Synbiotic treatment reduces intestinal dysbiosis, prevets inflammation and infections in chronic kidney disease – an efficient supplement to dialysis and transplantation?

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Microbiota, when properly functioning, is known to provide the host with energy-rich metabolites – nutrients, antioxidants and vitamins, reduce general inflammation in the body, reduce the content of various toxic molecules – also uremic toxins, increase protein utilization, and urea breakdown, maintain immune homeostasis, and protects against infections and various chronic diseases & premature aging.
Dysbiosis, - malfunctioning intestinal microflora - is intimately associated with almost all chronic diseases and known to aggravate disease – especially in end stage renal disease (ESRD). Patients with ESRD are known to have significantly reduced innate immune functions and too often suffer not only from infections and other forms of complications, but also from serious chronic diseases such as Coronary Heart Disease (CHD).
Changing diet and supplementing synbiotics is likely to provide significant improvements for these patients. Supply of Synbiotics can in patients with Chronic kidney disease (CKD) / End Stage Renal disease (ESRD) be expected to eliminate uremic toxins and, at least partly, rducethe risk of infections and chronic diseases, as has been observed when treating other chronic conditions such as Chronic Liver Disease (CLD), and to me extent also HIV by:
Reducing the inflammatory pressure in the body

- Eliminating/reducing heat-produced toxic molecules
- Eliminating/reducing uremic toxins
- Eliminate/reducing the amounts of Endotoxin in the body
- Reducing the burden also of other waste products largely produced by a failing Intestinal microbiota

Microbiota – an important, but often neglected, organ
The huge microbial community residing in the intestinal tract (microbiome) represents a symbiotic ecosystem that confers trophic functions, provides protection against pathogenic organisms, contributes to energy metabolism by facilitating absorption of complex carbohydrates, and participates in nitrogen and micronutrient homeostasis by synthesizing important amino acids (e.g. lysine and threonine and vitamins (such as vitamin K and group B vitamins).

The structures, compositions, and functions of the microbial flora are heavily influenced by the biochemical milieu in which it resides. The integrity and proper function of the microbiome is critical for good health. In fact, changes in the structure or function of the microbiome contribute to the pathogenesis of various diseases including inflammatory bowel disease, chronic inflammation, dyslipidemia, diabetes, cardiovascular diseases, neoplasms, obesity, and atopic disorders and especially important in Chronic Kidney Disease (CKD).

Dietary modifications are often suggested in order to prevent hyperkalemia, which most often include recommendations of restrictions in intake of fruits and vegetables, which are rich in potassium. The unfortunate consequence of this is reduced intake of antioxidants and vitamin and particularly of minerals such as magnesium – especially magnesium being of the

greatest importance for growth and function of the beneficial often gram-negative flora and production of short chain fatty acids, which are essential for tightening the intestinal and preventing of leakage through the intestinal barriers.

Chronic kidney diesease strongly associated with oxidative stress

Until rather recently, little attention had been paid to the role of the intestine and its microbial flora in the pathogenesis of CKD-associated inflammation. Increased general inflammation aggravates all diseases, and in particular CKD. However, leaking toxins and pathogenic bacteria bacteria from the gut today known to play the leading role in the pathogenesis of chronic diseases and particularly chronic kidney disease – as they induce oxidative stress (OS), which in turn is inseparably linked to increased systemic inflammation, which play a major role in progression of chronic kidney disease (CKD) and numerous complications to this disease, including cardiovascular disease, cachexia, anemia, and numerous others.

Activating the redox-sensitive transcription factor nuclear factor kappa B (NF-κB), which is the master regulator of pro-inflammatory cytokines and chemokines triggers activation and recruitment of immune cells and thereby promotes health-threating chronic inflammation. Inflammation, in its turn, initiates and amplifies the oxidative stress via production of reactive oxygen, nitrogen, and halogen species by the activated immune cells. Several factors have been shown to contribute to oxidative stress and inflammation in CKD.

The ultimate outcome of longlasting systemic inflammation is mitochondrial dysfunction and insulin resistance, activation of tissue angiotensin system, hypervolemia, hypertension, dyslipidemia, retained uremic toxins and metabolites, and infections (blood access, peritoneal dialysis catheters, hepatitis, etc.) - all accompanied by a series of comorbid conditions and especially by diabetes and auto-immune diseases.

Malfunctioning microbial flora with overgrowth of pathogens, dysbiosis, and leaking intestinal membranes – a great problem in Chronic Kidney Disease (CRD).

Recent studies provide solic documents that support the role of deranged gastrointestinal tract (GI) microflora, always in small intestine and large bowel, and sometimes involving the whole GI tract including the oral cavity. The observed disruption of the intestinal epithelial barrier complex constitute an important part in the development of systemic inflammation by enabling influx into the body of endotoxin, produced in large amounts by the pathogenic colonic microbiota, but also many other noxious luminal products such as uremic toxins into the systemic circulation. The primary source of several well known pro-inflammatory/pro-oxidant uremic toxins and many other as-yet unidentified retained compounds is undoubtedly the GI tract. In fact, CKD is always associated with disruption of the intestinal barrier structure and marked alteration of its microbial flora, with significant dramativ reductions in the preventive beneficial microbial components and an overwhelming overgrowth of pathogenic, often gram-negative microbes, not rarely in the entire gastro-intestinal tract - events that certainly play the dominating role in the pathogenesis of inflammation and uremic toxicity.[1]

Whole, live or dead pathogenic bacteria enter the body in malfunctioning microbiota - dysbiosis.

