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Use of Prebiotics, Probiotics and Synbiotics in Clinical Immunonutrition

- Invited Paper -

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Abstract

It is a recent observation that about 80 per cent of the body's immune system is localized in the gastrointestinal tract. This explains to a large extent why eating right is important for the modulation the immune response and prevention of disease. In addition it is increasingly recognized that the body has an important digestive system also in the lower gastrointestinal tract where numerous important substances are released by microbial enzymes and absorbed. Among these substances are short chain fatty acids, amino acids, various carbohydrates, polyamines, growth factors, coagulation factors, and many thousands of antioxidants, not only traditional vitamins but numerous flavonoids, carotenoids and similar plant- and vegetable produced antioxidants. Also consumption of health-promoting bacteria (probiotics) and vegetable fibres (prebiotics) from numerous sources are known to have strong health-promoting influence. It has been calculated that the intestine harbours about 300,000 genes, which is much more than the calculated about 60,000 for the rest of the human body, indicating a till today totally unexpected metabolic activity in this part of the GI tract. There are seemingly several times more active enzymes in the intestine than in the rest of the body, ready to release hundred thousand or more of substances important for our health and well-being. In addition do the microbial cells produce signal molecules similar to cytokines but called bacteriokines and nitric oxide, with provide modulatory effects both on the mucosal cells, the mucosa-associated lymphoid system (MALT) and the rest of the immune system. Identification of various fermentation products, and often referred to as synbiotics, studies of their role in maintaining health and well-being should be a priority issue during the years to come.

Key words: prebiotics, probiotics, synbiotics, immune system

INTRODUCTION

It is not long since the main function of the lower gastrointestinal tract was said mainly to be reabsorption of water and electrolytes. Now, we know that the body, in addition to the digestive system based on gastrointestinal secretions, possesses an important digestive system based on fermentation of food components by bacterial enzymes, which system is localized in the lower small intestine and the large intestine. Probably a much larger number of substances are released and absorbed at this level than in the rest of the gastrointestinal tract. An indication of this is the fact that the intestine harbors about 300,000 bacterial genes, to be compared with about 60,000 in the rest of the body.

Numerous fibers received from eating pulses, tubers, cereals, fruits and vegetables are disintegrated in the lower gastrointestinal tract and important substances released and absorbed; short chain fatty acids, polyunsaturated fatty acids, amino acids, polyamines, various carbohydrates, growth factors, coagulation factors, antioxidants and other many other important molecules. It is likely that several hundred thousand such molecules are released and absorbed at this level. Furthermore, flora, supplied probiotics, supplied prebiotic fibers, have all their own bioactivity and are involved in a variety of gastrointestinal and immune functions, through both antioxidative actions and through production of cytokine-like molecules, often referred to as bacteriokines.

Health and well-being is said to be dependent on some two million various chemical molecules supposed to exist in precise amounts, which creating an important equilibrium, homeostasis, which is of the greatest importance. Many of these molecules cannot be synthesized by the body, which makes us dependent on outside sources. The need of variation in supply of foods has been especially emphasized. It is suggested that the diet of our Palaeolithic forefathers was much richer than that of the Western diet of today (1). Not only did our ancestors eat considerably more plant fibers they did also eat food from many more sources compared to the foods available today. It is suggested that their annual diet included about 500 different plants. Mod-

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ern man, especially the Westerners, has reduced this to a dousin or two and allowed potato, rice and corn to totally dominate the diet.

The obvious increased incidence of diseases such as allergy, arthritis, diabetes, arteriosclerosis, coronary heart disease, and cancer but also neuro-degenerative diseases such as Parkinson's disease and Alzheimer (2) are increasingly recognized as associated with modern Western food habits. Metabolic syndrome X (MSX), common in Western countries, and manifesting itself in obesity, hypertension, hyperlipidemia, glucose intolerance and insulin resistance, and often mental depression, is increasingly recognized as associated with endemic and chronic diseases. And, accumulating evidence suggests that lack of physical activity, stress, lack of sufficient intake of plant fibers and probiotic bacteria, over consumption of refined and processed foods particularly cholesterogenic saturated fats and refined sugars, but also exposure to various toxic substances, including alcohol, tobacco and pharmaceutical drugs are responsible for this dangerous syndrome (3). There is increasing evidence that persons with this syndrome expresses an exaggerated "chronic phase response" (4), often seen in "Western diseases" such as rheumatoid arthritis, cardiovascular diseases, inflammatory bowel diseases, diabetes but also with conditions such as mental depression (5) or Alzheimer's disease (2).

Western diet (Table 1A,B) is also known to be deficient in inflammation-reducing omega-3 fatty acids, and to contain too much of inflammation-promoting omega-6 polyunsaturated fatty acids (sun flower oil frequently used in Western cooking contain 250 times more of omega-6 fatty acids than omega-3 fatty acids). Foods such as beans, peas, lentils, seeds, and nuts, known to be rich in amino acids (Table 2A~D), minerals (Table 3A,B), vitamins and antioxidants (Table 4A,B) and phytoestrogens are in Western countries consumed today to a much lesser extent than in the past.

Table 1. Negative features of Western foods (A). Comparison made with Paleolithic foods (B)

A. Western food - What is negative?

It contains:
1. Too much saturated fat.
2. Too little polyunsaturated fat.
3. Too much sodium salt.
4. Too little fermentable fibres.
5. Too much refined sugar.
6. Too little antioxidants - cooking, canning and freezing destroys vitamins and antioxidants.
7. Too much mutagens - frying and cooking produces mutagens.
8. Too much animal-derived hormones and growth factors, which delays apoptosis and enhances tumour development.
9. Too little probiotic microorganisms.

B. Paleolithic diet

Contained more of: (× = times more)	
Minerals	2×
Fibre	4×
Antioxidants	10×
Omega-3 FA	50×
Lactobacilli	$>10^{10}$×
Contained less of: (× = times less)	
Protein	2×
Saturated FA	4×
Sodium	10×

Modified after Bengmark (17).

