict rules, and I have the feeling that the rules in Europe are now going much more in the direction of the American system, especially regarding health claims. For instance, companies are trying to imitate breastfeeding as much as possible with so-called functional components, i.e. lactoferrin, prebiotics, probiotics, all sorts of long-chain polyunsaturated fatty acids and oil mixtures. In order to get their health claim they have to supply a portfolio on a phase-1, a phase-2 study, etc., like a drug, and as a result they will try to get most of the products that they make patented, which of course makes it more difficult for other companies to imitate, so it is going more into a sort of drug organization. Do you have that feeling and do you like the way it is going?

Dr. Aggett: It may well be that there are vulnerable sectors that do need to be protected in some way like this. When we first started to suggest some of these ways of increasing knowledge and taking a systematic approach to the nutritional assessment of formulas, there certainly were times when some issues arose about safety assessment and the justification of claims for new products, this has been called the pharmaceuticalization of the food industry. I think that is almost one for the politicians and society to decide, and those of us who are concerned with good sound evidence-based nutrition would like to feel that there are good quality guidelines for the data to substantiate a claim. That is one of the exercises currently in progress in the EU, looking at the process for assessing scientific evidence to support a claim for a food stuff, with

the view that once a company has provided a portfolio to be accepted by an appropriate advisory review body, it will be able to make a claim. It will be expensive. So what will certainly be needed before one can evocate any way in there, the pharmacy or the type of approach you are talking about, is that there has got to be some protection of intellectual property. But as you know that doesn't even exist for infant formulas or anything of that nature yet. It is a problem. I think the larger food companies regard this responsibly and have ways of being able to cope with it, but it does mean that smaller innovative companies may well not have an opportunity to join the market.

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Recommendations for Physicians and Parents

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Introduction

In the post-war period in the Western world, consumption of food has changed from a daily activity to fill the stomach to a social event. Daily meals play an important part in family life. Diners are used to tie friendships, relations, to create a suitable climate for business deals and to celebrate. But at the same time concern about food as a factor that will influence health and disease has been growing rapidly. The media are informing the public about the dangers of overweight and its consequences for the development of diabetes and coronary heart disease. Many somatic complaints are considered to be related to food and food products. Food allergy is in the focus of interest. Public discussion has turned its attention to the possible hazards of food additives, insecticides and genetically manipulated food products.

Parents, on the one hand, like to gain pleasure from the mealtimes with their family and, on the other hand, feel a compelling need to get the right food in the right quantity during the right period into their children. It is this compelling need that sometimes puts pressure on the educational process with consequences for nutritional behavior and subsequently the nutritional intake of their children.

Guidelines for Healthy Feeding in the First Years of Life

It is generally accepted that breast milk is the ideal food for human babies. When it is not available a variety of formulae are on the market to cover the nutritional needs for the first 4–6 months of life [1].

Physicians are frequently confronted with questions by parents and caregivers concerning the transition to complementary foods in infancy. When

can complementary foods be started and what are the best foods to start with? But also questions concerning the way new food products should be introduced are recurrent topics in baby health clinics. At the end of 2002 the American Dietetic Association together with the Gerber Products Company developed an evidence-based approach to establish healthy feeding guidelines for infants and toddlers that were published in 2004 [1, 2]. Simple questions, such as when is an infant's gastrointestinal tract capable of handling complementary foods, when are renal functions sufficiently mature to allow introduction of complementary foods or when do all gross and fine motor skills required for complementary feeding emerge, were answered in a clear, evidence-based way.

A healthy infant's gastrointestinal tract is mature enough for the handling of complementary foods by 3 or 4 months of age [3–5]. Despite some renal immaturity, most children will have no problems in maintaining water balance even during the introduction of food products with a relatively high solute load [6]. Motor development, however, varies considerably among infants but skills necessary to be able to handle complementary foods will be present in the majority between 4 and 6 months of age [7–9].

Breast and/or fortified formulas will be sufficient to provide the nutritional requirements for at least the first 6 months of life [1]. Taking these different aspects into account the introduction of complementary foods should not take place before 4 months of age.

