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Authors: Stig Bengmark

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Gut microbiota, immune development and function

Stig Bengmark,
Member Academia Europeae,
Emeritus professor Lund University, Lund, Sweden,
Honorary Visiting Professor to University College London (UCL), London University,
Affiliation to Departments of Surgery

Professional address:
Division of Surgery & Interventional Science,
University College London, 4th floor,
74 Huntley Street,
London.
WC1E 6AU
United Kingdom

Correspondence to the author:
Stig Bengmark
185 Barrier Point Road
Pontoon Docks
London
E16 2SE
United Kingdom
Tel & fax +44 20 7511 6842
stig@bengmark.se

Abstract
The microbiota of Westerners is significantly reduced in comparison to rural individuals living a similar lifestyle to our Paleolithic forefathers but also to that of other free-living primates such as the chimpanzee. The great majority of ingredients in the industrially produced foods consumed in the West are absorbed in the upper part of small intestine and thus of limited benifit to the microbiota. Lack of proper nutrition for microbiota is a major factor under-pinning dysfunctional microbiota, dysbiosis, chronically elevated inflammation, and the production and leakage of endotoxins through the various tissue barriers. Furthermore, the over comsumption of insulinogenic foods and proteotoxins, such as advanced glycation and lipoxidation molecules, gluten and zein, and a reduced intake of fruit and vegetables, are key factors behind the commonly observed elevated inflammation and the endemic of obesity and chronic diseases, factors which are also likely to be detrimental to microbiota. As a consequence of this lifestyle and the associated eating habits, most barriers, including the gut, the airways, the skin, the oral cavity, the vagina, the placenta, the blood-brain barrier etc, are increasingly permeable. Attempts to recondition these barriers through the use of so called 'probiotics', normally applied to the gut, are rarely successful, and sometimes fail, as they are usually applied as adjunctive treatments, e.g. in parallel with heavy pharmaceutical treatment, not rarely consisting in antibiotics and chemotherapy.

It is increasingly observed that the majority of pharmaceutical drugs, even those believed to have minimal adverse effects, such as proton pump inhibitors and anti-hypertensives, in fact adversely affect immune development and functions and are most likely also deleterious to microbiota. Equally, it appears that probiotic treatment is not campatable with pharmacological treatments. Eco-biological treatments, with plant-derived substances, or phytochemicals, e.g. curcumin and resveratrol, and pre-, pro- and syn-biotics

offers similar effects as use of biologicals, although milder but also free from adverse effects. Such treatments should be tried as alternative therapies; mainly, to begin with, for disease prevention but also in early cases of chronic diseases. Pharmaceutical treatment has, thus far, failed to inhibit the tsunami of endemic diseases spreading around the world, and no new tools are in sight. Dramatic alterations, in direction of a paleolithic-like lifestyle and food habits, seem to be the only alternatives with the potential to control the present escalating crisis. The present review focuses on human studies, especially those of clinical relevance.

Keywords: microbiota; microbiome**;** microbial translocation; probiotic bacteria; lactobacillus; lactobacillus plantarum; lactobacillus paracasei; microbial translocation; inflammation; infection; toll-like; neutrophils; pharmaceuticals; biological; eco-biologicals; nutraceticals; curcumin; resveratrol; antibiotics; chemotherapeutics; barriers; leakage; gut; airways; oral cavity; skin; vagina; placenta; amnion; blood-brain barrier; growth; replication; apoptosis; mucosa; endothelium; plaques; cytokines; IL1; NF-kB; TNF; growth factors; insulinogenic; IGF-1; prebiotics; plant fibers; greens; fruits; vegetables; minerals; fat diet; refined carbohydrate diet; advanced glycation end products (AGEs), advanced lipoxidation end products (ALEs); endotoxin; LPS; proteotoxins; casein; gluten; zein; Western lifestyle; Paleolithic; Schimpanzee; ADHD; AIDS; Allergy; ALS; Alzheimer; Arteriosclerosis; Atheroma; Autoimmune; Autism; Bipolar; Cancer; Celiac disease; COPD; Coronary Heart Disease; Chronic Fatigue Syndrome; Chronic Renal Disease; Cognitive; Diabetes; HIV-1; Encephalopathy; Irritable Bowel Disease; Inflammatory Bowel Disease; Liver cirrhosis; Liver steatosis; Obesity; Osteoarthritis; Osteoporosis; Pancreatitis; Paradontosis;Parkinson; Polycystic Ovary Disease; Rheumatoid Disease; Schizophrenia; Stress; Stroke; Uveitis;

An epidemic of obesity and chronic diseases

The global incidence of obesity and various endemic chronic diseases from ADHD, Alzheimer, and autism to osteoarthritis and stroke is rapidly increasing. For some decades the epidemic was mainly a problem of the Western world, with its modern agricultural techniques, mass production and easy access to and large consumption of agricultural foods, including those frequently industrially manipulated and processed such as meat, dairy and wheat [2-4]. However, similar development is now observed also in other parts of the world, largely in parallel to the adoption of a "modern"/Western lifestyle. Seemingly this epidemic of obesity and associated diseases has its epicenter in Southern United States [1], states like Alabama, Louisiana and Mississippi having the highest incidence of obesity and chronic diseases in the US and the world. These diseases spread, with the pace of a tsumani, across the world; to the West to New Zeeland and Australia, to the North to Canada, to the East to Western Europe & the Arab world and to the South, particularly Brazil.

Recent studies forecast by the year of 2050 a doubling of the incidence of diabetes [5] and a tripling of the incidence of ADHD, Alzheimer disease [6] and cancer [7] in most countries, including the US. A most interesting recently published study looked at the US and UK, together representing approximately 5 % of the world´s population [8], two countries which already have the highest rates of obesity and chronic diseases. The study suggest that these countries combined will, by the year of 2030, see another 76m obese adults, additional 6–8.5ml cases of diabetes, 6-7m cases of cardiovascular disease, 492,000–669,000 cases of cancer, leading to loss of 26–55m quality-adjusted life years and a dramatic increase in costs of care (calculated to be $50–68b/year) [8]. Other studies suggest that the increase will continue beyond 2030, if dramatic preventive measures are not instituted. While predicting future disease, especially cancer, might be fraught with uncertainty; predictions are necessary aids to health planners and others and must be done. The general experience is that the statistical models have, over the years, been refined and today these models are capable of providing accurate predictions.

A consequence of large consumption of insulinotrophic foods?

Although sharing an almost identical genome, difference of lifestyle and food habits between modern man and our forefathers living some 200,000 years ago, are huge. These individuals consumed only a small fraction of the insulinotrophic food consumed by modern man, especially in those living in the Western World. The so-called Paleolithic diet was almost identical to the food of the wild chimpanzee of today, with which we share about 99.4 of the genome. The Neolithic Revolution, and introduction of agriculture some 10 000 years ago, has provided increasing access food to insulinotropic and IGF-1-raising foods including sugar, dairy products and grains, a process significantly augmented by the Industrial Revolution, i.e. during the last 150 years. Many agree that the human genome has not, and may never, adapt to the high levels of insulin/IGF-1 signaling (IIS) that drives the Western diet, which supports the argument that modern man should attempt to develop a more Paleolithic-type diet [9].

The association between high intake of high IIS foods and the development of chronic diseases is strongly supported, especially by more recent observations in individuals with congenital deficiency in IGF-I (Laron syndrome, GH gene deletion, GHRH receptor defects and IGF-I resistance), who demonstrate a dramatic reduction in pro-aging signaling [10], rate of cancer [10-12], diabetes [10] and other chronic diseases. It is most interesting to note that these individuals, despite their dwarfism, marked obesity, and severely impaired metabolism (> 50 % of the individuals suffer nonalcoholic fatty liver disease (NAFLD)) experience longevity, the greater majority being alive at the ages of 75-78 years, some reaching even more advanced ages. Studies in both invertebrates (C-elegans flies, *Drosphila*) and rodents (mice and rats) with induced IGF1 deficiency (the IGF1 gene or the GH receptor being inactivated), demonstrate a significant prolongation of lifespan, particularly in females [13].

Reducing IGF signaling is presently regarded as a most promising strategy to reduce so-called proteo-toxicity and to delay/inhibit the development of chronic diseases similar to Alzheimer's disease, as recently demonstrated in a mouse study [14], and supported by 'calorie restriction' studies in humans [15,16]. However, many other factors seem to contribute to development of obesity and chronic diseases, among them vitamin D in serum deficiency and accumulation in the body of high temperature-induced, strongly proinflammatory products, known as 'advanced glycation end products' (AGEs) [2-4] Both Vitamin D-deficiency and accumulated AGEs in the body are factors highly suspected to potentiate the processes leading to obesity and chronic diseases. It is a fact that IGF1 plays a major role in childhood growth, has profound anabolic effects in adults, and promotes alterations in aging later in life. Individuals eating Western type food will normally show higher levels of IGF1 in serum, induced by large consumption of high 'glycemic index' food; in Western countries > half of the consumed calories consist in such foods, which constitute strong inducers of liver synthesis of IGF. Meanwhile, other foods provide a significant additional source of the peptide, dairy foods being especially rich in IGF1. A recent study in young boys fed casein, demonstrated a significant 15% ($P<0.0001$) increase IGF1/s but no changes in fasting insulin ($P=0.36$), while boys fed whey instead had a 21% ($P=0.006$) increased fasting insulin, and no change in IGF-1 ($P=0.27$)[17].

Western food and and its effects on microbiota and disease.

A major aim of this chapter is to review documented effects of Western lifestyle on the microbiota, its diversity and numbers of strains but also to investigate the role of Western foods on induction of inflammation and the role of dysbiosis in the pathogenesis of obesity and chronic diseases. The gut microbiota of individuals consuming a Western diet are likely to be reduced as the lower digestive tract is seriously depleted of metabolic fuels, probably leading to a sub-optimal gut microbial profile. The most obvious and harmful consequences of reduced microbiota are malfunction, dysbiosis, and the often observed high levels of endotoxin in plasma (endotoxemia), which in both experimental and clinical studies is strongly associated with inflammation and risk of obesity and chronic diseases. Endotoxins are integral components of the outer membrane of Gram-negative bacteria like Enterobacteriaceae and Pseudomonadaceae, composed of proteins, lipids, and lipopolysaccharides (LPS). LPS, which is

responsible for most of the biological properties of bacterial endotoxins, is known to have exceptionally strong ability to induce inflammation via the so called Toll-like receptors 2 (TLR2) and 4 (TLR4).

Volunteers living for 1 month on a Western-style diet demonstrated, in a crossover study, a 71% increase in plasma levels of endotoxin activity (endotoxemia) when compared to those consuming what the authors called a prudent-style diet, who, in turn, demonstrated a 31% reduced level of endotoxin(s) [18]. A positive correlation between sedentary lifestyle and higher levels of endotoxin levels and a negative correlation to the degree of physical exercise has also been reported [19]. High fat content of food, rather than high carbohydrate content correlates with high levels of endotoxemia. Fat in foods is likely to negatively influence microflora and its replication, but the most pronounced effects are expected from their translocation-facilitating ability i.e. to serve as vehicle for translocation of endotoxin, embedded in fat, though the mucosa and into the circulation, a process referred to as transcellular transportation. Mice fed a high-energy diet (either high-fat diet or high-carbohydrate diet) demonstrate a significant increase in plasma LPS, however, again, a high fat diet correlates to a higher degree than a high carbohydrate diet [20]. Strong correlations between plasma levels of endotoxin and numbers of parameters of metabolic syndrome and between persistant levels of high endotoxin/plasma and 'prospect of life' have been noted – large differences have been reported between the first and fourth quartiles [19]. Among the diseases associated with increased endotoxin/plasma are, particularly, Alzheimer´s disease [21,22] and cognitive impairment [23], arterio-coronary disease [23-25] and stroke[26], diabetes 1 [27] and 2 [28] and cancer [30] but also allergy [31], ALS [32], autism [33], autoimmune diseases [34], bipolar disease [35], chronic fatigue syndrome[36], COPD [37], minimal encephalopathy [38,39], fibromyalgia [40], HIV [41], liver cirrhosis [38,39], macular degeneration [42], nephropathies [43], obesity [44,45], osteoarthritis [46] , paradontosis [47], Parkinson's disease [48], rheumatoid disease [49], schizophrenia [50], stress [51] and uveitis [52.

Proteotoxins induce and enhance inflammation.

Humans are known to be extremely sensitive to endotoxin exposure and reported to show signs of inflammation at a dose of LPS that is at least 250-fold lower than that required in, for example, mice [53]. This is important as modern humans, as an unfortunate consequence of modern living, much more than other primates, are exposed to LPS both outdoors and indoors, to a large extent through the dust inhaled at the home, workplace and at school. Agriculture, textile and wood industries are especially recognized for their bad environment with extremely high levels of endotoxin exposure. Tobacco smoking is also recognized as a major source of LPS. Often neglected is the fact that the food we eat often contains unacceptably high levels of endotoxin. Cooking makes little difference as LPS is heat-resistant while both LPS and dead bacteria remain capable of inducing inflammation. The majority of fresh and raw whole vegetables should normally contain only minimal or undetectable levels of stimulants of TLR2 or TLR4 [54]. However, certain raw and minimally processed vegetables (MPVs) are very sensitive to storage and might occasionally contain quantities of bacteria and endotoxins; among these are bean sprouts, diced onions, and chopped root vegetables such as carrots and onions [55]. Beef, pork and turkey increase their content of TLR2- and TLR4-stimulants within a few days, even when stored at 5^{o} C, and especially if exposed to air [56]. The accumulation of TLR2- and TLR4-stimulants is minimized by storage of meat in its intact rather than in minced forms, and when stored under a modified atmosphere, rather than exposed to air [56]. Very little data exists about the health hazards of game meat, which despite its favourable nutritional profile when compared to farmed meat, is often kept hanging for weeks and, as a consequence, is especially rich in endotoxins.

Different food ingredients and particularly proteins may enhance or diminish the inflammatory properties of meat diets. Many peptides, to which modern humans are daily exposed, possess the ability to induce inflammation, activate TGF-β and Toll-like receptors (TLRs). Among these are various lectins, especially glutenoids, and caseins. Molecules induced by heating of food or longer storage at room temperature are molecules collectively called Maillard products e.g. the 'advanced glycation end products' (AGEs) and

'advanced lipoxidation end products' (ALEs). The invention of fire increased dramatically the possibilities for these food products to be produced, and the introduction of gluten-containing grains, which occurred about 10,000 years ago with the advent of agriculture, further increased exposure to dysfunctioning proteotoxins - pro-inflammatory molecules - developments, which, in a way, might be regarded as unfortunate "mistakes of evolution". The main reason for the large exposure to glutens might have been that it was mainly the members of the Triticeae grass tribe (wheat, rye, barley) and the Pooideae subfamily (including even oats) that grew well at higher latitudes. Modern plant breeding technology exacerbated the situation as modern bread contains 15-20 times more gluten, when compared to bread from the past. As an unexpected consequence, modern man suffers a series of highly unwanted human disorders that relate to exposure to glutenoids, particularly gluten (wheat), but also secalins (rye) and hordeins (barley), which all seem to induce inflammation and increase intestinal permeability [55]. Oats, on the other hand, are more distantly related to wheat, rye and barley and its active peptides, the avenins, are rarely reported to give stronger reactions of inflammation and allergy [56].