MODERN FOOD CONTAINS LITTLE BACTERIAL COMPONENTS

Modern food as eaten today in the Western countries, are poor in bacterial contents. Our Paleolithic forefathers are calculated to have consumed up to a billion times more of various bacteria, and especially of lactic acid bacteria (LAB). Their most common method to preserve food was to keep it in the soil, where it rapidly became fermented and rich in LAB with strong fiber-fermenting abilities. Modern food industry has really done its best to eliminate bacteria from foods, and in addition, fermented foods such as sauerkraut are not consumed as much as before, with the only exception of fermented milks / yogurts. However, most of bacteria used in yogurts seem to have rather weak health-promoting capacity compared to the LAB, which ferment fibers.

Several studies seem to demonstrate that modern Westerners have a much less rich commensal flora compared to those who live under more natural conditions, especially in rural Africa and Asia (6,7). Lack in the food of substrate for bacterial fermentation, chemicals and pharmaceuticals consumed, stress and disease seem to contribute to a significant reduction in commensal flora in modern man. Astronauts returning to Earth demonstrate a much-reduced protective flora at the same time as the numbers of potentially pathogenic micro organisms (PPMs) have dramatically increased (8). It has also been shown in animals with induced pancreatitis that the anaerobic flora is significantly reduced as early as after 4 to 8 hours, again paralleled by a significant overgrowth of PPMs, especially *E. coli*, and subsequent translocation to various sites in the body, especially to mesenteric lymph nodes and to the pancreas (9-11).

PROMOTION OF HEALTH THROUGH CONSUMPTION OF PRE- AND PROBIOTICS

Lactic acid bacteria are known to possess strong effects

Table 2. Content in foods of some key amino acids with special comparison with typical Western foods: Hamburgers, french fries and tomato ketchup

A. Arginine in foods (mg/100 g)		B. Glutamic acid in foods (mg/g)	
Gelatine	6000	Parmesan cheese	9570
Pumpkin seeds	4030	Gelatine	9150
Soya protein	3760	Low fat cheese	6490
Pea nuts	3600	Soya beans	6440
Sesame seeds	3330	Peanuts	6350
Soya beans	2730	Almonds	5930
Almonds	2500	Sunflower seeds	5580
Sunflower seeds	2400	Sesame seeds	4940
Brazil nuts	2390	Lentils	3900
Peas, lentils	2050	High fat cheese	3770
Shrimps	2000	Peas	3490
Baker's yeast	2000	Oat	3000
Parmesan cheese	1560	Beans	2840
Meat, fish	1500	Cereals	appr 2500
HAMBURGERS	950	HAMBURGERS	2060
Cereals	appr 500	Meat, fish	2000
FRENCH FRIES	140	Baker's yeast	1950
KETCHUP	125	KETCHUP	600
Vegetables	appr 100	FRECH FRIES	460
Pulses	appr 100	Vegetables	appr 100
Fruits	appr 50	Pulses	appr 100
		Fruits	appr 50
C. Histidine in foods (mg/g)		**D. Tryptophan in foods (mg/100 g)**	
Soya protein	2500	Parmesan cheese	570
Parmesan cheese	1630	Sesami seeds	470
Tuna fish	1220	Dry yeast	430
Sardines	950	Pumpkin seeds	430
Pheasant	940	Cheese, 10%	400
Cheese 17%	940	Wheat germs	330
Soya beans	930	Peanuts	310
Eel	880	Tuna fish	270
Dry yeast	770	Turkey	250
Beef	770	Feta cheese	240
Pea nuts	750	Chicken	240
Pumpkin seeds	680	Beef	220
Sesame seeds	680	Hazelnuts	220
Lentils	650	Salmon	210
Wheat germs	610	Walnuts	190
HAMBURGERS	590	Soya bean sprouts	180
Gelatine	580	HAMBURGERS	165
FRENCH FRIES	60	Alfa sprouts	135
KETCHUP	25	Soya, cooked	135
		Beans, cooked	108
		Tofu	94
		Yellow Peas, cooked	87
		FRENCH FRIES	53
		KETCHUP	35

Modified after Bengmark (4).

Table 3. Content in foods of two key minertals with special comparison with typical Western foods: Hamburgers, french fries and tomato ketchup

A. Calcium in foods (mg/100 g)		B. Magnesium in foods (mg/100 g)	
Parmesan cheese	138	Soyabeans	265
Sesami seeds	980	Cashewnuts	260
Cheese	750	Peanuts	190
Nettles	500	Beans	190
Persil	340	Peas	150
Dill	340	Lentils	80
Peas	300	Banana	35
Beans	300	Cheese	35
Sunflower seeds	265	FRENCH FRIES	35
Brazil nuts	180	HAMBURGERS	20
Cream	135	KETCHUP	18
Milk	120	Milk	15
Digestive biscuits	110		
Fish	100		
Spinage	90		
Black current	90		
Butter	18		
HAMBURGERS	10		
FRENCH FRIES	9		
KETCHUP	7		

Modified after Bengmark (4).

at molecular and cellular level (Table 5), which can be expected to be effective treatments in a whole series of medical conditions (Table 6). For more detailed information see Bengmark (12-20). These observations make it attractive to try LAB both to prevent and to treat disease, in humans as well as in animals. Furthermore, the occurrence of antibiotic-resistant bacteria is increasingly creating a large problems around the world and constituting serious threat to human health. This is why World Health Organisation (21) and national health authorities advocates a dramatic decrease in the use of antibiotics. Instead it is recommended that non-pathogenic bacteria such as an LAB be used to control pathogens, a treatment concept called microbial interference treatment (MIT).

The clinical effects are not equal to all LAB. One must remember that most LAB are not genetically related. It has been said that the genetic difference between one LAB and another can be much greater than the difference between a giraffe and a fish. It is also evident from data that emerge from experimental and clinical studies around the World that the potency of different LAB tried varies extensively. Table 7 summarizes much personal evaluation of some LAB as I judge their clinical efficacy from studying the literature. It must be pointed out, that the research on pre-, pro-, and synbiotics is in its infancy, and that the clinical effects of most LAB still remains to be investigated. There was a time when adherence to mucosa was regarded as essential for good clinical effect, but it seems not necessarily be so. Ability to adhere to mucus might be more important. It is my personal opinion that qualities such as ability to ferment strong and semi-resistant fibers such as inulin, antioxidant capacity and specific immunological effects are important factors of the highest relevance to clinical outcome.

