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Inflammation and Microbiota and Gut Reconditioning*Stig Bengmark*

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“Everything in excess is opposed to Nature. All disease begins in the gut.”
Hippocrates

64.1 A Dramatic Increase in Incidence of Chronic Diseases

The burden of chronic diseases is steadily increasing all around the world and is forecasted to continue to do so for at least another 50 years. While the increase in chronic diseases in Western countries, although accelerating in the last 50 years, had been ongoing for more than 100 years, it is mainly in the period after the last world war that more pronounced increases in incidences of chronic diseases have occurred, especially outside the Western hemisphere. Pronounced increases are, for example, reported from Japan from the period of the first 50 years after the last world war (1948–1998) – dramatic increases in incidences of diseases such as breast, ovarian, prostatic, and testicular, much in parallel to the Japanese population adapting Western food habits, to a large extent complementing or eventually also replacing traditional horticultural and aqua-cultural foods with processed Western-type agricultural foods. During these 50 years, for example, prostatic cancer in Japan increased by no less than 25 times, much in parallel to an increase in intake of egg 7 times, meat 11 times, and dairy products 20 times [1,2]. Similar, although slightly delayed, developments are also reported both from other continents and from neighboring Asian countries to Japan [3]. Seemingly this epidemic dominated by obesity and associated diseases has its epicenter in the southern United States,¹ with states such as Alabama, Louisiana, and Mississippi having the highest incidence of obesity and chronic diseases in the United States

1 <http://www.cdc.gov/obesity/data/trends.html> and <http://www.cdc.gov/diabetes/data/trends.html>.

and the world. These diseases are rapidly spreading across the world – to the west to New Zealand and Australia, to the north to Canada, to the west to Western Europe and the Arab world, and to the south, particularly Brazil, and Mexico.

64.2 The Epidemic Forecasted to Continue – Also in the Western World

Recent studies forecast that by 2050 at least a doubling has occurred in incidence of diabetes [4] and a tripling in incidence of attention deficit hyperactivity disorder (ADHD), Alzheimer disease [4], and cancer [5] in most countries, including both the United Kingdom and the United States, already heavily burdened by obesity and chronic diseases. A most interesting recently published study looked at the future development of disease pattern in the United States and the United Kingdom, two countries together representing approximately 5% of the world's population and two countries that already have the highest rates of obesity and chronic diseases. The study suggest that these countries combined will, by 2030, see another 76 mill obese adults, additional 6–8.5 mill cases of diabetes, 6–7 mill cases of cardiovascular disease, 492 000–669 000 new cases of cancer, leading to loss of 26–55 mill quality-adjusted life years, and a dramatic increase in costs of care – calculated to be \$50–68 billion/year [6]. Other rather recent 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.

64.3 Developing Countries Soon to Take Over the “Yellow Jersey”

During the last two to three decades, noncommunicable diseases (NCD) have taken over as the leading cause of death in countries such as Mexico, Brazil, Saudi Arabia, and India. Today the same development is seen in sub-Saharan Africa. It is promising that the rates of cardiovascular disease (CVD) including coronary heart disease (CHD) and stroke, have in recent years been declining in some industrialized countries, including the United States and the United Kingdom, but instead they are fast rising in most developing countries. For example the rate of mortality in stroke in urban Tanzania is, much in contrast to rural Tanzania, reported to be threefold higher than in the United Kingdom [7,8]. Recent studies suggest that 75% of the world's hypertensive population are to be found in developing countries [9] – a severe condition, which 40 years ago did not almost exist in developing countries [10]. Today more than one-third of adults living in Africa are hypertensive [11].

64.4 Hypertension Associated with Dysbiosis

Lifestyle-induced hypertension is today often accompanied by obesity and deranged sugar and fat metabolism and is today close to smoking, the leading cause of death on the Earth. Lifestyle-induced diseases are together responsible for the largest number of deaths in the United States [12] as probably in most other countries around the world, especially and certainly so in most developing countries. Increasing evidence suggests that for example hypertension is accompanied by a chronic low-grade chronic inflammation that perpetuates the hypertensive state and contributes to the often observed end-organ damage [1,13,14].

Hypertension, like most other chronic disorders, is often preceded by and always accompanied by malfunctioning intestinal microflora – a condition named dysbiosis. A recent experimental study reports significant decrease in microbial richness, diversity, and evenness in rats with spontaneous and induced hypertension [15]. Similar observations are done in a small cohort of human hypertensive patients ($n=10$), who compared to control patients ($n=7$) demonstrate a much reduced richness and diversity of microflora, characterized by significant increase in the ratio of Firmicutes/Bacteroidetes but also a significant decrease in acetate- and butyrate-producing bacteria [15], important to prevent a leaky gut.

64.5 Inflammation – A Mother of Disease

A few years after having achieved the status of Professor Emeritus, I felt a strong desire to better understand the pathogenic mechanisms underpinning various chronic diseases, the incidence of each having increased significantly during my lifetime years. I did spend endless hours during almost one year to study and try to understand the pathogenesis of various diseases from ADHD, allergy and Alzheimer's disease to Zollinger–Ellison syndrome and Zuska's disease. It became a great surprise to me that the processes leading to the various diseases were almost indistinguishable – the great majority of the chronic diseases demonstrated clear signs of a most often discrete chronic inflammation accompanied by increased activation of proinflammatory cytokines such as Il-6, acute phase reactants such as C-reactive protein (CRP), PAI-1 and increased levels of free fatty acids much similar to what is seen in acute diseases, such as trauma or sepsis. This observation led me at that time to call the process leading to the various chronic diseases “chronic phase reaction” and to publish a review entitled “Acute and “chronic” phase reaction – a mother of disease” [16,17]. Observations already in those days indicated a strong involvement of the gut and microflora in the process leading to development of chronic inflammation and associated metabolic manifestations. Among the informations early available was the observations that key proinflammatory cytokines are synthesized by enterocytes in response to the damaging endotoxin, to the largest extent produced by the gut microflora [18–20].

64.6 The Gut Harbors the Majority of the Immune System

The intestine is not only a major site for entry of pathogens, it is also an organ exposed to an enormous amounts of food antigens. It contains under normal conditions trillions of commensal bacteria and a collective genome at least 150 times larger than our own. Immunoglobulin A plays a major role in neutralization of invasive pathogens and maintenance of a strong presence of noninvasive commensal bacteria, is crucial for maintaining good health. Multiple mechanisms are suggested to be involved [21,22]. The Norwegian immunologist Peter Brandtzaeg suggested in 1989 that the gut, in contrast to what was earlier believed the spleen and the lymph nodes, is the major location of “the immune system” – 70–90% of IgA immunocytes (plasma cells and plasma blasts) are found in the enteric mucosa [23].

The intestinal mucosa is densely populated by various recognition cells of which the dendritic cells (DCs) in the lamina propria (LP) are especially and strongly associated with both adaptive and innate immunity. The recognition that dietary nutrients and microbial communities in the intestine influence both mucosal and systemic immune cell development and function and development of immune-mediated disease has in recent years led to a growing interest in the functionality of intestinal recognition cells, particularly in DCs, and an explosion in information in mucosal immunology.

64.7 Strong Association Between Composition of Microbiota and Chronic Diseases

The epidemic of a plethora of chronic inflammatory NCD, all documented to have in common an underlying low-grade inflammation, constitutes a major threat to future development and particularly to the economy of modern Societies. The epidemic include early-onset NCDs, such as allergy, asthma, and some auto-immune diseases, as well as later-onset NCDs, such as CVD, metabolic disease, and neurodegenerative disorders, which all appear to share common environmental risk factors and genetic risk variants – see Reference [24] for further detail. Although the pathways to disease are multifactorial, the lifestyle and eating habits and associated severely impaired gut colonization pattern with decreasing microbial numbers and diversity are clearly central to the observed physiologic, immunologic, and metabolic dysregulations seen in most NCDs. A dysbalance between two main phyla is observed in almost all NCDs, a dysbalance observed between Bacteroidetes, comprising gram-negative bacteria, and Firmicutes, comprising gram-positive bacteria [25]. As Firmicutes and Bacteroidetes account for more than 90% of the bacterial population in the colon [26], alterations within and between these two phyla are most likely of the greatest pathogenic significance. It was originally believed that the composition of the intestinal microbiota was relatively stable from early childhood; however, recent evidence suggests that poor diet can rapidly – within 24 h – cause dysbiosis, and alterations in the composition of the microbiota could fast lead to aberrant and most negative immune responses. Studies in recent years have repeatedly demonstrated a great

sensitivity of microbiota to lifestyle changes, particularly food habits; studies performed on large supply of fat, large supply of sugar, large supply of fat and sugar, fiber-reduced, calorie-restricted, complex carbohydrates, refined sugar, vegetarian diet, high n-6 PUFA, animal milk fat, all demonstrating significant alterations in composition of microflora – for review see [27]. It is of great interest that a dysbalance in favor of gram-positive Firmicutes is associated with a significantly increased extraction of energy [28].

64.8 Paleolithic Forefathers Had a Much Different, Much More Diverse and Richer Microbiota

The microbiota of some ancient remains on the Earth with lifestyle similar to our human ancestors, having lifestyle and food habits, which in most aspects date back thousands if not millions of years are especially precious to investigate. The most representative of such groups was until recently most likely the Hadzas, living in the Rift Valley ecosystem around the shores of Lake Eyasi in north-western Tanzania, who belong to the few last remaining hunting and gathering communities in the world. The Hadza diet consists of wild foods – meat from hunted animals, berries, fruit and seeds from the baobab tree, honey, and tubers.

The Hadzas practice no cultivation or domestication of plants and animals and eat minimal amounts of agricultural products (< 5% of calories) from external sources. They consume 100–150 g of fiber each day, which is about 10 times more than typically Americans and Europeans eat. A detailed study of the microbiota of 27 Hadza, aged 8–70 years, mean age 32 years and compared to 16 urban Italians age 20–40 years, mean age also 32 years, was recently published [29]. Although Firmicutes and Bacteroidetes are the dominant phyla in both Hadza and Italian gut microbiota, Hadza are characterized by a relatively higher abundance of Bacteroidetes and a lower abundance of Firmicutes. The two gut microbiota ecosystems are remarkably different with respect to subdominant phyla (< 10% relative abundance). The microbiotas of the Hadzas are largely enriched in Proteobacteria and Spirochaetes, which are extremely rare in the Italian gut microbiota, while Actinobacteria, an important subdominant component of the Italian gut microbiota, are almost completely absent from the Hadza microbiome. It is important to stress that in addition to those mentioned above many hitherto unclassified genera belonging to Bacteroidetes, Clostridiales, and Ruminococcaceae were observed in the Hadza gut microbiota. Furthermore, it was observed that the Hadza gut microbial ecosystem is profoundly depleted in Bifidobacterium and comprises an unusual arrangement of Clostridiales. This arrangement is defined by a general reduction of well-known butyrate producers, members of the Clostridium clusters IV and XIVa22 and a corresponding increase in unclassified Clostridiales and Ruminococcaceae. Detailed recent information on the health status of Hadza is not available but early studies suggest that the Hadza have low rates of infectious disease, metabolic disease and nutritional deficiencies in comparison to other settled groups in the northern Tanzania and south-eastern Uganda region [30,31].