What will be the nutritional requirement? The dietary reference intake (DRI) provides balanced recommendations for nutrient intakes in healthy infants [1]. After 6 months the majority of breastfed infants need some complementary foods to meet the DRI for energy and different micronutrients like manganese, iron, fluoride, vitamin D, vitamin B_6, niacin, zinc, vitamin E, magnesium, phosphor, biotin, thiamin [10].

Special attention should be paid to the iron intake in the Western world, since the prevalence of iron deficiency is highest among children in the first 2 years of life [11, 12]. In those infants with a high risk of food allergy (family history with at least 1 first-line family member with atopy), breastfeeding is recommended for at least the first 6 months, whereas it is recommended that the introduction of potentially allergic food products such as eggs, milk, wheat, soy, peanuts, and fish be delayed until the end of the first year of life [13, 14].

Attention should be paid to the way parents establish a healthy and effective feeding relationship. The importance of an appropriate and nurturing feeding environment together with the provision of appropriate healthy food needs to be stressed. It is important for parents to recognize that 'the child decides whether and how much to eat' [1]. Responsive parenting appears to be the core of a healthy feeding relationship involving: (1) recognition of the child's developmental abilities; (2) balancing the child's needs for assistance with encouragement of self-feeding; (3) allowing the child to initiate and guide feeding directions, and (4) responding early and appropriately to hunger and satiety cues.

Table 1. Nutrient: energy (calories) and estimated energy requirement (EER)

DRIs	
0–3 months	(89 × weight of infant [kg] − 100) + 175 (kcal for energy deposition)
4–6 months	(89 × weight of infant [kg] − 100) + 56 (kcal for energy deposition)
7–12 months	(89 × weight of infant [kg] − 100) + 22 (kcal for energy deposition)
13–35 months	(89 × weight of infant [kg] − 100) + 20 (kcal for energy deposition)

Sources of nutrients for infants: human milk or iron-fortified infant formula, and complementary foods.

Sources of nutrients for toddlers: variety of foods from all the food groups, whole milk, other dairy products, fortified cereal, whole grains, fruits, vegetables, margarines/vegetable oils, meat, meat alternatives.

Adapted from Butte et al. [1].

It is important for parents to learn how their child communicates hunger and fullness. Parents should be supported in the way they introduce complementary foods to their children, the order of introduction and quantity [15]. Repeated exposure to foods will enhance acceptance and, although no strict evidence exists, it is generally recommended that first solid foods should be single ingredients that can be started one at the time and at intervals of 2–7 days [16]. Table 1 provides information about the DRI of energy during the first years of life.

Feeding Problems

A variety of feeding problems, including a lack of appetite, eating small amounts, picky eating and strong food preferences, occur in 25–45% of healthy toddlers [17].

Serious feeding problems are seen in 3–10% of all children with a higher frequency in infants with developmental disabilities (33%) [18]. Feeding problems are defined as deficit in any aspect of taking nutrition resulting in undernutrition, poor growth, stressful mealtimes for children as well as caregivers. The scale of feeding problems which have been described vary from inappropriate mealtime behavior, lack of self-feeding and extreme food selectivity to food refusal, oral sensory motor immaturity or dysfunction and swallowing problems with frequent gagging and/or vomiting [19].

For clinical practice, feeding problems in the first years of life can be classified as shown in table 2.

Table 2. Classification of feeding problems in young children

Feeding problems due to:
Somatic diseases
Poor appetite due to disease
Aversive consequences of eating (stomach ache, dysphagia)
Physical impediment for eating/drinking
Prolonged tube feeding
Pedagogical problems
Overestimation nutritional needs
Behavioral problems
Pathological food refusal
Eating aversion
Swallow fear
Swallow phobia

In feeding problems due to somatic diseases, poor appetite can be caused by chronic disorders that are associated with anorexia like inflammation and infection but also by neoplastic and metabolic disorders [20]. Especially as increased nutritional requirements are present at the same time, as in cystic fibrosis and celiac disease the combination may result in failure to thrive.

