Obesity – Extending the Hygiene Hypothesis

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Abstract

The hygiene hypothesis proposes that the growing epidemic of atopic eczema, allergic rhinoconjunctivitis and asthma is related to reduced exposure to microbes at an early age as a result of environmental changes in the industrialized world. These include improved sanitation and living conditions, vaccinations and antimicrobial therapy, together with declining family size and changes in dietary intake. Recent scientific advances demonstrate that the hygiene hypothesis needs to be extended in three respects. Firstly, rigorous research in the field of probiotics points to the importance of the collective composition and the compositional development of the gut microbiota in consolidation of healthy immune responsiveness. Secondly, immunomodulatory and suppressive immune responses have been shown to complement the original immunological basis of the hygiene hypothesis, the so-called T helper 1/T helper 2 paradigm. Thirdly, host–microbe interaction appears to affect the risk of developing not only atopic disease but also other inflammatory Western lifestyle diseases, including obesity. The results of experimental studies suggest that deviations in gut microbiota composition predispose to excessive energy storage and obesity, and, more recently in humans, aberrant compositional development of the gut microbiota has been shown to precede overweight, inviting enormous possibilities to reach preventive and therapeutic applications in weight management.

The Hygiene Hypothesis: The First Extensions

In 1976, Gerrard and colleagues found an inverse relationship between the incidence of infections and atopic disease and concluded that the relative freedom from diseases due to viruses, bacteria and helminths caused the latter [for review see, 1]. Again in 1989, Strachan detected an inverse correlation
between family size and the prevalence of allergic rhinitis and suggested that infections acquired from older siblings might confer protection against the development of atopic disease. Subsequent research concerning the association of childhood infections with atopic sensitization or atopic disease have offered conflicting results. Indeed, our understanding of the critical window when, the mechanism how, and the infections which might carry protective potential is by no means satisfactory.

Concomitantly with the mainstream explanatory theories linking the prevalence of allergic diseases to improved sanitation and living conditions, declining family size, vaccinations and antimicrobial therapy, a matching progression is seen in dietary intake. A shift in food preservation from drying and natural fermentation to industrial processes including heat treatment and even radiation treatment has taken place during the last century.

T helper (h) 1-type (Th1) immune response is typically elicited against intracellular microbial pathogens. According to the cross-regulatory properties of the Th1/Th2 cells, the microbial stimulation would be needed to hinder the consolidation of the atopic Th2-skewed responder type. This is supported by the demonstration that, due to the immunological balance prevailing in utero, the Th2 phenotype is universal at an early age. Consequently, a significant overlap in the concentrations of interleukin (IL)-4, the key Th2 cytokine, and immunoglobulin (Ig) E antibodies prevails between atopics and non-atopics at an early age [2]. Healthy infants exhibit a decline in Th2 responses during the early postnatal period, whereas a converse pattern is characteristic of infants developing atopic disease. An important criterion for infectious disease to prevent allergic disease is that the infection also be prevalent in developed countries, as the majority of infants, even those genetically predisposed, are still spared from allergic diseases. Moreover, exposure to infection should occur very early, as the first expression of the atopic immune responder type frequently occurs within the first months of life, thus disputing the simple Th1/Th2 paradigm of the hygiene hypothesis.

In the same vein, according to the cross-regulatory properties of Th1 and Th2 cells, one would hypothesize that together with the rising prevalence of atopic diseases there would be a decline in the prevalence of autoimmune diseases, many of which are considered to arise from excessive Th1-type immunity. Also this simplistic assumption has been challenged [3]. Indeed, atopic diseases together with chronic inflammatory bowel disease, including Crohn’s disease and ulcerative colitis, and type-1 diabetes represent chronic diseases of rising importance in industrialized countries worldwide. Finally, not only has the risk of developing these chronic immunological diseases increased, but also the burden of infectious disease continues to present a clinical problem worldwide [1].