Gluten-sensitivity a common and "new" disorder.

!t has become increasingly apparent that "classic" celiac disease (CD) represents only "the tip of the iceberg" of an overall large glutenoid-associated disease burden. We are increasingly aware that there frequently exists, besides those who suffer celiac disease (CD) and classical wheat allergy, many individuals who often suffer discrete reactions to glutenoids. In these individuals, neither allergic nor autoimmune mechanisms seem to be involved, particularly when exposed to wheat, but also to rye and barley [57]. This phenomenon, many times more common than classical CD, is defined as gluten sensitivity (GS) [57]. Although LA-DQ8 is present in almost all CD patients, these genes are only present in about half of patients with GS. Some of the individuals with GS may suffer well-defined chronic diseases, others 'only' more ill-defined distresses; fatigue, depression, encephalopathy/'foggy mind', lack of energy, diffuse abdominal pain, bloating, diarrhea, eczema and/or rash, undefined headache, numbness in the legs, arms or fingers, joint pain and many other manifestations. More or less all report, when turning to gluten-free diets, increased well-being and frequently also improved clinical signs and symptoms. Experiences like these have made the gluten-free diet the number one health trend in the US, growing faster than both low carbohydrate diets and "fat-free" diets while fueling a market for gluten-free products approaching $2.5 billion (US) in global sales in 2010 [58]. Among the alternatives flours used for bread-baking are ancient grains, several of them known to grow particularly, if not exclusively, well in Africa; amaranth, arrowroot, brown rice, buckwheat, chia, chickpea, corn, hemp, maize, millet, oat, potato, quinoa, sesame, sorghum, soya, tapioca, teff and white rice, of which sorghum is the 5th commonest grain in world and especially attractive due to its extremely high content of antioxidants, low content of energy [59], and ability to resist heat-induced protein glycation [60], but also due to its high versatility and cost-effectiveness.

Proteotoxin-induced low threshold for immune response.

Glutenoids, which demonstrate endotoxin-mimicking abilities, are capable of lowering the threshold for immune responses, attracting leukocytes and increasing their reactive state, in similar manner to that of endotoxin e.g.10 μg/ml of wheat gluten induces the equivalent effects of 1 ng/ml LPS [61], while increasing dendritic cell maturation and chemokine secretion [61]. A study investigating fecal samples from 76 symptom-free, non-celiac, first degree CD relatives and compared to samples from 91 aged-matched healthy controls reported significantly lower level of acetic acid and total SCFAs as well as significantly increased level of i-butyric acid and fecal tryptic activity in the asymptomatic CD-relatives [62]. The information that removal of gluten from the diet of the non-obese diabetic mouse could attenuate the intensity of autoimmunity and reduce the incidence of diabetes led to a cross-over study, where 17 first-degree relatives were kept on a gluten-free diet for the first 6 months followed by another 6 months on a standard gluten-containing diet [63]. The acute insulin response to iv glucose tolerance test rose significantly in 12/14 subjects after the first 6 months of gluten-free diet when complying with the diet (P= 0.04) and decreased again in 10/13 during the following 6-month period on a standard gluten-

contaning diet (P= 0.07)[63]. A similar outcome was recently reported from a crossover study in which 100 individuals suffering ADHD, aged 4–8 years, were randomly assigned to 5 weeks on a restricted elimination diet including a restricted consumtion of gluten-containing bread (at the most twice a week) and compared to what was referred to as a healthy diet, followed by another 5 weeks on the alternative diet. All parameters' total score, inattention, hyperactivity and abbreviated Connor scale scores (ACS) improved significantly on the restricted diet but deteriorated significantly during the subsequent period on normal, although supposedly healthy diet [64]. Another study focused on thirty-four individuals with irritable bowel syndrome syndrome, 56 % of them with human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8 genes, during 6 weeks exposed to a gluten-free diet [65]. Statistically significant improvements, compared to controls, were reported in the gluten-free group in 3/6 parameters studied: abdominal pain (P = 0.02), satisfaction with stool consistency (P = 0.03), and tiredness (P = 0.001); no improvment was observed in overall symptoms (P = 0.15), wind (P = 0.08), and nausea (P = 0.69), and no differences observed between individuals with or without DQ2 / DQ8 genes [65].

Inclusion of industrially made foods ingredients and particularly those of a protein nature might both enhance and diminish the inflammatory properties of the diet. This is also true for various lectins and frequently observed with various bovine milk-derived proteins, and particularly with powdered milk. The synthesis in the brain of serotonin (5HT) and melatonin is dependent on access to their precursor amino acid, the essential amino acid L-tryptophan (TRP), normally released in the gut by microbial fermentation of plants rich in this amino acid [66]. As it is believed that some food proteins might block such a release, an animal study was undertaken to compare the effects of five different such proteins: zein (corn), gluten (wheat), soy protein isolate, casein, lactalbumin or no protein. An 8-fold variation was observed in levels of cortex tryptophan: a marked decline followed zein ingestion, modest reductions after casein or gluten, which were paralleled by reductions in cortical and hippocampal hypothalamic 5-hydroxytryptophan (5HTP) [66]. A recent publication reports disappearance of signs of malabsorption and intractable therapy-resistent seizures in three young girls (ages 18, 19, 23) when placed on gluten free diet [67]. Similarly individuals with similar symptoms as in neuropsychiatric diseases; Alzheimer`s disease/cognitive decline [68], autism [69] and schizophrenia [70] are also reported in the literature.

Heat- and storage induced inflammation-inducing proteins.

Heat- and storage-induced dysfunctioning proteins are of special interest due to their strong ability to induce inflammation [71,72]. Among foods rich in AGEs and ALEs are dairy products, especially powdered milk (frequently used in enteral nutrition and baby formulas, as well as in numerous industrially produced foods), in high-temperature produced fried and grilled meat and poultry, but also fish (especially deep-fried and oven-fried), drinks including coffee and colas, Asian sauces, such as Chinese soy sauces, balsamic products and smoked and cured foods in general [73-75]. The consumption of such foods, often main constituents of fast foods, have increased dramatically in recent decades, much in parallel to the endemic of chronic diseases. Higher levels of AGEs such as methylglyoxal derivatives in serum (sMG) and/or other AGEs, are strongly associated with a faster rate of cognitive decline in elderly individuals [76], neuro-degenerative diseases [73], premature aging and cognitice decline [74], diabetes type 1 and 2 [75], diabetic nephropathy [76], obesity [77] and liver disease, particularly liver steatosis and liver fibrosis [78], lung disease, particularly COPD [79] and various cancers including breast cancer [80], colorectal cancer [81], esophageal [82], gastric [83], lung [82],ovarian [82], pancreatic [85], prostatic [86], renal [87], and leukemia [88].

A wide range of pro-inflammatory mediators, including TNF-α, IL-1b, IL-6, IL-8 and the nuclear protein high mobility group box-1 (HMGB1), are implicated in the pathogenesis of the above-mentioned chronic diseases. HMGB1 is one of the important mediators known to signal by way of the advanced glycation end products, particularly RAGEs, and through the Toll-like receptors TLR2 and TLR4. Activation of these receptors will ultimately result in activation of NF-kB, known to induce up-regulation of leukocyte

adhesion molecules and production of pro-inflammatory cytokines and angiogenic factors in both hematopoietic and endothelial cells, thereby promoting inflammation [89]. A recent review suggests that particularly HMGB1, TLR and RAGE constitutes a functional tripod with high ability to promote inflammation [90]. However, many other molecules are also involved in the extremely complex processes, which are behind the development of both infectious and sterile inflammation. Among them are molecules such as heat shock proteins (HSPs), S100s, and hyaluronan, which play important roles, all known to trigger immune responses. For practical purpose it has been suggested that these, together with other mediators such as defensins, cathelicidin, eosinophilic-derived neurotoxin (EDN) and several others should be grouped together under the name of alarmins [91]. Recently recognized such alarmins are Activin A, a member of the TGF-β superfamily as well as its binding protein follistatin (FS), which during acute and chronic inflammatory processes are released by various cell types in the body [92]. The importance of these proteins to the inflammatory processes has, although known to biomedical science since the 1980s, only emerged recently [93]. The rapid release during the acute phases of inflammation into the circulation of Activin A is particularly noteworthy, placing it as one of the earliest factors in the systemic cascade of inflammatory events. But it is equally involved in the pathogenesis of chronic diseases, especially in rheumatoid arthritis, in IBD and in other diseases known for their association to pathological fibrotic events [93]. It also contributes to the proinflammatory macrophage polarization triggered by granulocyte-macrophage colony-stimulating factor (GM-CSF) whilw limiting the acquisition of the anti-inflammatory phenotype in a Smad2-dependent manner, skewing macrophage polarization towards a proinflammatory phenotype [94].

The triggering effects of several, if not all, of the alarmins are promoted by deficiency in vitamin D. A significant negative correlation has been observed between vitamin D levels and high-sensitivity C-reactive protein, NFκB activity, and TLR4 expression ($P < .05$), while monocytes, when preincubated with vitamin D are shown to significantly decrease lipopolysaccharide-activated TLR4 expression and also cytokine levels ($P < .05$) [95]. A recent study looked at seasonality of vitamin D status in healthy individuals and its relation to TLR-4-mediated cytokines [96]. Circulating concentrations of 25(OH)D(3) and 1,25(OH)(2) D(3) were, as expected, higher during summer ($P<0·05$) and also significantly associated with a down-regulation of the TLR-4-mediated cytokines, IL-1β, IL-6, TNF-α, interferon (IFN)-γ and IL-10 more in summer than during winter ($P<0·05$). The variation in cytokine response upon TLR-2 (Pam3Cys) stimulation was, compared to TLr-4, moderate throughout all the four seasons [96].

Each body surface has its own typical microbiome.

Virtually every surface of the human body exposed to the environment; mouth, hair, nose, ears, vagina, lungs, skin, eyes etc has its own unique, specific and very complex microbial assemblage constituted by very different microbial species each with their distinct functions, collectively referred to as microbiota or microbiome [97]. The genomic pool of human microbiota is claimed to be at least 150 times larger than the eukaryotic human nuclear genome, together harboring more than nine million specific genes [98], and contributing to the enrichment and modulation of numerous human functions. The microbiome is extremely sensitive to external influences and easily deranged. This is well demonstrated by the catastrophic changes induced on intestinal homeostasis by antibiotic treatment, according to a recent study, affecting over 87% of all metabolites detected, and deranging most metabolic pathways of critical importance to host physiology, including bile acid metabolism and eicosanoid and steroid hormone synthesis [99].

Studies on the largest microbiome of the human body - that of the gastrointestinal tract, and particularly that of the lower digestive tract - has until now received most the scientific interest, while the microbiome at other sites of the body remain largely unexplored. About 60% of the luminal content of lower GI tract consists in commensal bacteria, which has been described as ”an organ within an organ” and also as “a virtual super-organ” weighing up to 2 kg. The commensal bacteria play a key role in preservation of

intact integrity of the mucosal barrier function at all surfaces, and particularly at that of the lower part of the digestive tract. Impaired microbiome function, dysbiosis, has inevitably serious consequences for health, and is, sooner or later, associated with severe pathological implications. The gut microbiota also plays a major role in the modulation of both the intestinal and general immune system and is essential for preservation of functions such as maturation of gut-associated lymphatic tissue (GALT), secretion of IgA and production of important antimicrobial peptides. The gut microbiota exerts important trophic and developmental functions on the intestinal mucosa. More than anything, the enteric microbiome functions as a potent bioreactor, which controls numerous metabolic functions, of which many still remain unrecognized [100], while producing thousands of important and unique substances of the greatest benefits to the body, as indigestible food substances are converted by fermentation to simple sugars, short-chain fatty acids, various nutrients, antioxidants and vitamins.

Numerous mechanisms to control intestinal homeostasis.

Dysbiosis and impaired barrier functions are associated with several negative consequences; translocation of lipopolysaccharides (LPS) and whole microbial cells, accumulation of endotoxin in the body (endotoxemia) and hyperactivation of the immune system. The microbiota controls intestinal homeostasis through numerous mechanisms in which substances such as lipopolysaccarides, flagellins, peptidoglycans, and formylated peptides are involved. It interacts with intestinal cell receptors such as Toll-like receptors and activates important intracellular signaling pathways with ability to modulate processes such as cell survival, replication and apoptosis as well as inflammatory response. Among the challenging molecules are NF-κB, caspases, mitogen-activated protein kinases. The host immune system controls microbial composition through release of molecules such as β-defensins, cryptidins, lectins, angiogenin 4, reactive oxygen species, IgA and so called bacteriocins, which effectively limits the expansion of various pathogenic microorganisms [see further 101,102].

The enteric flora is mostly represented by strict anaerobes (70–90%), which predominate over facultative anaerobes and aerobes (10–30%) [102]. Recent studies suggest that the gut microbiota might be classified as belonging to one of three principal variants, or ‘‘enterotypes,’’ defined by a dominant presence of Bacteroides, Prevotella, or Ruminococcus species [103]. However, increasing evidence suggests that these enterotypes are more microbial gradients than, although discrete, defined microbial communities as most of the observed differences are largely explained on the basis of long-term dietary intake [104,105]. Diet is the most powerful influence on gut microbial communities in healthy human subjects [106-108]. A study of human subjects and 59 other mammals revealed clusters in which the effects of diet (carnivorous, omnivorous, or herbivorous almost always outweigh host phylogeny [106]. Bacteroides species are prevalent with long-term protein and animal fat diets, whereas Prevotella species are associated with long-term carbohydrate diets [108]. 45,000 of the presently identified >800,000 rDNA sequences (microbial species) and about 5 of the about 50 bacterial phyla identified are found in the lower GI tract [102]. Two of these phyla are totally dominating: *Firmicutes* (65-80% of the clones) and *Bacteroidetes* (about 23%), while *Actinobacteria* (about 3%), *Proteobacteria* (1%) and *Verrumicrobia* (0.1%) exists only in smaller amounts [102,109,110]. Of special interest is that *Actinobacteria* and *Firmicutes,* to which the genus *Lactobacillus* belongs, are almost exclusively Gram-positive, while *Bacteroidetes* and *Proteobacteria* are mainly Gram-negative [see further 111]. Recent attempts to study the microbiota at other sites within the digestive tract report that the mouth harbours the greatest phylogenetic diversity, the stomach the lowest, and diversity to increase from stomach to the stool [112].

Great differences in microbiota between rural and urban areas.