Aversive consequences of eating as in food allergy, esophagitis or certain metabolic disorders will result in a negative association with certain food products resulting in an aversive reaction and sometimes fear of eating. Physical impediments for eating and/or drinking as in oral motor/pharyngeal dysfunction, anatomic malformation or severe neurological or respiratory diseases will result in interference with the normal swallowing process. In the clinical setting prolonged tube feeding will result in feeding problems as the necessity to eat decreases and continuous enteral feeding will result in a decrease in stomach capacity [21].

A variety of pedagogical problems can form the basis of feeding difficulties. Overestimation of the nutritional needs of a small eater, growing and developing well, will result in forced feeding leading to feeding aversion in the end. Behavioral problems such as insufficient intake during the mealtime whereas although the total intake is still adequate can easily result in compulsive feeding with irritability, crying, fussing, tension and stress during mealtimes resulting in a kind of vicious circle.

The most difficult feeding problems to handle are those due to pathological food refusal. It consists of refusal of food or certain food products, with a struggle during meals which quite frequently leads to a decrease in intake and failure to thrive. Such problems can be resistant to pedagogical approaches. In the majority of cases the child will not exhibit appetite or hunger and no organic etiological causes can be found. Food refusal can be subdivided into different forms including [20, 21]: (1) eating aversion or the refusal of food; (2) swallow fear caused by a strange sensation when food enters the mouth

with a fear to swallow (for instance after prolonged tube feeding), and (3) swallow phobia which is fear of swallowing food (for instance after a traumatic experience resulting in aspiration and/or choking).

The majority of patients with pathological food refusal will present food refusal and mealtime distress in combination with failure to thrive.

One should keep in mind that behavioral problems causing feeding difficulties will sometimes have a physiological underpinning whereas physiologically based feeding problems can evolve behavioral-based feeding problems that will need a special approach [22, 23]. Many examples can be encountered in clinical practice. After multiple surgical interventions, infants fed by tube sometimes encounter so many negative experiences concerning bad taste, manipulations in the mouth and problems with swallowing that a serious aversion to oral feeding can easily result in the long term.

Diagnostic Approach to Feeding Problems

Extensive data collection including a caregiver interview, extensive history, physical examination, observation of caregiver and child interaction, dietary inventory and, for special indications, motor and swallow function testing will form the basis of the diagnostic approach to feeding problems [24]. An interview with the caregiver will provide basic information on the course of the feeding problem, the present status of the child and strategies used previously. But it will also reveal information about food acceptance and refusal, the amount consumed and the duration of a typical mealtime. It is important to pay attention to aspects like environment, behavior, and stress surrounding the normal feeding process. Physical examination should reveal information on growth and development and the nutritional status of the child. Extra attention should be paid to the neurological status and the presence of dysmorphic features pointing towards a genetic/syndromal background of the feeding problems. In addition to an extensive dietary inventory, observation of the interaction between caregiver and child can reveal important information. Are parents/caregivers eating with the child; how are they reacting on food refusal, and what kind of tools are used for distraction, encouragement and support. On indication further laboratory investigations (blood, urine, X-ray-examinations) can be performed [25].

Therapeutic Goals in Feeding Problems

During regular controls in baby health clinics, attention should be paid to the first signs of feeding difficulties with anticipatory guidance to prevent more serious feeding problems [26]. Early recognition should result in early interventions preventing deterioration, while established feeding problems

Table 3. Food rules for caregivers

Scheduling	Regular mealtimes (only planned snacks) Mealtime maximum 30 min Nothing between meals (except water)
Environment	Neutral atmosphere (no forced feeding) Sheet under chair (prevent mess) No game playing Food not as reward or present
Procedures	Small portions Solids first, fluids last Self-feeding Playing without eating (remove after 15 min) wiping mouth, cleaning up only after meal is completed

Adapted from Arvedson [27] and Chartoor et al. [24].

should be referred to dedicated centers able to provide specific multidisciplinary support, for instance by a feeding team consisting of a nutritionist, speech therapist, psychologist, pediatrician/pediatric gastroenterologist. A feeding team can provide support both in a clinical as well as in an ambulatory setting [25]. Their integrated approach is aimed at a steady weight gain with slow but stable initiation of oral feeding, by steadily weaning from tube feeding and increasing oral intake, while stress during mealtimes is reduced. In the final phase attention is paid to the acceptance of a variety of flavors and textures resulting in a steady normalization of oral intake [24]. If pedagogical problems predominate, extra attention should be directed to caregivers, providing them with support and practical food rules for daily mealtime practice. In more difficult cases a video intervention program can be used in which their interaction with the child can be visualized, discussed and modified. An example of simple practical food rules is provided in table 3.