On this basis, an extended hypothesis has been put forward [1] to provide an explanation for the coexistence of infectious, allergic and chronic inflammatory diseases in Western societies: overexpression of Th1 or Th2 respon-
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siveness could thus be ascribed to a defective immune regulation rather than a direct cause of such development. These conditions may reflect an inability of individuals residing in affluent hygienic conditions to provide their immune responses to antigens and allergens with adequate anti-inflammatory stimulus. Indeed, recent experimental and clinical studies put forward the conclusion that Th1- or Th2-skewed immune type may be balanced by regulatory T cells such as Tr1, Th3 or CD4+CD25+FOXP3+ cells which are induced by, e.g., indigenous intestinal microbes. Tolerogenic regulatory T-cell responses may inhibit both Th1- and Th2-type inflammatory reactions and hence complement the Th1/Th2 paradigm.

Regulatory T-cell responses are mediated to a large extent by anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-\(\beta\) [4], which modulate both innate and adaptive immune responses and also alter dendritic cell function to favor further induction of regulatory T cells thus creating an anti-inflammatory immune milieu [4]. The importance of regulatory T-cell responses is also reflected clinically: defective production of both IL-10 and TGF-\(\beta\) by T cells has been reported in children with food allergy [5, 6], and establishment of tolerance in children suffering from cow’s milk allergy coincides with an increase in milk-specific regulatory T cells [7].

**The Microbiota Hypothesis**

The earliest and most massive source of microbial exposure is derived from the acquisition of the gut microbiota. The microbiota of a newborn develops rapidly and is initially strongly dependent on the mother’s microbiota, mode of delivery, and birth environment. A recent study, evaluating a broad range of external influences to the gut microbiota composition in early infancy [8], confirms these observations. Infants born by cesarean section show lower numbers of bifidobacteria and *Bacteroides*, but more frequent colonization by *Clostridium difficile*, compared with vaginally delivered infants. Hospitalization and prematurity were associated with elevated *C. difficile* counts, and formula feeding with colonization by *Escherichia coli*, *C. difficile*, *Bacteroides* and lactobacilli. The differences in culturable microbiota between vaginally born infants and infants born by cesarean section have been shown to still be observed at 6 months of age, and appeared to be associated with the maturation of humoral immune mechanisms [9]; infants harboring *Bacteroides fragilis* and *Bifidobacterium* species had more circulating IgA- and IgM-secreting cells.

Recent studies challenge the general view of a sterile intrauterine existence so that colonization of the gut begins after delivery; the exposure to microbes may take place via several routes before and after birth [10–12]. It is well documented that specific pathogens can be present in the placenta, cord blood and amniotic fluid. Commensal intestinal bacteria, such as
Bifidobacterium and Lactobacillus and their DNA, have been detected in the human placenta [10], regardless of the mode of delivery. Presently their origins remain unknown calling for more experimental and clinical research to fully determine the precise colonization pattern in healthy neonates, in view of the importance of the first inoculum on later microbiota development.

Major postnatal changes in the composition of the gut microbiota occur during breastfeeding, weaning, and introduction of solid foods [8]. Infants who are breastfed have a natural predominance of bifidobacteria, while the formula-fed have a more complex profile similar to the adult microbiota with enterobacteria, lactobacilli, bacteroides, clostridia, bifidobacteria and streptococci. However, recent advances in formula composition appear to diminish these differences; the modifications include lower protein concentration, use of pre-hydrolyzed proteins, together with inclusion of polyunsaturated fatty acids, probiotics and prebiotics.

Recent studies also point to breastfeeding as a source of Bifidobacterium biota [11, 12]. With distinct strains present, breast milk may act as a natural synbiotic containing both health-promoting bacteria and their optimal substrates. Further, studies of regulatory mechanisms operating in human milk [13] show that human milk enhances host–microbe interaction on the intestinal mucosa by specific Toll-like receptors (TLRs), which may be imperative in the maturation of the gut immune system.