Table 4. Content in foods of some key vitamins and antioxidants with special comparison with typical Western foods: Hamburgers, french fries and tomato ketchup

A. Folic acid in foods (g/100 g)

Yeast	1000	Orange	30
Black eye beans	635	Banana	20
Chicken peas	560	FRENCH FRIES	17
Beans	425	Fish	15
Lentils	425	Potato	13
Soya beans	375	Pasta	10
Wheat germs	330	Rice	10
Wheat bran	260	HAMBURGERS	7
Spinage	195	KETCHUP	5
Peanuts	105	Milk	5
Cereals	50	Sausage	4

B. Antioxidant flavonoids, phenylpronoids and carotenoids

Antioxidant	Sources	TEAC (mM)
Vitamins:		
Vitamin C	Fruits, vegetables	1.0±0.02
Vitamin E	Grains, nuts, veg. oils	1.0±0.03
Flavonoids:		
Quercitin	Onion, apple skin, black grapes, berries, broccoli, teas	4.7±0.10
Epigallocatechin	Teas	3.8±0.06
Epicatechin	Black grapes/red wine	2.4±0.02
Flavonoids:		
Anthocyanidins	Grapes, raspberry, strawberry, aubergine, skin	4.4±0.12
Oenine	Black grapes/red wine	1.8±0.02
Hydrocinnamates:		
p-Coumaric acid	White grapes, tomatoes, spinach, asperagus, cabbage	2.2±0.06
Ferulic acid	Grains (oat), tomatoes, Spinach, asperagus, cabbage	1.9±0.02
Carotenoids:		
Lycopene	Tomatoes	2.9±0.15
β-Carotene	Carrots, sweet potato, tomatoes, paprika	1.9±0.10
Xanthophylls:		
β-Crytoxanthine	Mango, papaya, peaches, paprika, oranges	2.0±0.02

Modified after Bengmark (4) and Rice-Evans & Miller (85).

WHY A REGULAR BOOSTER DOSE OF PROBIOTICS ?

A good digestion of food during the passage through the gastrointestinal tract is depending on enzymes from the two sources, gastrointestinal secretions and flora bacterial enzymes. Various short chain fatty acids, amino acids, polyamines, vitamins, antioxidants, growth factors, coagulation factors and other nutritional factors are released and absorbed after microbial fermentation. At least 10% of the calories or 20% of the food volume is suggested to be colonic food, e.g. food supposed to be transported undigested through the small intestine.

Table 5. Suggested molecular effects from probiotics

stimulates sIgA production
inhibits IgE production
modulates Th1/Th2 response
modulates cytokine response
stimulates NO production
stimulates macrophage function
stimulates NK cell activity
activates the MALT system
stimulates apoptosis
promotes growth and regeneration
controls PPMs
reduces endotoxin production
reduces mutagenicity
produces antioxidants, nutrients (synbiotics) and various growth and clotting factors

Modified after Bengmark (86).

It is often questioned why daily supply of a few milligrams of lactobacillus should have any effect on the health of a person, who already has one to two kilogram of bacteria in his large intestine. Although this question remains largely un-answered, it has been suggested that a regular small (daily?) booster dose might be especially important for the upper part of the gastrointestinal tract; the stomach and the upper small intestine, e.g. parts of the digestive tract with low degree of microbial colonization. Possible targets could be the Peyer's patches of the mucosa-associated lymphoid tissue (MALT), and its covering epithelium, which has the highest proportion of specialized, so called M-cells. The increased antibacterial activity observed after administration of yogurts in the lymphocytes of Peyer's patches supports such an assumption. Experience from countries like Korea with its kimchi seem to support the value of daily consumption of LAB and fibers.

SUBSTRATE FOR MICROBIAL FERMENTATION (PREBIOTICS) ARE AS IMPORTANT AS BACTERIA (PROBIOTICS)

The Western Societies consume much less of fruit and vegetable fibers (Table 8). Instead Westerners have adapted the habit to eat a lot of processed food, sugars and saturated fats, which has been shown to induce increased serum levels of cholesterol (Table 9) and of saturated fats (dyslipidemia), conditions associated with metabolic syndrome X / Western Disease - see above.

A large proportion of the food eaten in some countries, especially in Asia and Africa, are even today based on fermentation by lactic acid bacteria (LAB). Well known examples are leafy vegetable-based foods such as kimchi (Korea), gundruk (India), pulses / bean-based foods such as iru (Africa), ugba (Africa), idli (India) and dhokla (India),

Table 6. Suggested clinical indications for probiotics

Clinical nutrition:
To supply antioxidants and nutrients to persons and patients who cannot eat normally, do not eat the recommended amount of fresh fruits and vegetables, are on total parenteral nutrition or on enteral nutrition with factory-produced artificial nutritional formulas with or without residue.
Allergology:
To reduce allergic manifestations.
Immunology:
To stimulate the immune system in immuno-depressed patients.
To reduce early rejection in transplant patients.
Intensive care:
To reduce morbidity in critically ill patients, especially those on immuno-depressing antibiotics and other pharmaceuticals. Topically applied around entrances through the skin of foreign material such as venous or arterial lines, drainage and tracheostomy tubes and voice prostheses in order to avoid biofilm development and infection.
Neonatology:
To prevent or reduce development of topic diseases.
To prevent and reduce rate and severity of infections premature and newborns.
Gastroenterology:
To prevent and reduce diarrhoea.
To treat antibiotic-associated diarrhoea.
To prevent and reduce *Helicobacter* infections.
To suppress or cure *Clostridum difficile* infections.
To reduce symptoms and prevent recurrence in inflammatory bowel disease.
Hepato-pancreatology:
To prevent infection in biliary obstruction.
To prevent infection in toxic liver injuries.
To prevent secondary infections in liver cirrhosis and portal hypertension.
To improve liver blood flow in portal hypertension and hereby reduce incidence of variceal bleedings.
To reduce hepatic encephalopathy.
To prevent sepsis in acute and chronic pancreatitis.
Hematology:
To prevent complications in patients with severe hematological diseases.
Rheumatology:
To reduce further development and symtoms of rheumatoid arthritis.
Nefrology:
To prevent infections in patients om hemo or CAPD dialysis.
Oncology:
To prevent cancer development.
To limit progress of malignant growth.
To improve quality of life in cancer patients.
Stomatology:
To prevent and reduce stomatitis in critically ill and cytostatic-treated patients.
To prevent infection in connection with teeth extraction or replacement of mercury-containing fillings.
Surgery:
To reduce surgical morbidity: sepsis, thrombosis, adhesion formation.
Gynecology:
To reduce bacterial vaginosis and sexually transmitted diseases incl. HIV.
To reduce complications such as premature membrane rupture and preterm labor.
Infectious diseases:
To reduce morbidity and improve quality of life in HIV/Aids patients.