Not only toxins but also whole pathogenic bacteria or debris of such bacteria pass into the body through through the digestive tract membranes, when the gut is leaking. Bacterial DNAs are in fact detected in the blood in 20 % (6/30) of the end stage renal disease (ESRD) patients, [2] and most likely in much higher numbers in the cells of the sicj persons. All the observed microbial genera observed in blood (Klebsiella spp, Proteus spp, Escherichia spp, Enterobacter spp, and Pseudomonas spp) of ESRD patients are as a matter fact present in the guts of all ESRD patients, who all are the object of a dramatic pathogenic overgrowth. Markers of pathogenic overgrowth, dysbiosis, such as increased Plasma D-lactate, High-sensitive C-reactive protein, and interleukin-6 levels are shown to be significantly elevated in patients with bacterial DNA in blood than those without. [2]

The problem of organic wastes.

Identifying and removing organic waste solutes that normally are excreted by the kidneys is the primary goal of modern treatment of CKD. The list of uremic toxins is long, some ninety GI-tract derived toxins identified – the most important mentioned in table 1. The majority of the toxins derived from amino acids, to a large extent tryptophane and tyrosine, which are often provided in excess by the foods we eat, and maldigested by pathogenic bacteria in the always dysbiotic gut microbiota of CKD patients. The most commonly studied colon-derived solutes are indoxyl sulfate (IS) and p-cresol sulfate (PCS) – commonly used as indicators of level of uremic toxins in the body.

Table 1 | Colon-derived uremic solutes

Indole compounds
Indoxyl sulfate
Indoxyl glucuronide
5-Hydroxyindole
Indole-3-propionic acid
Phenyl compounds
p-Cresol sulfate
p-Cresol glucuronide
Phenyl sulfate
Phenyl glucuronide
α-*N*-phenylacetyl-L-glutamine
Phenylpropionylglycine
Cinnamoylglycine
4-Ethylphenyl sulfate
Hippuric acid

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Western/industrially produced/processed diet – a major challage to optimal health

The Western industrialized' diet constitute, even under normal conditions with a well-functioning colonic microbiota a large burden to the microflora, and also to the kidneys, as it contains extremely large amounts of amino acids, reaching the microbiota in form of incompletely digested proteins, sloughed intestinal cells, and secretions. As a matter of fact, about 80 % of modern processed foods is absorbed in the small intestine and less than 20 % left for colon and the colonic microflora, compare to the foods consumed by our paleolithic forefathers and that rural populations today like Abkhasians, Vilcabambas and Hunzas.

Before dialysis became available, uremic symptoms were relieved peimarily by dietary protein restriction and the reduced production of colon-derived solutes may have contributed to the effectiveness of the treatment of that time. Protein restriction, however,

is difficult to implement and might, when practised to the extreme that was neccessary cause negative nitrogen balance in the body.

The yet unanswered and totally unstudied question is whether modern dialysis romoves all uremic toxins to the extent that might be dereable for optimal health. It might still be that a microbiota-friendly diet could complement dialysis and add to optimal health. It is clear that reducing the intake of animal fat and proteins and increasing the dietary intake of fresh green vegetables provide great health benefits also to healthy individuals. In the, in addition to a healthier lifestyle supplementing plant fibers and with health-promoting microbes (Synbiotics) could provide simple and safe means to reduce the production of colon-derived uremic solutes and hereby contribute to better health..

Insulin resistance and metabolic syndrome – a product of gut-derived uremic toxins?
CKD is frequently associated with peripheral insulin resistance, wn to major cardiovascular and diabetes risk factor. Fasting hyperglycemia, abnormal glucose tolerance, and hyperinsulinemia, hallmarks of insulin resistance, are common features in patients with CKD.

It is increasingly suggested the uremic are responsible for this development.[3,4] One particular compound - p-cresyl sulfate (PCS), a protein-bound uremic toxin that originates from tyrosine metabolism by intestinal microbes is the prime suspect. A study administering PCS for 4 weeks to mice with normal kidney function was found to trigger insulin resistance, loss of fat mass, and ectopic redistribution of lipid in muscle and liver, much mimicking features associated with CKD.[4]Furthermore, treatment of these mice with the prebiotic arabino-xylo-oligosaccharide, was shown to significantly reduce serum intestinal production of p-cresol and prevent the with insulin resistance associated metabolic derangements.[4]

Foods heated to higher temperatures (fried, grilled, baked etc) increase inflammation
The French scientist Louis Camille Maillard (1878 – 1936) undertook early studies of the chemical reactions between amino acids and sugars, when heated together., He suggested already in 1912 a strong association between foods heated to higher temperatures (temperatures above (TEMPERATURES ABOCE 100 C/ 212 F) and the development of chronic diseases, especially renal diseases. The work was at that time considered a major contribution, the reaction was named after him, Maillard reaction, and he was awarded several prices, including the French Academy of Medicine award (1914). However, the information was neglected by the public as well as by the physicians. It was first with the emergence of molecular biology some eighty years later his hypothesis was re-evaluated and confirmed. Today it is fully recognized that foods heat to above 100 C are rich in inflammation-inducing molecules and should ideally be restricted.

Plant-derived and antioxidant- and mineral-rich fibers are ideal foods for microbiota.
The term fiber includes both nonstarch polysaccharides and resistant starches that escape digestion in the small intestine, and reaches the lower GI tract – a favorite food for microbiota. Industrialized diets contain significantly less fiber than primitive diet, Dialysis patients consume often even less fibers than their healthy neighbors.