Indeed, in infancy, the main components of the gut barrier are immature and hence the intestinal surface is relatively permeable, which may partly explain the proneness of infants to inflammatory responses. The intestinal microbiota, again, has a profound impact on the development of oral tolerance and IgA antibody responses, which constitute an instrumental part of the immunological barrier functions. Disturbed microbiota succession has been linked to the risk of developing infectious, inflammatory and allergic diseases later in life [14], and also overweight and obesity [15]. Indeed, experimental and clinical follow-up studies suggest that constant stimulation by the indigenous intestinal microbiota, its complex composition and function, outweighs the importance of occasional infections in providing the essential immunological provocation for the maturation of the gut barrier functions [14].

The relationship between intestinal bacteria and the host is referred to as host–microbe crosstalk, implying peaceful coexistence and mutual benefit. To sense the myriad of microbial motifs found in the gastrointestinal microbiota, the gastrointestinal epithelium and different cell types in the gut-associated lymphatic tissue are equipped with TLRs which recognize specific conserved molecular patterns found in both pathogens and commensals [16]. Each TLR has a specific ligand, for example, TLR2 recognizes the bacterial lipopeptides and lipoteichoic acid of gram-positive bacteria, whereas TLR4 is the major receptor for the lipopolysaccharide (LPS) found in gram-negative bacteria. Ligand binding to TLRs initiates signaling cascades that ultimately result in activation of gene expression. Intestinal epithelium, however, contains sev-
eral negative regulators of the signaling cascades thus maintaining intestinal homeostasis by preventing uncontrolled inflammation detrimental to the host [16]. Of note, a recent study with transgenic mice found that a constant activation of TLR4 in the intestinal epithelium does not lead to unbalanced inflammation but elevated production of secretory IgA, i.e. increase in the gut barrier function [17].

Alterations in all the important denominators of the initial compositional development of the gut microbiota, the mother's microbiota as the first inoculum, the mode of delivery and early feeding practices may envisage a decline in the host defense mechanisms in the gut which is primed to assimilate potentially harmful challenges during the critical maturational period of life. The microbiota hypothesis may be reconciled with the absence of inflammation causing deviance of normal immunity and tolerance mechanisms.

**Gut Microbiota and Obesity**

Western societies are faced with an epidemic of metabolic disorders including obesity and diabetes. This also holds true for the pediatric population, about 20% of children and adolescents being overweight and a third of these obese [18]. The excessive maternal weight gain during pregnancy not only exposes the mother to a heightened risk of obesity in the long-term, but also the fetus to macrosomia, thereby creating the vicious circle of metabolic disorders: obese children often become obese adults and maternal obesity over-nourishes the fetus, thereby programming adult size and health with a heightened risk of obesity later in life [19].

Obesity can be defined as a disease state in which excess body fat has accumulated to the extent that health may be impaired. The underlying denominator is positive energy balance and weight gain. Obesity is also characterized by low-grade systemic inflammation resulting from the production of a variety of proinflammatory and anti-inflammatory factors such as adipokines, cytokines and chemokines [20].

Based on experimental studies, an innovative hypothesis has recently been proposed whereby the composition of the gut microbiota could influence energy homeostasis [21–24]. Intestinal microbes enable hydrolysis of indigestible polysaccharides to easily absorbable monosaccharides; conventionalized germ-free mice showed twice the uptake of a test dose of glucose into distal intestinal cells compared with germ-free mice. Fermentation of carbohydrates to short-chain fatty acids in the distal gut is increased as well. A larger amount of monosaccharides becomes transported to the liver more efficiently, where lipogenesis is stimulated and fat deposition is augmented. Activation of lipoprotein lipase by direct action on the villous epithelium increases fatty acid uptake and triglyceride concentration by de novo synthesis. Consequently, glucose is rapidly absorbed and fatty acids excessively stored, both processes
which boost weight gain. Furthermore, AMP-activated protein kinase is activated in metabolic stress, as well as by leptin and adiponectin, leading to suppression of anabolic pathways and induction of catabolic pathways.