As lifestyle, and particularly food intake, has a profound influence on the composition of the microbiota it should be of the greatest interest to understand more about the Paleolithic microbiome, to which humans have been adapted during millions of years. A recent study compared the fecal microbiota of European (Italian) children (EU) with that of children from a rural African village of Burkina Faso (BF) in Central

Africa. In this environment, the high fibre diet diet is, in most respects, the closest we can get to that of early human settlements at the time of the birth of agriculture. Significant differences in both biodiversity and richness of microbiota to the favour of BF children ($P < 0.01$) were a general and most characteristic observation [113]. BF children showed a significant enrichment in Bacteroidetes and depletion in Firmicutes ($P < 0.001$), with a unique abundance of bacteria from the genus Prevotella and Xylanibacter, genuses known to contain a set of bacterial genes for cellulose and xylan hydrolysis. These genuses were completely lacking in the EU children [113]. In addition, significantly higher levels of short-chain fatty acids ($P < 0.001$) were observed in BF than in EU children. Also Enterobacteriaceae (Shigella and Escherichia) were significantly under-represented in BF compared to EU children ($P < 0.05$) [113]. Of somewhat greater surprise was the observation, that Gram-negative bacteria (mainly Bacteroidetes) were more abundant (58.5%) than Gram-positive bacteria (37.4%) in the BF population, whereas Gram-positive (mainly Firmicutes) were more abundant than Gram-negative bacteria (70.4% versus 29.1% respectively) in the EU population, resulting an Gram-positive to Gram negative ratio of 37 to 59 in the BF population compared to 70 to 29 in the EU population [113]. A further study compared the fecal microbiota of monozygotic (MZ) and dizygotic (DZ) twin pairs living in South Korea and the United States; thirty-one MZ (n = 62) and 23 DZ (n= 46) European- and African-ancestry twin pairs from the Missouri Adolescent Female Twin Study, and 9.5 MZ (n = 19) twin pairs from the Korea Twin Family Cohort [114]. Alpha diversity (within-sample) measurements of the fecal microbiota did not show any significant overall difference between the Korean and U.S. cohorts, but a greater inter-individual separation between American and Korean subjects was observed in the lean sub-population than in the obese sub-population as well as the the total population.Furthermore, the diversity in obese US twins was found to be significantly smaller than in lean US twins; a similar trend was observed in the much smaller Korean sample, which consequently did not reach statistical significance. No significant differences were found between those of African or European origin in the American lean population. Finally, it was observed within both Korean and the US populations that the differences in fecal microbiota were significantly greater between individuals from different families than between those of the same family. The family-level taxa that discriminated between the Korean and US cohorts included *Bacteroidaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, Prevotellaceae, Rikenellaceae, Ruminococaceae, Streptococcaceae, and Veillonellaceae* [114].

Clear association between level of fiber intake and obesity and obesity-related diseases.
It is an old observation that some individuals, despite similar intake of calories and nutrients, and comparable levels of daily activity, are more susceptible to weight gain than others. This observation is usually explained on the basis of real, although discrete, differences in content of dietary fibers in the foods. Equally, though it could the result of differences in composition of microbiota, and consequently due to differences in production of nutrients and calories to be absorbed [115]. A study demonstrates that the small intestine of dogs fed fermentable fiber has a 28% greater surface area, a 37% larger mucosal mass, is 35% heavier, and has 95% higher capacity for glucose uptake than that of dogs fed a diet without access to fermentable fibers (in the study given only non-fermentable cellulose) [116]. Furthermore, it was observed that the anatomic differences were most pronounced in the proximal portion of the small intestine, where salvage of up to 10 % more energy from the eaten food could occur [116].

However, it is not unlikely that as much or even more calories will be produced through the colonic "bioreactor" – i.e. produced by the fermentation of otherwise indigestible components of the diet, e.g. fermentable fibers, a process referred to as "energy harvest" [117]. As a matter of fact, it has been observed that the cecal concentrations of just short-chain fatty acids (SCFA) - important energy sources for the host - could account for as much as 10% of daily energy intake [118]. It has also been noted that production of SCFAs is significantly higher in obese than in lean animals [119], which correlates well with the pronounced phylum-level bacterial changes observed, which includes decreased Bacteroidetes and increased Firmicutes levels, in subjects on a weight-reduction diet [120]. The wide spectrum of prebiotic fibres possess varying influences on microbiota, gastrointestinal function and health. Some such

fibers are reported to increase Firmicutes and decrease Bacteroidetes, a profile often associated with a leaner phenotype but also with positive effects on energy intake, blood glucose, insulin release, satiety hormones, and hepatic cholesterol and triglyceride accumulation [121]. Some Bifidobacteria and Lactobacillus species seem, though, to remain within the very obese, where they can exist in normals and sometimes increased numbers. A group of obese patients were recently reported to have low levels of Bacteroidetes, but also Firmicutes compared to their lean controls, but still an abundance of Lactobacillus species within the Firmicutes seemed characteristic of obesity [122].

A study in obese adolescents (average age 15) undergoing lifestyle intervention (reduced food intake and regular physical exercise) found definite changes in gut bacteria and in associated IgA production, which clearly related to the success in body weight reductions. This supports the concept of interactions between diet, gut microbiota, host metabolism and immunity [123,124]. Reductions in *Clostridium histolyticum* and *E. rectale-C. coccoides* correlated significantly with weight reductions in the whole adolescent population. Proportions of *C. histolyticum*, *C. lituseburense* and *E. rectale-C. coccoides* dropped significantly, whereas the Bacteroides-Prevotella group increased significantly after the intervention in the adolescents who lost more than 4 kg. The total fecal energy was nearly significantly reduced in this group of adolescents, but not at all in the group that lost less than 2.5 kg. Proportions of gA-coating bacterial decreased most significantly in those who, during the intervention, lost more than 6 kg significantly in parallel to reductions in the *C. histolyticum* and *E. rectale-C.* coccoides populations [123,124]. Twenty 4-5 year old overweight or obese children were compared to twenty children of the same age but with normal body mass index [125]. The concentration of the Gram-negative family Enterobacteriaceae was significantly higher in the obese/overweight children and the levels of *Desulfovibrio* and *Akkermansia muciniphila*-like bacteria were significantly lower in the obese/overweight children. No significant differences were found in content of Lactobacillus, Bifidobacterium or the *Bacteroides fragilis* group. It was also observed that the diversity of the dominating bacterial community tended to be less diverse in the obese/overweight group, although the difference was not statistically significant [125].

Vitamin D, physical exercise and other factors of importance.

Although lifestyle and dietary habits seem to have a dominant influence on the composition of the microbiota, immune development, immune functions and numerous other factors associated with inflammation seem to play important roles for the microbiota to grow and function well. Among key participants are vitamin D and its receptor (VDR), as well as the level of physical activity of the individual.

Vitamin D deficiency is increasingly recognized as associated with early-life wheeze, reduced asthma control [127 - 129) and allergic diseases [126-128] and most chronic diseases. Mice that lack the VDR receptor show signs of a chronic, low-grade inflammation, especially affecting the gastrointestinal tract, but also signs of decreased homing of T cells in the gut and low levels of IL-10 and increased inflammatory response to normally harmless commensal flora [129]. Commensal as well as pathogenic bacteria are demonstrated *in vivo* to directly regulate colonic epithelial VDR expression and enhance bacterial-induced activation of intestinal NF-κB and attenuate the response to microbial infection [130].

Increasing evidence suggest that voluntary regular physical exercise lowers the risk of diseases such as colon cancer, diverticular disease, cholelithiasis as well as constipation [131]. Rats who exercised in a wheel an average of 3,500 miles/d [really? check] demonstrated after 5 weeks, when compared to sedentary controls, not only lower body weight (318 g vs 364 g), but also significantly larger caecum, increased cecal weight (021 g vs 0.17 g) and significantly higher concentrations of caecal n-butyrate (8.14 mmol/g vs 4.87 mmol/g caecal content) and a significantly altered caecal microbiota [132]. No human study investigating exclusively the effects of physical exercise on microbiota has thus far been published,

all efforts to date have concentrated on the combined effects of exercise *and* controlled food intake [see for example 123,124]. Increased systemic inflammation is almost, if not always, a sign of dysbiosis and increased translocation of toxins of bacterial origin, such as endotoxin [131]. A number of observational and interventional trials have demonstrated significant positive effects of physical exercise on parameters of inflammation, such as C-reactive protein (CRP), TNF-alpha, IL-1 alpha, IL-1 beta, IL-4, IL-10, IL-6 and transforming growth factor-beta-1 (TGF-β1), which drive the cytokine balance to an "anti-inflammatory" state," [132], paralleled by significant signs of improved health; reduction in triglycerides and apolipoprotein B, increased high-density lipoprotein, altered low-density lipoprotein particle size, increase in tissue plasminogen activator activity, and decrease in coronary artery calcium [133]. Brisk walking is a form of exercise which fits most middle-aged and elderly individuals, demonstrated to have seemingly miraculous effects on health. A recent study reports that men, who walk briskly for 3 h/wk or more, demonstrate a 57% lower rate of progression of prostatic cancer compared to those who walked at an easy pace for less than 3 h/wk [134]. The positive effects of brisk walking observed in breast cancer patients include: reduced risk of breast cancer [135], significant reductions in insulin-like growth factor-I (IGF-I) and its binding protein (IGFBP-3)[136], decreased body fat, increased lean mass and maintained bone mineral density (BMD) [137]. Similar positive effects are reported in, for example, common diseases such as Alzheimer´s disease [138], cardiovascular disease [139], diabetes [140] and obesity [141], all diseases associated with endotoxemia and consequently also with deranged microbiota and dysbiosis [142,143]. Similar improvements are reported in less frequent conditions such as sleep apnea [144] and polycystic ovary syndrome [145].

Dysbiosis and leaky barriers.
Most interest has, thus far, focused on translocation from the lower gastrointestinal tract. However, increasing evidence suggests that leakage from other barriers; the oral cavity, the upper GI tract, the airways, the skin, the vagina and female reproductive tract, the placenta, the eye cavity etc., but also the blood-brain barrier, might be of equal importance in the pathogenesis of disease.

Leaky gut: [loss of gut barrier integrity] The gut meets the exterior world across a surface suggested be approximately 7-8,000 m^2 - equivalent to the size of a soccer field. This surface is the object of extreme challenges with at least half, if not more, of individuals living a Western-type lifestyle suggested to suffer impaired microbiota and more or less permanent leaky gut. Increased translocation of toxic or infectious molecules and even whole microorganisms is a frequent phenomenon in a comprehensive series of diseases. The transfer of these elements and other occur paracellularly e.g. through the intercellular space referred to as 'tight junctions', but also trans-cellularly, and then encapsulated in fat molecules from the consumed foods. The tight junctions, once regarded as static structures, are now known to be extremely dynamic and ready to adapt to a variety of developmental, physiological, and pathological circumstances, and regulated by several molecules including the interesting endogenous modulators named zonulins [146,147]. The tightness of the GI mucosa is largely dependent on consumed foods and its effects on intestinal microflora and is thus strongly associated with dysbiosis and subsequent inflammation. Life-style factors such as physical activity,intake of alcohol and cigarette smoking play important roles but the dominant regulatory factors are processed and refined food, sugars and content of insulinotrophic molecules, proteotoxic and dysfunctining molecules such as AGEs and ALEs, molecules especially common in modern food/industrially produced foods - all disadvantageous to barrier integrity. High temperature-produced foods are prevalent in the Western world, commonly produced in processes such as bread baking, and the preparation of fast foods dependent on frying and grilling [2-4,148]. Storage of foods for longer periods, even at room temperature, as well as flavoring of foods, is known enhance the availability of these molecules in the foods [2-4,148]. These molecules play important roles in the pathogenesis of diseases including Alzheimer´s disease [149], cardiovascular diseases [150,151], chronic liver diseases [152-154], chronic kidney disease [155,156], chronic obstructive pulmonary diseases (COPD)[157], diabetes [158], inflammatory bowel diseases(IBD) [159,160], irritable bowel syndrome(IBS) [161], paradontal diseases such as paradontosis [163] and polycystic ovary

syndrome (PCOS) [163]. Leaky gut is also seen in a large variety of other conditions, such as alcoholism [164], autoimmune diseases [165), chronic encephalopathy [166], chronic fatigue syndrome [167,168], mental depression [169,170] and other, idiopathic, conditions, which are mainly observed in the Western world. Not only translocated endotoxin, but also viruses [171,172] live bacteria [173,174] and debris of bacteria not only translocate, but can remain intracellularly in various cell types; these may be particularly observed in the adipocytes in obesity, where they seem to enhance inflammation and further storage of fats.

Leaky oral cavity: The oral cavity comprises different mucosal sites, anaerobic pockets, and teeth, each harbouring a unique and diverse microbial assemblage. Great interpersonal variation in pattern of microbiota exists; some oral communities are dominated by Streptococcus species and others by Prevotella, Neisseria, Haemophilus, or Veillonella species. Accumulation of pathogens and inflammatory cells in the vascular wall and the subsequent release of pro-inflammatory cytokines are thought to exacerbate atherogenic processes. Studies published over the last two decades suggest that coronary artery disease may be due to an infection-induced inflammation, but also that the impact of infection on atherogenesis relates to numbers of aggregated pathogens within the endothelial walls/plaques, a concept referred to as ”pathogen burden” [175]. Several studies published thereafter confirm an oral source of bacteria associated with atherosclerotic plaques [176-178]. A recently published study of 15 individuals identified Chryseomonas in all atherosclerotic plaque samples studied, and Veillonella and Streptococcus in the majority of them [179].The combined abundances of Veillonella and Streptococcus in atherosclerotic plaques correlated well with their abundance in the oral cavity. Several additional bacterial phylotypes in the same individual were common both to the atherosclerotic plaque and oral or gut samples. Interestingly, several bacterial taxa in the oral cavity and the gut also correlated with levels of plasma cholesterol [179].

Special interest has been paid to *Chlamydia pneumonia*, the first bacteria to have been identified in atherosclerotic lesions [180], a species known to possess the ability to promote lipid body formation in human macrophages [181]. Recently a diverse range of bacteria have been identified in human atheroma (181), the most frequently observed being Gram-negative, including *Acenetobacter baumannii, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Pseudomonas diminutive* and *Proteus vulgaris* and Gram-positive; *Staphylococcus aureus, Staphylococcus epidermidis, and Streptococcus salivarius* [181]. Each of these bacteria, when heat-killed, in common with many other bacteria, is known to stimulate Toll-like receptors and have demonstrated ability to, in a dose-dependent manner, induce lipid body formation and cholesterol ester accumulation. Microbial debris in atheroma, in the past largely considered harmless, might well play a major role in the formation of lipid bodies in the arterial wall but also in the continuous progress of the artherioscerotic disease [181]. It is not yet fully verified if the translocation occurs predominantly in the oral cavity or further down the GI tract. The present belief is, however, that it occurs directly through the gingiva and that brisk tooth-brushing, [with eventual smaller bleeding], might enhance the process.

Leaky airways: The surface of adult human airway is, after the gut, the second largest in the body, thought to cover up to 200 m^2 (size of a tennis court). Exposure of sensitive individuals to antigens can induce allergic responses, mainly apparent in the respiratory tract but also in the skin and eyes, manifesting as vasodilatation, plasma leakage, leukocyte influx, and bronchoconstriction. Endothelial gaps have been identified through which leakage of plasma and inflammatory mediators occur [182], accompanied by leukocyte influx and accumulation of plasma proteins in the airway mucosa. Far less interest has been paid to the process of leakage from the airways through the airway epithelium and into the circulation, despite the fact that such leakage is a very common phenomenon, probablt as frequent as leaky gut. Such leakage is known to influence expression of pattern recognition receptors that detect environmental stimuli and secrete endogenous danger signals, activate dendritic cells and innate and adaptive immunity [182].