In the clinical setting, in children with somatic diseases that force the use of enteral feeding, attention should be paid to the prevention of feeding problems on the long term, by continuous oral support and stimulation of oral motor development during the period of enteral nutrition.

Concluding Remarks

Well balanced nutrition is of great importance for health, especially in growing and developing children. In infancy and childhood it involves the interaction between caregivers and their children in which different environmental aspects play a part. A pleasant and safe feeding environment will help the child to develop eating skills and a positive attitude towards eating that will form the basis of an accepted social behavior. Feeding should

never be directed towards just 'getting food into the child'. A successful learning process concerning feeding will result in an acceptance of a variety of foods with different tastes and developmentally appropriate texture. It will also help the child to develop necessary motor skills, safe sucking, chewing, propelling and swallowing. So successful feeding progress in infancy can become a joy forever.

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Discussion

Dr. Aggett: We have all touched on getting information across and somehow generally improving people's understanding of what nutrition is about. You have many thoughts about this. Would you like to share them with us please, so we can perhaps get a feeling for how we can start to try to get over some of these general aspects on understanding feeding behaviors, normal behavior, and what normality actually is, and what people's expectations should be rather than what they are lead to expect.

Dr. Heymans: I think that is a major question. If you watch TV, and every one of us would say that he doesn't do that on a regular base, but you see that health care and hospitals are one of the main targets of interest. People are watching it and most people are only interested in emergencies, the ER. We are not using our time and influence to show what is really important. The problem is that we don't exactly know, and still have differing opinions. In this country we have baby health clinics, we see more than 90% of all the children that are born. I think we should use these baby health clinics to provide parents with the information that will help them make the right decisions in the right time, for instance to prevent feeding and/or behavioral problems. In the Netherlands pediatricians are not involved enough in preventive health care. We don't have child health as part of our interest; we are working inside hospitals, and I think that is something which hasn't been beneficial in the last years. I would like to see a change that might influence preventive health care in such a way that we really are able to get our ideas and views across, and we don't do that enough. How are things in England?

Dr. Aggett: Possibly even worse; I don't know. I think like you that pediatricians, as a profession, are not that well engaged in this. We have this predilection to be involved with the acute services, and the appreciation of the value of preventive medicine and public health, particularly public health nutrition, is under-invested and is not necessarily seen as being a rewarding area. It may be changing, as public health initiatives are recurring. Public health in nutrition, particularly in the UK as well as everywhere else, may give people responsibility but it doesn't always need pediatricians; of course it does need other informed health professionals with access to the families to be able to implement things.

Dr. Gracey: I would like to pursue this just a little more. Dr. Aggett I think you are absolutely right when you say that most ministries of health or governments are preoccupied with disease care rather than health care, certainly the government in

Australia is. We as pediatricians have a responsibility to try to change this imbalance. I would like to ask Dr. Heymans to follow up the British approach to banning or restricting advertising of junk food, which seems to me in many ways to be a very negative approach to improving health. For too long we have taken approaches like, don't do this, it is bad for you, don't eat that, don't do that and so forth. I think we really need to have much more positive approaches to improving health, and I wonder how we can do that in association with industry, with marketing, with advertisers, with governments, with politicians and with the community. I would really appreciate your comments on those things.

Dr. Heymans: It is a difficult question; let me give you an example. In this country the government tried to forbid soft drink machines in high schools because they had decided that lots of calories go into children because of those soft drinks. But we are a permissive society, so who is going to decide that you are not free to drink whatever you want whenever you want, and that is an ongoing discussion. What we haven't done yet is give the information to the people so that they can make the right decision themselves, and that should precede new regulations, we should do that first. In schools good information should be given about nutrition and also about lifestyle. Things that should be done beyond not eating too much and too fatty foods, and too many high calorie snacks, things like moving, cycling, walking stairs instead of taking elevators and escalators. We are in a society in which everything is changing, so do not only concentrate on food alone, but also on lifestyle as a whole. People are not aware of the fact that we are on the wave of a huge epidemic, but perhaps we have to be in the middle of the epidemic before we are willing to do something. Is it different in Scandinavia?