64.9 Different Microbiota in Infants in Hunting and Gathering Communities

Fecal microbiota of 14 healthy children, aged 1–6 years, from the Mossi ethnic group living in the small village of Boulpon in Burkina Faso (BF) were studied and compared with that of 15 healthy European children (EU) living in the urban area of Florence, Italy [32]. A significant depletion in Firmicutes accompanied by a unique abundance of bacteria from the genus *Prevotella* and *Xylanibacter*, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, was observed in the BF children, but completely lacking in the EU children. A significantly greater production of short-chain fatty acids (SCFAs) was also observed in BF children in comparison to the EU children. The microbiota of the BF children was significantly richer in potentially anti-inflammatory gram-positive bacteria, while the microbiota of the EU children had a much greater component of potentially inflammation-inducing gram-negative bacteria. It was especially observed that Enterobacteriaceae (*Shigella* and *Escherichia*) are significantly underrepresented in BF compared to EU children. As concluded by the authors, gut microbiota seems to have coevolved with the polysaccharide-rich diet of BF individuals, allowing them to maximize energy uptake from fibers but also protecting them from inflammation-induced noninfectious diseases.

The gut microbiota of 44 rural Malawian 6-month-old infants was in another important study compared to that of 31 Finnish infants of the same age [33]. The Finnish children were breast-fed to 68%, the Malawian to 100% (no formula, no complementary food at 6 months of age, just as the World Health Organization (WHO) and other authorities recommend today. The Malawian microbiota comprised *Bacteroides-Prevotella* (17 vs. 5%) and *Clostridium histolyticum* (4.4 vs. 2.8%; $P \leq 0.01$), respectively. The species *Bifidobacterium adolescentis*, *Clostridium perfringens*, and *Staphylococcus aureus* were absent in Malawian but not in Finnish infants. Bifidobacteria were dominant at 6 months of age in all of the infants, although to a greater proportions in Malawian (71%) than in Finnish infants (47%). The preponderance of these special bacterial species might reflect the risk of developing allergy; it is well known that allergic infants are more often colonized by *C. difficile* and *S. aureus* and less by bifidobacteria. Higher levels of clostridial species and staphylococci, common in Western microbiota, are known to be associated with obese and overweight infants.

64.10 The Most Robust Microbiota Ever Seen

A most recent report describe the microbiome of a previously unknown group – the Yanomamis [34], a group of villagers living in complete isolation in the Amazon, and as suggested, with no contact with the outside world for an estimated 11 000 years. The Yanomami were originally mountain people, who were first contacted in the mid-1960s and who continue to live a semi-nomadic hunter-gatherer lifestyles in the Amazon jungle in the high Orinoco state of the vast mountainous Yanomami territory, Venezuela, uninfluenced by anybody from

the outside world. These villagers are observed to eat wild bananas and seasonal fruits, plantain, palm hearts, and cassava. Their hunting for food focuses mostly on birds and small mammals but also small crabs, frogs, small fish from nearby streams, and also, although very occasionally, peccary, monkey, and tapir. Samples of the microbiota of oral cavity, forearm skin, and feces were recently obtained from 34 of 54 villagers at the time of the first medical expedition in 2009 to this isolated and previously uncharted village.

The microbiotas of the villagers were analyzed and reported to have the highest diversity ever reported in humans (~40% more in Westerners), but also when compared to the rural Africans discussed above [34] – an important observation indicating that a large proportion of important ingredients of our early microbiota have become extinct during the process of Westernization. The Yanomamis harbor high amounts of *Prevotella*, *Helicobacter*, *Oxalobacter*, and *Spirochaeta* but also high levels of parasites that are absent or significantly reduced in industrialized humans. A most unexpected finding was that the gut and oral bacteria also contained genes coding for antibiotic resistance. Though these genes were inactive, they confer resistance, not only to natural antibiotics found in the soil but, and most likely, also to other chemicals including pharmaceuticals. The observations support the assumption that a large proportion of Western diseases, rare in rural Africa and South America, are results of lifestyle and profound diet changes. The medical workers reported that villagers were healthy and did not suffer from autoimmune disorders, diabetes, or high blood pressure.

64.11 Rural Lifestyle Prevents Western Diseases

The British physician Dennis Burkett should be credited for alerting the world to the need of a much higher intake of fiber to maintain health. He reported some 40 years ago a strong association between low-fiber content in the diet and a significantly higher risk of colorectal cancer, but also of other diseases such as CHD and diabetes, all based on his observations in Uganda, Africa. He reported that the shortage in plant fibers in Western diet was associated with poor gastrointestinal function – both a 10 times reduction in stool weight (United Kingdom 60 g/day, Uganda 600 g/day) and a 5 times increase in transit time (United Kingdom: 100 h., Uganda: 20 h) in Western Societies (United Kingdom) in comparison to what was observed in Uganda [35–39]. The lack of fibers in diet was at that time reported especially low in special groups of the Western Societies and especially so in the foods served at hospitals – a shocking transit time of more than 14 days observed and reported in half of the investigated British geriatric patients [40].

Studies in other ancient remains of humans of the world, such as Abkhazia, Vilcabamba och especially Hunza – all living almost totally on nonprocessed plant food: Hunza och Vilcabamba 99% and Abkhazia on 90% provide most interesting results. They seem to harvest energy better than Westerners as they despite hard work seem to live and maintain weight on only 17–1800 calories, obtained from 100% nonprocessed plant foods. Obesity is said to never be seen, the incidence of disease is remarkably low and the numbers of centenarians are greater than observed in most places on the Earth – for review, see Reference [41].

64.12 Endotoxin (LPS) – A Major Inducer of Inflammation and Disease

The most obvious and harmful consequences of reduced microbiota, dysbiosis, are high levels of endotoxin in plasma (endotoxemia), which both in experimental and clinical studies are strongly associated with inflammation and risk of obesity and almost all chronic diseases. Endotoxins are integral components of the outer membrane of gram-negative bacteria such as *Enterobacteriaceae* and *Pseudomonadaceae*, and essentially composed of proteins and lipids, in addition to the toxic and inflammation-inducing lipopolysaccharides (LPS). LPS 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, and a “prudent-style diet,” who, in turn, demonstrate a 31% reduced level of endotoxin [42]. A positive correlation between sedentary lifestyle and higher levels of endotoxin levels, and a negative correlation to the degree of physical exercise is reported [43]. High-fat-content foods, rather than high-carbohydrate-content foods, correlate with high levels of endotoxemia. Mice fed with 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 contributes more than a high-carbohydrate diet to a high endotoxin level [44].

64.13 Meals Rich in Long-Chain Fatty Acids Induce Endotoxemia

Fat in foods influences negatively microflora and its replication, but the most pronounced negative effects are observed with high consumption of animal-origin fat, which facilitates translocation of LPS – the chylomicrons serving as vehicle for translocation of endotoxin, through the mucosa and into the circulation, a process referred to as transcellular transportation. This process facilitates higher concentrations and a long-lasting presence of both long-chain fatty acids and endotoxin in the general circulation – see further below. Strong correlations between plasma levels of endotoxin and numbers of parameters of metabolic syndrome as well as between persistent levels of high endotoxin/plasma and “prospect of life” have been observed – especially large differences being reported between the first and fourth quartiles [42]. Among the diseases associated with increased presence of fat and endotoxin in plasma are particularly; Alzheimer’s disease [45,46] and cognitive impairment [47], arteriocoronary disease [47,48] and stroke [49], diabetes 1 [50,51], diabetes 2 [52], cancer [52,53], but also allergy [54], ALS [55], autism [56], autoimmune diseases [57], bipolar disease [58], chronic fatigue syndrome [59], chronic obstructive pulmonary disease (COPD [60], minimal encephalopathy [61,62], fibromyalgia [63], HIV [64], liver cirrhosis [62,65], macular degeneration [66], nephropathies [67], obesity [68,69], osteoarthritis [70], paradontosis [71], Parkinson’s disease [72,73], rheumatoid

diseases [74,75], psychiatric and cognitive disorders [47,76,77], stress [78,79], and uveitis [80].

64.14 Humans Are Especially Sensitive to Endotoxin

Humans are known to be extremely sensitive to endotoxin exposure and reported to show signs of inflammation at extremely low doses of LPS, reported to be at least 250-fold lower than what is required, for example, in mice [81]. This is especially important to remember as modern humans, much more than other primates, as an unfortunate consequence of modern living, are exposed to high amounts of LPS both at outdoor and indoor living. Tobacco smoking is a major source of LPS but also dust from home is rich in endotoxin, often occurring at home, workplace and at school. Industries such as agriculture, textile, and wood industries are especially recognized as bad environment with often extremely high levels of endotoxin exposure.

64.15 Fresh Fruits and Vegetables Contain Minimal Levels of Stimulants of Toll-Like Receptors (TLRs)

The fact that the food we eat often contains unacceptably high levels of endotoxin is often neglected. Cooking the food makes little difference as LPS is heat-resistant and both LPS and dead bacteria maintain their ability to induce inflammation. The majority of fresh and raw whole vegetables should normally contain only minimal or undetectable levels of stimulants of TLR2 or TLR4 [82]. However, certain raw and minimally processed vegetables (MPVs) are very sensitive to storage and might occasionally contain considerable quantities of bacteria and endotoxins; especially bean sprouts, diced onions, and chopped root vegetables such as carrots and onions [82]. Beef, pork and turkey increase within a few days of storage their content of TLR2- and TLR4-stimulants remarkably, even when stored at below 5 °C, especially when exposed to air [83]. The accumulation of TLR2- and TLR4-stimulants is minimized by storage of meat in intact rather than minced forms, and when stored under a modified atmosphere, rather than exposed to air [83]. Very little data exists about the health hazards of game meat, which despite its favorable nutritional profile, compared to farmed meat, is often kept hanging for weeks and, as a consequence, is especially rich in bacteria and endotoxins.

64.16 Several Proteotoxins Stimulate Also Toll-Like Receptors (TLR) and Induce/Enhance Inflammation

Different food ingredients and particularly proteins may enhance the inflammatory properties of human diets. Many peptides and proteins, to which modern humans are daily exposed, possess the ability to induce inflammation and activate

transforming growth factor- β (TGF- β) and TLRs. Among these are various lectins, especially glutenoids, and also caseins. Novel molecules, induced by heating of food or longer storage at room temperature, are collectively called Maillard products and consist in so-called “advanced glycation end products” (AGEs) and “advanced lipoxidation end products” (ALEs). The “invention of fire” in Stone Age increased dramatically the possibilities for proinflammatory food products to be produced, and the introduction of gluten-containing grains, which occurred about 10 000 years ago with the advent of agriculture, did further increase human exposure to dysfunctioning proteotoxins – proinflammatory molecules – developments, which might be regarded as unfortunate “mistakes of evolution.”