For some reason healthy airways have, until recently, been regarded as sterile but now we know that it has both a rich and diverse microbiota. Most recent studies of microbiota have tended to focus on microbiota in individuals with airway diseases, such those with asthma [183,184], cystic fibrosis (CF) [185, 186], obstructive lung disease (COPD) [187,188], mechanically ventilated preterm infants [189], whith less information being available regarding normal microbiota in healthy nose and lungs. In CF for example, in addition to previously recognized pathogens typical for the disease, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, another 460 phylogenetically diverse bacterial genera, not previously associated with the disease, have now been reported [185]. However, much as in the gut, the airway microbiota of patients with CF are not only polymicrobial but also spatially heterogeneous, few taxa being common to all microbial communities in the different anatomical regions of the airways [186]; consequently treatment based only on cultivation of sputum might not always be adequate. Future studies will most probably try to further explore the microbiota of different microbial communities in the airways in healthy individuals, as well as the mechanisms behind leaky airways, and the extent and consequences of such leakage for health, not only associated to the airways, but to the whole body.

Leaky skin: The skin, compared to the gut and the airways, a quite modest surface area – less than 2 m^2, corresponding to approximately half a table tennis board. Non-invasive techniques to study the barrier function of the skin have long been available. It is well known that a number of human skin conditions and disorders are associated with defects in skin permeability. Most of the skin barrier function resides in the cornified layer, while most immune cells, especially the dendritic cells/Langerhans cells. are located slightly below. The human skin harbors myriad bacteria, fungi, and viruses, these microbial communities intricately linked to human health and disease. Recent findings suggest that a dysfunctional epidermal barrier is pathologically involved in a variety of common, antigen-driven skin diseases, allergic diseases such as atopic dermatitis (AD) as well as psoriasis [190], and probably contributes to several general health disorders. Genomic approaches reveal a great diversity of organisms predominantly within the four main phyla: *Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria* [191]. Great differences in the pattern of microbiota are observed between individuals and also between different anatomical regions of the skin, largely associated with differences in structure and physiology of the various skin sites but also depending on factors including hygiene and character of the skin with moist, dry or sebaceous microenvironments [191,192]; *Staphylococcus* and *Corynebacterium spp*. being the dominant colonizing organisms of moist areas. The greatest diversity of microbes is, however, found in the dry areas with a mixed representation from all four phyla [191]. It is most interesting that Gram-negative organisms, previously thought to rarely colonize the skin, are found in abundance in the dry areas, an observation which might have great implications for disease development not only within the skin but in the whole body[191].

The transfer of chemicals through the skin is so effective and reliable that it is increasingly used for drug delivery of analgesics, such as Buprenorphine, Caisapsin, Fentanyl and Lidocaine, hormones, such as estradiol, progesterone and testosterone, drugs against motion sickness and nausea, such as Scopolamine and Granisetron, anti-inflammatory drugs, such as Ketoprofen, Piroxicam, Piclofenac, antihypertensives, including Clonidine, Rivastigmine, and Rotigotine to be used in Alzheimer´s and Parkinson´s diseases, Selegiline for mental depression, Oxybutynin for hyperactive bladder, and antihypertensives like Clonidine and Methylphenidate prescribed for ADHD [193], in total some 40 products as registered in 2010 [193]. The fact that at least half of the drugs are meant to target the central nervous system means that they not only have ability to transfer through the skin but also through other barriers, including the blood-brain barrier. If these chemicals can easily pass the skin barriers, and also the blood-brain, it is most likely that other chemicals, such cosmetics will do the same.

Translocation of chemicals and microbes in individuals with intact skin occurs mainly through the hair follicles. In burn patients, however, where the protective layer has been eliminated, it occurs directly through the skin. Microbial translocation, sepsis and eventually multiple organ failure (MOF) was for long time thought to happen via a leaky gut. Increasing evidence suggest, however, that to a large extent, such translocation occurs directly through the burned skin surfaces, especially as cultivations from blood and septic skin areas are dominated by pathogens typical for skin. A recent study looked at the microbial pattern in blood and at burn surfaces in a group of 338 patients with thermic injuries. The microbes most commonly simultaneously cultivated in both blood and at the burned skin surfaces were *Acinetobacter baumannii* (47%) and *Pseudomonas aeruginosa* (37%) [194]; other frequently isolated microorganisms identified in this study were the Gram-positive *Staphylococcus epidermidis* MRSE (20%) and *Staphylococcus aureus* MRSA (19%) [194].

Leaky vagina (incl. the whole female reproductive tract): The vaginal microbiota provide a vital and highly effective defense mechanism against a whole range of microbial infections [104]. The predominant phyla of bacteria identified in the vagina belong to *Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria* [195]. No single bacterium has been identified as a specific marker for healthy over diseased conditions, but three phyla - *Bacteroidetes, Actinobacteria* and *Fusobacteria*, and eight genera including *Gardnerella, Atopobium, Megasphaera, Eggerthella, Aerococcus, Leptotrichia/Sneathia, Prevotella* and *Papillibacter* are strongly associated with bacterial vaginosis (BV) ($p < 0.05$) [195]. The vaginal bacterial communities of 396 asymptomatic North American women, representing four ethnic groups (white, black, Hispanic, and Asian), were recently characterized by pyrosequencing of barcoded 16S rRNA genes [196]. The communities clustered into five groups: four dominated by *Lactobacillus ine*rs, *L. crispatus*, *L. gasseri*, or *L. jensenii.* The proportions of each community group varied significantly among the four ethnic groups ($p < 0.0001$). Moreover, the vaginal pH of women in different ethnic groups also differed being higher in Hispanic (pH 5.0 ± 0.59) and black (pH 4.7 ± 1.04) women than in Asian (pH 4.4 ± 0.59) and white (pH 4.2 ± 0.3) women [196].

The tight junction protein, occluding, is to a large extent under control of estrogens and the tightness of the vaginal mucosa will for that reason vary significantly with age [197], as well as with the menstrual cycle. Not only the vagina but the whole female reproductive tract (FRT) has unique structures for the regulation of immune protection, especially as it must deal with not only with sexually transmitted pathogens, but also with allogeneic spermatozoa, and the immunologically very different fetus. To meet these challenges, the FRT has evolved unique immune mechanisms to protect against potential pathogens without compromising fetal survival or maternal health [198].

More than twenty pathogens are transmissible through sexual intercourse, and an estimated 340 million new cases of sexually transmitted infections (STI) are reported each year; bacteria such as (group B streptococcus, Neisseria gonorrhoeae, *Chlamydiatrachomatis*, *Treponema pallidum*), parasites (*Trichomonas vaginalis*), and viruses (HerpesSimplex, Human Papilloma, Human Immunodeficiency) are commonly identified [198]. The epithelial cell structures of vagina and FRT possess intracellular and extracellular pathogen recognition receptors (TLR, NOD, RIG, MDA-5, etc), and have the abilitie to secrete chemokines and cytokines that initiate, regulate and link together innate and adaptive immune responses, present antigens to T cells, produce polymeric immunoglobulin receptors for transporting mucosal IgA antibodies from tissues into luminal secretions, and produce intracellular and secreted anti-microbial factors aimed to kill invading microbes – see further [198].

Leaky blood brain barrier (BBB) (and the blood-cerebrospinal fluid barrier (BCSFB): These two barriers constitute a tight seal between the circulating blood/cerebrospinal fluid and the central nervous system (CNS), both consisting of brain microvascular endothelial cells surrounded by basement membranes, astrocytic endfeet, and pericytes. The brain microvascular endothelium is characterized by

the presence of tight junctions (TJs) and a lack of fenestrae, meant to limit the entry of plasma components, as well as red blood cells and leukocytes, into the CNS. These anatomical structures confer a low paracellular permeability and high electrical resistance to the deposition of molecules such as amyloid beta (Ab) into leptomeningeal and cortical brain vasculature, characteristic of Alzheimer´s disease.

Interplay between dozens of connecting transmembrane proteins (occludin and claudins) are as essential to these barriers, in their tight junction formation and function, as they are to all other barriers in the body, and demonstrated to malfunction when leakage occur. Clearly dysfunction of these barriers and their efflux and influx transporters constitute a major factor in the pathogenesis of degenerative neuronal disorders. Complex interactions between AGEs, advanced lipoxidation end products (ALEs), the receptor for advanced glycation end products (RAGE), oxidative stress, inflammatory mediators, common proinflammatory pathways and amyloid-beta (A beta) peptide contribute to BBB dysfunction in a series of degenerative disorders [2-4,199, 200]. Malfunction of other transporters such as the organic anion transporter (OAT) 3 and organic cation transporter (OCT) 3 are essential to leakage of toxic injurious material [201]. Endotoxins, originating from a leaky gut, induce disruption in tight junction (TJ) functions, increase paracellular permeability and alters the functions of the TJ proteins occludin ZO-1, and ZO-2, and thereby increase transcellular leakage as observed in sepsis-induced barrier leakage [202,203], and also in ecephapathies in general – see further below.

Leaky placenta: For the last two decades it has been known that not all babies are born in sterile conditions [204,205]. More recent studies describe an association between infection, within the amniotic cavity, and low birth weight. These studies reveal the presence of various opportunistic pathogens in the amniotic cavity, most of them from outside the genitourinary tract, often of oral origin but also sometimes coming from other body sites of the mother, such as the gut, which are all thought to contribute to the development premature labor and birth. A number of bacteria have been cultured in amniotic infections including *Fusobacterium nucleatum*, a common oral species, being the most frequently isolated species from amniotic fluid, but also *Fusobacterium nucleatum, Peptostreptococcus spp*, *Porphyromonas* and *Prevotella spp*. *Eubacterium spp.* and *Eikenella corrodens* are sometimes found the amniotic fluid of women with preterm labour [205,206]. When umbilical cord blood was cultivated from healthy neonates born by cesarean section, a shocking 9/20 (45 %) demonstrated positive growth and the following species identified on 16S rDNA sequencing: *Enterococcus faecium*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Streptococcus sanguinis* [207].

Chorioamnionitis is a new entity, defined as the inflammatory response of the membranes, placenta and amniotic fluid in response to a microbial invasion of the amniotic cavity, frequently seen and associated with a greatly enhanced risk of adverse neonatal outcome [208,209]. It is likely, although the pathogenesis of this condition is yet not fully investigated and understood, that in accordance with what we know about leakage of other membranes, that these conditions are associated with Western lifestyle, and especially with Western food habits. Chorioamnionitis is most often clinically silent or diagnosed in the presence of signs of inflammatory reactions of the mother, often very early in pregnancy and more or less always associated with microbial invasion of the amniotic cavity, as documented by microbial cultures of amniotic fluid and histologic analysis of the placenta and its membranes [208-210].

Barker *et al*, in their classical series of studies, presented mainly between 1996 and 2004, provide evidence of a link between intra-uterine programming of the immune system of the infant and later in life chronic diseases, particularly cardiovascular events [211-216]. Since then, numerous clinical and experimental studies have confirmed the early developmental influences, with and without alterations in birth weight, on not only later in life cardiovascular but also pulmonary, metabolic, and psychological diseases. The intrauterine environment is dramatically and almost exclusively impacted by the overall maternal lifestyle and health. Both premature birth and low or high birth weights are most often

associated with maternal conditions including alcohol, drug and pharmaceutical consumption, use of tobacco or other toxic substances, over- as well as under-nutrition, dys-nutrition of other reasons, metabolic conditions including obesity, diabetes and hypercholesterolemia, chronic maternal stresses, infections and inflammations of other reasons [217]. A recent study suggests that the beta-cell adaptive growth, which normally occurs during gestation, does not, under the above-mentioned conditions, take place in the offspring, with risk of gestational diabetes and propagation of diabetes to the new generation [218]. Women with high degree of systemic inflammation, such as seen in psoriasis, are reported to suffer a two-fold risk of chronic diseases. It is most likely that leaky placenta and subsequent chorioamniotis will provide a satisfactory explanation for such a development, occurring at a time when the immune system of the fetus is in its most sensitive phase of "calibration" – the third trimester of pregnancy.

Effect of foods on microbiota and leaky barriers

It is almost half a century since Burkitt reported an up to 90 % decrease the in intake of plant fibers to have occurred in Western societies between 1880 and 1970, paralleled by an approximately four-fold increase in intake of calories derived from animal fat and refined sugars [220), a dramatic deterioration of eating habits, which seemingly has continued during the years up until today. More than 50% of today's diet is made up of refined carbohydrates, e.g. foods which are absorbed in the upper GI tract, which will not reach and benefit the microbiota in the lower GI tract. Another 2% of the diet is comprised of meat and refined oils, also not ideal foods for microbiota; less than 20 % of the foods consumed contain plant fibers e.g. fruits, vegetables and greens. Burkitt also reported an up to 5-fold increase in GI transit time (app 100 vs 20 hours) and 10-fold reduction in stool weight when comparing rural Africans with Europeans (600 g/day vs app 60/day) [220]. Another study at the same time, undertaken in British geriatric patients, reported transit times of a shocking >14 days in > half of geriatric patients [221].

Burkitt emphasized the association of low intake of fiber, high GI transit times, and low weight of stool not only with increasing problem of constipation, but also with the endemic of various acute and particularly chronic diseases in Western societies; including appendicitis, coronary heart disease and some cancers, particularly colorectal cancers, diverticulosis/diverticulitis and gallbladder diseases [222], as well, as was later shown, with obesity and diabetes. The dysbiosis induced by Western food habits is strongly associated with a dramatic reduction in both total numbers and diversity of bacteria at body surfaces, particularly in the gut, in comparison to individuals who live in rural areas and who most likely have lifestyle and eating habits closer to our Paleolithic forefathers. Similar differences as observed between Westerners and rural Africans are also observed when comparing microbiota sequenced from chimpanzees in the wild and in captivity, where there is a far greater presence of plant polymers and clostridia, ruminococci, and eubacteria being described in the stools of wild chimpanzees [223].

Finegold reported in 1983 that *Lb plantarum*, a lactic acid bacteria (LAB) always present in rural stools, were found in only 25% of healthy omnivorous Americans but in 65 % of healthy vegetarian Americans [224], A similar study, performed in healthy Scandinavians and published some 15 years later, reported a significant lack of common LAB normally found in rural stools; *L plantarum* 52 %, *L rhamnosus* 26 %, and *L paracasei ssp paracasei* 17 % [225], while a recent study reported significant reductions in LAB in obese Europeans compared to individuals with normal weight, in fact, no *Lb. plantarum* at all was found in the obese compared to 18% in controls (p= 0.0004) and *Lb. paracasei* in only 14.7% of the obese vs. 38% in controls (p=0.004) [226]. *L Reuteri* was associated with obesity (p=0.04) and *Bifidobacterium animalis (B. animalis,* p=0.056) and *Methanobrevibacter smithii* (M. smithii, p=0.03) with normal weight, no differences observed in *L rhamnosis, L ruminis and L salivarius* [226].

An *in vitro* study published 50 years demonstrated significant inhibition of lactobacillus growth in the presence of purified casein or wheat gluten with some LAB growing poorly and others not at all [227]. More recent observations suggest that both diets rich in protein or fat are detrimental not only to long-term health but also to microbiota. Both high protein and moderate carbohydrate diet (HPMC) and and

high protein and low carbohydrate (HPLC) diets increase proportions of branched-chain fatty acids but also the concentrations of phenylacetic acid and N-nitroso compounds [228]. The low carbohydrate diet version (HPLC) in particular resulted in significant decrease in proportions of butyrate in fecal short-chain fatty acid concentrations and in a reduction in the *Roseburia/Eubacterium* rectal group of bacteria, in parallel to greatly reduced concentrations of fiber-derived antioxidant phenolic acids, such as ferulate and its derivatives [228]. Cani and Delzenne have, in a series of studies, demonstrated that feeding high-fat diets changes the gut microbiota profile and that particularly the levels of *Bifidobacterium* spp. and *E rectale/Cl Coccoides* group are significantly reduced in animals fed a high fat diet when compared to animals receiving a standard high carbohydrate diet [229]. It is noteworthy that, as demonstrated in rodents, that *Bifidobacterium* spp. possesses unique abilities to reduce the levels of intestinal endotoxin, and thereby improve or fully restore mucosal barrier function [230,231].