Dr. Hernell: We have exactly the same problem, but I also think that if we are going to do something about it we need to begin earlier than in school age. We need actually to work through the parents and through the well-baby clinics or whatever system we have, I mean if you are successful in getting rid of the vending machines with soft drinks from the schools it does not help much if the children go home to find their parents drinking these soft drinks every now and then.

Dr. Kleinman: I think there are some hopeful things happening. For example in the US the National Institutes of Health has focused a certain amount of money specifically towards this question, and has asked for proposals regarding novel approaches that primary care physicians and health care providers can bring towards the prevention of obesity. Ultimately this will provide the evidence needed to guide national recommendations. Second, in the US, insurers are now increasingly aware that there is value in paying for preventive medicine in childhood, and they have built financial incentives for pediatricians to do things like monitor the body mass index. Another advance is the use of the electronic medical record, which is becoming more widespread in the US, and that kind of record allows automatic reminders as well as automatic letters to be generated to parents saying that it is time for your child's immunization, please show up in 2 weeks; or as your child has not been weighed and measured in the last 6 months it is time to come in for that. So I think technology is going to help move forward this whole issue of the amount of time spent on preventive measures. Then the very last comment has to do with parents' responsibility, and I agree entirely with all these comments about starting early and giving parents the support that they need to be able to deal with these problems early. But it is very difficult for a parent who is dealing with an older child or an adolescent to deal with all the pressures that they have in their environment that work towards making their child fat. In fact to some degree it is almost impossible. We talked about advertising before and it is quite obvious that USD 6–7 billion are spent in the US towards promoting the consumption of food every year, and the amount of money spent on health messages,

in educational programs to support parents, is less than one tenth of that. It is not a fair fight and the only way it is going to be a fair fight is for the food industry to step up and take some responsibility for this, and many now are doing it, from McDonalds to other corporations, but if they don't do it there is no choice but to ask government to intervene because it is not a level playing field. With regard to vending machines in schools, many school districts in the United States have banned vending machines in schools or they have required that the vending machines be filled with fruits and milk and other healthier foods, or they leave the vending machines there but they don't allow them to be turned on 2 h before the lunch time, up until 2 h after lunch time. They do that very successfully, and it gives the child an opportunity to make healthier choices.

Dr. Mexitalia: Although in Indonesia obesity is increasing, malnutrition still remains. When parents come with a malnourished child, we give them advice on how to give their child better food, more milk, but most of the time they cannot afford to buy it. So the problem is not only regulation.

Dr. Heymans: It is also an astonishing fact that the number of malnourished children in the world is equalizing the number of over-fed children, so we have a very strange problem in trying to divide things in an equal way, and I can imagine what you say.

Dr. Verloove: I am very happy to hear about the USA; things are going in the right direction of prevention in an early age. I think in the Netherlands at least we always follow USA.

Dr. Heymans: 25 years later.

Dr. Verloove: No, it is shorter nowadays, it is only 5 years I think. But I quite agree with Dr. Heymans that we should work together with preventive child health care to get the message across, but not forget about the low compliance. We shouldn't idealize the though that whatever we give as information or advice will be followed because half of the people don't. Having said that, it is worthwhile to try and get the other half at least to be knowledgeable about nutrition. My impression is that a lot of the information that professionals, pediatricians, child health people or industry give is in percentage of fat or 30% of intake should be fats or whatever, or in carbohydrates or in proteins, and people just don't understand. They want to have information about cups of milk or spoons of vegetables and what vegetables and how. So maybe our information just doesn't get there because a lot of calculations must be done afterwards. I don't know what kind of information we could give people or lists of things so that it can be done positively, and not always state you should or should not eat that but this is what a child of that age should eat. Is that an approach that is taken in some countries? What do you know about Holland that I am not aware of?