Modified after Bengmark (19).

maize-based foods such as ogi (Africa), kenkey (Ghana), pozol (Mexico), millet-based foods such as kunu-zarki (Africa), and cassava-based foods such as gari (Africa), lafun (Africa), agbéli mawè (Bénin), peujeum (Indonesia). The last trace of fermented food in Western countries; sauerkraut (fermented cabbage) seem during the last 50 years to have almost disappeared at the North American continent and is also rapidly disappearing in Europe, especially in West Europe and among the young.

Animal food products such as milk and meat are also dramatically affected by the type and quality of food we supply our domestic animals and seem to contain much less of some important nutrients than corresponding products from wild so called game animals. The content in animal and human food products of important nutrients such as polyunsaturated fatty acids, amino acids, vitamins and antioxidants can be considerably improved if the animals, in lack of access to pasture, are supplemented with silage

Table 7. Personal evaluation of clinical efficacy as judged from the literature

LAB with expected strong probiotic effects:
(suitable also for pharmacological use)
Lactobacillus casei Shirota
Lactobacillus plantarum
LAB with modest probiotic effects:
(suitable for functional food application)
Lactobacillus rhamnosus (GG)
Lactobacillus Reuteri
Lactobacillus Johnsonii (LA-1)
LAB with small or questionable probiotic effects - good palatability:
(suitable for food application)
Bifidobacteria
Lactobacillus acidophilus
Yogurt bacteria
Some LAB of special interest for future research:
Enterococcus faecium
Lactobacillus fermentum
Lactobacillus paracasei subsp. paracasei
Lactococcus
Leuconostoc
Pediococcus

Modified after Bengmark (87).

Table 8. Fiber consumption (g/day)

Recommended	20~30
Actual intake	<20
Rural Chinese	77
Paleolithic ancestors	>100
Native Americans > 100 years ago	>100
Rural Africans	120
Chimpanzee	>200

Table 9. Cholesterol in serum

	mg/dL	mM/L
Westerners	<200	<5.18
Rural Chinese	127±15	3.3±0.4
Hunters-gatherers	123± 7	3.2±2.2
Non-human Primates	90~135	2.3~3.5

products containing special LAB and other supplements (22).

LARGE AMOUNTS OF HEALTH-PROMOTING BACTERIA NEEDED

Lactobacillus plantarum, but also *lactobacillus casei*, are reported to be the dominating LAB in Asian and African foods, and were most likely also the dominating species in the food of our ancestors. *Lactobacillus plantarum is* the dominating species in fermented food products such as sourdough, sauerkraut, green olives, natural wines and ecological beers. A study undertaken in the US demonstrates, that these lactic acid bacteria (LAB) are more common component of the commensal flora of vegetarians, in whom app. 2 of 3 persons are colonized with *Lb plantarum* compared to appr 1 in 4 persons of omnivourous (23). Most of the lactobacillus species colonizing our intestines are sensitive to stress and food habits. It reported that astronauts on return to earth from space flights have lost most of their commensal flora, *Lb plantarum* being lost to 100%, *Lb casei* to almost 100%, and *Lb fermentum, Lb acidophilus, Lb salivarius and Lb brevis* reduced by 43, 27, 22, and 12% respectively (8). Instead the flora of *Enterobacteriaceae* is significantly increased, which most likely is associated with the stress and poor eating (dried foods, no fresh fruits and vegetables) on space flights. One could speculate that this might also be the reason why many living on earth exhibit the same changes in flora. It has also been shown that both Pakistani children and Estonian children have a much richer commensal flora than Swedish children (24).

It was calculated that humans living in so called developed countries have a commensal flora weighing about 1.3 kg, and it is suggested that people living in rural areas in developing countries and consuming large quantities of living lactobacillus and vegetable and fruit fibers carry a flora of more than two kilogram. Furthermore it is calculated that the human body consists of ten times more bacterial cells (10^{14}) than eukaryotic cells (10^{13}). About 400 bacterial species have been identified in the faecal / intestinal micro flora, but only 30 to 40 species seem to constitute 99% of the flora (25). A recent study suggest that the largest LAB taxa to be found on the rectal mucosa in healthy humans living a Western lifestyle are *Lb plantarum, Lb rhamnosus* and *Lb paracasei* ssp *paracasei*, isolated in 52%, 26%, and 17% respectively of studied Swedish individuals (7). The colonization rate of commonly milk-born probiotic bacteria such as *Lb casei, Lb reuteri* and *Lb acidophilus* was in the same study only 2%, 2% and 0% respectively.

GASTROINTESTINAL TRACT OF GREAT IMMUNOLOGICAL IMPORTANCE

We are increasingly aware of the fact that up to 80% of the human immune systems is localized in the gut. Although the importance of immunoglobulins for the local immune defense has been known since quite some time, it is only during the last decades that it has been fully recognized, that most of the body's IgA is produced by the gut. Deficiencies in IgA are associated with a pronounced increase in morbidity and mortality after major surgery and increased rejection after liver transplantation (26). The synthesis of IgA is highly dependent on T-cells and several cytokines, and particularly TGF-β is known to influence the IgA differentiation (27). Nutrition, physical activity, sleep,

mood, age, gender, circadian rhythm, use of drugs, medical illness and other innate changes are all known to influence the lymphocyte function and the Ig-production and hereby also resistance to disease. Several studies suggest that LAB has a strong ability to enhance the IgA response both in experimental animals and in man (28,29).