The main reasons for the large exposures during human history to glutenoids 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 has made it worse as modern bread contains 15–20 times more gluten, compared to bread produced in the past. An unexpected consequence of high consumption of wheat, rye, and barley, is that modern will suffer from a series of highly unwanted human disorders that relate to exposure to glutenoids, particularly gluten (wheat), but also to secalins (rye) and hordeins (barley), which all have strong ability to induce inflammation and increase intestinal permeability [84]. Oats is distantly related to wheat, rye and barley, but its active peptides, the avenins, are rarely reported to give stronger reactions, either inflammation or allergy [85].

64.17 Gluten-Sensitivity a Common and Newly Detected Inflammation-Inducing Disorder

It 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, besides those rather few, who suffer CD and classic wheat allergy, most individuals, if not all, also suffer very discrete, but always negative reactions to glutenoids. Despite the fact that few direct and immediate allergic reactions or autoimmune mechanisms are involved with exposure to wheat, rye, or barley, consumption of these foods will almost always or always add to the general burden of inflammation in the body. A rather recently made observation has led to recognition and acceptance of a new disorder called gluten sensitivity (GS) [86]. 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 have no symptoms at all, others will suffer well-defined chronic diseases, and the majority “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 affected individuals report that, when turning to gluten-free diets, increased well-being and frequently also improved clinical signs and symptoms will occur.

64.18 Gluten-Free Foods Have Led to a New and Successful Industry

Experiences such as these have made the gluten-free diet the number one health trend in the world,² growing faster than both low carbohydrate diets and “fat-free” diets, and rapidly fueling the gluten-free product market, which has grown in the United States with 44% in less than 3 years, from 2011 to 2013, and is expected to continue to grow further – the US market from \$10.5 to \$15.6 billion, and the UK market from \$395 to \$931 million by 2017. A parallel development is also observed in the growth of alternative milk – the global growth of soy, oat, almond, and so on based alternative milks constitute seemingly around 6% per year of the combined milk and alternative milk products.³

A fast increase in alternative sources of flours is also observed. Among the alternatives flours 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 fifth most common grain in world and especially attractive due to its extremely high content of antioxidants, low content of energy [87], and ability to resist heat-induced protein glycation [88,89], but also due to its high versatility and cost-effectiveness.

64.19 Glutenoids Show Endotoxin-Mimicking Abilities

Glutenoids, which demonstrate endotoxin-mimicking abilities, are capable of lowering the threshold for immune responses, attracting leukocytes and increasing their reactive state, in a manner similar to that of endotoxin. Nearly 10 µg/mL of wheat gluten induces the equivalent effects of 1 ng/ml endotoxin LPS [90], increasing DC maturation as well as chemokine secretion [90]. A study investigating fecal samples from 76 symptom-free, nonceliac, first-degree CD relatives and compared to samples from 91 aged-matched healthy controls reported significantly lower level of acetic acid and other SCFAs) in parallel to significantly increased levels of *i*-butyric acid and fecal tryptic activity in the asymptomatic CD-relatives [91].

The information that removal of gluten from the diet of the nonobese 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 [92]. The acute insulin response to intravenous glucose tolerance test rose significantly in 12/14 patients 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-containing diet ($P=0.07$) [92].

2 <https://www.glutenfree.com/#/articles/gluten-free-a-global-perspective>.

3 <http://blog.euromonitor.com/2014/09/whitewaves-acquisition-of-so-delicious-dairy-free-should-cement-its-lead-in-non-dairy-milk-alternati.html>.

64.20 Similar Effects Observed on Gluten Restriction in Individuals with ADHD

A similar outcome is reported from a crossover study in which 100 individuals suffering ADHD, aged 4–8 years, who were randomly assigned to 5 weeks on a restricted elimination diet (not planned as but in fact a gluten-restricted diet) or 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 again during the subsequent period on a "normal," although supposedly healthy diet [93]. Another study focused on thirty-four individuals with irritable bowel syndrome, 56% of them with human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8 genes, during 6 weeks on a gluten-free diet [94]. 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 ($P=0.001$), but no improvement being 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 [94]. Another rather recent study reported disappearance of signs of malabsorption and intractable therapy-resistant seizures in three young girls (ages 18, 19, 23) when placed on gluten-free diet [95]. Similarly, individuals with symptoms of neuropsychiatric diseases; Alzheimer's disease/cognitive decline [96], autism [97], and schizophrenia [98] are reported in the literature as symptom-free on a gluten-free diet.

64.21 Gluten Sensitivity Associated with Severe Dysbiosis, Leaky Gut and Increased General Inflammation

Typical symptoms of nonceliac GS are fatigue, sleep disturbances (insomnia and/or nonrestorative sleep), morning stiffness and cognitive problems, but also gastrointestinal symptoms are frequent – a clinical picture almost identical to fibromyalgia, 50–70% of patients with fibromyalgia are reported to suffer non-specific gastrointestinal symptoms such as abdominal distension (59%), dyspepsia (50%), nausea (21%), alternating diarrhea-constipation (63%), constipation (12%), and diarrhea (9%) [99].

All gluten-sensitive diseases as mentioned above are strongly associated with severe dysbiosis, leaky gut and permanent general chronic inflammation. A recent study was aimed to clarify how cultured peripheral blood mononucleated cells (PBMC) obtained from nonceliac gluten-sensitive (NCGS) patients responded to contact with wheat proteins. Wheat protein was observed to induce an over-activation of the proinflammatory chemokine CXCL10 and decrease the trans-epithelial resistance of monolayers of normal colonocytes by diminishing the mRNA expression of cadherin-1 (CDH1) and tight junction protein 2 (TJP2), two primary components of the tight junction strands. Furthermore, the induction of

inflammation was greater with proteins extracted from modern compared to when extracted from ancient wheat genotypes [100].

64.22 Proteotoxin-Induced Low Threshold for Immune Response

Inclusion of industrially made foods ingredients and particularly those of 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 [101]. As it is believed that some food proteins might block release of some amino acids such as L-tryptophan, 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 eightfold variation was observed in levels of cortex tryptophan: a marked decline followed zein ingestion, slightly more modest reductions were observed after casein or gluten application, all paralleled by reductions in cortical and hippocampal hypothalamic 5-hydroxytryptophan (5HTP) [101].

Fermented milk such as Kefir contains more than 250 peptides, mainly released from β -casein, of which several have significant biological effects: ACE-inhibitory, antioxidant, antithrombotic, mineral binding, antimicrobial, immunomodulating, and opioid activity [102]. It is most likely that the same peptides are released during microbial fermentation in the gut, which might explain why intake of various dairy products are associated with dysbiosis, increased general inflammation and also why dairy-free diet often leads to reduced general inflammation and clinical improvements, and also explain the observed association between larger intake of dairy and increased incidence of diseases including Alzheimer's disease and dementia [103–107].

64.23 Heat- and Storage-Induced Inflammation-Inducing Proteins

Heat- and storage-induced dysfunctioning proteins are of special interest, as they are shown to have strong ability to induce inflammation [108,109]. It is thought that certain dietary AGEs are indigestible by human gut enzymes and most likely enter the colon, where they will act as a growth substrate for detrimental bacteria such as some *Clostridium* and *Bacteroides* species [110,111]. Therefore, it is most conceivable that individuals who consume highly processed diets (which contain large quantities of AGEs) may adversely alter their colonic microbial composition and potentially enhance the risk for the development of metabolic diseases such as obesity and type 2 diabetes [112].

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 other industrially produced foods), and also especially rich in high-temperature prepared 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 as well as smoked and cured foods in general [113–115]. Consumption of such foods, often main constituents of fast foods, has 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 [116], neurodegenerative diseases [113], premature aging and cognitive decline [114], diabetes type 1 and 2 [115], diabetic nephropathy [116], obesity [117], liver disease, particularly liver steatosis and liver fibrosis [118], lung disease, particularly COPD [119], various cancers especially breast cancer [120], colorectal cancer [121], esophageal [122], gastric [123], lung [122], ovarian [122], pancreatic [124], prostatic [125], renal [126], and leukemia [127].

64.24 Numerous Proinflammatory Mediators Involved

A wide range of proinflammatory 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 effects of the AGEs, particularly RAGEs, and through the TLR2 and TLR4. Activation of these receptors will ultimately result in activation of NF- κ B, known to induce up-regulation of leukocyte adhesion molecules and production of proinflammatory cytokines and angiogenic factors in both hematopoietic and endothelial cells, thereby promoting inflammation [128]. A recent review suggests that particularly HMGB1, TLR and RAGE constitute a functional tripod with high ability to promote inflammation [129].

64.25 Short-Chain Fatty Acids – A Magic Bullet?

The gut microbiota is, as already discussed, the key to gut/immune homeostasis and health to the host. Increasing evidence suggests that this is achieved, at least to a large extent, through the release SCFAs, which are the main microbial metabolites produced by specific colonic anaerobic bacteria in the gut fermentation of dietary fiber and resistant starches. Deficiencies in SCFAs will strongly affect the pathogenesis of a diverse range of diseases, from allergies and asthma to cancers, autoimmune diseases, metabolic diseases, and neurological diseases.

Therapeutic manipulation of the gut microbiota and restoration of normal microbiota (a condition called eubiosis) could potentially reduce the levels of circulating AGE levels and improve the metabolic health of individuals at risk for the development of type 2 diabetes or other metabolic syndrome-associated diseases. Regular consumption of fiber-rich/prebiotic-rich foods to promote the growth of beneficial gut bacterial flora is a most promising possibility. Prebiotics/

nondigestible plant-derived carbohydrates confer health benefits to the host by acting as a fermentation substrate in the colon, stimulating the preferential growth and activity of a limited number of beneficial microbial species [130]. Prebiotic-induced stimulation of gut fermentation increases the levels of intestinal Bifidobacterium species, known to attenuate the production of ROS and markers of inflammation in individuals consuming high fat diets [131]. Supplementation of the human diet with prebiotic fructans such as inulin or fructooligosaccharides alters the bacterial composition of the large intestine and favors a selective proliferation of beneficial lactic acid-producing species such as bifidobacteria and lactobacilli.

