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LIST OF ABBREVIATIONS

ADD	Antibiotic-Associated Diarrhea
ASA	Aminosalicylic acid
B. adolescentis	Bifidobacterium adolescentis
B. bifidum	Bifidobacterium bifidum
B. longum	Bifidobacterium longum
B. pseudocatenulatum	Bifidobacterium pseudocatenulatum
C. coccoides	Clostridium coccoides
C. leptum	Clostridium leptum
DGGE/TGGE	Denaturing or temperature-gradient-gel-electrophoresis
DNA	Deoxyribonucleic acid
DSS	Dextran Sodium Sulphate
E. rectale	Eubacterium rectale
E.coli	Escherichia coli
E.faecalis	Enterococcus faecalis
EEN	Exclusively Enteral Nutrition
EN	Enteral nutrition
F. prausnitzii	Faecalibacterium prausnitzii
FAO	Food and Agriculture Organization of the United Nations
Fiaf	Fasting induced adipocyte factor
FISH	Fluorescent in situ hybridisation
FOS	Fructooligosaccharides
GALT	Gut associated lymphoid tissue
GI	Gastrointestinal
GRAS	Generally recognized as safe
HDL	High-density-lipoprotein
IBD	Inflammatory bowel disease
IBS	Inflammatory bowel syndrome
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G

IL	Interleukin
ILSI	International Life Science Institute
INF	Interferon
IκB	Inhibitor of NF-κB
KO	Knockout
L. bulgaricus	Lactobacillus bulgaricus
L.acidophilus	Lactobacillus acidophilus
L. casei	Lactobacillus casei
L. gasseri	Lactobacillus gasseri
L. plantarum	Lactobacillus plantarum
L. rhamnosus	Lactobacillus rhamnosus
LcS	Lactobacillus casei Shirota
LDL	Low-density-lipoprotein
LGG	Lactobacillus rhamnosus GG
LPL	Lipoprotein lipase inhibitor
LPMCs	Lamina propria mononuclear cells
LPS	Lipopolysaccharide
M. smithii	Methanobrevibacter smithii
n-3	Omega 3
n-6	Omega 6
^{NAC}MAAS	National Asthma Campaign Manchester Asthma and Allergy Study
NF-κB	Nuclear factor kappa light chain enhancer of activated B-cells
NOD	Nucleotide Oligomerization Domain
NSAR	Non-steroidal anti-inflammatory drugs
PAMPs	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
PEN	Partial enteral nutrition
PSPG complex	Polysaccharide-peptidoglycan complex
PUFAs	Polyunsaturated fatty acids
RNA	Ribonucleic acid
S. boulardii	Saccharomyces boulardii
S. thermophilus	Streptococcus thermophilus

SCFA	Short chain fatty acid
TGF	Transforming growth factor
TLR	Toll like receptor
TNF	Tumor necrosis factor
Treg	T regulatory cells
UC	Ulcerative Colitis
UNICEF	United Nations International Children's Fund
VLDL	Very-low-density-lipoprotein
VSL#3	Lactobacillus acidophilus, L. bulgaricus, L. casei, L. plantarum, Streptococcus thermophilus, Bifidobacterium breve, B. infantis, and B. longum
WHO	Worl Health Organization

1. INTRODUCTION AND QUESTION

1.1. MICROBIOTA IN THE GASTROINTESTINALTRACT AND THEIR IMPACT ON HEALTH

The composition of the gut flora differs from human to human and is therefore like a fingerprint unique. Postnatal, microorganisms colonize the gastrointestinal tract. The highly acidic stomach fluids are a chemical barrier for microorganisms and therefore the stomach holds low bacterial counts (Madigan and Martinko 2006). Mainly facultative anaerobes like Lactobacilli, Streptococci and yeasts are present. The bacterial load increases in the small intestine, especially that of the Enterobacteria, Lactobacilli and Streptococci which are facultative anaerobes yet anaerobes like Bacteroides, Bifidobacteria and Clostridia are also predominant. The colon has enormous numbers of bacteria as well as the greatest diversity of all. Dominant are strict anaerobes like Bacteroides spp., Clostridia, Ruminococcus spp., Butyrovibrio spp., Fusobacterium spp., Eubacterium spp., Peptostreptococcus, Bifidobacterium spp., Atopobium spp., and Pepptococci. Facultative anaerobes also occur but in smaller amounts like, Lactobacilli, Enterococci, Streptococci and Enterobacteriaceae (Rastall 2004). The diversity of bacteria in the human colon is very high but until now it has not been possible to detect all of them. Currently, millions of microbial species exist that are not cultivated (Achtman and Wagner 2008).