With low-fiber intake the supply of carbohydrates to the colonic microbes will be low. With limited substrate for fermentation, microbial growth is reduced and so also the volume of the stool, of which microbes make up a major portion.

A consequence of reduced volume of the stool is delay in intestinal passage time (from normally about 24 hours to several days), increased transfer of not fully digested foods and toxic substances and pathogenic microbes into the circulation of the sick. Furthermore,the availability of amino acids and minerals, most often of plant origin, needed for synthesis of microbial protein and the replication of particularly beneficial gram-positive disease-preventive microbiota is significantly reduced, overgrowth of the disease-inducing pathogenic microbiota significantly increased as instead larger portions of animal proteins and peptides are delivered to the colon and converted into uremic solutes.

Microbial fermentation of polysaccharides in fibers yields large amounts of short chain fatty acids (SCFAs), which are neccessary to keep the membranes active and tight. Another consequence of shortage in plant fiber in the diet is a significantly prolonged transit time through the colon, increased exposure time to the pathogenic microbiota and increased conversion of amino acids to uremic wastes. Increasing dietary fiber intake is knpwn to have the opposite effects and reduce the conversion of amino acids to uremic solutes, .while increasing ”the normalization” of microbiota by promoting replication of beneficial preventive gram-positive microorganisms. The high fiber in combination with no intake of animal foods is credited for the significantly urinary excretion of indoxyl sulfate and p-cresol sulfate observed in vegetarians.

Supplementing non-digestible carbohydrates and Lactobacillus to dialysis patients has promissing effects.

It is suggested that the problem of leaky gut is eventually a larger problem in hemodialysis than in peritoneal dialysis. An interesting, but but yet explained, finding is that production of the colon-derived solutes indoxyl sulfate and p-cresol sulfate appears to be lower in patients on peritoneal dialysis than in patients on hemodialysis.[5]

Attempts to restore the GI barrier functions by reconstitution microbiota have, In sharp contrast to other chronic diseasis, been very late. However, it is recently demonstrated in dialysis patients, that supplementing a diet with a content of fiber/ non-digestible carbohydrates already at the rather low level of 10–20 g/day will significantly reduce plasma levels of p-cresol sulfate,[6] which should encourage further and more forceful efforts in this direction.

Althoughy overgrowth of pathogenic bacteria also in the small bowel was early observed in end-stage kidney failure, and already some decades ago considered responsible for producing uremic toxins and responsible for decreased well-being of the patients surprisingly few attempts have been made to restore a normal microbiome. Possible the enthusiasm of the emerging possibilites for treatments like dialysis and transplantation contributed to an attitude that no other measures were needed.

Supplementing lactobacillus with or without provide new treatment options in patients under dialysis and transplanted patients.

In a small study,[7] published about 20 years ago, eight hemodialysis patients were treated with oral supplementation of unidentified Lactobacillus acidophilus (LBA) in an attempt to reduce the Small Bowel Microbial Overgrowth (SBBO).The treatment did effectively reduce serum dimethylamine (DMA) levels, which at the end of LBA treatment had dropped with approximate one third (from 224 +/- 47 to 154 +/- 47 micrograms/dl ($p < 0.001$). It also reduced the level of Nitrosodimethylamine, a wellknown toxic substance and carcinogen, that dropped with more than 50 % (from 178 +/- 67 (untreated) to 83 +/- 49 ng/kg). However, the general nutritional status, serum albumin, body weight, caloric intake, midarm muscle area (MAMA) and appetite did only improve modestly. Most likely was that due to the very short period of treatment, the very low lactobacillus dose and the lack of simultaneous supply of plant fibers, providing building stones for anabolism.. No adverse effects of the treatment were observed.[7]

Another small study at the same time looked at the effects on uremic patients of oral administration of a preparation consisting in rather low dosis of antibiotic-resistant lactic acid bacteria.[8] The levels of fecal putrefactive metabolites were reported be reduced to levels more comparable to those of healthy individuals. As an example, the plasma level of indican/ Indoxyl sulfate, a uremic toxin, was significantly decreased in the lactobacillus-treated patients and analysis of the fecal microflora revealed that, what they at that time called a ”normalisation of the disturbed composition of the microflora”.

First ever synbiotic treatment study in Chronic Kidney Disease published first in 2011.

The first study[8] ever undertaken to investigate the effects of combined fiber/probiotic (synbiotic treatment) was recently published. Nine dialysis patients received three times a day during 2 weeks a supplementation of a combination of 10^8 *Lactobacillus casei strain* Shirota, 10^8 *Bifidobacterium breve,* (Yakult) and 4 g galacto-oligosaccharides – wellknown prebiotics. The serum p-cresol level were significantly decreased during the treatment. Furthermore, the patients with a high serum p-cresol level tended in general to have hard stools and difficulty with defecation. The quantity of stools increased significantly during the treatment and the hard, muddy or sometimes soft stools tended to be replaced by normal ones.[6]

Probiotic and Synbiotic formulations have proven a high ability to remove or reduce the content in the the intestine of numerous toxic sustances such as endotoxin, gluten, casein, aflatoxin, ochratoxin A and zearalenone, microcystin-LR, fumonisin B1 and B and numerous other toxins and possess ability to remove toxins also in patients with other chronic diseases, particularly chronic liver disease and chronic kidney disease.