64.26 Consumption of Plant Fibers Crucial to Eubiosis and Good Health

It is increasingly understood that different fibers have different impacts on microbiota composition and metabolism. When the effects on human fecal microbiota of six dietary fibers: pectin, guar gum, inulin, arabinoxylan, b-glycan, and resistant starch was investigated, pectin was found to induce increases in Actinobacteria (more specifically bifidobacteria), guar gum increased levels of Bacteroidetes and reductions in Firmicutes and Proteobacteria, resistant starch increased levels Bifidobacterium adolescentis type-2 and Blautia wexlerae, while inulin increased Actinobacteria [132]. Supplementations of guar gum and inulin resulted in the largest production of butyrate [132]. Apple pectin is reported to be an effective apple component improving the fecal Environment – stimulating growth of Bifidobacterium, Lactobacillus, Enterococcus, and the Bacteroides fragilis group but not microbes such as *Escherichia coli*, *Collinsela aerofaciense*, *Eubacterium limosum*, and *C. perfringens* [133].

Fibers such as pectin and guar gum are known to inhibit weight gain, adiposity, fatty liver, and high blood glucose levels in experimental animals fed a high-fat diet. A recent study demonstrated significantly increased levels of butyric acid in cecum and blood after supply of guar gum with medium and high viscosity as well as of high methoxylated pectin [134]. These fibers reduce to varying extent levels of blood cholesterol, liver steatosis, and blood glucose levels – much depending on the degree of methoxylation and viscosity of the particular fiber. The medium viscosity guar gum was the most effective fiber to prevent a diet-induced hyperlipidemia and liver steatosis. Guar gum is shown to have distinct bifidogenic effects independent of viscosity, and increases the cecal abundance of bifidobacterium about 10-fold [134].

64.27 Prebiotic Fibers Generally Resistant to Digestion in the Small Intestine

It is characteristic of prebiotic fibers that they, to the greatest extent, are resistant to digestion in the human small intestine and that they consequently will largely

pass intact into the colon, where they increase viscosity and bulking of the fecal matters. It is preferably in the proximal half of the large intestine that the dietary fibers undergo fermentation by the resident anaerobic colonic microbiota and produce SCFAs. SCFAs consist in a subset of saturated fatty acids containing six or less carbon molecules that include formic acid (C1), acetate (C2), propionate (C3), butyrate (C4), pentanoic (valeric) acid (C5), and hexanoic (caproic) acid (C6). Recent advances in the information about SCFAs, favorably acetate, propionate, and butyrate, highlight their much important effects on metabolism at both cellular and molecular levels.

Butyrate is mainly utilized by colonic mucosa, while acetate and propionate reach the liver solely through the portal vein. Propionate is fastly metabolized by hepatocytes, while acetate will either remain in the liver or be released to the peripheral venous system and tissues. Of the SCFAs only acetate can be detected as circulating in the body.

64.28 It Is the Luminal Levels of SCFAs, Which Make the Difference

A significant association exists between composition of the microbiota and luminal levels of SCFAs. Higher luminal concentrations and lower colonic pH are observed in proximal colon where fermentation is highest (pH = 5.5–6.5), compared to the distal colon with its significantly higher pH (pH = 6.5–7.0). SCFAs, especially butyrate – the primary energy source for the colonic mucosa – is credited for its unique and important ability to inhibit growth of potential pathogens, gram-negative Enterobacteriaceae, especially pathogens such as *Salmonella* spp. and *E. coli* [135,136].

Diets containing low amounts of dietary fibers are strongly associated with distinct long-term negative effects on composition of the intestinal microbiota and production of SCFAs. All SCFAs have significant anti-inflammatory and antiproliferative properties. Consumption of a diet rich in meat and low in fiber promotes proteolytic fermentation and production of uremic compounds and branched-chain fatty acids, which all are proinflammatory and increase the risk of development of disease, particularly cancer.

64.29 Not only SCFAs but MCFAs Have Strong Ability to improve Immunity and Prevent Disease

The documented strong ability of SCFAs to control inflammation, reduce excessive production of radical oxygen species and mitochondrial dysfunction [137], and to regulate immune functions particularly regulating T-cell functions [138] should offer hope for various therapeutic applications, not only by SCFAs (1–6 carbon atoms) but also by medium-chain fatty acids (MCFAs) (6–12 carbon atoms), which are more easy to administer. However, attempts to infuse various SCFAs and MCFAs have not been met with the success,

which was primarily expected. The most significant benefits obtained this far were obtained by direct application of bioactive fibers – administer to facilitate derived production of SCFAs at the mucosal surface. However, intake of plant fibers is not always possible, especially in critically ill, which somewhat limits the applicability of this otherwise successful treatment alternative.

64.30 Stress Increases Microbial Growth and Virulence

It is well documented that the immune system and central nervous system are involved in cross-talk to maintain homeostasis under normal and disease conditions. Acute and chronic stress induces release of a variety of hormones, neurochemicals, and neuropeptides, which directly or indirectly lead to severe impairment of immune functions [139]. Nearly all types of cells, especially immune cell possess receptors for the stress-related neurohormones adrenaline and noradrenaline. Sympathetic nerve fibers do also extensively innervate “immune”/lymphoid organs, such as the bone marrow, thymus, spleen and lymph nodes, and terminate in close proximity to lymphocytes [139]. The large intestine has by far the greatest number of nerve fibers when compared to other organ in the body – the total number of enteric neurones estimated to be at least 500 million [140]. Stress hormones such as norepinephrine and dopamine are shown to dramatically increase the virulence of pathogenic hormones [141]. Noradrenaline, adrenaline, dopamine, and the synthetic catecholamine inotropes dobutamine and isoprenaline are all able to increase virulence and microbial growth by up to 100 000-fold over controls [142].

64.31 Early-Life Conditions Influence Long-Term Health

Early-life conditions play important role in the establishment of microbiota and immune functions, which often influences stress reactions: allergic and immunological response neurodevelopmental later in life. Babies born via cesarean section are born by mothers with high levels of stress. Meta-analyses suggest a 20% increase in incidence of asthma among babies born with cesarean section [143]. Cesarean section, especially unplanned cesarean section, is perceived as severely stressful [144,145] with significantly increased glucocorticoid and catecholamine neuroendocrine hormone levels [146,147]. Breast-fed babies, fed by a different and unique prebiotic human milk fibers, oligosaccharides [148], acquire maternal intestinal bacteria from breast milk [149] to digest the fibers and have during this period a much different, more “beneficial” gut microbiota [150,151]. Maternal separation, a common early-life stressor, has been shown to cause a significant decrease in fecal lactobacilli on day 3 post separation, returning to normal by day 7 [152]. It is suggested that early-life stress might have long-term effects on the microbiome [153]. Analysis of the 16 S rRNA diversity in adult rats exposed to maternal separation for 3 h/day from post-natal days 2–12 reveal a significant and permanently altered fecal microbiome, when compared to nonseparated control animals [154].

64.32 Psychological Stress Impairs Microbiota

Psychological stress has long been known, both clinically and experimentally, to cause significant bowel problems. In a study published in 1976 the fecal contents of three astronauts were examined during a 5-month period, when housed under significantly stressful conditions isolated in Skylab [155]. *Bacteroides fragilis* subspecies *thetaiotaomicron* was found to be significantly increased in all three astronauts investigated. A slightly later similar study in astronauts reports a distinct decrease in the numbers of *Bifidobacterium* and of *Lactobacilli* and a substantial increase in numbers of the pathogens *E. coli* and of *Enterobacteria* during preparation for flight, particularly significant within the period immediately before take-off. The numbers of potentially pathogenic *Enterobacteria* and *Clostridia* were substantially increased and numbers of *Lactobacilli* decreased also after the flight. Other studies at the same time conclude that preparation for flights is associated with about 50% reduction in beneficial bacteria, *lactobacilli* and *Bifidobacteria* [156]. A detailed analyses of the microflora of 24 Russian astronauts during preparation for flight performed a few years later reported dramatic reductions in various *Lactobacilli* and significant increases in potentially pathogens [157]. The composition of intestinal [157], oral [158,159] and nasal [158] microbiota was significantly deranged after spaceflights, changes that remained at least for weeks after spaceflights [160], and most likely also for months if not years. See further a recent review [161]. Another interesting study collected saliva samples and fecal samples from 23 healthy undergraduate students during two separate 1-week periods: during the beginning of semester (low-stress baseline condition) and during the first week of exams (high-stress condition). The density of microbes in feces fell in connection with exams from a baseline level of $6 \times 10^7 - 1 \times 10^7$ and remained at this low level during the following 5 days [162].

64.33 Physical Exercise Increases Microbiota Numbers and Diversity

Numerous studies report that physical activity decreases the risk of many chronic diseases and increases longevity. Current US, UK, Australian, and WHO guidelines recommend that significant health benefits can be obtained through about 150 min of moderate activity or 75 min of vigorous activity per week – see further a recent review [163]. Large meta-analyses of cohort studies have reported an app 30% difference in mortality when comparing groups with the lowest and highest amount of physical activity [164,165]. Both volume and intensity of physical exercise have been shown to exert significant influence on gastrointestinal health status. It is demonstrated that exercise reduces the transient time of foods in the gastrointestinal tract, hereby reducing the prolonged contact of pathogens with the gastrointestinal mucus layer and also with circulatory system [166]. Exercises have been shown to have capacity to change gut microbiota diversity both quantitatively and qualitatively and a significant increase of butyrate concentrations is reported in animals after voluntary running exercise [167]. A recent study

in elite rugby players reports significant increases in gut microbiota richness and diversity after physical exercise [168].

It is clearly demonstrated that the diversity of gut microbiota correlates positively with protein intake and creatine kinase concentrations, suggesting that diet and exercise are drivers of biodiversity in the gut. As expected, athletes and controls differ significantly in levels of plasma creatine kinase (a marker of extreme exercise), as in levels of inflammatory and metabolic markers. Furthermore, athletes are shown to have significantly higher diversity of gut microorganisms, representing 22 distinct phyla, which in turn positively correlate with protein consumption and creatine kinase. These results provide evidence for a beneficial impact on gut microbiota diversity of exercise, but it is most likely also influenced by the better diet, often rich in fibers characteristic to elite athletes. For further information – see recent review [169].

64.34 Exercise Has Positive and Negative Effects on Immunity Depending on Its Intensity

Exercise might though have both positive and negative effects on immune function and also health, much depending on intensity. Regular moderate-intensity exercise enhances immune functions significantly above those typically found in sedentary individuals [170]. These functions include the potentiation of T-cell-mediated immunity, natural killer (NK) cell cytotoxicity, proinflammatory cytokine production, and a stronger Th1 reaction as demonstrated both in human and animal models. Very prolonged strenuous bouts of exercise with periods of intensive, very strenuous training and strong competition may, however, impair immune functions and increase susceptibility to acute disorders such as upper respiratory tract infections (URTI) by decreasing saliva secretory immunoglobulin A (S-IgA) secretion, NK-cell activity, and proinflammatory cytokine production. Comparisons were recently made between patients, who reported that they exercised 3–6 h/week (LOW), 7–10 h/week (MED) or 11 or more h/week (HIGH). The HIGH and MED groups had more URTI episodes than the LOW group (2.4 ± 2.8 and 2.6 ± 2.2 vs. 1.0 ± 1.6 , respectively: $P < 0.05$) [171]. The HIGH group demonstrated approximately threefold higher levels of interleukin (IL)-2, IL-4 and IL-10 (all $P < 0.05$) in antigen-stimulated whole blood cultures compared to the LOW group while the MED group showed a twofold higher IL-10 levels than the LOW group ($P < 0.05$) [170]. Although to my knowledge no studies exist on alterations in composition of microbiota in immediate connection with strenuous exercise, it is likely that increasing load of exercise is accompanied by increasing exhaustion also of the microbiome.