Dr. Heymans: We have nutritional advice based on a survey that was performed some years ago, but for all schoolchildren.

Dr. Verloove: But it is 30 years old.

Dr. Heymans: In the coming months it will be renewed, but our society is rapidly changing. Just to give you an idea, in the bigger inner cities in this country about 64–65% of children aged from 0 to 14 years have non-Dutch parents and there is a variety of ethnical backgrounds with a variety of different food and food products. In hospitals this really needs to be taken into account because most of the children are not able to eat normal hospital food. I think it is difficult to reach the young population in this country.

Dr. Aggett: I think what you raised is very important. In the UK we try to convert this advice into examples of diet, what something really should or could be. This was done for the first time about 10 years ago with cardiovascular disease for example. When the advice went out about reducing the amount of saturated fatty acids down to 10% or so of energy fat, 30% of energy or something like that, examples of how many potatoes should

be in the diet, just really to try and translate into objective advice, it was immediately hit by a very strong campaign in the media about a nanny state and over-mothering state treating people in a patronizing way as if they can't understand. It is not difficult to find that this was emanating from various sectors of the agro food industry, and it can be tracked back and is certainly one of the reasons why measures were taken both in Europe and the UK to try to enforce this advice from other invested interests that might be around. Yesterday I talked about the nanny state and this type of attitude that we must overcome, we must make people aware of the public health perspectives and their importance.

Dr. Lemay: I would like to ask two questions. First of all, I am hoping that eventually we will have positive international campaigns on the benefit of good nutrition in our diet, especially for children under the age of 5. In your study on children between 9 and 12 years of age you focused on how many children were following a diet. Do you have any data showing how many children were to some extent following an inappropriate diet or eating appropriately? My second question is that, especially in North America, when educating future pediatricians, students in residence, the content of the nutritional domain is still quite light, compared to a lot of other subjects that are being taught. What would be the best way to teach our physicians, and are we teaching enough about nutrition?

Dr. Heymans: Rather good questions. In our study we didn't ask about the content of the normal diet, but I showed you that there is some information about what children are eating in this country. I have seen the recent report from 2002 on toddlers and it is quite reassuring, there is no over-consumption but that is until 3 years of age, so it is very difficult to draw conclusions in the long-term [1]. There has been a study on what people in the Netherlands are eating but I am not aware of information about the age group. In our study we asked all the schoolchildren whether they were on a normal diet, we didn't ask them what they were eating. So that is something we should get information about, although this is a rural part of the country and I don't know if it will be feasible to use the information from there for the whole of the Netherlands. So I can't tell you if they had a decent diet. Do we invest enough in nutritional education? No. In the normal curriculum of the universities in this country too little attention is paid to nutrition. In the curriculum of pediatricians in this country, there is also only very little attention paid to nutrition. Together with Dr. Taminiau, Dr. Lafeber and I started a 2.5-day nutritional course for pediatricians in a very little village in this country where they couldn't run away. We tried to provide them with information that they as pediatricians need to have on nutrition. Presently the epidemic of obesity that we are seeing is a major problem. The results of the epidemic will become clear in the coming 12, 20, 30 years, but we have to address it now. So I agree with you, it is something we have to change in the program of our universities, and look how much nutritional education people get there, practically nothing.

Dr. Lafeber: So it will be useful if, in a future workshop, we could share different methods that are used to teach nutrition. That would be a great start because eventually we will need to address this issue.

Dr. Heymans: Nutritional education.

Dr. Hardiono Djoened: You said that behavioral problems could cause feeding problems in children. I want to ask the reverse, is there any evidence that some food can cause behavioral problems? If there is no evidence then how should we convince the parents about this because in one of your slides you showed that a large percentage of parents still believe that food can cause behavioral problems.

Dr. Heymans: That is a very interesting question and it will be hard to answer. There is no proof that certain food products, for instance the Feingold diet [2] or having all kind of substances in the food, will cause behavioral problems. There have

been many very well-controlled studies that could not show that this was the case. Still there are individuals who feel that their child improves enormously if they change the diet. For instance in our study in the northern part of the Netherlands, more than 50% of the diets used were not prescribed by doctors but by all kinds of advisors.