Some pathogenic bacteria in the microbiota produce more damage to the body and particularly the kidneys than others – one such pathogen being *Escherichia coli* because it ha one of the highest observed activities of the enzyme that produce IS - tryptophanase, indicating a strong need it at least eliminate/reduce the *E. coli* component from the pathogenic microbiota.

Pro-/Synbiotics reduces effektively uremic toxins – but convincing studies are still lacking.

Only one probiotic study provide this far more detailed information on the effects on the microbiota and levels of uremic toxins.[9] This study demonstrated not only that the fecal flora before supplementation with probiotics contained a significantly greater proportion of

aerobic bacteria (specifically *Escherichia coli*) - 100 times higher than that in healthy matched controls, and significantly lower levels of the beneficial *Bifidobacteria*. It also reported a significant decrease in both pathogenic Enterobacteria and in serum indoxyl sulphate (IS) levels.

However, the effects are not longlasting, both protein-bound uremic toxins, p-cresyl sulphate (PCS) and indoxyl sulphate (IS) did return to preintervention levels two weeks after conclusion of the studies.[10] Most likely future should aim to, in addition to radical diet changes, to supply pre-, pro- and synbiotics on regular basis as the disease remains, and most likely also provide both pre- and probiotics (synbiotics) in significantly larger doses.

A recent meta-analysis at the effectiveness of pre-, pro-, and synbiotics on reducing two protein-bound uremic toxins, p-cresyl sulphate (PCS) and indoxyl sulphate (IS) – summarizes all studies published before 2012. Eight studies were found to investigate prebiotics, six probiotics, one synbiotics, one both pre- and probiotics, and three studies trialled all three interventions. The quality of the studies ranged from *moderate* to *very low*. 12 studies were included in the meta-analyses with all four meta-analyses reporting statistically significant reductions in IS and PCS with thevarious therapies. Their conclusion was that ”there is a limited but supportive evidence for the effectiveness of pre- and probiotics on reducing PCS and IS in the chronic kidney disease population,” but that ”further studies are needed to provide more definitive findings before routine clinical use can be recommended.” [10]

It is undisputable that gut microbial symbiots are critical for normal digestion and immune defense. It is also without doubt that play an important role in development of disease and in metabolizing orally ingested therapeutics.[11] Furthermore, it is is increasing recognized that intestinal bacteria metabolize drugs and will alter an individual’s response to drug treatment much depending on the presence of specific bacterial strains. It is alsosuggested that probiotics, by altering intestinal microflora, might have the capacity to alter the entire enterohepatic circulation of secondary bile acids.[12] Clearly microbiota plays an important role in pathogenesis and further development of chronic diseases, on example being the recent observation of frole of enteric bacteria in phosphatidylcholine metabolism and in the pathogenesis of cardiovascular disease.[13]

Microbiota and probiotics sensitive to chemicals - does all pharmaceuticals impair microbiota?

It is becoming increasingly obvious that almost all pharmaceutical drugs have a negative influence on immune development and functions and probably on the microbiota, too. As discussed above, antibiotic treatment will dramatically destroy intestinal homeostasis and introduce changes that affect almost 90% of the functions of microbiota, including critical metabolic functions such as bile acid metabolism and eicosanoid and steroid hormone synthesis.[14] Similar negative effects on microbiota have been reported in association with chemotherapy treatment for cancer; for decades this has been known to significantly damage the rapidly generating GI mucosal cells, disrupts the ecological balance, induce dysbiosis, and allow pathogens such as *Clostridium difficile* to grow,[15] a bacterium found to be the causative agent in at least 20% of antibiotic-associated diarrhea (AAD) cases.[16] During chemotherapy treatment, as observed in a pediatric patient material, the total number of bacteria in fecal samples is reduced to only 10^9 per gram of dry weight feces, which is 100-fold lower than normally seen in healthy individuals, and on fluorescent in situ hybridization analysis

shown to consist in an up to 10,000-fold decrease in anaerobic bacteria and a 100-fold increase in potentially pathogenic enterococci.[17]

The negative effects of pharmaceutical drugs on microbiota is not only limited to antibiotics and chemotherapeutics. Negative effects on microbiota also occur with other drugs including those that, in the past, have been assumed have no or limited side effects, such as proton pump inhibitors and anti-hypertensive drugs. As examples, the offspring of mothers consuming proton pump inhibitors during pregnancy have a significantly increased risk of acquiring asthma later in life,[18] while users of hypertensive drugs suffer not only significantly reduced salivation and severe mouth dryness (xerostomia) but also a documented profound oral dysbiosis.[19]

New information concerning intimate cross-talk between the intestinal microbiota and the host immune system has opened new avenues. Alterations in the microbiota are known to immediately induce increased translocation of bacterial antigens and dramatically alter the host immune reaction, leading to a chronic inflammatory state and impaired metabolic function, including insulin resistance, hepatic fat deposition, insulin unresponsiveness, and excessive adipose tissue development.[20] Consequently, each decision to use pharmacological treatment may, in the future, need to be based on weighing the need of pharmacological treatment against the importance of maintaining microbiota homeostasis and preventing leakage at body surfaces. Clearly, the impact of newly developed pharmaceuticals on microbiota and immune functions, neglected in the past, should be fully investigated before products are licensed for public use.

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

Considerable progress with symbiotic therapy in other chronic diseases.

Severe dysbiosis exists in chronic diseases such as diabetes, chronic liver disease, chronic lung diseases, especially chronic obstructive pulmonary diseases (COPD), as discussed above chronic kidney disease and in chronic infections such as HIV.