A high-fat diet has been shown to increase LPS-containing microbes in the gut which leads to hyperinsulinemia and whole-body, hepatic and adipose tissue weight gain, characterized as metabolic endotoxemia [23]. Furthermore, induction of a Bifidobacterium-dominant microbiota by prebiotic supplementation prevented metabolic endotoxemia in mice fed a high-fat diet, suggesting a preventive role for bifidobacteria in weight gain and associated conditions [24].

A recent NAMI (Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota) research group study tends to substantiate the notion that the composition of the gut microbiota and body weight are linked [25]. Mother's weight and body mass index prior to pregnancy correlated with higher numbers of Bacteroides, Clostridium and Staphylococcus in fecal samples. In general, the microbial numbers increased from the first to third trimester of pregnancy. Specifically, weight gain during pregnancy was shown to be associated with the mother's microbiota; high Bacteroides numbers being associated with excessive weight gain over pregnancy, while women with normal weight gain tended to harbor higher numbers of bifidobacteria in their gut microbiota.

The hypothesis is further supported by our recent finding demonstrating decreased numbers of fecal bifidobacteria, determined by fluorescent in situ hybridization with flow cytometry, in infants who later became overweight or obese [15]. These 25 overweight children were selected from a prospective follow-up study with a population size of 159 at the age of 7 years and identified according to the International Obesity Task Force criteria. Normal-weight children were selected from the same cohort and matched for gestational age and body mass index at birth, mode of delivery, probiotic supplementation, duration of breastfeeding, use of antibiotics during infancy, and frequencies of atopic diseases and atopic sensitization. The finding was confirmed by two other molecular methods of microbiota analysis. Moreover, the microbiota aberrancy during infancy was also associated with a greater number of Staphylococcus aureus in children becoming overweight than in children remaining normal weight.

Cytokines and TLRs participate in coordinated adaptive function and maintenance of systemic low-grade inflammation necessary for formation of tolerance. However, when sustained or unbalanced release of inflammatory mediators occurs, undesirable pathological consequences become evident. Indeed, the underlying denominator of conditions such as obesity and allergic disease appears to be decreased immunological tolerance as a consequence of immunological changes induced by adipokines and cytokines [26]. The gut microbiota and related inflammatory processes may provide a unifying hypothesis for the raising prevalence of allergic diseases as well as obesity and its comorbidities. The generation of inflammation is a corollary of over-
weight. Leptin and proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, diminish the activity of regulatory cells. Leptin induces oxidative stress and an increase in adhesion molecules. The generation of TNF-α, again, is directly associated with insulin resistance. A link to the dietary fat arises from similar signaling pathways [27]. Although the major function described for soluble (s)CD14, a pattern recognition receptor, is binding of lipopolysaccharides and thereby in execution of host–microbe communication, its binding specificity is not restricted to bacteria. It also binds phospholipids, thus providing a lipid transfer system. This innovative function of sCD14 may prove significant in execution of the immunomodulatory action. Further, such immuno-irregulatory processes may contribute to the metabolic conditions affecting glucose metabolism [28].

**Gut Microbiota and Glucose Metabolism**

A higher-than-optimal glucose level is now acknowledged to be more common than anticipated and may involve long-term effects on metabolism, particularly cardiovascular health [29]. For children, long-term health benefits may be conferred by a balanced maternal glucose metabolism during pregnancy. We have provided the first clinical evidence in humans demonstrating that combined dietary counseling, targeting the low-fiber and high-fat intake comprising the major problem in dietary intake in Western societies, and probiotics intervention, *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis*, yielded consistently improved plasma glucose concentrations and insulin sensitivity in healthy women during pregnancy and 12 months after [30]. Previous evidence regarding the effects of probiotics on glucose metabolism has been limited to experimental studies, specifically in mice with existing alterations in glucose metabolism. The effect afforded by these clinical and experimental studies may be rationalized by an active dialog between host and microbiota in glucose metabolism.