Minerals, especially magnesium (Mg), are important for immune functions, for cellular replication, and also for microbes, particularly the Gram-positives. Mg is involved in > 300 biochemical processes and subclinical hypomagnesemia is known to increase the severity of the systemic inflammatory response, worsen the systemic response to endotoxins, increase the levels and the effects of endotoxemia and increase insulin resistance, thereby promoting the development of the organ injuries commonly seen in critically illness but also in various chronic diseases. Mice deprived of dietary magnesium demonstrate, within just two days, an increased systemic and intestinal inflammation, which after 4 days is accompanied by significant reduction in gut bifidobacteria content (21.5 log), a 36–50% lower mRNA content of factors known to control gut barrier function, particularly in the ileum (zonula occludens-1, occludin, proglucagon), and increased mRNA content (app 2-fold) in both the liver and intestine of tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), CCAAT/enhancer binding protein homologous protein, and activating transcription factor 4, all reflecting inflammatory and cellular stress [232]. Magnesium deficiency, clinical as well as subclinical, is commonly observed in humans as well as in farm animals. In man it is associated with common disorders such as obesity, body aches, muscle twitches, leg cramps, headaches and migraines, fatigue or low energy, restless sleep, premenstrual syndrome, chronic bowel problems, insulin resistance, hypertension, heart disease, stroke, type 2 diabetes and osteoporosis**.** It is worth observing that typical Western foods contain only small amounts of Mg (cheese 35, French fries 35, bread 24, hamburgers 20, milk 15, cream 14, butter 3 mg/100 g food) in contrast to foods more commonly consumed in rural cultures similar to Paleolithic foods (e.g. pumpkin & squash seeds each 540, cacao 20-22% 520, wheat bran 355, sesame seeds 350, wheat germs 290, almonds 280, soya beans 265, cashew nuts 260, rosehip 240, peanuts 190, beans 190 and peas 150 mg/100 g food), foods, which seemingly constitute a better substrate to enhance the growth and function of microbiota. Iron-deficient rats have significantly lower concentrations of cecal butyrate (-87%) and propionate (-72%), shown to be accompanied by significant modifications of the dominant microbial species including greater numbers of lactobacilli and *Enterobacteriaceae* but also a significant decrease of the *Roseburia spp./E. rectale* group, known as major butyrate producers. Repletion with 20 mg $FeSO_4 \cdot kg\ diet^{-1}$ did not only significantly increase cecal butyrate concentrations, it also, at least partly, restored normal bacterial populations [233]. However, substitution of iron must be done with care as a recent study in aenemic African children observed that to heavy iron fortication can be accompanied by increased risk of inducing a more pathogenic profile to gut microbiota, characterized by significant increase in the number of enterobacteria ($P < 0.005$), decrease in lactobacilli ($P < 0.0001$) and increase in fecal calprotectin concentration ($P < 0.01$), changes known to be associated with increased gut inflammation [234].

Effect of pharmaceutical drugs on microbiota and leaky barriers

It is becoming increasingly obvious that almost all pharmaceutical drugs have a negative influence on immune development and functions and probably on the microbiota, too. As discussed above, antibiotic treatment will dramatically destroy intestinal homeostasis and introduce changes that affect almost 90% of the functions of microbiota, including critical metabolic functions such as bile acid metabolism and

eicosanoid and steroid hormone synthesis [99]. Similar negative effects on microbiota have been reported in association with chemotherapy treatment for cancer; for decades this has been known to significantly damage the rapidly generating GI mucosal cells, disrupts the ecological balance, induce dysbiosis, and allow pathogens such as *Clostridium difficile* to grow [235], a bacterium found to be the causative agent in at least 20% of antibiotic-associated diarrhea (AAD) cases [236]. During chemotherapy treatment, as observed in a pediatric patient material, the total number of bacteria in fecal samples is reduced to only 10^9 per gram of dry weight feces, which is 100-fold lower than normally seen in healthy individuals, and on fluorescent in situ hybridization analysis shown to consist in an up to 10,000-fold decrease in anaerobic bacteria and a 100-fold increase in potentially pathogenic enterococci [237].

The negative effects of pharmaceutical drugs on microbiota is not only limited to antibiotics and chemotherapeutics. Negative effects on microbiota also occur with other drugs including those that, in the past, have been assumed have no or limited side effects, such as proton pump inhibitors and anti-hypertensive drugs. As examples, the offspring of mothers consuming proton pump inhibitors during pregnancy have a significantly increased risk of acquiring asthma later in life [238], while users of hypertensive drugs suffer not only significantly reduced salivation and severe mouth dryness (xerostomia) but also a documented profound oral dysbiosis [239].

New information concerning intimate cross-talk between the intestinal microbiota and the host immune system has opened new avenues. Alterations in the microbiota are known to immediately induce increased translocation of bacterial antigens and dramatically alter the host immune reaction, leading to a chronic inflammatory state and impaired metabolic function, including insulin resistance, hepatic fat deposition, insulin unresponsiveness, and excessive adipose tissue development [240]. Consequently, each decision to use pharmacological treatment may, in the future, need to be based on weighing the need of pharmacological treatment against the importance of maintaining microbiota homeostasis and preventing leakage at body surfaces. Clearly, the impact of newly developed pharmaceuticals on microbiota and immune functions, neglected in the past, should be fully investigated before products are licensed for public use.

It is very unfortunate that pharmacological treatment and bioecological treatments are in general not compatible. It is a frequently observed that pre-, pro- and synbiotic treatments are more successful in experimental animals than in man. Until today most, if not all, clinical trials using probiotic treatment have had to accept being applied merely as adjunctive interventions, i.e. in parallel to existing pharmaceutical treatment, and never having the chance to be tried as a truly alternative treatment. Particularly in critically ill patients, trials involving probiotics have always been influenced, and most likely, strongly handicapped by a parallel application of heavy antibiotic, chemotherapeutic and other similar regimens. In many, if not most, incidences the supplied probiotics have been dramatically compromised before reaching their target organs, which could well explain the absence of positive results observed, especially in the critically ill.

Over-reacting neutrophils.

Dysbiosis frequently occurs in severe trauma, major surgery and severe sepsis, often in parallel with a significant decrease in lymphocytes, a significant, sometimes disproportionate, increase in circulating and tissue neutrophils, and a persistent decline in T-4 helper lymphocytes and elevation of T-8 suppressor lymphocytes [241]. It is suggested that a T-4/T-8 lymphocyte cell ratio of < 1 is a sign of severe immunosuppression and prediction of poor outcome in conditions such as multiple and severe trauma, multiple organ dysfunction syndrome, severe acute pancreatitis but also in myocardial infarction, and in chemotherapeutic treatments, especially with oncology patients [242]. A large early increase in circulating neutrophils is always accompanied by tissue infiltration of neutrophils and is responsible for common post-trauma/postoperative dysfunctions such as paralytic ileus [243,244], bone marrow suppression, endothelial cell dysfunction, and leads to tissue destruction and organ failure, particularly in

the lungs [245-248], intestines [248], liver [249] and kidney [250]. Neutrophil infiltration of distant organs [251], particularly the lungs [245], is significantly aggravated by mechanical therapeutic efforts such as handling of the bowels during operation [243], and ventilation of the lungs [252]. Poor nutritional status, preexisting immune deficiency, obesity, diabetes and high levels of blood sugar [253] contribute to immune deterioration and to increased expressions of molecules such as NF-κB, COX-2, LOX and iNOS [253,254]. It is important to remember that a disproportionate increase in circulating neutrophils can, to a large extent, be successfully inhibited by the supplementation of antioxidants [255-257] as well as specific probiotics [258]. Supplementation of probiotics is also shown to effectively prevent neutrophil infiltration of the lung and also to reduce the subsequent tissue destruction as demonstrated in studies with inflammation induced by cecal ligation and puncture (CLP) – see further below.

Bioecological reduction of inflammation, neutrophil infiltration and tissue destruction.
Experimental animals, subjected to induced infections through cecal ligation and puncture (CLP), were treated with prophylactic supplementation using a synbiotic cocktail, Synbiotic 2000 Forte (see further below). The treatment consisted of the four LAB comprising the cocktail being injected subcutaneously at the time of trauma [259] or being supplied as an oral pretreatment for three days before the induced trauma with the whole composition, both LAB and fibers [260]. Both treatments effectively prevented both neutrophil accumulation in the lung tissues (Table 1) and pulmonary tissue destruction (Fig 1). Significant reductions in parameters associated with the degree of systemic inflammation, such as myeloperoxidase (MPO, Table 2), malondialdehyde (MDA, Table 3) and nitric oxide (NO, Table 4), indicated a significant suppression of trauma-induced inflammation, all differences between the treatment and placebo groups in the two studies being statistically significant (< 0.05) [260].

Personal experience with pro- and synbiotics
My personal interest in microbiota and probiotics started in the early 1980's. Since 1963 I have been involved in the development of liver extensive surgery and active in the search for new tools to combat the unacceptably high rate of peri-operative infections, which was and still is associated with major surgery in general and in particularl with extensive liver resections. At that time it was standard practice to treat patients with an antibiotic umbrella for at least the first five post-operative days, in the belief that this treatment would reduce the rate of post-operative infections. However, a review of our last 81 liver resections gave unexpected information, which directed my interest to human microbiota and the possibility of using probiotics as an alternative infection prophylaxis. From this study it was shown that only 57/81 patients had, in fact, received antibiotic treatment; this prophylaxis had been neglected in the remaining 24/81 patients [261,262]. It was surprising that there were no cases of sepsis in the group of patients, who had not received prophylactic antibiotics with sepsis incidence confined to the antibiotic-treated patients. There was at that time a growing awareness of the importance of human microbiota [263] and to contemporaneous published studies that had attempted to recondition the gut through the supply of lactobacilli [264]. There was also at that time a growing understanding that not only disease but lifestyle and prescribed chemicals and pharmaceuticals, could impair microbiota immune defense. The use of probiotic treatment, as an alternative means of preventing unwanted infections in disease in general but particularly in surgical and medical critically ill patients, appeared an attractive option. This was the reason why I established collaborative efforts with experts in microbiology, chemistry, nutrition and experimental and clinical science to seek, develop and test probiotics both experimental and clinically, which could be expected to constitue powerful tools to prevent sepsis of various kinds.

Interdisciplinary collaboration in the early 1990's lead to the identification of some *L plantarum* strains that demonstrated strong anti-inflammatory capacities. *L plantarum* 299, later used together with oatmeal in a synbiotic composition [265-267], is produced and marketed by Probi AB, Lund, Sweden. I participated heavily in this program until 1999, when I decided to re-direct my interest towards development and studies of a more complex synbiotic composition, designed not only to supplement four newly identified bioactive LABs in combination but also four different prebiotic fibers, already known for

their strong bioactivity. Our aim was to provide this composition in much larger doses than was the practice at that time. Furthermore, knowing that most of the important LABs rarely exist in the microbiota of Westerners encouraged us to seek potent probiotic bacteria normally growing on plants instead of selecting bacteria normally found in human microbiota

Since 1999, all my efforts in this field have concentrated on a four LAB/four fiber composition, consisting of either a mixture of 4 x 10^{10} (40 billion LAB, Standard version - Synbiotic 2000™) or a mixture of 10^{11} (400 billion Forte version - Synbiotic 2000 Forte™) based on the following four LAB: *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* subsp paracasei 19, and *Lactobacillus plantarum* 2362 in combination with 4 x 2.5 g of each of the following four fermentable fibres: betaglucan, inulin, pectin and resistant starch, in total 10 gr of prebiotic fibers per dose [268,269], a formula that is currently marketed by Synbiotic AB, Höganäs, Sweden.

Perioperative prophylaxis in elective surgery.
L plantarum 299 in a dose of 10^9 plus a total of 15 gram of oat and inulin fibers was tried, under research condition, in patients undergoing extensive abdominal surgical operations. The patient were mainly derived from those undergoing liver, pancreatic and gastric resections, equally distributed between three groups and supplemented with either: 1. live LAB and fiber, 2. heat-inactivated LAB and fiber, and 3. standard enteral nutrition [271]. Each group comprised 30 patients. The 30-day sepsis rate was 10 % (3/30 patients) in the two groups receiving either live or heat-inactivated LAB, compared to 30 % (9/30 patients) in the group on standard enteral nutrition (p=0.01) [270]. The largest difference was observed in incidence of pneumonia: Group1 2 patients; Group 2 1 patient; Group 3 6 patients. The beneficial effects of treatment were seemingly most pronounced in gastric and pancreatic resections; the sepsis rate being: Group 1 - 7 %, Group 2 - 17 % and Group 3 - 50 %. The same pattern was observed for non-infectious complications: Group 1 - 13% (4/30) Group 2 17% (5/30); Group 3 - 30 % (9/30). The supply of antibiotics to Group 1 was significantly less (p=0.04) than to the other two groups, with the mean length of antibiotic treatment also considerably shorter: Group 1 - 4$\pm$3.7 days; Group 2 - 7$\pm$5.2 days; Group 3 - 8$\pm$6.5 days.

In a prospective, randomized, double-blind trial 80 patients undergoing pylorus-preserving pancreatoduodenectomy (PPPD) received, twice daily, either Synbiotic 2000™ (2x40 billion LAB, i.e. 80 billion LAB per day) or only the fibers in composition from the day before surgery and during the first seven postoperative days [271]. A highly significant difference in infection rate (p=0,005) was observed as only 5/40 patients (12.5 %) in the Synbiotic 2000-treated group suffered infections (4 wound and one urinary tract infection) vs 16/40 (40 %) in the fiber-only group (6 wound infections, 5 peritonitis, 4 chest infections, 2 sepsis, and one of each of urinary tract infection, cholangitis and empyema). The number of infecting microorganisms were also statistically and significant reduced – see table 5. Statistically significant differences between the groups were also observed regarding the use of antibiotics (mean: Synbiotic 2000; 2$\pm$5 days, Only-fibers; 10$\pm$14 days) [271].

In another randomized controlled study 45 patients undergoing major surgery for abdominal cancer were divided into three treatment groups: 1. enteral nutrition (EN) supplemented with Synbiotic 2000 (LEN), 2. EN supplemented with only the fibers in the same amounts (20 g) (20 g) as in Synbiotic 2000™ (FEN) and 3. standard parenteral nutrition (PN). All treatments lasted for 2 preoperative and 7 days postoperative days. The incidence of postoperative bacterial infections was 47 % with PN, 20 % with FEN and 6.7 % with LEN (p<0.05). The numbers of infecting microorganisms were also statistically and significantly reduced – see table 6. Significant improvements were also observed in prealbumin (LEN, FEN), C-reactive protein (LEN, FEN), serum cholesterol (LEN, FEN), white cell blood count (LEN), serum endotoxin (LEN, FEN) and IgA (LEN) (Han Chun Mao, personal information).