It has been recognized in recent years that a balance between Th1 and Th2 lymphocytes is essential to health and well-being. It has been observed that a reduced exposure to bacteria and a reduced bacterial stimulation during infancy and childhood is associated with a slower postnatal maturation of the immune system, and a delayed development and dysbalance between Th1 and Th2 immunity (30). Allergic disease is thought to be caused by inappropriate generation and activation of Th2 cells, a process known to be inhibited by INF-γ and IL-12 (31). Some LAB species has been shown both to inhibit Th2-immune response and promote Th1-immune response. Among these LAB are *Lb plantarum*, *Lb rhamnosus*, *Lb casei* and *Lb bulgaricus* (32-34). Other LAB such as *Lb johnsonii* are shown to have no such regulatory effect (33).

LAB-INDUCED STIMULATION OF MACROPHAGE FUNCTION IMPORTANT

The ability of some special cells in the body to engulf, kill and eliminate invading micro organisms and / or defective cells, but also eliminate toxins, mutagens and other poisonous substances is extremely important to maintenance of health. Certain Western foods containing large amounts of saturated fatty acids, but also excessive amounts of polyunsaturated fatty acids especially omega-6 fatty acids, are known to inhibit the macrophages and thereby this important functions. Mice on a high fat diet exhibit higher IgM and IgG antibody levels; significantly increased proteinuria and also shorter life span than mice fed a low fat diet. The group on high fat diet showed a significantly higher release of IL-6, TNF-α and PGE_2 when LPS in vitro stimulated peritoneal macrophages from the two groups (35). Also chemicals, pharmaceuticals and particularly antibiotics do reduce or inhibit the macrophage function, both the ability of the macrophage to produce and secrete cytokines and the bactericidal function. Both live and nonviable LAB are known to stimulate the macrophage function. However, not all LAB are capable of activating macrophages. As an example pronounced macrophage activation was observed in experimental animals after intraperitoneal administration of *Corynobacterium parvum* and *Lb casei*, but not after administration of *Lb fermentum* (36). A recent study compared the *in vitro* ability of strains commonly used in yogurt production to induce cytokine stimulation. *Streptococcus thermophilus* stimulated macrophage and T-cell cytokine production to a somewhat greater extent than did *Lb bulgaricus, Bifidobacerium adolescentis* and *Bifidobacterium bifidum* (37). A significant variability was, however, observed between various *Streptococcus thermophilus* strains.

CONSUMPTION OF LAB STIMULATES APOPTOSIS

Larger consumption of diary products, rich in saturated fat and various growth factors including insulin growth factor 1 (IGF-1), various cow estrogens and xeno-estrogens (from pesticides) is claimed to be associated with increased morbidity in topic diseases, diabetes, cardiovascular disease and certain cancers such as large intestinal, breast and prostate cancers. Both saturated fatty acids and growth factors such as IGF-1 are known to significantly inhibit apoptosis, programmed cell death. Recent studies suggest that for each one per cent of intake of saturated in the diet the risk of dying in breast cancer will increase by 10%, and when on treatment for breast cancer, the risk of treatment failure increase by 8%. Restriction in food intake (caloric restriction), consumption of colonic foods such as pectin, oat, wheat, rye, chicory fibre (inulin) and also LAB increase the rate of apoptosis and hereby enforces the cancer prevention. Short chain fatty acids (SCFAs) produced in the colon during bacterial fermentation of fibres are known to have a strong apoptosis stimulatory effect (38). It has been shown in experimental animals that feeding beans increases the production of SCFAs seven times, and feeding oligofructans (onion / artichoke / chicory fibers) inhibits the induction of colonic preneoplastic lesions (39).

Health is complex and depending numerous interactions between factors that control appetite, energy balance, metabolic rate, stress response, apoptosis, cell proliferation and repair (40). It seems rather unlikely that one or two synthetic pharmaceutical drugs, or a few nutritional components can significantly influence this complex system. Food is based on hundreds of thousands of molecules of which most are important to health. It is said that the human body contains at least 2 million different molecules on which our health is dependent. Only of flavonoids do we consume with food some four thousand compounds, and of carotenoids almost six hundred. And, some of the flavonoids have an up to ten-time stronger antioxidant effect than vitamin C or vitamin E. It should be remembered that many of the important amino acids, polyamines, antioxidants etc are normally released through microbial fermentation at the level of the large intestine. However, so far neither fiber nor probiotic bacteria are routinely provided with the formulas used in the sickest patients, and claimed to be immunostimulatory.

WHY THE POOREST FOOD TO THE SICKEST PATIENTS

It is a dilemma that the sickest patients most often are supplied the worst food. One should always remember that no formula can ever compare with and replace a comprehensive and balanced diet based on natural foods. If daily supply of "five to eight fresh fruits and vegetables" is important for healthy individuals, it is most likely critical for the very sick. It is clearly not easy to arrange such supply in the very sick, but at least attempts should be made. Successful administration even in ICU patients of fresh fruit and vegetable juices are today reported from a few medical centres. Such a policy should not exclude attempts to produce more effective nutrition solutions, as these will most likely always be needed for supply of the bulk of calories, nutrients, and antioxidants.

The reaction of the body to physical and mental stress - often referred to as acute phase response seems to reflect both the extent of the trauma and the size of the body's response to it. There is accumulating evidence that this response is exaggerated and prolonged in Westerners - for review see Bengmark (4). One of many changes occurring during the acute phase response is a state of hypercoagulability. It well known that the coagulation is different in animals and human, who consume large amounts of fruit and vegetable fibres as well as live lactobacilli (41). Such individuals demonstrate a significantly longer coagulation time, softer jelly-like clots and do rarely suffer thrombosis.