Archaea, Eukarya and Bacteria are the main domains which are present in the human gut, bacteria making up the largest amount (Backhed, Ley et al. 2005). Within the domain bacteria, Firmicutes and Bacteroidetes are the most abundant phyla in the gut. Firmicutes are butyrate producing bacteria and among others, belong to the Clostridia Class. In small amounts, occur Proteobacteria, Actinobacteria, Fusobacterua and Verrucomicrobia phylas (Eckburg, Bik et al. 2005). Clostridium coccoides group (clostridial cluster XIVa), Clostridium leptum subgroup (clostridial cluster IV) and Bacteroides-Prevotella group are quantitatively dominant bacteria in the faeces of healthy individuals (Sokol, Lay et al. 2008).
About one third of faeces are made up of bacteria. The bacteria which live in the lumen of the large intestine are continuously washed out with the flow of the material and replaced through new growth (Madigan and Martinko 2006).

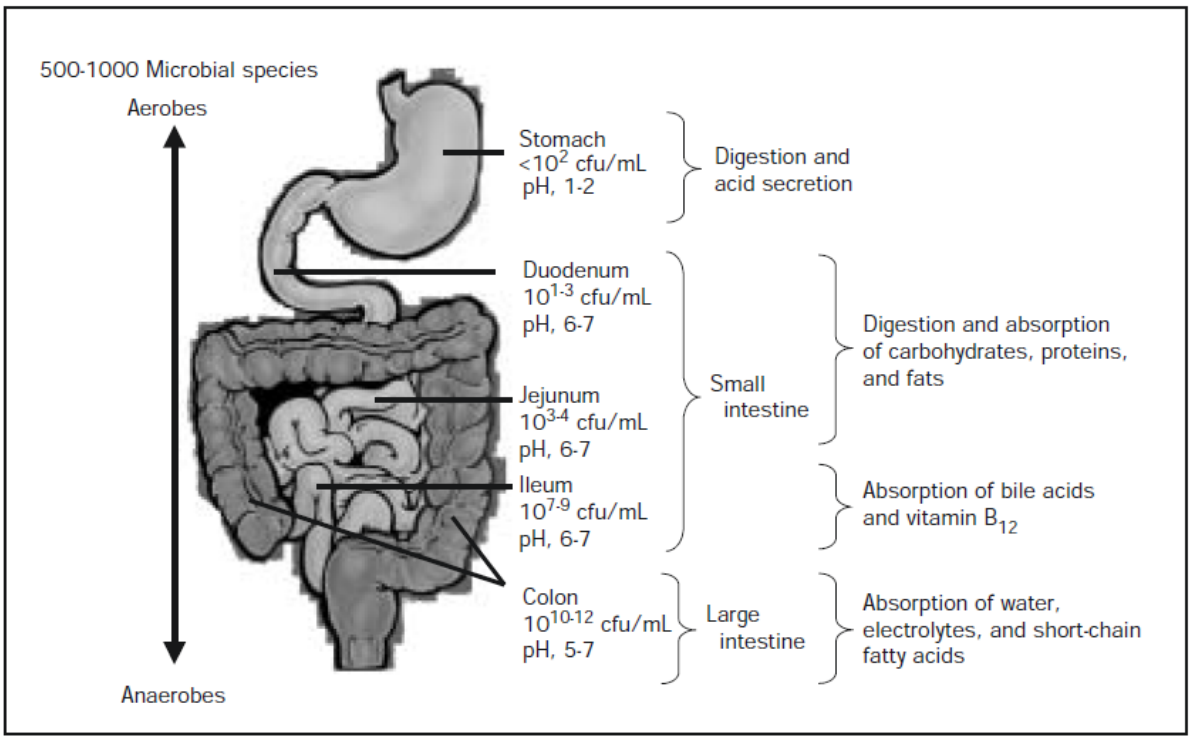


Figure I. Key physiologic and microbiological features of the gut.
Relative concentrations of bacteria and the PH at various locations within the adult gut are also noted. Cfu = colony forming unit.
(DiBaise, Zhang et al. 2008)

With the development of methods for identifying gut microflora they have noted that about 10^{12} microorganisms per millimetre of luminal content colonize the gastrointestinal tract in an adult human. The diversity is very high; about 500-1000 different bacterial species are detected. Meanwhile it is not possible to verify all occurring bacteria. Current studies suppose that the numbers are actually much higher, from up to 15000 to 36000 species of bacteria. Several studies have shown that the lower portion of the gastrointestinal tract has a higher bacterial count with basically anaerobic bacteria and the upper portion with lower counts is primarily colonized with aerobic bacteria (DiBaise, Zhang et al. 2008).