64.35 Postprandial Inflammation a “Deadly” Threat to Long-Term Health

Postprandial lipemia has emerged as a potential candidate to induce postprandial inflammation. It is well demonstrated that ingestion of a high-fat meal causes

systemic increases in a wide range of inflammatory mediators. Frequent episodes of postprandial inflammation has for years been recognized as a key factor behind the development of arteriosclerosis [172] and various other chronic diseases, especially metabolic syndrome [173], diabetes [174], and hepatosteatosis [171]. Every meal rich in fat is associated with significant derangement of microbiota [27], cascades of markers of inflammatory and oxidative stress [175], particularly tumor necrosis factor- α (TNF- α) [176] and significant endothelial dysfunction [177]. Plasma endotoxin levels were studied in 12 healthy men (20–58 years, mean age: 32 years) after exposure to no meal, three cigarettes, a high-fat meal, or a high-fat meal plus three cigarettes [176]. The high-fat meal with or without cigarettes, but not the no meal or only smoking, reduced significantly ($P < 0.05$) the plasma endotoxin neutralization capacity – an indirect measure of endotoxin exposure. The levels of endotoxin/plasma increased with a mean of 50% ($P < 0.05$) and remained significantly elevated for about one and a half-hour, while the triacylglycerol concentrations remained statistically significantly elevated as long as 4 h after the meal [176]. This study provides strong support to the hypothesis of endotoxemia and postprandial inflammation as major contributors to endothelial activation and to the development of atherosclerosis. It is also observed that simultaneous intake of sugars significantly potentiates the postprandial inflammation induced by a high fat meal [177]. The inflammatory responses of the vascular endothelial cells are shown to vary directly and much in parallel to the patient's postprandial serum triglyceride level and waist circumference [178].

64.36 Abdominal/Visceral Obesity Enhances Postprandial Inflammation

Visceral obesity was extremely rare among our forefathers and remained so until only a few decades ago. When anthropometric studies using NMR were performed in 54 otherwise healthy female volunteers with BMI ranging from 19 to 57 kg/m², it was demonstrated that visceral fat content can vary from only a few ml in lean individuals to over 6 L in morbidly obese [179]. A strong correlation exists between visceral amount of fat, waist circumference and waist-to-hip ratio [179]. It is of the greatest interest that when in stress, visceral adipocytes will per time unit, compared to subcutaneous fat cells, secrete significantly more of free fatty acids and also about three times as much of proinflammatory factors such IL-6 and PAI-1 per gram tissue, observations that might well explain the high risk of acute disease episodes in individuals with visceral obesity [180]. As a matter of fact, the stress-induced load on the vascular endothelium and particularly those the brain, heart and lungs can be extreme, as proinflammatory and procoagulant molecules can vary up to 1000 times in blood concentrations. Observations such as these provide strong support to what has been called the “portal theory,” proposing that the liver when exposed to larger amounts of free fatty acids and proinflammatory factors, released from visceral depots of fat into the portal vein especially in obese patients, promotes development of liver steatosis, hepatic insulin resistance, and enhances the development of metabolic

syndrome, and subsequent development of type 2 diabetes [181]. Dysbiosis and metabolic endotoxemia is strongly associated with obesity and insulin resistance both in mice and humans, supporting the strong linkage between gut microbes, gut barrier function, acute and chronic inflammation, adipose tissue inflammation, endothelial inflammation and dysfunction, diet-induced obesity, chronic inflammation and insulin resistance and various chronic diseases.

64.37 Postprandial Inflammation Are Induced by Long-Chain but Not Medium-Chain Fatty Acids

The metabolism in the body of consumed MCFAs (C6–C12) is fundamentally different from that of long-chain fatty acids (LCFAs) (C12–C21) – observations that have not yet received enough interest. Medium-chain triglycerides (MCT) are, much in contrast to long-chain triglycerides (LCT), much more readily hydrolyzed. MCFAs are known to be absorbed from the small intestine by a more direct and much more rapid pathway, the portal vein than that of LCFAs. Since several decades ago it is well known that MCTs undergo a rapid hydrolysis by the gastric, salivary or pancreatic lipases, most likely due to much better solubility and motility of the MCT lipid droplet. MCFAs are therefore rapidly transported as nonesterified fatty acids into the portal blood stream to reach the liver, while LCFAs after long-lasting transport as chylomicrons via the thoracic duct and after hours remaining in the general circulation are redistributed as LCTs by hepatic lipoproteins to nonhepatic tissues – see References [182,183] and Figure 64.1 for further detail.

The clinical effects of chain length were studied in a most interesting experimental study. Wale Wistar rats were fed isocaloric high-fat diets containing triacylglycerols composed of either MCFAs or LCFA. Insulin sensitivity was

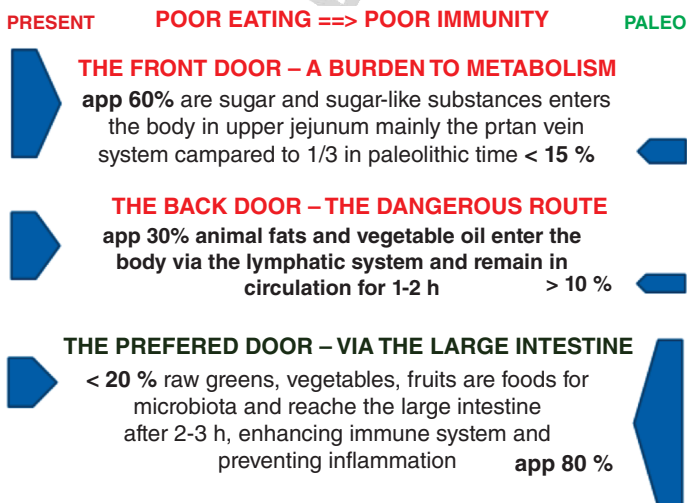


Figure 64.1 Comparing overview of eating habits and immune system.

demonstrated to already after 4 weeks be reduced by 30% in the LCFA group, while it remained unaffected in the MCFA group [184]. Triacylglycerol concentrations in muscle were higher in both the high-fat groups in comparison to controls. No diet-induced changes were found in acyl-CoA oxidase (ACO) activity (liver and muscle) in the MCFA group, while feeding LCFAs significantly raised carnitine palmitoyltransferase activity. It was concluded that the chain length of saturated fatty acids clearly affect whole-body insulin sensitivity and mitochondrial fatty acid uptake even in absence of obesity. It was also observed that MCT compared to LCT feeding resulted in lowered fasted and post-prandial triglyceride concentrations [184]. Feeding sows with diets containing 15% MCTs resulted in a significantly lower mortality of newborns and promoted development, particularly of underweight piglets [185,186].

64.38 Reduced Intake of LCFAs and Increased Intake of MCFAs Good for Microbiota and Health

Numerous studies have demonstrated significant beneficial impacts of feeding MCFAs on both composition of the intestinal microbiota and inhibitory effects on bacterial concentrations in the digesta, mainly of Salmonella and coliforms. Apart from the specific nutritional and metabolic effects of MCTs and MCFA, their rapid digestion, passive absorption and obligatory oxidation, provide these fatty acids with especially strong immunomodulatory effects. While LCTs and LCFAs have meat and dairy as their dominating food source, MCTs and MCFAs are typical plant fats. The content of these fats is especially high in coconut oil – caprylic acid (C8), 3.2–15% of capric acid (C1), and 41–56% of lauric acid (C12). High contents of caprylic (2.4–6.2%), capric (2.6–7.0%), and lauric acid (41–55%) are also found in palm kernel oil. Cuphea seeds (family of loosestrife) have a broad species-dependent diversity in MCFAs. Oils from Cuphea seeds are known for their extraordinarily high content of MCFAs; Cuphea lanceolata and Cuphea ignea oils containing over 80% capric acid and are often used for nutrition of domestic animals, particularly piglets [186].

This far too few studies are undertaken in humans. One study reports impressive effects in patients with severe hypertriglyceridemia after 7-day treatment with a formula diet rich in omega-3 fatty acids and medium-chain triglycerides: plasma triglycerides decreased from a mean of 1601–554 mg/dL ($P < 0.05$), total cholesterol levels reduced from 417 to 287 mg/dL ($P < 0.001$), and slight decreases also observed in fasting glucose (–8%) and uric acid levels (–12%) [187]. Significant improvements of supplementation of MCTs and MCFAs are also reported in conditions such as:

- Obesity with significantly reduced body weight, waist line and insulin resistance [188,189]
- Mild to moderate dementia in Alzheimer [190] – improved cognition without adverse effects, and improvements in adrenergic or symptomatic responses to hypoglycemic
- Type 1 diabetic patients [191]

- Premature aging and arteriosclerosis [192]
- Inherited cardiomyopathy [193,194]
- Chronic pancreatitis [195]

64.39 Gut Reconditioning – An Important Tool to Maintain Optimal Health

Supplementing live bioactive bacteria constitutes in addition to eating large amounts of raw plant fibers a powerful instrument to control inflammation and protect health. It is important to stress that only a small minority of bacteria sold as probiotics on the market have any significant probiotic clinical effects, even if they sometimes carry the same name as one demonstrated to be effective. Only a few products on the market have done proper preclinical and clinical studies – the three leading being, in addition to my own synbiotic product, Synbiotic 2000 – see below – are Lactobacillus GG (Valio Ltd), Lactobacillus reuteri (Biogaia Ltd), and VSL 3 (Sigma-Tau Ltd.). Exciting results are obtained when probiotics, which are properly tested preclinically; *in vitro*, in experimental animals are tried in controlled clinical trials. About two dozens human controlled studies, most of them published in the last few months are presented below. They demonstrate statistically significant and impressive clinical effects: improved gut colonization and immune system [196], reduced allergic manifestations [197], reduced risk of ADHD [198], reduced disease activity in rheumatoid arthritis [199], reduced clinical symptoms and improved quality of life in colorectal cancer survivors [200], reduced diarrhea in children with tropical diarrhea [201], clinical improvement in full-term infants with critical illness [202], improved safety and clinical effects in very low-birth-weight infants (VLBW) [203], reduced translocation and inflammation in HIV patients [204], reduced insulin sensitivity, blood lipids, and inflammation in metabolic syndrome [205], relief of symptoms in irritable bowel syndrome (IBS) [206,207], improved eradication of *Helicobacter pylori* when used as supplement to antibiotic therapy [208], reduced inflammation in CD [209], reduced negative effects when exposed to hepatotoxins and carcinogens [210], reduction in fatty infiltration and inflammation in non-alcoholic fatty liver disease (NAFLD) [211], reduced accumulation of urea in chronic kidney disease [212], reduced inflammation in dialysis patients [213], increased reduction of uremic waist products in patients undergoing renal dialysis [214], reduced number of days with URTIs [215,216], reduced degree of inflammation in cystic fibrosis (CF) [217], and prevention of toxic effects of environmental toxic metal exposure [218] – just to mention a few of very many interesting studies presented in the literature.