It is my conviction that emphasis on supply with nutrition of these compounds should dramatically improve outcome. Recent studies in surgical patients suggest that enteral nutrition is more important as a tool to control the acute phase and immune responses than provision of calories and nutrients. Among the nutritional factors that control cytokine production and oxygen actions are glutathion, other dietary antioxidants and vitamins, omega-3 fatty acids, various dietary fibres and protective (probiotic) bacteria. When parenteral hyperalimentation (PN) was compared with enteral nutrition (EN) in liver resection patients, no differences were observed in nutritional parameters, but significant differences when studying immunological parameters such as natural killer cell activity, lymphocyte numbers, response to phytohemagglutinin (PHA) and natural killer cell activity (42). Most important the incidence of infectious complications was 8% in the EN group compared to 31% in the PN group. A similar study in acute pancreatitis did reach similar results (43). Here disease severity scores (APACHE II), C-reactive protein (CRP), IgM anticore endotoxin antibodies (EndoCAB) and total antioxidant index (TAC) was significantly better in the EN group compared to PN. In addition the systemic inflammatory response (SIRS), sepsis rate of organ failure and stay in the intensive care unit was significantly better in the EN group.

CLINICAL EFFECTS OF PROBIOTICS STILL LARGELY UNEXPLORED

It should be emphasized that most of the present experience with use of LAB for treatment is often and sometimes only based on studies in experimental animals. There is no condition in humans is which LAB (and fibres) have been as extensively tried as in diarrhoea of various kinds, varying from rather simple tourist diarrhoea to severe and life-threatening conditions such as antibiotic-associated and radiotherapy-induced diarrhoea. Several excellent reviews have been published about diarrhea in recent years (44-47). It clear from all these studies that LAB provides a simple, inexpensive and effective tool, with no documented side effects, to be used in prevention and treatment of all forms of diarrhoea. It is also obvious that LAB are effective in controlling diarrhoea of both bacterial and viral origin, but seem to be slightly more effective in virus-induced diarrhoea. This is promising, as an increasing number of infections today both in connection with extensive surgery such as transplantation and in severe chronic disease such as HIV are of viral origin. But all LAB are not equally efficient.

PROBIOTICS - AND PREBIOTICS- IN DIARRHEA IN CHILDREN

Several millions of children die each year in diarrhoeal dehydration. A larger European multi-center trial in children one month to three years of age was recently reported (48). One hundred and forty children were randomly allocated to oral rehydration and placebo, another 147 children to oral rehydration and daily supply of 10^{10} cfu of *Lactobacillus* GG. Clinical signs of diarrhoea lasted 58.3 $\pm$27.6 hours in the LAB-treated group to be compared to 71.9$\pm$35.8 hours ($p = 0.03$) in the placebo group. Diarrhoea lasted in rotavirus-positive children treated with LAB 56.2$\pm$16.9 hours compared to 76.6$\pm$41.6 in the control group ($p = 0.008$).

The same lactobacillus was tried with the aim to prevent diarrhoea in a placebo-controlled trial performed in 204 undernourished Peruvian children, age 6 to 24 months (49) The treatment was given during 15 months. The lactobacillus-treated children had fewer episodes if diarrhoea (5.21 episodes / child and year compared to 6.02 in the placebo group, $p = 0.028$). The therapeutic gain, as pointed out by du Pont (50) and others, must be regarded as modest. It is likely that use of other and more potent LAB, or com-

binations of LAB, would lead to more significant therapeutic success.

A study of 1237 newborn Columbian children with risk of developing severe diarrhoea (inpatients and transfer patients) and receiving prophylactically during one week or until they were discharged a daily supply 250 million live *Lactobacillus acidophilus* and 250 million live *Bifidobacterium infantis* was recently reported (51) and the outcome compared to the outcome for similar children treated during the year before. The incidence of necrotizing enterocolitis was with probiotic prophylaxis reduced to one third (18 vs 47, $p<0.0005$) in the inpatient group, and by half (19 vs 38, $p<0.03$) in the patients transferred from other hospitals (which most likely came late under treatment). No complications could be attributed to the use of probiotic preparations even in very sick newborn children, weighing in average 2600 g (range <1000 to >4000 g), of which one third suffered from severe conditions such as sepsis, pneumonia or meningitis. It was incidentally observed that the LAB-treated children suffered significantly less diaper dermatitis.

The unmature green banana, rich in pectin and amylase-resistant starch, or pure pectin, is increasingly used in the ICU to prevent stress ulcerations and is in this capacity equally effective as H_2 blockers and proton inhibitors. It was recently tried in a double blind study in Bangladesh for treatment of persistant diarrhea in children (52). A rice-based diet containing either 250 g/L of cooked green banana (equal to about two uncooked fruits) or 4 g/kg pectin or the rice diet alone was provided daily for seven days. Both green banana and pectin reduced amounts and frequency of stools, diarrheal duration, numbers of vomiting and use of oral rehydration or iv fluid solutions. The effects were seen already on the third day when 59% in the green banana group, 55% in the pectin group and 15% in the rice diet alone group had recovered from diarrhea ($p<0.001$). These results are equal to what so far has been achieved by treatment with various LAB - see above.

PROBIOTICS IN ANTIBIOTIC-ASSOCIATED DIARRHEA

Diarrhoea is a common side effect of antibiotic therapy (48,53). Up to 40% of children receiving broad-spectrum antibiotics experience diarrhea (54). Given the large numbers of pediatric patients, who receive antibiotic therapy each year, preventing even a proportion of the cases of antibiotic-associated diarrhoea may have a large impact. The efficiency of *Lactobacillus* GG (LGG) to prevent diarrhoea was tried in a series of 202 antibiotic-treated children. 25 placebo-treated (26%) and 7 LGG-treated developed diarrhea (55). The mean duration of diarrhoea was 4.7 days in the LGG group vs 5.88 days in the placebo group. Again, the efficacy of the treatment is not impressive, and as pointed out by Saavendra, "the reduction of 1 day of two liquid stools over a 10 day period in a child might be questioned" (56).