The mechanisms whereby the microbes may have an impact on glucose metabolism incorporates processing of dietary polysaccharides, indigestible by human enzymes, and thereby adding to the pool of gastrointestinal absorbable glucose [22]. The gut microbiota may also enhance glucose storage in adipose tissue by suppressing the fasting-induced adipocyte factor gene transcription with ensuing enhanced lipoprotein lipase activity. Probiotic consumption, by generating a less saccharolytic microbiota, may diminish both fermentation of polysaccharides and induction of fasting-induced adipocyte gene transcription [30]. Uniformly, regulation of inflammatory pathways by probiotics may be of importance due to the fundamental involvement of inflammation in the evolution of insulin resistance.

The concomitance of elevated blood glucose concentrations, insulin resistance and dyslipidemia with activation of inflammation pathways is related
to an enhanced risk of a range of metabolic disorders, including obesity and cardiovascular disease. Probiotic intervention with modification of gut microbiota and controlling the systemic and local gut inflammatory responses complement the more traditional strategies, modulation of dietary energy, fat and fiber intakes as well as increase in physical activity, in the prevention of Western lifestyle diseases.

**Extending the Hygiene Hypothesis of Allergy to Western Lifestyle Disease**

Initial contact with environmental antigens may be decisive in determining the type of immune responsiveness, i.e. inflammation of either Th1 or Th2 type or tolerance, elicited by the mucosal immune system and reflected systematically. Failure to establish anti-inflammatory and tolerogenic responses to environmental or self antigens may thus increase the risk of developing a chronic inflammatory disease. As discussed above, indigenous intestinal microbes appear to be causally related to the generation of obesity. Recently surfaced data indicate that, in addition to digesting dietary carbohydrates and thus increasing the amount of absorbable glucose, intestinal microbes directly modulate host energy metabolism. The pathophysiological process involved in obesity, insulin resistance and dyslipidemia includes a low-grade inflammatory state maintained by cytokines and other proinflammatory mediators such as TNF-α and leptin. Given the association between gut microbiota and generation of overweight, we hypothesize that the changing pattern of microbial encounter, including intestinal microbiota composition, related to a Western lifestyle might also underlie the obesity epidemic in industrialized societies.

The ‘hygiene hypothesis’ was originally introduced to explain the rising prevalence of allergic disorders by lack of microbial, mostly pathogenic, immune stimulation leading to a deviant immune responder phenotype and allergic disease. Subsequently, considerable revisions have been made to the hypothesis [14]. We currently hold that, given the early origins of immunoinflammatory conditions and the newly appreciated importance of indigenous intestinal microbes to healthy immune maturation, gut microbiota may be the most important source of maturational signals to the developing mucosal and systemic immune systems. Indeed, altered gut microbiota composition has been associated with development of allergic and autoimmune disorders [14] and recently, as alluded to above, with obesity [15].

From an immunological point of view, an impressive body of evidence published over the past decade has expanded our understanding of the mechanisms primed to control and suppress potentially detrimental inflammatory responses directed against environmental and self antigens, including those derived from indigenous gut microbes. Importantly, there are convincing data to suggest that gut microbes are instrumental in generating and maintaining
tolerance through induction of regulatory T-cell function [1]. We suggest that the same regulatory immune mechanisms which suppress allergic or autoimmune inflammation may also be involved in controlling the low-grade inflammatory tone implicated in the pathogenesis of obesity. Taken together, a yet expanded view of the connections between the Western lifestyle, early microbial contact and origins of human disease may be compiled (fig. 1): Lifestyle-related changes in hygienic, dietary and medical practices have altered the pattern of microbial exposure and particularly the composition of the gut microbiota. The modern infant may therefore lack sufficient stimulation of the mucosal immune system to generate a tolerogenic immune milieu and be prone to develop chronic inflammatory conditions, which may take the form of allergic or autoimmune disease, or a low-grade inflammatory state affecting energy metabolism which predisposes the child to obesity, insulin resistance and dyslipidemia. If this hypothesis proves plausible, it opens new avenues in combating the obesity epidemic with interventions aiming to modulate early gut microbiota composition and generation of tolerogenic immune responses.