Most interest has this far focused on chronic liver diseasewhele the experience from the other disease are scares but promising. Larger studies are also ongoing, particularly in chronic liver disease.

Here follows a short information of the results:

Chronic liver disease: Fifty-five patients with severe derangements of the gut micro-ecology and significant overgrowth of potentially pathogenic *Escherichia coli* and *Staphylococcal* species received Synbiotic 2000 for 30 days. The tratment lead to significant reductions in mucosal pH, pathogenic bacteria in stool(*E. coli* $p<0.001$, *Staphylococcus* $p<0.01$ and *Fusobacterium* $p<0.05$ but also in level of endotoxin/s, ammonia/s, ALT/s, and bilirubin/s and in improved liver functions as indicated by significant increases in albumin/s and prothrombin.[21] The severity of liver disease improved, as

measured with the Child-Turcotte-Pugh scale improved in about 50% of cases. A significant reversal of encephalopathy, foggy mind, often associated with chronic liver disease was also observed in half of the treated patients. In a second study, 30 cirrhotic patients were randomized to receive either Synbiotic 2000 or placebo during only 7 day.[22] Significant improvements were observed in viable fecal counts of Lactobacillus species, plasma retention rate of indocyanine green (ICGR15), whole blood tumour necrosis factor alpha (TNF-a) mRNA and interleukin-6(IL-6) mRNA, serum TNF-a, soluble TNF receptor (sTNFR)I, sTNFRII and IL-6 and plasma endotoxin levels and inliver function tests. [22] Minimal encephalopathy is common not only in liver cirrhosis but is also seen in other chronic diseases such as diabetes. The observations in patients with liver cirrhosis provides hope that Synbiotic treatment may also be effective in other chronic diseases. Leading American hepatologists commented it favourably: the authors "have made a major contribution to the application to gut flora therapy to humans with liver disease" "merits attention as a big step forward".[23] They also suggested applications in other areas such as chronic kidney disease: " Preliminary work suggests that such effects help to explain why gut-directed therapies also improve uremia in hemodialysis patients" – referring to the work of Hida et al – see above.[9] These studies did also encourage an Australian group to undertake a major study, which now is ongoing since about 3 years.

HIV. 38 women with HIV, all on ongoing highly active antiretroviral therapy (HAART), were recently supplemented with Synbiotic 2000 Forte orally for 4 weeks.[24] A surprising and very encouraging observation was that the supplemented formula showed ability, despite heavy pharmaceutical treatment, to survive during the passage through the GI tract, and also demonstrated ability to colonize the gut and contribute to a significantly elevated level in the stool of the supplemented LAB group. Furthermore, the T-cell activation phenotype was altered by exposure to the Synbiotic formula and accompanied by a slightly elevated HLA-DR expression of a minor population of CD4+ T-cells, which normally lack expression of HLA-DR or PD-1. These significant changes occurred in the context of unaltered microbial translocation, as measured by plasma bacterial 16S ribosomal DNA. It is especially encouraging that the LAB supplemented with Synbiotic 2000, despite heavy medication/highly active antiretroviral therapy (HAART), were able to colonize the gut and seemingly, and although only slightly, improve immune functions. It is the hoper that significantly more pronounced positive effects might be obtained the day we are ready to try the treatment, not only as complementary treatment, but as a real alternative to pharmaceutical treatment.

Synbiotic 2000 reduces accumulation of pathogens in stool

As demonstrated byhese three examples, Synbiotic 2000 has a remarkable capacity to reduce pathogens in stools of sick persons – see further[25-28]

After liver transplantation[29]

Isolated bacteria:	Synbiotic 2000	Fibres only
Enterococcus faecalis	1	11
Escherichia coli	0	3
Enterobacter cloacae	0	2
Pseudomonas aeruginosa	0	2
Staphylococcus aureus	0	1
Total	1	18

After pancreatectomy for cancer[30]

Isolated bacteria:	Synbiotic 2000	Fibres only
Enterobacter cloacae	2	8
Enterococcus faecalis/faecium	1	7
Escherichia coli	0	7
Klebsiella pneumoniae	2	2
Proteus mirabilis	1	1
Staphylococcus aureus	0	2
Total	6	27

In severe acute pancreatitis[31]

Isolated bacteria:	Synbiotic 2000	Fibres only
Pseudomonas aeruginosa	1	4
Enterococcus faecalis	1	2
Enterobacter spp	1	1
Streptococcus spp	2	-
Staphylococcus aureus	1	1
Enterococcus faecium	1	-
Candida spp	-	2
Staphylococcus haemolyticus	-	1
Serratia spp	-	2
Klebsiella spp	-	1
Escherichia coli	-	1
Stenotrophomonas maltophilia	-	1
Citrobacter freundii	-	1
Total	7	17

Synbiotics will also reduce complications, particularly infections in severe acute diseases/intensive care treated patients

Liver transplantation[29]

Synbiotic 2000 or Only fibres supplemented daily from the day before surgery + during 14 postop days

30 day-infection rate:

Synbiotic 2000 1/33 - 3 %

Only fibres 17/33 - 51 %

After pancreatectomy for cancer[30]

Infections	Synbiotic 2000	Fibres only
General infections	/40 = 13 %	16/40 = 40 %
Wound infections	4	6
Peritonitis	0	5
Pneumonia	0	4
Urinary	1	1
Sepsis	0	2
Cholangitis	0	1
Empyema	0	1
Total	**5**	**20**

In severe acute pancreatitis[31]