Here follows brief summaries of these selected studies.

64.39.1 Allergies

Improved gut colonization and development of the immune system – 241 mother-infant pairs with history of allergic disease and atopic sensitization were randomly

assigned to receive either (1) *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 (LPR + BL999), (2) *L. paracasei* ST11 and *B. longum* BL999 (ST11 + BL999), or (3) placebo, beginning 2 months before delivery and continuing during the first 2 months of breast-feeding [216]. Altogether 205 infants completed the follow-up up to 24 months and were included in the analyses. The risk of developing eczema during the first 24 months of life was significantly reduced in infants of mothers who received LPR + BL999 ($P < 0.001$) and ST11 + BL999 ($P < 0.001$), the odds ratios (ORs) for chronically persistent eczema described to be 0.30 ($P = 0.016$) and 0.17 ($P = 0.003$), respectively [196].

Reduced allergic manifestations – 48 patients were randomized to receive for 12 weeks a combination of two probiotics: *Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03 or maltodextrin as placebo and studied at baseline, at the end of therapy, and 2 months later [197]. The patients receiving probiotics showed a significant improvement in clinical parameters, Severity Scoring of Atopic Dermatitis Index (SCORAD) ($P < 0.0001$) and in Dermatology Life Quality index (DLQ) ($P = 0.021$). The probiotics reduced significantly microbial translocation ($P = 0.050$), immune activation ($P < 0.001$), improved T-helper cell – regulatory T cell (Treg) ($P = 0.029$) and Th1/Th2 ($P = 0.028$) ratios. None of these changes were observed in the placebo group [197].

64.39.2 ADHD

Reduced risk of developing/elimination of ADHD – 75 infants were randomized to receive *L. rhamnosus* GG (ATCC 53103) or placebo during the first 6 months of life and were thereafter followed for 13 years [198]. The children were re-investigated at the age of 13 years by specialist in child neurology or child psychiatry. ADHD and Asperger's syndrome (AS), based on ICD-10 diagnostic criteria, were found in 6/35 (17.1%) of the children in the placebo-treated and in none in the perinatally probiotic-treated children ($P = 0.008$). The mean numbers of *Bifidobacterium* species bacteria in feces during the first 6 months of life was found to be significantly lower in the children who developed ADHD and AS – 8.26 (1.24) log cells/g compared to healthy children 9.12 (0.64) log cells/g; ($P = 0.03$) [198].

64.39.3 Autoimmune Diseases

Reduction in disease activity in Rheumatoid arthritis – 46 female patients with established rheumatoid arthritis (RA) for more than 1 year, and on stable medication for at least 3 months, were in a randomized double-blind clinical trial randomly allocated to receive for 8 weeks either 10^8 colony-forming units (cfus) of *L. casei* 01, or a placebo. Twenty-two patients received the probiotic and 24 patients the placebo [199]. *L. casei* 01 supplementation decreased significantly serum high-sensitivity C-reactive protein (hs-CRP) levels, and reduced tender and swollen joint counts, and improved global health (GH) score and Disease activity score-28 (DAS28) ($p < 0.05$). Significantly more patients in the *L. casei* 01 group, at the end of the study, as based on the European League Against Rheumatism (EULAR) criteria, demonstrated clinical response to the probiotic treatment ($P < 0.01$). Statistically significant differences in favor of the probiotic

group were also demonstrated in activity of IL-10, IL-12, and TNF- α ($P < 0.05$) [199].

64.40 Cancer

Reduction of bowel symptoms and improving quality of life in colorectal cancer survivors – A double-blind, randomized, placebo-controlled trial was undertaken with 32 participants receiving twice daily for 12 weeks Lacidofil (Institut Rosell's *L. helveticus* and *L. rhamnosus*.) and 28 participants receiving placebo [200]. Lactofil did significantly decrease the numbers of patients suffering from irritable bowel-like symptoms ($P = 0.03$), improve cancer-related quality of life ($P = 0.04$), and improve fatigue-related quality of life ($P = 0.02$) as well as patients' health-9 (PHQ-9) scores ($P = 0.01$) [200].

64.41 Childhood Diarrhea

Reducing tropical childhood diarrhea – An open-labeled, randomized controlled trial was undertaken in 2000 children with acute severe watery diarrhea, aged between 6 months and 5 years [201]. The children received for 5 days either *Lactobacillus* GG (LGG) in dose of 10 billion cfus/day or no probiotic medication. Median duration of diarrhea was significantly shorter in children in LGG group (60 vs. 78 h, $P < 0.001$) and the consistency of stool returned to normal significantly faster than in the control group (36 vs. 42 h, $P < 0.001$). Furthermore, significant reduction in the average number of stools per day was observed in the treated children ($P < 0.001$). The benefits of probiotics were also seen rotavirus-positivite children [201].

64.41.1 Critical Illness

Improved safety and clinical effects in critically ill neonates – A double-blind, randomized controlled trial was conducted in 100 full-term critically ill infants, assigned at birth to during 8 days three times daily receive a total of 30 billion of *L. casei*, *L. acidophilus*, *Bacillus subtilis*, and *Enterococcus faecalis* or placebo [206]. Significant reduction in the rate of nosocomial pneumonia (18 vs. 36%) and in multiple organ dysfunction syndrome (6 vs. 16%) was observed in the probiotic-treated group compared with the placebo group ($P < 0.05$). Significantly higher levels of IgA than the placebo group was observed in the probiotic-treated infants ($P < 0.05$). Furthermore, a significantly reduced hospital stay was reported in the probiotic-treated group ($P < 0.05$) [202].

Prevention or reduction of disease activity in necrotizing enterocolitis (NEC) in VLBW – A multicentre, prospective, randomized, controlled trial was conducted in 400 VLBW infants, who during 8 weeks and until discharge or death were supplemented either *B. lactis*, the prebiotic inulin, or a combination of *B. lactis* and inulin (= synbiotics) [203]. Maternal exposure to antibiotics increased significantly the risk of the babies developing NEC. The rate of NEC was lower

in both *L. lactis*-treated (2.0%) and *L. lactis* plus inulin-treated (4.0%) compared to only inulin (12.0%) or placebo (18.0%) treated children ($P < 0.001$). The time to reach full enteral feeding was significantly shorter ($P < 0.001$), the rate of clinical nosocomial sepsis was lower ($P = 0.004$), the stay in the neonatal intensive care unit shorter, ($P = 0.002$), and the mortality rate lower ($P = 0.003$) in the infants receiving active treatments compared to controls [203].

64.41.2 HIV – Virologic Suppression

Reduce translocation and inflammation in HIV patients – double-blind, randomized, placebo-controlled trial was conducted in 44 nonconsecutive HIV-1-infected patients with viral load of < 20 copies/mL during the last 2 years, who received either *Saccharomyces boulardii* or placebo [204]. A significant decrease in lipopolysaccharide-binding protein (LBP) was observed (57.9 vs. 6.2% $P = 0.002$) and also in IL-6 (-0.60 vs. $+0.78$ pg/mL, $P = 0.002$) [204].

64.41.3 Metabolic Syndrome and Diabetes

Reduced insulin sensitivity, blood lipids, and inflammation – A probiotic composition consisting in 112.5×10^9 cfus of three strains of bifidobacteria (*B. longum*, *B. infantis*, and *B. breve*), four strains of lactobacilli (*L. acidophilus*, *L. paracasei*, *L. delbrueckii* subsp. *bulgaricus*, and *L. plantarum*), and one strain of *Streptococcus salivarius* subsp. *thermophilus*. (VSL 3) with or without added omega-3 fatty acid was tried in 60 overweight (BMI > 25), healthy adults, aged 40–60 years, who for 6 weeks received either placebo, only omega-3 fatty acid, only probiotic VSL 3, or combination of omega-3 and VSL 3 [205]. Patients with low HDL, higher insulin resistance, and high hsCRP had significantly lower total lactobacilli and bifidobacteria count and higher *E. coli* and bacteroides count. VSL 3 supplementation resulted in significant reductions in total cholesterol, triglyceride, LDL, and VLDL, and increase in HDL ($P < 0.05$). The VSL 3 treated patients demonstrated significantly improved insulin sensitivity ($P < 0.01$), decreased hsCRP, and an improved gut microbiota. Also the Omega-3 treated demonstrated significant effect on insulin sensitivity and hsCRP, but no effects on gut microbiota. Combination of VSL 3 with omega-3 fatty acid attenuated the effects on HDL, insulin sensitivity, and hsCRP [205].

64.41.4 Gastrointestinal Disorders

Relief of symptoms in patients with irritable bowel syndrome (IBS) – 50 patients suffering from IBS were randomized to receive daily for 4 weeks either *L. casei rhamnosus* LCR35 in a dose of 6×10^8 cfus ($n = 25$) or placebo ($n = 25$) [206]. No general improvements were reported, but the subgroup dominated by diarrhea demonstrated significant improvement with LCR35 treatment (reduction with 37%) compared to placebo (reduction with 3%) and also significant reduction in abdominal pain severity score (reduction with 36%). On the other hand, a slight improvement in favor of placebo was observed in patients with severe constipation; more reduced with placebo reduction with 41%) than in the LCR35-treated

(reduction with 20%) [206]. Another randomized, double-blind, placebo-controlled trial was undertaken in adult patients with symptomatic IBS with a probiotic composition consisting in *L. rhamnosus* NCIMB 30174, *L. plantarum* NCIMB 30173, *L. acidophilus* NCIMB 30175, and *Enterococcus faecium* NCIMB 30176 or placebo (1 mL/kg/day), administered for 12 weeks [207]. Total 152 patients completed the study. The mean reduction in IBS scores was 63.3% in the probiotic-treated versus 28.3% in placebo ($P=0.019$). No improvements in IBS quality of life scores could, however, be seen [207].

Improved clinical effects as supplement to antibiotic therapy to eradicate H. pylori. – A multicenter, prospective, randomized, placebo-controlled, and double-blind study in totally 650 patients, who received for 14 days, twice a day, at least 2 h prior to or after the administration of antibiotics, capsules with probiotics containing either 10^8 – 10^{10} of *L. rhamnosus* GG (LGG®) and *Bifidobacterium* (BB-12®) or placebo capsules [208]. A significantly larger proportion of successfully cured patients were observed with probiotics added to the antibiotic treatment than without (87.38 vs. 72.55%; $P<0.001$). The average intensity for symptoms induced by the antibiotic therapy was significantly reduced when probiotics were added (0.76 vs. 0.55; $P<0.001$). Supplementation of probiotics reduced in average 7 of the following 10 symptoms: presence and intensity of epigastric pain, bloating, flatulence, taste disturbance, loss of appetite, nausea, vomiting, heartburn, rash, and diarrhea [208].