**References**


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**Fig. 1.** The extended hygiene hypothesis. Firstly, the importance of infections in causing immune deviance may be outweighed by stimulation from the gut microbiota. Secondly, immunomodulatory and suppressive immune responses complement the Th1/Th2 paradigm. Thirdly, host–microbe interaction appears to provide endogenous protection not only against allergic disease but also against other Western lifestyle diseases, including obesity. Modified from Rautava et al. [1].


Discussion

Dr. Gibson: As I understand, what you are asking us to believe is that the gut microbiota may be causative in obesity. This is based on the experiments that you carried out in pregnancy where there were 3 groups: a control group, a low-fat group, and a low-fat group plus probiotics, is that correct?

Dr. Isolauri: We were counseling healthy diet and supported this approach by giving, for instance, food products to promote normal intake according to the current recommendations. Then we also had an intervention group which was subdivided in a double-blind manner into two, supplemented with either probiotics or placebo [1, 2].

Dr. Gibson: So there wasn’t a group without dietary intervention and probiotics?

Dr. Isolauri: We had a control group which did not receive this extensive dietary counseling but had a placebo.

Dr. Gibson: How big was this study? What were the numbers per group?

Dr. Isolauri: The study population was 250 in total, something like 70–80/group.

Dr. Gibson: You just said that there was an increase in the polyunsaturates. Was that intake or the status of the women as a result of the dietary intervention?

Dr. Isolauri: Both.

Dr. Gibson: Which class of polyunsaturates increased: n-6 or n-3?

Dr. Isolauri: Actually this part of the analyses is still ongoing. The paper on dietary intake has been published [3]. Dr. Laitinen, would you like to comment? Dr. Laitinen is a nutritionist involved with this study.

Dr. Laitinen: If you are talking about the intake, we increased the intake of both monounsaturated and polyunsaturated fatty acids, and because the food products that were given were mainly rapeseed-based, it was mainly n-3 that was increased.

Dr. Prescott: Thank you very much for your novel hypothesis. This is certainly not my field, but my understanding of the current or traditional model of in utero programming of obesity is that of mismatch, and that actually the greatest risk of obesity is in the undernourished fetus, which is then overfed in the postnatal period, and that is what sets the scene for metabolic syndrome, obesity and all of the things that follow. I am not sure how your model would fit in with that other model.

Dr. Isolauri: It doesn’t fit because these experiments were done in a different population. Maternal nutrition constitutes a decisive factor in the in utero environment. Infants of small birth weight reflecting poor intrauterine nutrition have a heightened risk of such chronic diseases, a phenomenon called programming. In Western societies, in contrast, the fetus is often overnourished due to the mother’s excessive intake of saturated fat, also creating a risk to the child in later life.

Dr. Davies: The body mass index distribution in children isn’t just all shifting to the right, it seems to be that the extremes are becoming more extreme. So it’s not simply a matter of every child gaining a few kilos, the extreme levels of overweight are increasing.
**Dr. Isolauri:** I agree that those extremes create the risk and the problem is that we have more of them today.

**Dr. Davies:** I wonder whether that information could be built into your model somehow?

**Dr. Isolauri:** We have evaluated those extremes in this child population in a case-control study [4].

**Dr. Björkstén:** You mentioned allergy and inflammatory bowel disease and then obesity, but there is also diabetes. There are very interesting studies in Helsinki on diabetes and the hygiene hypothesis. Could the risk of diabetes influence obesity? Is there any relationship or have you looked into diabetes or markers of imminent or late developing type-1 diabetes?

**Dr. Isolauri:** We have evaluated one entire birth cohort in a register study in Finland, and have shown the coexistence of the Th1 and Th2 diseases [5]. The data discussed here, together with recent experimental studies, indicate obesity is linked to poor glycemic control, insulin resistance and metabolic syndrome and type-2 diabetes.

**Dr. Björkstén:** My second question is based on the Barker hypothesis suggesting fetal enzyme induction. Could that have any relevance to your findings with regard to obesity here and microbiota? Could Barker's hypothesis even really be differences in microbiota rather than enzyme induction?