Infections	Synbiotic 2000	Fibres only
Total infections	9/33 = 27 %	15/29 = 52 %
Pancreatic abscesses	2	2
Infected necrosis	2	6
Chest infections	2	4
Urinary infections	3	3
SIRS	3	5
MOF	5	9
SIRS + MOF	8	14
Late (>48h) MOF	1	5
Complications	9/33	15/29
Surgical drainage	4/33 = 12 %)	7/29 = 24 %
Mean hospital stay	14.9 ±6.5	19.7±9.3
Dead	2/33 = 6 %	6/29 = 18 %

In severe trauma treated with commercial nutrition solutions[32]

TOTAL NUMBER OF INFECTIONS:		
Alitraq Abbott-Ross (glut+arg)	16/32	50 %
Nova Source Novartis (+guargum)	17/29	58 %
Nutricomp peptide Braun (+peptide)	13/26	50 %
Nutricomp standard (+Synbiotic 2000)	4/26	15 %

NUMBER OF CHEST INFECTIONS:		
Alitraq Abbott-Ross (glut +arg)	11/32	34 %
Nova Source Novartis (+guargum)	12/29	41 %
Nutricomp Braun (peptide)	11/26	42 %
Nutricomp standard (+Synbiotic 2000)	5/26	19 %

In colitis-induced diarrhea[33]

Rectal application, 10 patients, studied before, and after 7, 14 and 21 days of treatment:

	Day 0	Day 7	Day 14	Day 21
Urgency	1.9	⇒ 1.2	⇒ 1.0	⇒ 1.0
Episodes of diarrhoea	2.4	⇒ 1.3	⇒ 0.9	⇒ 0.8
Nightly diarrhoea	0.5	⇒ 0.1	⇒ 0	⇒ 0
Visible blood	2.2	⇒ 1.2	⇒ 0.8	⇒ 0.8
Consistency of stool	1.1	⇒ 0.9	⇒ 0.7	⇒ 0.8

The composition of Synbiotics 2000

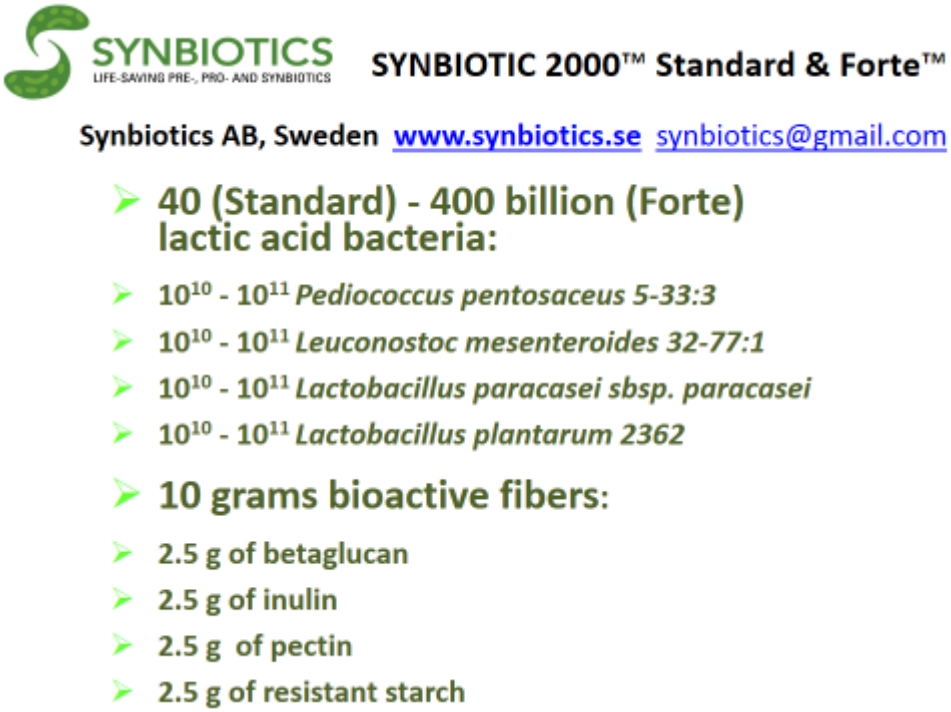
Synbiotic 2000™ is multistrain/multifibre composition, consisting in a mixture of 10^{10} and a Synbiotic FORTE™ with 10^{11} of each of four lactic acid bacteria (LAB): *Pediacoccus pentosaceus* 5-33:3,, *Lactobacillus paracasei* subsp paracasei 19, and Lactobacillus *plantarum* 2362, *Leuconostoc mesenteroides* 32-77:1 and 2.5 g of each of the four fermentable fibres (prebiotics): betaglucan, inulin, pectin and resistant starch (Synbiotic AB, Höganäs, Sweden), which in recent years has been extensively studied in clinical trials. The choice of LAB for the formulation was done after extensive studies of > 350 human[33] and >180 plant microbial strains[34] and based especially the ability of the LAB to produce bioactive proteins, transcribe NF-κB, produce pro- and anti-inflammatory cytokines, produce antioxidants, and most important, to functionally complement each other. In recent studies both the Synbiotic 2000 FORTE™ but also a Probiotic 2000 FORTE™ (no fibre added), containing 10^{11} of each of the four LAB, e.g. 400 billion LAB per dose have been tried.

Plantarum, paracasei and pediococcus exhibit unique probiotic properties.