Dr. Hardiono Djoened: But how can we convince the parents about this? You said that about 70% of parents still put their children on a diet because of this belief in Holland.

Dr. Heymans: What we try to do is to show them in a kind and clear way the results of studies that implicate that there is no proof of any beneficial effect. We have an information center in our hospital which provides the parents with information from the literature, shows them how to find their way, how to select information that can be beneficial to their child. But sometimes dietary interventions are based on belief, and belief is very difficult to discuss.

Dr. Exl-Preysch: I would like to come back to what was discussed before and Dr. Lemay took up what I wanted to say. I think we are already discussing a little bit the end of the line here; we really have to start at the beginning and those children that see the pediatrician because of feeding problems are already at the end of the line. So what we want is to establish healthy feeding habits and healthy children with no problems in the beginning, and it is exactly as you pointed out, nutrition is not only not in the curriculum, it is absolutely not sexy for them, nobody is interested. Everybody is interested in molecular biology and whatever is in fashion nowadays, but nutrition is boring, and I think that is the major problem we are facing. Most of the pediatricians are not really interested, only those who are here, but those who are not here or at those congresses are not at all interested. I don't know if it is different in different countries, but in a lot of countries children are not even seen by a pediatrician, they are seen by a general practitioner who knows even less about health and nutrition. He knows exactly the same that the parents know, he has no idea, and I think that is the point where we really have to start to think what to do. Again in different countries there are different systems, for instance in Switzerland we have a system of so-called mother consultancy, so the parents visit the mother consultant with their children and it is this consultant who gives the nutritional advice and discusses with the parents. I think we really have to start an overall system to sensitize these people, and also the government and the politicians. They spend huge amounts on alcohol, drugs, HIV and smoking, and they spend literally nothing on nutrition and nutrition prevention, and that is the point where we really have to start and build up a huge and strong community to convince these people that it is a real problem. Finally, as has already been said, the industry has to do something together with non-government and government organizations. To give you a good example: in Switzerland we set up a joint venture of the Swiss Society of Nutrition together with the financial support of the government and set up an association called Nutrikid to create material for the nutritional education of normal children and normal parents. It started in 2001 and the sets are really running very well. We have already sold 4,000 sets. I think this is the way we should go to really educate both children and their parents. I think it is the best way to set up a balanced nutrition and then the children can make their own choices. They are not so stupid, they like learning.

Dr. Steenhout: In my point of view, nutritional recommendations to mothers should already start before birth and be done by the obstetrician in collaboration with the future pediatrician. This is the only way to continue to obtain better results in terms of breastfeeding policy implementation.

Concerning your suggestion to hold a workshop on the problems of nutritional education, this was already been organized by Nestlé in 1988 (vol 20: Changing Needs in Pediatric Education). Nestlé has always been committed to providing the material and the support for programs concerning nutritional education.

On the other hand, and unfortunately, with their current budget constraints, most European countries are not increasing the expenses for education, and less and less nutritional programs are present in the medical curriculum!

Now concerning the role of industry and the collaboration between industry, health and regulatory authorities, or medical and scientific societies, I don't see a problem. If a clear consensus, a clear message issued by the scientific and medical community exists, and moreover, if this is converted in recommendations or rules by the health authorities (FDA, EFSA, etc.), the food industry will obviously move in this direction and apply the new regulation. Political authorities must also be involved to establish legislation and to implement the right competitive rules.

So we have to work together and this is certainly one of the key messages I would like to leave at the end of this workshop.

Dr. Heymans: First I agree with everything, I don't want to comment on anything you said, but for those who are interested in research on how to change behavior, particularly with regard to nutrition and physical activity, there will be an international meeting here in Amsterdam, it is to be lead by Dr. Buch from the Free University and is under the auspices of the International Society of Behavioral Nutrition and Physical Activity. This will be the 4th meeting of the society, and it is quite an interesting group. It brings together investigators in the area of behavioral change, specifically around nutrition and physical activity, and if you are interested in it, the web site is www.isbnpa.org.

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