**Dr. Isolauri:** As mentioned, the nutritional status of the population evaluated is important in programming child health and also in impacting on the compositional development of the gut microbiota. We are evaluating this specific question with Dr. Salminen.

**Dr. Stanley:** I believe there has been a study in which fetal sheep were put under stress and born growth-restricted. Then 2 groups of sheep were studied: one which was not growth-restricted, and the other which was. Half of each group was swapped so that the lambs were fed by a mother who had no problems during pregnancy, and converted them from fat-metabolic sheep into normal sheep as a result of that postnatal experience. I am not sure if this has been published, but it might actually link in with the work you are doing that perhaps the postnatal environment is enough to reverse the high rate of obesity.

**Dr. Isolauri:** This model is very similar to the one that Cani and Delzenne [6] are working on in their glucose control experiments. Unfortunately in these studies the overall microbiota composition has not been evaluated.

**Dr. Sartor:** I am intrigued with the results you are showing and I wonder about the selectivity of the bifidobacteria. You mentioned that several different species of bifidobacteria were related to lack of weight gain. Did you look at other groups of bacteria, lactobacilli, clostridia, or did you home in selectively on bifidobacteria?

**Dr. Isolauri:** We have done other experiments as well, but I invite Dr. Salminen to comment on the methods.

**Dr. Salminen:** So far we have actually published the data on PCR and FISH looking at the major groups of bacteria [1] and what Dr. Isolauri presented is really interesting and significantly different compared to the others. There are other differences but analysis is still under way so the profiles are not yet ready and we will publish them as soon as possible, so there will be more data. I think the idea actually comes from the work of Turnbaugh et al. [7]. The work they have done in experimental animals very nicely defines the potential mechanism. We have tried to take this into the real situation in human subjects from early life onwards and see how a small change may influence weight gain in the longer term. It is important to say that we are not saying that this is the way to stop the obesity of neonates, but it is one way of influencing it.
Dr. Vaidya: We heard about the microbiota hypothesis and how it changes the metabolism and causes obesity. Do you think it could work in the other direction as well, that the mothers who gain less weight in pregnancy will have children who grow slowly? Do you think that microbiota can play a role?

Dr. Isolauri: May I say immediately that this is a very complex situation because, for instance, obese women gain less weight during pregnancy than normal weight women. Therefore we presented the changes in gut microbiota associated with excessive weight gain and then the pre-pregnancy weight separately.

Dr. Vaidya: Do you think that this could work in the other direction as well because this does influence metabolism? So if you have an abnormal flora, is it possible that it would also cause less weight gain or poorer growth?

Dr. Isolauri: This has been studied in diabetic mice; so when you make them lose weight the gut microbiota composition changes as well [7].

Dr. Haschke: How did you select the 25 obese and normal children from the overall cohort? Was this a random selection?

Dr. Isolauri: No, the population size was 159 and we chose 25 overweight or obese children at the age of 7 for whom we had fecal samples available at the age of 6 and 12 months. For these children we chose a control matched for several confounding factors such as use of antibiotics, breastfeeding, allergies, and mode of delivery.

Dr. Haschke: You commented on the microbiota at 6 and 12 months, was there still a difference at 7 years of age?

Dr. Isolauri: We didn’t do that because we saw that the gut microbiota changes and the differences we have in early infancy disappear later when they start to use regular food. So it appears that also here we have a window of opportunity to control metabolic programming, immunological programming and microbiological programming to promote health later in life.

Dr. B. Koletzko: My question relates to the same aspect of your presentation where you showed differences in bifidobacteria at 6 and 12 months in children who later were overweight and obese. The question arises what comes first the hen or the egg; is the bifidobacteria count causal for later weight or are there other factors that are associated with later overweight and obesity which also relate to early colonization, for example, the duration of breastfeeding, complementary feeding, lifestyle factors, etc?