Perioperative prophylaxis in liver transplantation

A prospective, randomized, study in 95 liver transplant patients supplemented *L plantarum* 299 in a dose of 10^9 plus 15 gram of oat and inulin fiber [272]. Three groups of patients were studied: 1. selective digestive tract decontamination (SDD) four times daily for six weeks, 2. *L plantarum* 299 (LLP) in a dose of 10^9 plus 15g of oat and inulin fibres supplied postoperatively for 12 days, and 3. identical to group 2 but with heat-killed *L plantarum* 299 (HLP). Identical enteral nutrition was supplied to all patients from the second postoperative day. The numbers of postoperative infections were SDD 23, LLP 4 and HLP 17. Signs of infections occurred in SDD 48 % (15/32), in LLP 13 % (4/31), p=0.017 nd HLP 34 % (11/32) respectively. The most dominant infections were cholangitis (which occurred: SDD 10, LLP in 2, and HLP in 8) and pneumonia (which occurred: SDD in 6, in LLP in 1, and HLP in 4). There was a statistically significant reduction in the numbers of infecting microorganisms, the most often isolated microbes being *Enterococci* and *Staphylococci*. Patients requiring haemodialysis were SDD: 8; LLP: 2 and HLP: 4 and the number of re-operations SDD: 6; LLP: 4 and HLP: 2 respectively. There were no deaths. The stay in ICU, the hospital stay and length on antibiotic therapy was shorter in the LLP group, but did not reach statistical significance. The CD4/CD8 ratio was alsohigher in the LLP group compared to the other two groups (p=0.06).

In a subsequenct study, 66 human orthotopic liver transplant patients were randomized to either receive Synbiotic 2000 or only the fibers in Synbiotic 2000. The treatment was started on the day before surgery and continued for 14 days after surgery. During the first postoperative month only one patient in the Synbiotic 2000-treated group (3 %) show signs of infection (urinary infection) compared to 17/33 (51%) patients in those supplemented with only the four fibers [273]. Only one infecting organism was cultivated in the Synbiotic-treated group, which was shown to be *Enterococcus fecalis*, in contrast to seventeen organisms in the fiber- only treated group – see table 7. The use of antibiotics was on average 0.1$\pm$0.1 d in the Synbiotic-treated patients and 3.8$\pm$0.9 d in the fiber- only treated group [273].

Early treatment in major trauma

Two prospective randomized trials with Synbiotic 2000 and Synbiotic 2000 Forte respectively were undertaken. The first study compared in patients with acute extensive trauma four types of treatment: 1. Synbiotic 2000 (40 billion LAB/d), 2. A soluble fiber, 3. A peptide diet (Nutricomp, Braun Inc Germany) and 4. Glutamine supplementation [274]. Treatment with Synbiotic 2000™ lead to a highly significant reduction in number of chest infections (4/26 patients - 15 %) compared to peptide diet (11/26 patients - 42 %, p<0.04), glutamine treatment (11/32 patients - 34 %, p<0.03) and only fiber treatment (12/29 patients- 41 %, p<0.002) [275]. Also the total number of infections were significantly decreased; Synbiotic 2000™ 5/26 patients (19 %), peptide 13/26 patients (50 %) glutamine16/32 patients (50 %) and only fibers 17/29 patients (59 %) [274].

In the other study 65 polytrauma patients were randomized to receive once daily, for 15 days following major trauma, either Synbiotic 2000 Forte (400 billion LAB + 10 gram of fibers, see above) or maltodextrine, as placebo. Significant reductions were observed between the groups in the number of deaths (5/35 vs 9/30, p<0,02), severe sepsis (6/35 vs 13/30, p<0.02), chest infections (19/35 vs 24/30, p<0.03), central line infections (13/32 vs 20/30, p<0.02), and ventilation days (average 15 vs 26 days [66]) [275]. A total of 54 pathogenic microorganisms were cultivated in the Synbiotic treated group compared to 103 in the maltodextrine group – see table 8 [275-277]. Repeat analyses also revealed that serum levels of endotoxin (LPS) were decreased and 'time to bloodstream infection' significantly prolonged in patients treated with Synbiotic 2000 Forte.

Early treatment in severe acute pancreatitis

In a further study, patients with severe acute pancreatitis were randomized to receive either a freeze-dried preparation containing live *L plantarum* 299 in a dose of 10^9 together with a substrate of oat fiber or a similar preparation but heat-inactivated, administered daily through a nasojejunal tube for seven days [278]. The study was concluded when, on repeat statistical analysis, significant differences in favour of

one of the two groups were obtained. This occurred when a total of 45 patients had entered the study. 22 patients had, at that time, received treatment with live, and 23 with the heat-killed, *L plantarum* 299. Infected pancreatic necrosis and abscesses were seen in 1/22 (4.5%) in the live LAB group vs. 7/23 (30%) in the heat-inactivated group (p=0.023). The only patient in the lactobacillus group, who developed infection, a urinary infection, did so on the fifteenth day, i.e. at a time when he had not received treatment for eight days. The length of stay was also considerably shorter in the live LAB group (13.7 days vs. 21.4 days) but the limited size of the material menat that statistical significance was not reached [278].

Sixty-two patients with severe acute pancreatitis (SAP) (Apache II scores: Synbiotic 2000-treated 11.7±1.9, controls 10.4±1.5) were given either two sachets/day of Synbiotic 2000™ (2x40 billion LAB/day and totally 20 g fibers) or the same amounts of fibers (20 g) as in Synbiotic 2000™ during the first 14 days after arrival at the hospital [279]. 9/33 patients (27 %) in the Synbiotic 2000-treated group and 15/29 patients (52 %) in the fiber-only treated group developed subsequent infections. 8/33 (24%) of the Synbiotic 2000-treated and 14/29 (48%) of the fiber-only treated patients developed SIRS, MOF or both (p<0.005). A total of seven pathogenic microorganisms were cultivated in the Synbiotic-treated group compared to seventeen in the fiber-only group – see table 9. Another, as yet unpublished, study in patients with severe acute pancreatitis compared 32 patients treated with Synbiotic 2000 Forte with 30 control patients. Eight patients in the treated group suffered septic episodes compared to 21 in the control group (Pupelis G, personal information). Late MODS occurred in 1 patient vs 9 in the control group, the mortality was 0% vs 17%, hospital stay 23 vs 36 days, and stay in ICU 8 vs 16 days. Three patients in the treated group vs 12 in the control group underwent surgical operations.

Effects on "mind clarity" – encephalopathy

Patients with critical illness, as well as patients with chronic disorders such as liver cirrhosis and diabetes, frequently suffer a mild but sometimes severe confusion, which often has its origin in the gut [280]. Increasing evidence suggest that probiotics, alone but also in combination with plant antioxidants and fibers, possess strong neuro-endocrine modulatory effects and can alleviate the effects of physical and mental stressors [281,282]. We undertook some studies to explore the effects of Synbiotic in patients with liver cirrhosis and minimal encephalopathy (MHE) [283]. Fifty-five patients with MHE were randomized to receive for 30 days: 1. Synbiotic 2000 (n=20), 2. the fibers in the composition alone (n=20), or 3. a placebo (n=15). All cirrhotic patients with MHE were found to have severe derangements of the gut micro-ecology and significant overgrowth of potentially pathogenic *Escherichia coli* and *Staphylococcal* species. Synbiotic treatment significantly increased the fecal content of non-urease-producing *Lactobacillus* species and reduced the numbers of potentially pathogenic micro-organisms. The treatment was also associated with a significant reduction in endotoxemia and in blood ammonia levels. A documented reversal of MHE was obtained in half of the treated patients, while the Child-Turcotte-Pugh functional class improved in about 50% of cases [283]. Treatment with fermentable fibers alone also demonstrated substantial benefits in a proportion of patients.

In a second study, 30 cirrhotic patients were randomized to receive either Synbiotic 2000 or placebo for only 7 days [284]. Viable fecal counts of Lactobacillus species, Child-Pugh class, plasma retention rate of indocyanine green (ICGR15), whole blood tumour necrosis factor alpha (TNF-a) mRNA and interleukin-6(IL-6) mRNA, serum TNF-a, soluble TNF receptor (sTNFR)I, sTNFRII and IL-6 and plasma endotoxin levels were measured, pre- and post-treatment. The treatment with Synbiotic 2000 was associated with significantly increased fecal lactobacilli counts and significant improvements in ICGR15 and Child-Pugh class. Significant increases in whole blood TNF-a mRNA and IL-6 mRNA, along with serum levels of sTNFRI and sTNFRII, were also observed and TNF-a and IL-6 levels correlated significantly, both at baseline and post-Synbiotic treatment. Synbiotic-related improvement in ICGR15 was accompanied by significant changes in IL-6, both at mRNA and protein levels, but this was unrelated to levels of plasma endotoxin. No significant changes in any parameter were observed following placebo treatment. This study concluded that even short-term synbiotic treatment significantly modulated gut flora and improved

liver function in patients with cirrhosis [284]. Minimal encephalopathy is common not only in liver cirrhosis but is also seen in other chronic diseases such as diabetes. The observations in patients with liver cirrhosis gives hope that Synbiotic treatment may also be effective in other chronic diseases.

Effects in HIV

It is well documented that disturbance of the microbiota occur early in HIV-1 infection, which leads to greater dominance of potential pathogens, reduced levels of bifidobacteria and lactobacillus species and increasing mucosal inflammation. Current and emerging studies support the concept that probiotic bacteria can provide specific benefit in HIV-1 infection. It was not until Brenchley *et al.* in 2006 identified translocation of microbes or microbial products without overt bacteremia, as a major cause of systemic immune activation in HIV-1 and SIV infection [285], that a greater interest in bio-ecological treatment emerged.

Impairment of the GI tract in HIV-positive patients is already present in the early phases of HIV disease and is associated with elevated levels of intestinal inflammatory parameters and definite alterations in the gut commensal microbiota, confirming a possible correlation between intestinal microbial alteration, GI mucosal damage, and immune activation status, further confirming that alterations at the GI-tract level are a key factor in the pathogenesis of chronic HIV infection [286]. The findings, in a recent study, of fairly mild changes in microbiota of HIV-infected individuals, before intiation of pharmacological treatment, might suggest that the later observed more profound alterations in microbiota could be pharma-induced, as only a trend to a greater proportion of Enterobacteriales compared to control subjects ($P = 0.099$) were observed, despite the significant negative correlations between total bacterial load and duodenal CD4+ and CD8+ T-cell activation levels [287]. As pointed out in a recent review, current and emerging studies appear to support the concept that probiotic bacteria can provide specific benefit in HIV-1 infection. Probiotic bacteria have proven active against bacterial vaginosis in HIV-1 positive women and have enhanced growth in infants with congenital HIV-1 infection [288]. Probiotic bacteria may also stabilize CD4+ T cell numbers in HIV-1 infected children and are likely to have protective effects against inflammation and chronic immune activation of the gastrointestinal immune system [288].

Recent studies at least partly support the assumption that *L rhamnosis* GR-1 and *L Reuteri* RC-14 tend to increase the probability of a normal vaginal flora (odds ratio 2.4; P=0.1) and significantly increase the probability of a beneficial vaginal pH (odds ratio 3.8; P=0.02) at follow-up [289,290]. However, later attempts using probiotic yoghurts have proven less successful [291]. In a recent pilot study 38 women with HIV, taking highly active antiretroviral therapy (HAART), were supplemented with Synbiotic 2000 Forte orally for 4 weeks [292]. In a surprising and very encouraging observation, the supplemented formula showed ability, despite heavy pharmaceutical treatment, to survive during the passage through the GI tract, and also the ability to colonize the gut and contribute to a significantly elevated level in the stool of the supplemented LAB group. The T-cell activation phenotype was altered by exposure to the Synbiotic formula and was accompanied by a slightly elevated HLA-DR expression of a minor population of CD4+ T-cells, which normally lack expression of HLA-DR or PD-1. These significant changes occurred in the context of unaltered microbial translocation, as measured by plasma bacterial 16S ribosomal DNA [292]. It is especially encouraging that the LAB supplemented with Synbiotic 2000, despite heavy medication/highly active antiretroviral therapy (HAART), were able to colonize the gut and seemingly, at least slightly, improve immune functions. Hopefully, significantly more pronounced positive effects will be obtained the day we are ready to try eco-biological treatment, not only as complementary treatment but as an alternative to pharmaceutical treatment.

Life-threatening systemic inflammation

A study of patients in intensive care suffering life-threatening extreme systemic inflammation - a systemic inflammation response syndrome (SIRS) - and its relation to gut microbiota was recently published. Twenty-five patients with severe SIRS and a serum C-reactive protein level > 10 mg/dL were studied

[293). Analysis of gut microbiota revealed markedly lower total anaerobic bacterial counts, particularly of the beneficial Bifidobacterium and Lactobacillus and higher counts of total facultative anaerobes such as Staphylococcus and Pseudomonas compared to healthy volunteers. In patients with bacterial translocation, Gram-negative facultative anaerobes were the most commonly identified microbial organisms in mesenteric lymph nodes and serosal scrapings at laparotomy. Gastrointestinal complications were strongly associated with a significantly reduced number of total obligate anaerobes and highly increased numbers of Staphylococcus and Enterococcus and significantly decreased numbers of total obligate anaerobes and total facultative anaerobes [293].

A more recent study in 63 similar patients suggests impaired gastrointestinal motility as a significant marker of poor outcome [294]. Patients with ≥ 300 mL per day reflux from nasal gastric feeding tube demonstrated significantly lower numbers of total obligate anaerobes including Bacteroidaceae and Bifidobacterium, higher numbers of Staphylococcus, lower concentrations of acetic acid and propionic acid, and higher concentrations of succinic acid and lactic acid ($P \leq 0.05$), accompanied by dramatically higher incidences of bacteremia (86%vs 18%) and mortality (64%vs 20%) than patients without gastric detension ($P \leq 0.05$) [294]. Furthermore, in 29 similar patients treatment with a synbiotic composition, consisting of *Bifidobacterium breve* and *Lactobacillus casei*, in combination with galactooligosaccharides, was attempted. Higher levels of *Bifidobacteria* and *Lactobacillus*, but also total organic acids, particularly short-chain fatty acids, were reported and the incidence, compared to historical controls, of infectious complications such as enteritis, pneumonia, and bacteremia, observed to be significantly lower in the treated group [295].

Studies with no or adverse effects

Ecologic 641™

In a multicenter randomized, double-blind, placebo-controlled trial, 298 patients with predicted severe acute pancreatitis and with APACHE II score > or = 8, Imrie score > 3, or C-reactive protein >150 mg/L) were, within 72 h of onset of symptoms, randomly assigned to receive either a multi-species synbiotic composition (n=153) or a placebo (n=145), administered enterally twice daily for 28 days [296]. The supplemented synbiotic composition, Ecologic 641 (Winclove Bio Industries, Amsterdam, Netherlands), consists of 10^{10} of each of six different strains of freeze-dried, viable bacteria: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* plus cornstarch and maltodextrins. Infectious complications occurred in 46 (30%) of patients in the treated group and in 41 (28%) in the placebo group (relative risk 1.06, 95% CI 0.75-1.51). Twenty-five (16%) patients in the synbiotic group died, compared to nine (6%) in the placebo group (relative risk 2.53, 95% CI 1.22-5.25) [296]. Furthermore, nine patients in the synbiotics group developed bowel ischemia, of which eight had fatal outcomes, compared to none in the placebo group (p=0.004).