Significant reduction of inflammation in CD – The potential effects of *B. longum* CECT 7347 in children with newly diagnosed CD were evaluated in a double-blind, randomized, placebo-controlled trial in 33 children, who received daily for 3 months in addition to a gluten-free diet either 10^9 cfus *B. longum* CECT 7347 or placebo [209]. The *B. longum* CECT 7347 treated demonstrated compared to placebo greater height percentile improvements ($P=0.048$), decreased peripheral CD3⁺ T lymphocytes ($P=0.004$), and slightly reduced TNF- α concentration ($P=0.067$). Significant decreases in CD3⁺ ($P=0.013$), in human leukocyte antigen (HLA)-DR⁺ T lymphocytes ($P=0.029$), and slightly reduced TNF- α concentration ($P=0.085$) were also reported in the *B. longum* CECT 7347-treated patients [209].

64.41.5 Liver Disease

Reduced uptake of hepatotoxins and carcinogens – 90 healthy young men were enrolled in a study aimed to determine whether administration of probiotic bacteria could block the intestinal absorption of a dangerous poison aflatoxin B(1) and reduce urinary excretion of aflatoxin B(1)-N(7)-guanine (AFB-N(7)-guanine), a marker of aflatoxin exposure [210]. The patients received for 5 weeks twice daily either a mixture of *L. rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp. *shermanii* strains or a placebo. The percentage of samples with negative AFB-N(7)-guanine values were after the 5-week intervention higher in the probiotic group than in the placebo group period ($P=0.052$), and the decrease in urinary AFB-N(7)-guanine was statistically significantly reduced in the probiotic-treated group compared to placebo ($P<0.05$). The observed reduction was as high as 36% after 3 and 55% after 5 weeks – the geometric means for the

probiotic and placebo groups reported to be 0.24 and 0.49 ng AFB-N(7)-guanine/mL respectively ($P=0.005$) [210].

Reduced fatty infiltration in NAFLD – A double-blind clinical trial supplementing a probiotic composition consisting in 112.5×10^9 cfus of three strains of bifidobacteria (*B. longum*, *B. infantis*, and *B. breve*), four strains of lactobacilli (*L. acidophilus*, *L. paracasei*, *L. delbrueckii* subsp. *bulgaricus*, and *L. plantarum*), and one strain of *Streptococcus salivarius* subsp. *thermophilus*. (VSL 3) or placebo, supplemented to obese children with biopsy-proven NAFLD [211]. Forty-four patients (22 VSL 3 and 22 placebo) completed the study. At baseline, moderate and severe NAFLD were present in 64% and 36% of placebo children and in 55% and 45% of VSL 3 children. The probability that children supplemented with VSL 3 had in comparison none, light, moderate, or severe fatty liver at the end of the study was 21 versus 0%, 70 versus 7%, 9 versus 76%, and 0 versus 17% group ($P<0.001$). No differences were detected in triglycerides, homeostasis model assessment (HOMA) and alanine transaminase (ALT) while BMI decreased and glucagon-like peptide 1 (GLP-1) and activated GLP-1 (aGLP-1) increased in the VSL 3 group ($P<0.001$ for all comparisons) [211].

64.41.6 Renal Disease

To reduce the accumulation of urea in chronic kidney disease – Patients with chronic kidney disease (CKD) show an increase in bowel aerobic bacteria that produce uremic toxins and decreased anaerobic bacteria as bifidobacteria and lactobacillus [212]. Two different strains of *L. casei shirota* (LcS) were tried for 8 weeks in CKD patients in a simple randomized, controlled clinical trial in two doses: Group A: 8×10^9 cfu and group B: 16×10^9 CFU. The larger dose was most effective, inducing a statistically significant no less than 10 times reduction in blood urea concentrations [212].

To reduce inflammation in dialysis patients – Peritoneal dialysis (PD) patients received daily for 6 months one capsule of a probiotic composition containing 10^9 cfu *Bifobacterium bifidum* A218, 10^9 cfu *Bifidobacterium catenulatum* A302, 10^9 cfu *B. longum* A101, and 10^9 cfu *L. plantarum* A87, or maltodextrin as placebo [213]. 39 patients completed the study. The serum levels of proinflammatory cytokines TNF- α , IL-5, IL-6, and endotoxin were significantly decreased after 6 months of treatment, while serum levels of anti-inflammatory cytokine IL-10 were significantly increased, whereas no significant changes in levels of serum cytokines and endotoxin were observed in the placebo group. Most importantly, the residual renal function was well preserved after 6 months in patients receiving probiotics [213].

To reduce uremic waist products in patients with in patients with chronic kidney disease – In a 6-month crossover trial 46 outpatients were supplemented a probiotic formulation consisting in a gel capsule containing a mix of *L. acidophilus* KB27, *B. longum* KB31, and *S. thermophilus* KB19, totally 1.5×10^{10} CFU/day [214]. Significant improvements were observed: enhanced well-being (quality of life – QLT) ($P<0.05$), absence of serious adverse effects and impressive reductions in blood urea nitrogen (BUN) ($P<0.05$ – BUN levels reported to be decreased by 63%, creatinine levels by 43% and uric acid levels by 33%. The improvement in QOL was estimated to be no less than 86% ($P<0.05$) [214].

64.41.7 Respiratory

To reduce the number of days with URTI in academically stressed students – Randomized, double-blind, placebo-controlled study examined the effect of three potentially probiotic bacteria on the proportion of healthy days over a 6-week period in academically stressed undergraduate students (n = 581) who received one of the following probiotics; *Lb helveticus* R0052, *B. longum* ssp. *infantis* R0033, *B. bifidum* R0071 or placebo [215]. The percentage of participants reporting ≥ 1 day of cold/flu during the 6-week intervention period was significantly lower only with *B. bifidum* ($P < 0.05$), while no effects were observed with supplementing *B. infantis* or *L. helveticus* [215]. The efficacy of daily dietary consumption of *L. brevis* KB290 (KB290) to prevent against influenza also in elementary schoolchildren, was studied in an open-label, parallel-group trial conducted 8-week periods [216]. The incidence of influenza was 16% with probiotic protection and 24 without ($P < 0.001$). The reduction in the incidence of influenza by KB290 consumption was especially pronounced in nonvaccinated individuals [216].

To reduce inflammation in CF patients – 22 children with CF children were enrolled in the study. The CF children were found to suffer severe intestinal dysbiosis with significant reductions in *Eubacterium rectale*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *B. adolescentis*, *B. catenulatum*, and *Faecalibacterium prausnitzii* [217]. Significant elevations in fecal calprotectin (CLP: 184 vs. 52 ± 46 $\mu\text{g/g}$, $P = < 0.01$) and in rectal nitric oxide (rNO) levels (184 vs. 52 ± 46 $\mu\text{g/g}$, $P = < 0.01$) were reported in children with CF compared to healthy controls. The degree of dysbiosis was especially increased in children with CF, who were taking antibiotics. A significant correlation was observed between the treated groups in favor of the probiotic-treated group both in degree of inflammation and increase of microbial richness of intestinal microbiota. Administration of 6×10^9 cfus of *Lactobacillus* GG (LGG) reduced significantly fecal CLP and restored, at least partially, the intestinal microbiota [217].

64.41.8 Toxic Exposure

To prevent effects of environmental toxic metal exposure – A study in Tanzania investigated the efficacy of probiotic foods to prevent consequences of high local toxic metal exposures. A group of 44 school-aged children was followed over 25 days, and 60 pregnant women were followed over their last two trimesters until birth [218]. A yogurt containing 10^{10} CFU *L. rhamnosus* GR-1 per 250 g was administered, while control groups received either whole milk or no special foods. Changes in blood metal levels were assessed, and the gut microbiomes of the children were profiled by analyzing 16S rRNA sequencing via the Ion Torrent platform. The children and pregnant women at the beginning of the study found to have significantly higher blood levels of lead and mercury when compared to age- and sex-matched Canadians. Consumption of probiotic yogurt provided a statistically significant tool to protect against increases in blood levels of both mercury (3.2 nmol/L; $P = 0.035$) and arsenic (2.3 nmol/L; $P = 0.011$) particularly in the pregnant women, a trend, which was not statistically significant in the children, at least not after only treated for 25 days [218].

64.42 Fecal Microbiota Transplantation (FMT) – A Giant Step Forward

Reconditioning of the gut by transfer of microbiota from a healthy individual to an often very sick patient, marks a new époque in the history of restitution a healthy gut and attempts to reduce the burden of disease. It is unfortunate that in the Western world most, if not all of the donors, also suffer a much reduced microbiota, compared to those still living a rural forefather-like lifestyle such as the Hadzas [29], the Burkina Fasons [32], the Yanomamis [34], whose microbiota demonstrate around 50% higher diversity – and worse, recent data suggest that the deterioration of human microbiota is a continuous process [219]. It seems reasonable to assume that the most effective health-promoting bacteria are already extinct in Western population due to lack of proper foods. Maybe one day such rich stools from groups such as those may be available deep-frozen for FMT. Under all circumstances it will not last long before deep-frozen stools are a reality – to begin with stools from the individual saved already at an early age. It must be emphasized though that the best results from reconditioning of the gut is obtained when combined with a dramatic change in lifestyle and food habits – and that forever. Recent studies demonstrate that it takes less than 24 h to destroy microbiota if the microbes do not receive for them suitable nutrients [220]. For information about recent progress with FMT – please read some recent excellent reviews [221–225].

64.43 Synbiotics – A Less Invasive Alternative

Oral or rectal supplementation of a synbiotic formula offers simple and less cumbersome alternatives – constitutes attempts to produce “synthetic feces.”

For the most recent 15 years my personal focus has been on trying:

- 1) To identify lactobacilli (LAB) on growing plants that might have unique inflammation-reducing abilities, based on the belief that the strongest anti-inflammatory microbes might be extinct in human microbiome.
- 2) To provide strong fibers with documented ability to serve as substrate for microbial fermentation (favored nutrients for microbes) and in doses of at least 10 g – adding about 40% more fiber to the average fiber consumption, which is very low in Westyerners.
- 3) To provide large doses of LAB than commonly used (exemption VSL 3).