Dr. Isolauri: The cases and controls were matched for these factors, mode of delivery, breastfeeding, antibiotics, etc. It appears that bifidobacteria control the inflammation at a certain age, which is the window of opportunity for programming. I did not say that bifidobacteria per se are the explanation, they are just mediating the situation on inflammation. In the same vein, concerning our hypothesis of allergic disease, the question is which is the primary factor, skin barrier and gut barrier dysfunction which leads to excessive antigen uptake, and inflammation locally. Inflammation locally in the gut disturbs the balance of gut microbiota which is proinflammatory and also disturbs the barrier function. So it fits this hypothesis as well. As Dr. Brandtzaeg put it, gut barrier function needs to be intact.

Dr. Makrides: My understanding is that those children came from a randomized trial during pregnancy and you used those to match. So did that also mix up the randomization?

Dr. Isolauri: That was one of the factors we controlled, it did not. So probiotic intervention was controlled, antibiotic treatment as well. Thank you for reminding me of this.

Dr. Exl-Preysch: I also always ask myself what comes first, the immune deviation or the microbiota deviation in the pregnant mothers. They are obese and then it is past
on to their children and, as you have shown very nicely, then they have completely different gut flora microbiota.

**Dr. Isolauri:** For this reason we separately evaluated excessive weight gain during pregnancy and excessive pre-pregnancy weight with less weight gain during pregnancy.

**Dr. Exl-Preysch:** Yes, but why do the obese mothers already have a different microbiota? The other thing I want to mention concerns the diabetes discussion; there is a huge German study where they showed very nicely that those babies with type 1 diabetes had significantly less atopic dermatitis. So there must be something, Th1, Th2? I don't know how this works, but I would like to have your comment on that.

**Dr. Isolauri:** There are several epidemiological studies on this question. Most publications report their coexistence but also opposite data are on record.

**Dr. Tobin:** I am always interested in your observations because often you make these observations before some of us have thought about it. I think we could spend the whole afternoon trying to understand the potential list of hypotheses to explain your findings. In generating our hypotheses, it would be easy to miss the role of the enteric nervous system in potentially sensing this new load of organisms that you are actually giving. As part of the process, are you giving these mothers an intestinal bypass whereby if their processing through the small bowel were sped up they might absorb less nutrients? Have you altered small intestinal transit?

**Dr. Isolauri:** We have looked at dietary intake, including fiber, but assessing transit was not possible in this cohort.

**Dr. S. Koletzko:** Did you measure fecal inflammation markers in your infants? I always wonder why we have these high calprotectin or lactoferrin levels in normal healthy infants: in some it is sky high and in others it is zero. I always ask myself what is normal. Do we need a certain degree of inflammation; is this perhaps beneficial or is this bad; does it correlate with fecal flora which might have a predictive value for later outcome? Why do we see this high degree of inflammation as indicated by these fecal markers?

**Dr. Isolauri:** We have used TNF-α in fecal samples and it is not positive in a normal situation, so what I present here is not based on calprotectin.

**Dr. Brandtzaeg:** Very intriguing hypothesis. You describe an indirect bacterial effect on the downregulation of inflammation. Is it your message that bacteria should downregulate the inflammation and therefore decrease obesity? I haven't followed this field too closely but quite recently I saw a paper on a mouse model where they actually describe the effect of the microbiota on energy consumption.

**Dr. Isolauri:** These papers are from Cani and Delzenne [6] and Turnbaugh et al. [7]. It appears that there are more energy harvest and storage, but also the low-grade inflammation controlled by specific strains of the gut microbiota. However, we have to remember that there are also specific dietary compounds which interact with microbiota like fatty acids, vitamins, zinc, vitamin D as was mentioned earlier.

**Dr. Howidi:** We learned a lot about bifidobacteria, etc., and they all fit under environmental factors, but I am still trying to tie this up with the genetic factors. We also know that obesity can be genetic; asthma can be genetic; allergies can be genetic. It seems as though we focus a lot on environmental factors but we miss the genetic elements.

**Dr. Isolauri:** Also the microbes within the microbiota contribute to the genetic factors here. However, we have to acknowledge now that gut microbiota is an active metabolic organ and so is the adipose tissue.

**Dr. Howidi:** Basically these two factors are needed for interaction.
References