Whenthe ability of 712 different LAB to ferment oligofructans was studied, only 16/712 were able to ferment semi-resistant fibres; the phleins and 8/712 inulin; *Lactobacillus plantarum, Lactobacillus paracasei subsp. paracasei, Pediococcus pentosaceus* and *Lactobacillus brevis*.[35] Interesting clinical results are also often obtained when these LAB are involved. When more than 100 LAB were compared Lb paracasei subsp paracasei were demonstrated to be the strongest inducer of Th1 & repressor of Th2 cytokines.[36] Several other studies have also documented the unique ability of Lb paracasei to iinduce cellular immunity, stimulate production of suppressive cytokines as TGFβ and Il-10, suppress CD4 T-cells, suppress Th2 activity, suppress splenocyte proliferation and decrease antigen-specific IgE and IgG1.[37-40]

The effect of *Lactobacillus paracasei* (NCC 2461), *Lactobacillus johnsonii* (NCC 533) and *Bifidobacterium lactis* Bb12 (NCC 362) on the induction and maintenance of oral tolerance to bovine beta-lactoglobulin (BLG) was investigated in mono-colonized germfree mice. The effects of *L. paracasei* were reported superior to those of the other two.[40] A study, which compared the ability of 50 different LAB to control 23 different pathogenic *Clostridium difficile* found more than half (27/50) totally ineffective, (18/50) antagonistic to some , but only five strains effective against all: two strains of *Lb paracasei* s. *paracasei* and five strains of *Lp plantarum*[41] Another study compared the effects in rats receiving during days 10 to 21 after *Trichinella spiralis* - induced infection either *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Bifidobacterium longum*, or *Bifidobacterium lactis; Lb paracasei* but not the other LAB attenuated muscle hyper-contractility, reduced the infection-associated Th- 2 response and muscle levels of TGF-β, COX-2 and PGE2.[42] A recent study compared in animals the effects of three probiotic strains: *Bifidobacterium lactis* NCC362, *Lactobacillus johnsonii* NCC533, and *Lactobacillus paracasei* NCC2461 on stress-induced changes in gut

permeability & on sensitivity to colorectal distension (CRD) Only *Lb paracasei* both prevented reduced significantly existing visceral pain and also restored normal gut permeability.[43]



References

1. Vaziri ND CKD impairs barrier function and alters microbial flora of the intestine: a major link to inflammation and uremic toxicity Curr Opin Nephrol Hypertens 2012;21:587-592
2. Wang F et al. Gut bacterial translocation is associated with microinflammation in end-stage renal diseasepatients. Nephrology 2012;17:733-738
3. Soulage CO, Koppe L, Fouque D, Protein-Bound Uremic Toxins. New Targets to Prevent Insulin Resistance and Dysmetabolism in Patients With Chronic Kidney Disease J Ren Nutr E-pub 2013
4. Koppe L, Pillon NJ, VellaRJ. Croze ML, Pelletier, Chambert et al p-Cresyl Sulfate romotes insulin resistance associated with CKD. J Am Soc Nephrol 2013;24: 88–99.
5. Pham NM, Recht NS, Hostetter TH et al. Removal of the protein-bound solutes indican and p-cresol sulfate by peritoneal dialysis. Clin J Am Soc Nephrol 2008;3:85–90
6. Meijers BK, De Preter V, Verbeke K et al. p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin. Nephrol Dial Transplant 2009;25:219–224
7. Simenhoff ML, Dunn SR, Zollner GP, Fitzpatrick ME, Emery SM, Sandine WE, Ayres JW Biomodulation of the toxic and nutritional effects of small bowel bacterial vergrowth in end-stage kidney disease using freeze-dried Lactobacillus acidophilus. Miner Electrolyte Metab 1996;22:92-96
8. Nakabayashi I, Nakamura M, Kawakami K, Ohta T, Kato I, Uchida K, Yoshida M. Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: a preliminary study. Nephrol Dial Transplant 2011;26:1094-1098

9. M. Hida, Y. Aiba, S. Sawamura, N. Suzuki, T. Satoh, Y. Koga, “Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oval administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. Nephron 1966;7:349–355
10. Rossi M, Klein K, Johnson DW, Campbell KL Pre-, pro-, and synbiotics: do they have a role in reducing uremic toxins? A systematic review and meta-analysis. Int J Nephrol 2012;2012:673631
11. Lindon JC, Holmes E, Nicholson JK Metabonomics in pharmaceutical R & D. Febs J 2007;274:1140–1151
12. Ridlon JM, Kang DJ, Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006;47: 241–259
13. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, et al. (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57–63
14. L. Caetano L, Antunes M, Han J, Ferreira RBR, Lolic´ P, Borchers CH et al. Effect of antibiotic treatment on the intestinal metabolome. Antimicrob Agents Chemother. 2011;55:1494-1503
15. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, Remely M et al. Changes in human fecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. PLoS One 2011;6:e28654
16. Koning CJ, Jonkers DM, Stobberingh EE, Mulder L, Rombouts FM, et al. The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxycillin. Am J Gastroenterol 2008;103:178–189
17. van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, de Bont ES et al. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis 2009;49:262-70
18. Andersen AB, Erichsen R, Farkas DK, Mehnert F, Ehrenstein V, Sørensen HT. Prenatal exposure to acid-suppressive drugs and the risk of childhood asthma: a population-based Danish cohort study. Aliment Pharmacol Ther 2012;35:1190-8
19. Nonzee V, Manopatanakul S, Khovidhunkit SO. Xerostomia, hyposalivation and oral microbiota in patients using antihypertensive medications. J Med Assoc Thai 2012;95:96-104
20. Burcelin R, Garidou L, Pomié C. Immuno-microbiota cross and talk: the new paradigm of metabolic diseases. Semin Immunol 2012;24:67-74
21. Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology 2004;39:1441-49
22. Riordan SM, Skinner NA, McIver CJ, Liu Q, Bengmark S, Bihari D et al. Synbiotic-associated improvement in liver function in cirrhotic patients: Relation to changes in circulating cytokine messenger RNA and protein levels. Microb Ecol Health Dis 2007;19:7-16
23. Solga SF, Diehl AM. Gut flora-based therapy in liver disease? The liver cares about the gut. Hepatology 2004;39(5):1197-200
24. Schunter M, Chu H, Hayes TL, McConnell DL, Crawford SS, Luciw PA et al. Randomized Pilot Trial of a Synbiotic Dietary Supplement in Chronic HIV-1 Infection. BMC Complement Altern Med.2012;12:84