Lactobacillus plantarum 299™ - ProViva™

One hundred and three critically ill patients were randomized to receive 1. an oral preparation containing L. plantarum 299v, ProViva, a fruit drink containing 5 % of LAB-fermented oat and live *Lactobacillusb plantarum* 299V with a density of $5x10^7$ (n=52) or 2, conventional nutrition therapy alone (n = 51). The treatment demonstrated no identifiable effect in terms of bacterial translocation (12 % vs 12 %; p=0.82), gastric colonization with enteric organisms (11 % vs 17 %, p=0.42), or septic morbidity (13% vs 15 %; p=074), serum CRP levels or mortality [297]. In another study, 11 patients undergoing elective abdominal surgery receive *L plantarum* 299v, (ProViva) for a median time of 9 days (range 5-18 days) to a total average amount of 3250ml (range 2100-9000 ml) and were then compared to 11 control patients. The authors found no significant differences between the *L plantarum* 299v group and the control group in terms of concentrations of plasma cells, IgA positive cells or IgM positive cells in the lamina propria

[298]. A significantly higher concentration of IgM at the mucosal surface was observed in the control group (P = 0.02, Fishers Exact test mid P) but no difference in terms of IgA.

Lactobacillus rhamnosus GG™
Sixty-one patients in a pediatric ICU study were randomized with 31 patients receiving treatment with one capsule of *Lactobacillus rhamnosus* strain GG in a dose of 10×10^9 and 30 receiving one capsule of inulin daily (control group) [297]. No differences in rate of infections were observed between the groups; the mean number of infections in the treatment and control groups was 1.83 and 1.33, respectively. 9/31 patients in the probiotic-treated group developed in total 15 nosocomial infections: 6 bloodstream infections (40%), 5 tracheo-bronchitis (33%), 2 pneumonia (13%), and 2 UTI (13%). There were six deaths in total during the study period; four in the placebo group and two in the treatment group. No cases of *Lactobacillus* bacteremia or other serious adverse effects were observed [299].

Synbiotic 2000™/Synbiotic 2000 Forte™
Two hundred and fifty nine enterally fed critically ill patients, expected to require mechanical ventilation for 48 hours or more were enrolled in a study; 130 patients received Synbiotic 2000 FORTE® (twice a day) and 129 patients, a cellulose-based placebo for a maximum of 28 days [230]. The oropharyngeal microbial flora and colonization rates were unaffected by the synbiotic treatment. The overall incidence of ventilator associated pneumonia (VAP) was lower than anticipated (11.2%) and no statistical difference was demonstrated between the groups receiving synbiotic or placebo; incidence of VAP (9 and 13%, P=0.42), VAP rate per 1000 ventilator days (13 and 14.6, P=0.91) or hospital mortality (27 and 33%, P=0.39), respectively. No negative effects of the treatment were observed [300].

Trevis™
A total of 90 patients admitted to an ICU were randomized to receive either a synbiotic or placebo (45 into each group) [301]. The synbiotic treatment consisted of the supply of a capsule of Trevis™ (Chr Hansen Biosystem, Denmark) three times a day, containing 4×10^9 colony forming units of each of *L. acidophilus* La5 (La5), *B. lactis* Bb-12 (Bb-12), *S. thermophilus* and *L. bulgaricus*. In addition, the prebiotic oligofructose (7.5 gm of RaftiloseTM powder, Orafti Active Food Ingredients, Belgium) was administered twice a day. The patients in the synbiotic group demonstrated, after 1 week of therapy, significantly lower incidence of potentially pathogenic bacteria (43% versus 75%, P = 0.05) and multiple organisms (39% versus 75%, P = 0.01) in their nasogastric aspirates, than the controls. However, there were no significant differences between the groups in terms of intestinal permeability, septic complications or mortality [301].

VSL#3™
Twenty-eight patients critically ill patients were enrolled and randomly assigned to one of 3 treatment groups: 1. placebo (n=9) 2. viable probiotics - 2 sachets daily of VSL#3™ (n=10) or 3. bacterial sonicates - non viable VSL#3 bacteria (n=9) [302]. Each sachet of the supplemented probiotic, VSL#3 (VSL Pharmaceuticals, Ft Lauderdale, FL) contained 900 billion viable lyophilized bacteria of 4 strains of *Lactobacillus* (*L. casei, L. plantarum, L. acidophilus,* and *L. delbrueckii subsp. Bulgaricus*) plus 3 strains of *Bifidobacterium* (*B. longum, B. breve, and B. infantis),* plus *Streptococcus salivarius subsp. Thermophiles,* totally eight strains. Intestinal permeability decreased in all treatment groups. The rate of severe sepsis and MODS were not significantly affected by the treatment, although a significantly larger increase in systemic IgA and IgG concentrations were observed in the group supplied live bacteria than in the patients who received placebo or sonicated bacteria (*P*_0.05) [302].

Why do studies fail?
Critical care units are generally highly artificial environments and the burden of environment-induced physical and mental stress and subsequent status of systemic hyper-inflammation on the patient,

enormous. Patients treated under these conditions are in many ways dys-functional; the whole microbiota has often, more or less, disappeared and probiotic bacteria supplied will usually be extinct before they reach one of their targets – the lower gastrointestinal tract. This artificiality seems to vary from country to country and sometimes also from hospital to hospital, an observation that might explain the great variation in outcome from studies undertaken in different countries and regions.

As discussed above, probiotic treatment has never been given the chance as a 'stand alone' alternative. It has, thus far, only been tried as an adjunctive treatment to heavy multi-drug pharmaceutical treatments. Many of the numerous drugs used in the ICU, including antibiotics are known to derange not only the microbiota but to dramatically derange the majority of immune functions. Some 25 years ago the use of an antibiotic, Mezlocillin (Bayer, 150 mg/kg body weight) was demonstrated to significantly suppress essential macrophage functions, derange chemiluminescence response, chemotactic motility, bactericidal and cytostatic ability and impair lymphocyte proliferation, impair macrophage functions and bactericidal efficacy as well as production and secretion of cytokines [303].

Artificial nutrition, both enteral and parenteral, is an important contributor to ICU-associated sepsis; catheter-related sepsis is reported to occur in about 25% of patients fed via intravenous feeding-tubes [304]. Other common perioperative practices, e.g. use of artificial feeding regimens, preoperative antibiotics [305], and mechanical bowel preparation [306,307] will, instead of preventing expected infections, contribute to increased rates of treatment-associated infections. Other measures in the ICU such as mechanical ventilation [308], treatment with various pharmaqceutical drugs, including antibiotics [309,310], chemical solutions for clinical nutrition and many others promote super-inflammation and, indirectly, infection.

Enteral nutrition formulas, most likely deleterious to microbiota, are known to induce loss of intestinal barrier function, promote bacterial translocation, and impair host immune defense [311], a phenomenon, observed in humans but also extensively elucidated in animal studies. In such studies the incidence of bacterial translocation to the mesenteric lymph node was significantly increased when the animals were fed nutrition formulas such as Vivonex (53%), Criticare (67%), or Ensure (60%) ($p < .05$) [310-312]. Dramatic elevations in pro-inflammatory cytokines have been observed in patients, when fed a standard enteral nutrition solution (Nutrison) following pancreat-duodenectomy, e.g. IL-1beta day 7 ($P<0.001$); day 14 ($P=0.022$), TNF-alpha- day 3 ($P=0.006$); day 7 ($P<0.001$) [313]. Of special interest are the observations that such changes are not observed when the standard nutrition is replaced with a formula whichis claimed to have immune-modulatory effects (Stresson). Instead anti-inflammatory cytokines were seen to be significantly elevated: IL-1ra/s : day 7 ($P<0.001$); IL-6: day 10 ($P=0.017$); IL-8: day 1 ($P=0.011$) days 3, 7, 10, and 14 ($P<0.001$), and IL-10: days 3 &10 ($P<0.001$) [313].

Choice of lactic acid bacteria as probiotics.

The choice of bacteria for probiotic purposes is critical. Only a few LAB strains have demonstrated an ability to influence the immune system, reduce inflammation and/or eliminate or reduce unwanted pro-inflammatory molecules from foods. Even strains that carry the same name can have different and even sometimes opposite effects. A recent study selected 46 strains of *Lactococcus lactis* from about 2,600 LAB and compared their ability to induce cytokines. It was demonstrated that the inter-strain differences in ability to produce pro- and anti-inflammatory cytokines was great [314], an observation that underlines the importance of extensive animal and preclinical studies before a LAB or combination of LAB is chosen as a probiotic. Strains that improve immune function by increasing the number of IgA-producing plasma cells, improve phagocytosis, and influence the proportion of Th1 cells and NK cells [315] are particularly desirable for probiotic purposes. Popular probiotic species that are usually reliable and available commercially are *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, *L. johnsonii*, *L. fermentum*, *L. reuteri*, *L. plantarum*, *Bifidobacterium longum* and *Bifidobacterium animalis* [111]. Among the strains

with documented stronger anti-inflammatory functions are *Lactobacillus paracasei* subsp *paracasei, Lactobacillus plantarum,* and *Pediococcus pentosaceus. Lactobacillus paracasei*, in particular, seems to have a solid record; it has been shown to induce cellular immunity and stimulate production of suppressive cytokines such as TGFβ and Il-10 and to suppress Th2 activity and CD4 T-cells [316,317], suppress splenocyte proliferation [318] and decrease antigen-specific IgE and IgG1 [319]. When more than one hundred LAB strains were compared *Lactobacillus paracasei* was shown to be the strongest inducer of Th1 and repressor of Th2 cytokines [320].

A recent study using rats compared the ability of four different strains: *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Bifidobacterium longum*, or *Bifidobacterium lactis* to control *Trichinella spiralis*-induced infection. *Lactobacillus paracasei,* alone was able to reduce infection-associated Th2 response, muscle levels of TGF-β, COX-2 and PGE2 and to attenuate infection-induced muscle hyper-contractility [321]. Another study compared the ability to reduce stress-induced changes in gut permeability and sensitivity to colorectal distension of three probiotic strains: *Bifidobacterium lactis* NCC362, *Lactobacillus johnsonii* NCC533, and *Lactobacillus paracasei* NCC2461. *Lactobacillus paracasei,* alone restored normal gut permeability, reduced visceral hyperalgesia and reduced visceral pain [322]. Several other important clinical effects of *Lactobacillus paracasei* subsp *paracasei* are summarized in a recent review; a strain called NTU 101 and its fermented products demonstrating the ability to reduce blood cholesterol, blood pressure, and prevent allergies, osteoporosis and inhibit accumulation of fat tissue [323]. *Lactobacillus plantarum* also has an excellent record. When the ability of fifty different LAB to control twenty-three different *Clostridium difficile* (C diff) strains were studied, *Lactobacillus paracasei* and *Lactobacillus plantarum* seemed to be equally efficient and the only strains of the fifty tried, to demonstrate the ability to effectively eliminate all *C diff* strains - more than half of the tried LAB strains were totally ineffective, and some effective only against a few [324].

Some LAB seem to be potentiated in their efficacy by simultaneous supply of prebiotic fibers (probiotics + prebiotics => synbiotics). However, there are great differences in the ability of different strains to ferment and utilize plant fibers, especially when it comes to semi-fermentable fibers such as oligofructans. Only a handful of 712 LAB strains tested demonstrated an ability to ferment inulin and phlein, namely : *L plantarum* (several strains), *L paracasei* subsp. p*aracasei, L brevis & Pediococcus pentosaceus* [325].

Molecular gene targeting – the future?

Ingredients specific to certain plants are known to exert profund effects on specific genes. Among these agents are curcumin (turmeric), resveratrol (red grapes, peanuts and berries), genistein (soybean), diallyl sulfide (allium), S-allyl cysteine (allium), allicin (garlic), lycopene (tomato), capsaicin (red chilli), diosgenin (fenugreek), 6-gingerol (ginger), ellagic acid (pomegranate), ursolic acid (apple, pears, prunes), silymarin (milk thistle), anethol (anise, camphor, and fennel), catechins (green tea), eugenol (cloves), indole-3-carbinol (cruciferous vegetables), limonene (citrus fruits), beta carotene (carrots), and dietary fiber [326,327].

Curcumin, for example, has demonstrated a profound ability to inhibit a whole series of cell-signaling pathways, including NF-kB, AP-1, STAT3, Akt, Bcl-2, Bcl-X(L), caspases, PARP, IKK, EGFR, HER2, JNK, MAPK, COX2, and 5-LOX [255,326,327]; supplemented probiotics are likely to exert similar effects. Gene expression of human duodenal mucosa cells were studied after exposure to one of the following four lactic acid bacteria; *Lactobacillus plantarum* WCFS1 [328], *Lactobacillus acidophilus* L10, Lactobacillus *casei* CRL-431 and *L. rhamnosus* GG [329], administered in a cross-over study to healthy volunteers in a dose of 10^{10}. Mucosal biopsies were taken from duodenum after 6 hours and compared to control biopsies. The interventions did not impair immune and metabolic homeostasis

but a fascinating and most distinct influence on the expression of several hundred genes (transcriptome) was reported after administration of each of the studied LAB. This is possibly the first time that different probiotic lactobacilli have been reported to induce more or less strain-specific and markedly different expression profiles, very similar to what is known to occur with ingestion of various foods, especially plant ingredients [326,327] and in many respects similar tp what is observed after supply of certain pharmaceuticals. *L. plantarum* was observed to specifically modulate overt adaptive immune responses [326], *L. acidophilus* to suppress inflammation, *L. casei* to stimulate Th1 response and improve the Th1–Th2 balance and *L. rhamnosis* to influence cellular growth and proliferation [327]. These effects were suggested to resemble, although in a distinctly milder form, those obtained by specific pharmaceuticals:

- *L. acidophilus* - antagonists of α-receptor activity, guanine antagonists, synthetic corticosteroids and flavonoids,
- *L casei* - modulators of GABA receptors, cholinergic blocking agents, antagonists of β-adrenergic receptors,
- *L. rhamnosus* - glycoside steroids, alkaloids, protein synthesis inhibitors and protein kinase C inhibitors.

The responsiveness to ingestion of various LAB seems to be strongly influenced, not only by eventual genetic background and existing resident microbiota, but also by lifestyle, and particularly by diet, which might explain the differences in person-to-person response, as observed in the above studies but also the differences in outcome, often encountered in clinical probiotic studies, and especially when tried with critically ill patients (see above).

It is all about inflammation.

Inflammation, an essential component of immune-mediated protection against pathogens and tissue damage, and uncontrolled immune responses, will commonly, especially in Westerners, institute a state of chronic inflammation, which will occur when immune response are activated despite the absence of 'danger' signals, fail to fully turn-off despite elimination of danger signals and/or fail to completely clear such signals. Numerous factors, in addition to genetic predisposition, trauma and various stress factors (physical and emotional) are known to contribute to increased discrete and long-lasting inflammation, among them age, diet and medications.