PROBIOTICS -AND PREBIOTICS- IN INFLAMMATORY BOWEL DISEASE (IBD)

We observed in the early nineties that humans with inflammatory bowel disease have a reduced LAB flora (57). We also observed in experimental animals with induced colitis that the inflammation could be significantly reduced by supply of pre- and probiotics in combination (synbiotics) (58). Subsequently it has been convincingly demonstrated that the concentrations of endogenous *Lactobacillus* and *Bifidobacteria* are significantly reduced in patients with active Crohn's disease, ulcerative colitis, pouchitis as well as in experimental colitis (59,60). A recent study quantified and characterized changes to a species level for aerobic and anaerobic bacteria from colonic biopsies in ulcerative colitis (UC) (61). A significant quantitative decrease in growth of Lactobacillus spp in colitis biopsies was observed. Total aerobic speciation revealed 32 different subspecies of which only 18 were found in UC. Anaerobic speciation revealed in average 4.7 subspecies in UC patients compared to 6.7 in controls. An incidental finding was that *Bacteroides thetaiotaomicron* could be identified in 8 / 10 UC biopsies compared to 4 / 10 controls, an observations, which significance remains to be explored.

A LAB cocktail called VSL#3 consisting in four lactobacillus strains, three bifidobacteria strains plus *Streptococcus salivarius* ssp *thermophilus* (5×10^{11} cells/g), and most probably chosen at random without any further documentation of the molecular/immonological effects for each of the LAB was recently tried in an uncontrolled study in patients with ulcerative colitis (62). The patients were given 3 gram a day during one year and 15 / 20 patients remained in remission, one was lost to follow up and 4 / 20 had signs of relapse. The same LAB cocktail was also tried in a small controlled study in patients with pouchitis (63). Twenty patients served as controls, all showed remission within 9 months. In sharp contrast to this did only 3 / 20 patients develop remission during the same time period, when supplied with VSL#3 probiotic cocktail. These results are surprisingly good and thought-provocative. They are most likely better than what is so far achieved by any conventional treatment, an assumption supported by a recent systematic review of the literature (64) concluding that this far "metronidazole is an effective treatment for active chronic disease" (odds ratio 12.34) but "oral probiotic therapy with VSL#3 for maintaining remission" (odds ratio 15.33).

Although the scientific basis for treatment of IBD seems reasonable and attractive, it must be emphasized that it is far too soon to recommend routine use of probiotics in IBD. Further studied are much warranted. Also prebiotics without additional supply of probiotics seem to alleviate colitis symptoms (65). The good results obtained in the two small studies cited above seem to suggest that combination of several LAB might have stronger clinical effects in IBD than use of single-bacteria treatments. It is tempting to anticipate that a cocktail consisting in LAB, where each of the bacteria has been chosen with the regard to their documented metabolic and immunological effects, should eventually be even more successful. The ideal treatment remedy will probably be complex, and much remains before the most suitable prebiotic(s), and the most effective probiotics have been identified.

PROBIOTICS TO CONTROL STOMACH ENVIRONMENT AND *HELICOBACTER PYLORI* INFECTIONS

It was suggested more than ten years ago lactic acid produced by *Lactobacillus acidophilus* is able to inhibit *Helicobacter pylori* (66). A recent study tested the antibacterial activity of seventeen strains of lactobacilli against ten different strains of *H. pylori* (67). All *Lactobacillus* strains were able to inhibit *H. pylori*, but the effect was lost if pH was adjusted to 6.0. However, the effect of *Lactobacillus acidophilus* CRL 639 remained even after pH was adjusted. The effect seemed less related to pH and more to release of a proteinaceous compound, with autolysin effects. One hundred and twenty *H. pylori* patients were randomised to, in addition to a 7-day triple therapy (Rabeprozole, Chlarithromycin, Amoxicillin), receive either placebo or a lyophilised and inactivated culture of *Lactobacillus acidophilus*. The eradication rate was significantly improved by supplementation of the LAB: 52 / 59 patients (88%) vs 42 / 58 patients (72%) ($p = 0.03$) (68). The effects of live *Lactobacillus* GG was investigated in subsequent studies and performed in a similarly sized material of patients receiving the same triple therapy (69,70). This study reports improved tolerability (reduced antibiotic-induced bloating, diarrhoea and taste disturbances), but in sharp contrast to the effect of *Lactobacillus* acidophilus, no improvement in eradication rate from the use of live *Lactobacillus* GG.

Daily oral consumption of 4×50 mL of the supernatant from a whey-based *Lactobacillus acidophilus* (La1) culture, combined with either omeprazole or placebo, was reported to show a significant reduction in breath test both with and without supply of omeprazole, immediately as well as six weeks after the treatment episode (71). It should be remembered that whey is extraordinarily rich in immunologically active and anti-infectious substances. It is thus, this far not clear whether the observed effects are due to the *lactobacillus* used, to the whey or a combination of both.

USE OF SYNBIOTICS IN THE SICKEST PATIENTS / CRITICAL CARE

Modern surgery is, despite significant advances in surgical techniques, far from safe. The incidence of the three leading causes of complications and sequelae; infections, thrombosis and adhesion formation seem to remain unchanged during the last fifty years. It is been calculated that about 2 million Americans (6% of the hospital patients) suffer each year from nosocomial infections, and most of the patients have reduced immune functions, and half of the patients are over the age of 65 (72). Infections are especially common in neutropenic patients (48%), after transplantation (appr. 50%) and after extensive operations such as liver or pancreas resections (appr. 33%), but the infection rates are also unacceptably high after gastric and colonic resections (appr 20%). The mortality in acute conditions such as severe pancreatitis is increased four times when the pancreatic tissue has become infected (appr 40%) with anaerobic gut bacteria (73).

ICU patients acquire nosocomial infections at a much greater rate than patients elsewhere in the hospital. For ICU patients the risk is as much as 5 to 10 times greater than for those on general medical wards (74,75). The most representative data on nosocomial infection rates are provided by the National Nosocomial Infections Surveillance (NNIS) system in the USA but similar systems are increasingly introduced in most Western countries. The major types of infection found in the European Prevalence of Infection in Intensive Care (EPIC) study (76) were pneumonia / lower respiratory infection (64.7%), urinary tract infection (17.6%) and blood stream infection (12%). An American study found four major systems to be frequently involved: respiratory tract (31%), urinary tract (24%), blood stream (16%) and surgical sites (8%) (77).