25. Bengmark S Lactic acid bacteria and plant fibers in treatment of acute and chronic human disease. In Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications Edited by Susan S Cho, Terry Finocchiaro CRC Press, Taylor and Francis Group, Boca Raton 2009
26. Bengmark, S. Synbiotics in human Medicine. In Therapeutic Microbiology: Probiotics and Related Strategies. Editors Versalovic J, Wilson M, ASM Press, Washington DC USA, 2008, pp 307-321.
27. Bengmark S. Gut microbiota, immune development and function. Pharmacological Research 2013;69:87– 113
28. Bengmark S. Integrative medicine and human health - the role of pre-, pro- and synbiotics. Bengmark Clinical and Translational Medicine 2012;1:6
29. Rayes N, Seehöfer D, Theruvath T, Schiller RA, Langrehr JM, Jonas S et al. Combined perioperative enteral supply of bioactive pre- and probiotics abolishes postoperative bacterial infections in human liver transplantation - a randomised, double blind clinical trial. Am J Transplant 2005;5:125-30
30. Rayes Rayes N, Seehofer D, Theruvath T, Mogl M, Wilke C, Schiller RA et al. Supply of pre- and probiotics reduces bacterial infection rates after pylorus-preserving pancreatoduodenectomy- a randomized, double-blind trial. Ann. Surg 2007;246:36-41
31. Oláh A, Belágyi T, Pótó L, Romics Jr L, Bengmark S. Synbiotic control of inflammation and infection in severe acute pancreatitis, a randomized double blind study. Hepatogastroenterology 2007;54:36-41
32. Pathmakanthan S, Walsh M, Bengmark S, *et al*.: Efficacy and Tolerability treating acute distal ulcerative colitis with synbiotic enema's: A Pilot Trial. Abstract. *Gut* 2002**;**51(suppl 1III)**:**A307
33. Ljungh Å, Lan J-G, Yamagisawa N. Isolation, selection and characteristics of *Lactobacillus paracasei* ssp paracasei isolate F19. Microb. Ecol. Health Dis 2002;Suppl *3*:4-6
34. Kruzewska K, Lan J, Lorca G, Yanagisawa N, Marklinder I, Ljungh Å. Selection of lactic acid bacteria as probiotic strains by in vitro tests. *Microecol. Ther. 29* (2002) 37-51 The proceedings of the XVI international Congress on Microbial Ecology and Disease, held Noordwijkerhout, The Netherlands, Oct 2001
35. Müller M, Lier D. Fermentation of fructans by epiphytic lactic acid bacteria. J Appl Bacteriol 1994;76:406-411
36. Fujiwara D, Inoue S, Wakabayashi H, Fujii T The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. Int Arch Allergy Immunol 2004;135:205-215
37. von der Weid T, Bulliard C, Schiffrin EJ. Induction by a lactic acid bacterium of a population of CD4(+) T cells with low proliferative capacity that produce transforming growth factor beta and interleukin-10. Clin Diagn Lab Immunol 2001;*8*:695-701
38. Ibnou-Zekri N, Blum S, Schiffrin EJ, von der Weid T. Divergent patterns of colonization and immune response elicited from two intestinal Lactobacillus strains that display similar properties in vitro. Infect Immun 2003;71:428-436
39. Nagler-Anderson C. Tolerance and immunity in the intestinal immune system. Crit Rev. Immunol 2000;20:103-120
40. Prioult G, Fliss I, Pecquet S. Effect of probiotic bacteria on induction and maintenance of oral tolerance to beta-lactoglobulin in gnotobiotic mice. Clin Diagn Lab Immunol 2003;10:787-792
41. Naaber P, Smidt I, Stsepetova J, Brilene T, Annuk H, Mikelsaar M. Inhibition of Clostridium difficile strains by intestinal Lactobacillus species. J Med Microbiol 2004; 53:551-554

42. Verdú EF, Bercík P, Bergonzelli GE, Huang XX, Blennerhasset P, Rochat F, Fiaux M, Mansourian R, Corthésy-Theulaz I, Collins SM Lactobacillus paracasei normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. Gastroenterology 2004;127:826-837
43. Eutamene H, Lamine F, Chabo C, Theodorou V, Rochat F, Bergonzelli GE, Corthésy-Theulaz I, Fioramonti J, Bueno L. Synergy between Lactobacillus paracasei and its bacterial products to counteract stress-induced gut permeability and sensitivity increase in rats. J. Nutr. 2007;137:1901-1907