Studies of human gene-related inflammation suggest that, of the approximately 25,000 human genes, approximately 5 %, or some 1,200 genes, are involved in inflammation [328-330]. It is increasingly understood that the human genome in itself will only explain a minority of chronic diseases, far less than changes in lifestyle, food habits and social behavior, factors which seem to have a dominating impact on human health. Clearly, the molecular mechanisms linking environmental factors and genetic susceptibility was first envisioned after the recent exploration of the, until recently hidden, source of genomic diversity, i.e. the metagenome with its more than 3 million genes [331]. Although the mechanisms behind the metagenome-associated low-grade inflammation and the corresponding immune response are not yet fully understood, there is no doubt that the metagenome has a dominating influence on altered body functions such as adipose tissue plasticity and diseases such as hepatic steatosis, insulin resistance and cardiovascular diseases, but also on disorders such as autoimmune diseases including rheumatoid arthritis, gastrointestinal and neuropsychiatric diseases and on development and progress of a number of cancers [332], as well as many other chronic disorders. When disease exacerbations occur, in trauma or in critical illness, the normally silent or discrete inflammation turn into a storm [333] as experienced in systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) [334]. In many severe conditions like MOF and SIRS components of cytokine-induced injury might be more damaging than the initial cause/trauma/early invasion of micro-organisms in themselves. Inflammatory cytokines, such as

TNF alpha and IL-1β, released by these events will destabilize endothelial cell-cell interactions and cripple vascular barrier function, producing capillary leakage, tissue edema, organ failure, and sometimes death [334].

Cytokine-inhibition, pharma and/or probiotics?

Inflammation is, as discussed above, extraordinarily complex. In rheumatoid arthritis (RA) for example, the joints are rich in cytokine-secreting cells containing a wide range of effector molecules including pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and IL-18, chemokines such as IL-8, IP-10, MCP-1, MIP-1 and RANTES, MMPs such as MMP-1, -3, -9 and -13 and metabolic proteins such as Cox-1, Cox-2 and iNOS, which interact with one another in a complex manner that is thought to cause a vicious cycle of pro-inflammatory signals resulting in chronic and persistent inflammation [335,336]. NF-ΚB is increasingly suggested to the master regulators of inflammatory cytokine production in RA. These mediators are also involved, although not in an identical manner, in other autoimmune disorders such as inflammatory bowel diseases [337]. This knowledge has led to development to a new generation of pharmaceutical drugs, generally referred to as biological, designed to inhibit the crucial mediators of pro-inflammatory signals and subsequent abnormal immune response. A whole series of revolutionary new drugs such as anti-TNF-α, anti-IL-1β, anti-HER2 etc are already successfully tried and new drugs such as antibodies targeting IL-12/IL-23 pathways, IFN-γ, IL-17A, IL-2 and IL-6, and also inhibitors of NF-ΚB more or less extensively tried in a variety of chronic inflammatory and autoimmune diseases. Some of these have already demonstrated initially promising result, while other treatments such as administration of the regulatory cytokines IL-10 and IL-11 have failed to induce reproducible clinical effects [337]. Significant benefits in quality of life and tissue/organ healing are encountered in at least something over 50%. These drugs are generally tolerated well, but adverse events such as infections including reactivating tuberculosis, tumours such as lymphomas and demyelinating diseases and infusion reactions are sometimes evident. These changes must be regarded as acceptable as long as they are used in diseases that have proven to be refractory to all other treatments but may be an issue when, as increasingly suggested, they are tried in early stages of diseases, as happened after widening indications for statins [338]

Single target or multitarget treatment?

Most biologicals are desinged to target single molecules, those regarded as mainly responsible for the etiology of disease, even if they in reality actually affect several other molecules. There are also indications that sometimes the results of selective targeting may be short-lasting and that the inflammation sooner or later will find other pathways and the disease consequently continue to progress. Most diseases involve a large variety of molecular abnormalities; for these broad-spectrum treatments might offer a good, and sometimes better, solution than that offered by pro- and symbiotic treatment. [check intented meaning]

It is unfortunate that no studies thus far have addressed the effects of the biologicals on microbiota and leaky barriers. Until done, one must assume that these drugs have the same devastating effects on microbiota and barrier efficacy as other drugs. Plant-derived mediators, or phytochemicals, such curcumin, resveratrol, genistein etc, (see further above) and plant fibers, particularly prebiotic fibers, and probiotic bacteria, which may be termed 'eco-biologicals' possess, can be expected, alone or in combination, to have the same molecular functions as biologicals - although much weaker - but also without known adverse effects. Compounds officially classified as GRAS, generally considered as safe, should be considered where the main indications are prevention, early in disease treatment but also where used as palliative treatment particularly in children and the elderly.

Several population-based studies indicate that people in Southeast Asian countries have a much lower risk of developing colon, gastrointestinal, prostate, breast, and other cancers than do their Western counterparts. They also have significantly reduced incidence of other chronic diseases such as coronary heart diseases, neuro-degenerative diseases, diabetes, inflammatory bowel diseases, etc. It is likely that their frequent use of dietary constituents containing chemopreventive molecules, as is the case with garlic, ginger, soybeans, curcumin, onion, tomatoes, cruciferous vegetables, chilies, and green tea and many others may play an important role in protection from these cancers, and other diseases, especially as these dietary agents might suppress transformative, hyperproliferative, and inflammatory processes [339].

Final remarks

An individual who wants to live in line with the present knowledge obtained from extensive research in recent years might want, in addition regular physical exercise, good sleep and spiritual harmony to consider:

1. Minimizing intake of insulinogenic foods such as refined carbohydrates; cereals, bread, sweats, cookies, rice, pasta, cooked tubers incl. potatoes, foods, which are absorbed high in the small intestine and of minimal benefit to microbiota.
2. Keeping a daily intake of fructose below 25 gram a day.
3. Minimizing their intake of dairy products especially butter, cheese and milk powder, rich in saturated fats, hormones and growth factors such as IGF1, and to reduce meat intake, especially inflammation-inducing processed and cured meat such as bacon and sausages, this far though only fat demonstrated to being detrimental to microbiota.
4. Dramatically increasing the intake of fresh and raw greens, fresh spices and vegetables, rich in antioxidants, fibers, minerals and nutrients, but also inflammation-controlling factors such as curcumin, resveratrol - some of which most likely are of great importance for diversity, replication, growth and functions of the microbiota and for immune development and immune functions of the body.
5. Minimizing intake of foods, which are heated above 100^{o} C known to be rich in the inflammation-inducing molecules AGEs and ALEs, foods heated above 130 $C^{o,}$ which with increase in temperature becomes increasingly rich in pro-inflammatory and carcinogenic substances such acrylamide and heterocyclic amines. This means avoiding fried and grilled foods but also toasted and high-temperature baked breads.
6. Minimizing exposure to microbe-derived highly inflammation-inducing endotoxin, especially rich in meat hung for several days, hard cheeses, pork and ice-creams.
7. Eliminating/minimizing intake of foods rich in proteotoxins such as casein, gluten and zein.
8. Seeking out and consuming ancient anti-oxidant-rich, high fiber, low-calorie containing grains such as buckwheat, amaranth, chia, lupin, millet, quinoa, sorghum, taro, teff etc, and also increasing the intake of beans, peas, chickpeas, lentils, nuts and almonds - all extraordinary rich in nutrients and minerals - all prepared for eating by low-temperature cooking - all most likely of importance for maintenance of a rich microbiota.
9. Restricting intake of chemicals including pharmaceutical drugs to only what is absolutely nessessary as most likely most chemicals are detrimental to microbiota.
10. Supplement of large doses of vitamin D and omega fatty acids, both important in control of inflammation and for function of microbiota.

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Fig 1. Hematoxylin-eosin of lung tissues from placebo, only fibers-treated and Synbiotic 2000-treated animals [259].

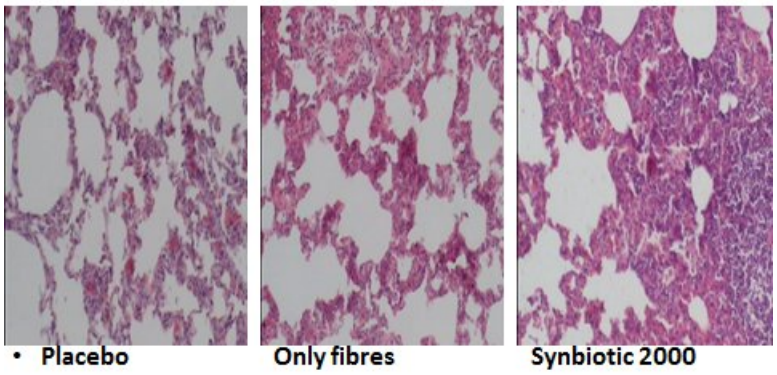

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Table 1. Neutrophil counts after treatment with Synbiotic 2000, only the LAB in Synbiotic 2000, only the fibers in Synbiotic 2000 and placebo [259].

Synbiotic 2000	9.00±0.44
Only LAB	8.40±0.42
Only the fibers	31.20±0.98
Placebo	51.10±0.70
p< 0.05	

Table 2. Myeloperoxidase (MPO) activity in the supernatant presented as U/g lung tissue, after treatment with Synbiotic 2000, only the LAB in Synbiotic 2000, only the fibers in Synbiotic 2000 and placebo [259].

Synbiotic 2000	25.62±2,19
Only LAB	26.75±2,61
Only the fibres	56.59±1,73
Placebo	145.53±7,53
p< 0.05	

Table 3. Lipid peroxidation in the lung tissue determined expressed as levels of malondialdehyde (MDA), measured in nmol/mg protein, after treatment with Synbiotic 2000, only the LAB in Synbiotic 2000, only the fibers in Synbiotic 2000 and placebo [259].

Synbiotic 2000	0.22±1,31
Only LAB	0.28±3,55
Only the fibres	0.48±5,32
Placebo	0.67±2,94
p< 0.05	

Table 4. Lung tissue nitrite (NO2) and nitrate (NO3), expressed as µmol/g wet tissue, after treatment with Synbiotic 2000, only the LAB in Synbiotic 2000, only the fibers in Synbiotic 2000 and placebo [259].

Synbiotic 2000	17.16±2,03
Only LAB	8.91±2,24
Only the fibres	47.71±3,20
Placebo	66.22±5,92
p< 0.05	

Table 5. Pathogens isolated from patients undergoing pancreatectomy treated with Synbiotic 2000 and only the fibers in Synbiotic 2000 resp. [271].

Isolated Microorganisms	SYNBIOTIC 2000	Fibers Only
Enterobacter cloacae	2	8
Enterococcus faecalis/faecium	1	7
Escherichia coli	0	7
Klebsiella pneumoniae	2	2
Proteus mirabilis	1	1

Staphylococcus aureus	0 (total 6)	2 (total 27)

Table 6. Pathogens recovered from patients undergoing surgery for abdominal cancer treated with Synbiotic 2000 and only the fibers in Synbiotic 2000 resp. (Han et al. personal communication).

Isolated Microorganisms	SYNBIOTIC 2000	Fibers Only
Pseudomonas aeruginosa	17	24
Staphylococcus aureus	8	11
Staphylococcus epidermidis	1	1
Staphylococcus faecalis	-	1
Enterobacter cloacae	4	-
Acinetobacter spp.	2	3
Staphylococcus haemolyticus	-	1
Serratia spp.	-	2
Klebsiella spp.	-	1
Proteus mirabilis	-	2
Candida albicans	2	6
Aspergillus spp.	-	-
Bacillus subtilis	-	1
Klebsiella spp.	-	1
	(Total 34)	(Total 54)

Table 7. Pathogens isolated from patients undergoing liver transplantation treated with Synbiotic 2000 and only the fibers in Synbiotic 2000 resp [272].

Isolated bacteria	SYNBIOTIC 2000	Fibers only
Enterococcus faecalis	1	11
Escherichia coli	0	3
Enterobacter cloacae	0	2
Pseudomonas aeruginosa	0	2
Staphylococcus aureus	0	1
	(total 1)	(total 18)

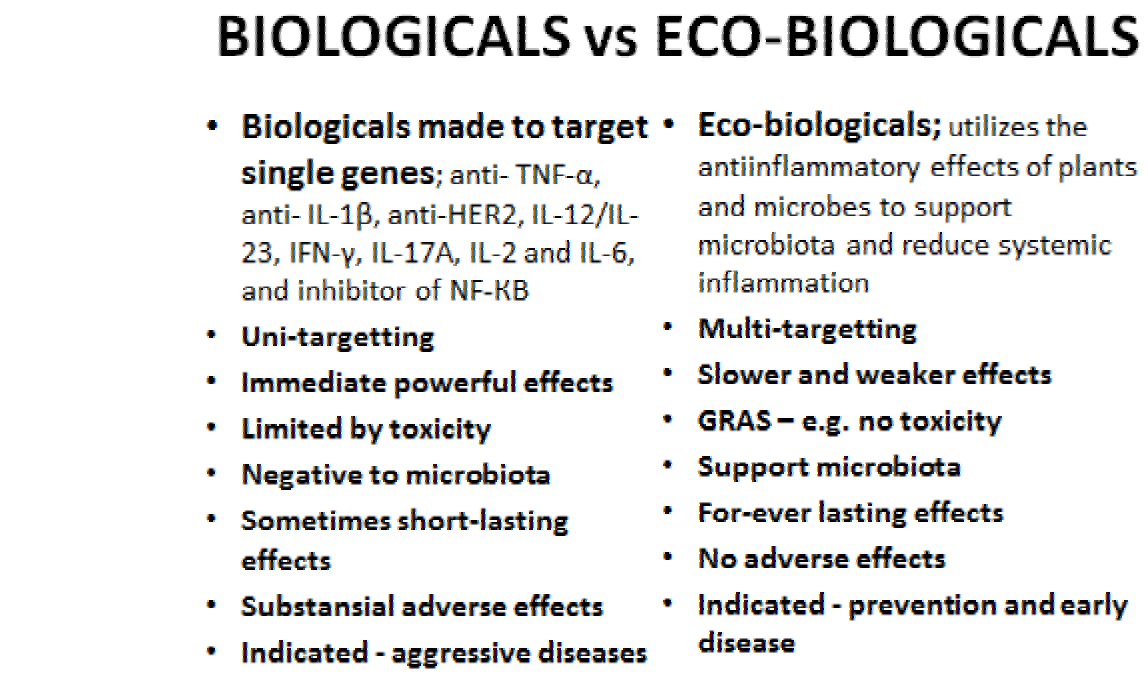
Table 8. Pathogens isolated from patients with polytrauma treated with Synbiotic 2000 and only the fibers in Synbiotic 2000 resp. [275].

Isolated Microorganisms	SYNBIOTIC 2000	Fibers Only
Acinetobacter baumanni	21	35
Candida albicans	7	17
Pseudomonas aeruginosa	15	14
Staphylococcus epidermidis	2	10
Staphylococcus aureus	4	7
Staphylococcus hominis	-	2
Enterobacter aerogenes	-	2
Staphylococcus haemolyticus	-	1
Serratia spp.	-	2
Klebsiella spp.	5	12
Proteus spp.	-	1
	(Total 54)	(Total 103)

Table 9. Pathogens isolated from patients with acute pancreatis treated with synbiotic versus receiving only fibers [279]

Isolated Microorganisms	SYNBIOTIC 2000	Fibers Only
Pseudomonas aeruginosa	1	4
Enterococcus faecalis	1	2
Enterobacter spp.	1	1
Streptococcus spp.	2	-
Staphylococcus aureus	1	1
Enterococcus faecium	1	-
Candida spp.	-	2
Staphylococcus haemolyticus	-	1
Serratia spp.	-	2
Klebsiella spp.	-	1
Escherichia coli	-	1
Stenotrophomonas maltophilia	-	1
Citrobacter freundii	-	1
	(Total 7)	(Total 17)

Comparison between biologicals and eco-biologicals



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