There are good reasons to believe that pre-, pro-, and synbiotics could dramatically change the outcome for critically ill patients, and be a good alternative to the use of antibiotics in ICU patients. It is regrettable that this far only a handful of studies have been performed in critically ill and postoperative patients, and, furthermore, most of these studies are under publication. However, the interest is fast increasing and several studies are presently conducted. The only study in a mixed ICU population known to the present writer was performed by a nurse in Hongkong and presented as a thesis for B Sc Degree in Health Studies (78). 19 patients received daily from within 12 hrs

of arrival to the ICU sachets containing 10^{10} of *Lactobacillus plantarum* 299 and oat fiber, and another 19 patients heat-killed *Lactobacillus plantarum* 299 and oat fiber (controls). 5 / 19 (26%) died in the treated group vs 8 / 19 (42%) in the control group, but the patient material was too small to allow statistical significance. However, it stimulated the physicians at the same unit to undertake a larger study, which is presently under way.

Acute severe pancreatitis

Contamination of the pancreatic tissue occurs frequently in severe pancreatitis, being reported to be 24% during the first week and amounting to 72% during the third week (79). Furthermore, pancreatic sepsis seems to be a strong determinant for complication such as multiple organ failure (MOF) and death. A recent study found a death rate of 24% in patients with infected necrosis compared to 1.8% with sterile pancreatic necrosis (80). It was also rather recently shown that infection of the pancreatic tissue is almost always preceded by about one week with colonization the large intestine with non-coli gram-negatives (*Pseudomonas*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Acinetobacter*, *Morganella*, *Serratia* or *Proteus*) (81). Prevention of such a colonization to happen could thus be expected to have a dramatic influence on outcome. A prospective double-blind randomized study was recently concluded in severe pancreatitis, comparing the influence of *Lactobacillus plantarum* 299 and oat fiber with heat-killed *Lactobacillus plantarum* 299 and oat fiber (control). The study was designed to be concluded when repeat statistical analysis demonstrated statistically significant differences between the two study groups (82). This happened at the time when all together 45 patients had entered the study. At that time 22 patients had received treatment with live LAB and 23 with heat-killed LAB, in both groups during seven days. Infected necrosis and abscesses occurred in 1 / 22 patients (4.5%) in the live LAB group and in 7 / 23 patients (30%) with heat-killed LAB ($p = 0.023$). Although the length of stay was 13.7 days in the treatment group vs 21.4 days in the control group, it did not reach statistical significant at the time when the study was interrupted. The only patient who developed sepsis in the treatment group did that after fifteen days, e.g. eight days after the treatment has been discontinued, implying that this treatment should be given for at least 14 days, or most likely as long as the patients are on antibiotics or have signs of gram-negative GI colonization.

Abdominal surgery patients

Another prospective randomized study compared the effect of live *Lactobacillus plantarum* 299 in a dosis of 10^9, heat-killed *Lactobacillus plantarum* 299 in the same dosis and parenteral nutrition in a mixed material of 3×30 patients undergoing abdominal operations such as liver resection, pancreas resection, gastric resection, colon resection and intestinal by-pass (83). The groups treated with either live or heat-killed LAB suffered less infections (3 / 30 in each group, e.g. 10%) compared to 9 / 30 (30%) in the parenteral group. However, the material was not large enough for statistical significance to be reached. When the subgroup of gastric and pancreatic surgery patients were analyzed separately an even larger difference was observed: 0 / 8 in the live LAB group, 1 / 8 (12%) in the heat-killed LAB group and 3 / 6 (50%) in the parenterally treated group suffered infections.

Liver transplantation patients

The same group did also recently conclude a study in human liver transplants (84). Also this study was a randomized controlled study involving an equally sized patient material. Comparison was made between selective bowel decontamination (SBD) + a standard enteral formula, live *Lactobacillus plantarum* 299 + oat and inulin fibers, heat-killed *Lactobacillus plantarum* 299 + oat and inulin fibers The total amount of fibers in the two last groups was about 11 gram. The LAB supplemented during the first five days. The sepsis rate was 48% in the selective bowel decontamination group, 34% in the group treated with heat-inactivated LAB and 13% in the group receiving live LAB. Also the mean duration of antibiotic therapy, the mean total hospital stay and the stay on ICU were shorter than in the groups with inactivated lactobacilli and fibre as well as with SBD. However, these differences did not reach statistical significance.

CONCLUSION AND FUTURE ASPECTS

The prevention and treatment disease, using pre-, pro- and synbiotics, despite being used for centuries, is still in its infancy. This old and rediscovered is not and will no be a panacea for everything. As summarized in Table 6 many authors have in recent years suggested use of pre- and probiotics as treatment in numerous conditions. The fact that the molecular functions of pre-and probiotics are so well documented by research both in animals and in man offers great hope for future clinical applications. It must, however, be emphasized that for most indications is clinical documentation still largely lacking. Many more studies must be performed before the concept has found its niche in the therapeutic armamentarium of the 21st century. It must also be emphasized that conclusions can never be drawn from one LAB strain to another. Genetically there are greater differences between one LAB strain and another than between a human being and a fish. All effects observed must be regarded as specific to the particular strain

investigated. Only a few of the several hundred LAB strains known have this far documented strong health-promoting effects (Table 7). In general LAB known for their ability to ferment fibers seem to be more effective in this respect than those known to produce palatability of various milk products. These LAB are often found in various ethnic foods such as kimchi, in silage, on the surface of various plants including grains, and in various fermented food products such as saurkraut. Finally there is increasing support for an assumption that combination of several LAB might be more effective than the most often today used "single and magic *Lactobacillus*" which today dominate the research efforts. There also much to support that future treatment concepts will include the parallel use of not only one, but several bioactive/prebiotic fibers.

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