I took initiative in 1999 to develop and produce a synbiotic formula based on these principles. Since 1999, all my efforts in this field have concentrated on a four LAB/four fiber composition, consisting of either a mixture of either 4×10^{10} (40 billion LAB in a Standard version – Synbiotic 2000™) or a mixture of 10^{11} (400 billion, Forte version – Synbiotic 2000 Forte™). From studies of about 500 lactobacillus species (about 200 collected from plants) the following four LAB, which demonstrating unique anti-inflammatory abilities [226,227] were chosen for the composition: *Pediococcus pentosaceus* 5–33:3, *Leuconostoc mesenteroides* 32–77:1, *Lactobacillus paracasei* subsp *paracasei* 19, and *Lactobacillus plantarum* 2362. To the four LAB was added 10g of documented bioactive fibers:

4 × 2.5 g of each of the following four fermentable fibers: betaglucan, inulin, pectin and resistant starch, a formula that is currently a product produced by Synbiotic AB, Sweden.

Below is summarized the experience from some of the human randomized controlled studies, using Synbiotics 2000:

To prevent postoperative sepsis in extensive pancreatic surgery – A prospective, randomized, double-blind trial was performed in 80 patients undergoing pylorus-preserving pancreatoduodenectomy (PPPD) who received, twice daily, either Synbiotic 2000™ (2 × 40 billion LAB, i.e., 80 billion LAB/day) or only the fibers in composition from the day before surgery and during the first seven postoperative days [228]. A highly significant difference in infection rate ($P=0,005$) was observed – only 5/40 patients (12.5%) in the Synbiotic 2000-treated group suffered infections (4 wound and one urinary tract infection) in sharp contrast to 16/40 (40%) in the fiber-only group (6 wound infections, 5 peritonitis, 4 chest infections, 2 sepsis, and 1 of each of urinary tract infection, cholangitis, and empyema). The number of infecting microorganisms were also statistically significantly reduced ($P=0,005$) as was the use of antibiotics (mean: Synbiotic 2000; 2 ± 5 days, only fibers; 10 ± 14 days) [228].

To prevent postoperative sepsis in abdominal cancer operations – 45 patients undergoing major surgery for abdominal cancer were randomized 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 only 6.7% with Synbiotic 2000-based LEN ($P<0.05$). The numbers of infecting microorganisms were statistically and significantly reduced. Significant improvements were observed with both Synbiotic 2000 and only-fiber-based treatments (LEN, FEN), in prealbumin, CRP, serum cholesterol, serum endotoxin, and with only Synbiotic 2000 in white cell blood count and in IgA (Han Chun Mao, personal information).

To prevent postoperative sepsis in orthotopic liver transplantation – 66 human orthotopic liver transplant patients were randomized to either receive Synbiotic 2000 or selective bowel decontamination (SBD) [229]. The treatment continued from the day before surgery until 14 days after surgery. The Synbiotic 2000 treatment did almost extricate postoperative sepsis. After 1-month follow-up had only one patient in the Synbiotic 2000-treated group developed an infection – a benign *E. faecalis*-based asymptomatic catheter-induced urinary infection 1/33 (3%) compared to 17/33 (51%) in the SBD-treated patients [229]. The use of antibiotics was on average 0.1 ± 0.1 days in the Synbiotic-treated patients and 3.8 ± 0.9 days in the SBD-treated patients [229].

To reduce infections and complications to severe acute pancreatitis – 62 patients with severe acute pancreatitis (SAP) were supplemented twice daily during the first 14 days after arrival at the hospital with Synbiotic 2000™ (80 billion + 20 g fibers) or the same amounts of fibers (totally 20 g/day) as in Synbiotic 2000™ [230]. 9/33 patients (27%) in the Synbiotic 2000-treated group and 15/29 patients (52%) in the fiber-only treated group developed 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 detected in the Synbiotic-treated group compared to seventeen in the fiber-only group [230]. Another study supplemented Synbiotic 2000 Forte (400 billion Lab + 10 g fibers) from within 24–48 hrs of symptoms of sickness to patients with severe acute pancreatitis and compared to 32 patients on a control formula considered as anti-inflammatory [231]. Synbiotic-2000 Forte treatment lead to a lower infection rate including less pancreatic and peripancreatic necroses; secondary infections (2 vs. 9, $P = 0.0001$), septicemia (2 vs. 7, $P = 0.03$), lower rate of surgical interventions, (3 vs. 12, $P = 0.005$), shorter stay in ICU (8 vs. 16 days $P = 0.05$), shorter hospital stay (23 vs. 36 days, $P = 0.03$), and reduced mortality (0 vs. 17 patients, $P = 0.02$) [231].

To reduce infections and complications to major trauma – Two prospective randomized trials with either Synbiotic 2000™ or Synbiotic 2000 Forte™ were undertaken. In the first study [232] 52 patients were randomly allocated into 4 groups with different commercial nutrition formulas: Group A: Alitraq (Abbott-Ross) B: Nova Source (Novartis) Group C: Nutricomp peptide (*B. braun*) plus Synbiotic 2000) Group D: Nutricomp standard (*B. braun*) plus Synbiotic 2000. Nova Source and Nutricomp peptide did not reduce the levels of proinflammatory cytokines at all, while Alitraq and Nutricomp standard + Synbiotic 2000 significantly down-regulated Il-6, but not Il-8 and TNF- α . The groups containing Synbiotic 2000 demonstrated significant reductions in both total infections and in chest infections; the total number of infections being reduced with about two thirds ($P < 0.005$) and the number of chest infections being reduced to about half ($P < 0.005$) [232]. In a series of studies 65 polytrauma patients were randomized to receive once daily for 15 days following major trauma, either Synbiotic 2000 Forte (400 billion LAB + 10 g of fibers) or maltodextrine as placebo [233–235]. 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 days on artificial ventilation (average 15 vs. 26 days). A total of 54 pathogenic microorganisms were cultivated in the Synbiotic treated group compared to 103 in the placebo group. Repeat analyses revealed that serum levels of endotoxin (LPS) were significantly decreased and the time to occurrence of bloodstream infection significantly prolonged in patients treated with Synbiotic 2000 Forte ($P < 0.02$) [233–235].

64.44 Less Suffering for the Patients – Great Savings for Society

Figure 64.2 summarizes the gains in reduced numbers of infections obtained after Synbiotic 2000 treatment in various severe conditions – reductions varying between 60 and 94% depending on disease/trauma/surgical treatment demonstrated. Figure 64.3 summarizes the savings in use of costly antibiotics, savings varying between 80 and 97%, savings on costly artificial respiration of about 20% savings on reduced time in costly ICUs vaying between 14 and 67% and also savings on the average in hospital time varying from 3 to 24% depending on treated condition.

REDUCTIONS IN INFECTIONS/POSITIVE BLOOD CULTURES**Liver transplantation – 66 patients²³⁶**

Patients with postop. infections	16 => 1 = 94%
Patients with pos. blood cultures	11 => 1 = 91%

Pancreatodudenectomy for cancer – 80 patients²³⁵

Patients with postop. infections	16 => 5 = 69%
Patients with pos. blood cultures	27 => 5 = 82%

Severe pancreatitis – 62 patient²³⁷

Patients with infections	15 => 9 = 40%
Patients with pos. blood cultures	17 => 7 = 59%

Severe trauma, treated with synbiotic 2000 standard – 52 patients²³⁹

Patients with infections	23/30 (77 %) => 17/35 (49 %)
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Severe trauma, treated with synbiotic 2000 Forte – 72 patients²³⁹⁻²⁴¹

Patients with post-trauma infections	13 => 5 = 62%
Patients with pos. blood cultures	13 => 5 = 62%

Figure 64.2 Reduction in infections/positive blood cultures after Synbiotic 2000 treatment.**REDUCTIONS IN USE OF ANTIBIOTICS, ARTIFICIAL RESPIRATION, TIME IN ICUs, AND IN HOSPITAL****Liver transplantation – 66 patients²³⁶**

Days on Antibiotics	3.8 => 0.1 = 3.7 (97 %)
Days in ICUs	10.2 => 8.8 = 1.4 (14 %)
Days in Hospital	27.9 => 27.8 = 0.1 (3 %)

Pancreatodudenectomy for cancer – 80 patients²³⁵

Days on Antibiotics	10 => 2 = 8 (80 %)
Days in ICUs	6 => 2 = 4 (67 %)
Days in Hospital	22 => 17 = 5 (23 %)

Severe acute pancreatitis – 62 patient²³⁷

Days in Hospital	19.7 => 14.9 = 4.8 (24 %)
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Severe trauma treated with synbiotic 2000 Forte – 65 patients²³⁹⁻²⁴¹

Days on Artificial Respiration	24 => 19 = 5 (21 %)
Days in ICUs	41.3 => 27.7 = 13.6 (33 %)

Figure 64.3 Reduction in use of antibiotics, artificial respiration, time in ICUs, and in hospital after Synbiotic 2000 treatment.**64.45 Ecobiological Functions – Same but Different Response Compared to So-Called Biological Drugs**

Inflammation is said to involve about 1200 genes and affecting an enourmously wide range of effector molecules; proinflammatory cytokines such as IL-1 β , 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, and many more. The so-called biological drugs are made to target exactly the same genes, but mainly one at the time. Ecobiologicals;

BIOLOGICAL vs. ECOBIOLOGICAL

- | | |
|--|---|
| <ul style="list-style-type: none"> • Biologicals aimed to target single genes; anti- TNF-α, anti-IL-1β, anti-HER2, IL-12/IL-23, IFN-γ, IL-17A, IL-2 and IL-6, and inhibitors of NF-kB • Uni-targetting • Immediate powerful effects • High degree of toxicity • Reduce microbiota/induce leakage • Often short-lasting effects • Frequent adverse effects • Indicated in aggressive disease | <ul style="list-style-type: none"> • Ecobiologicals; utilize anti-inflammatory effects of microbes and plants; greens, vegetables, fruits, and spices to support microbiota and reduce inflammation • Multi-targetting • Slower and weaker effects • GRAS – e.g. no toxicity • Support microbiota • Long-lasting effects • No adverse effects • For prevention and in early disease |
|--|---|

Figure 64.4 Comparison of effects of biologicals versus ecobiologicals.

probiotic bacteria, plant fibers, plant antioxidants and so on produce an accumulated response targeting all the genes much in parallel but slower and weaker (Figure 64.4).

Especially affected are the two special tissues, strongly involved in inflammation; the intestinal mucosa and the vascular endothelium organ, both effective barriers, effective metabolic, effective endocrine, and also important immune organs, both covering similarly wide surfaces: the intestinal mucosa that of a soccer field – greater than 7000 m² and consisting in greater than 10 000 billion cells and weighing greater than 1 kg each and both intimately involved in inflammations such as postprandial inflammation [171–185]. The total endothelial tissue of the body, which cover a similarly sized surface, has the unique ability to activate extraordinary many genes and factors involved in inflammation such as VCAM-1, ICAM-1, E-selectin, PDGF, TGF- β , TNF- α , IGF-1, MCP-1, CTGF, IL-6, PAI-1, RAGE, VEGF, ANG II-dependent cell activation factors, type IV collagen expression factors, toll-like stimulatory factors, cell cycle progression factors, and fibronectin [236].

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