Phyla	Representative genera
Bacteria	
Firmicutes	*Ruminococcus*
	Clostridium
	Peptostreptococcus
	Lactobacillus
	Enterococcus
Bacteroidetes	*Bacteroides*
Proteobacteria	*Desulfovibrio*
	Escherichia
	Helicobacter
Verrucomicrobia[b]	
Actinobacteria	*Bifidobacterium*
Cyanobacteria[b]	
Synergistes[b]	
Archaea	
Euryarchaeota	*Methanobrevibacter*

Figure II. Major Bacteria and Archaea Phyla and Genera Found in the Human Gut Microbiota[a].
[a] Prokaryotic phyla were identified by using an alignment of the 18,348-sequence dataset from (Ley, Backhed et al. 2005).
[b] Not related to any known genera.
(DiBaise, Zhang et al. 2008)

The latest study from Qin et al. led to the conclusion that a 'core' microbiome exists, which hypothesizes against previous study results. To analyse the microbial communites, investigators conducted the experiment by metagenomic sequencing which is an alternative to rRNA sequencing used in precedent studies (Qin, Li et al.; Riesenfeld, Schloss et al. 2004; Tringe and Rubin 2005; von Mering, Hugenholtz et al. 2007). Qin et al. genetically investigated faecal samples from 124 European adults. Subjects were either healthy, obese or affected by Inflammatory bowel disease (IBD). Investigators could identify 3.3 million genes and additionally found out that 38% of the total gene pool was equal in each individual. The subjects who were affected from Inflammatory bowel disease had 25% fewer common genes. This is consistent with previous studies who have already noticed that these individuals have a lowered bacterial diversity (Qin, Li et al.; Manichanh, Rigottier-Gois et al. 2006). To quantify, 57 bacterial species were present in ≥ 90% and 75 of the bacterial species were present in ≥50% and at least 18 species occurred in all

individuals. Like in previous studies Bacteroides and Firmicutes made up the highest amount (Qin, Li et al.).

A “microbiome” is the collective genome of microorganisms present in humans. The microbiome encodes 100-fold more unique genes than our own genome. The genomes of the microbiome possess traits of which humans have not evolved (Gill, Pop et al. 2006).

The ‘core human microbiome’ represents the set of genes occurring in all or in the vast majority of humans. Genes which are present in a smaller percentage of humans belong to the ‘variable human microbiome’. The ‘variable human microbiome’ is influenced by the host itself. The environment in which the host is exposed, the diet habits and other lifestyle factors of the host, the genotype of the host, the physiological status of the host, the host’s state of health, as well as transients organisms occurring in the host, all are factors which are decisive of the ‘variable microbiome’ (Turnbaugh, Ley et al. 2007).

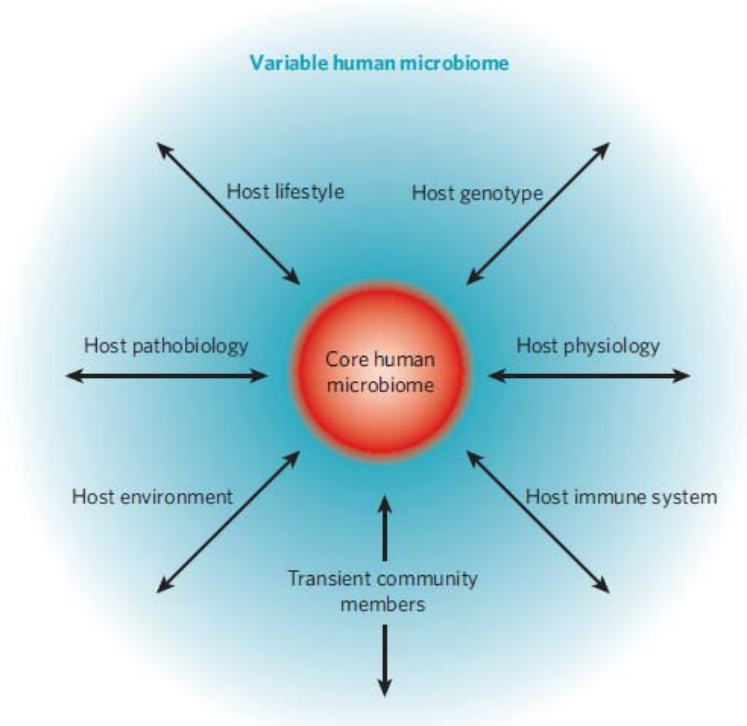


Figure III. The concept of a core human microbiome.
(Turnbaugh, Ley et al. 2007)

The Microbiota – BIRTH

At birth, the colon of an infant is sterile. The first colonization is influenced by the bacterial load of the environment as well as the mode of delivery and nutrition are deciding. Bifidobacteria and Lactobacilli are dominant in breastfed infants and have a positive impact. In Formula-fed infants the ratio of Bifidobacteria to Enterobacteria is 1:1 whereas in breastfed infants the ratio is 10:1 (Harmsen, Wildeboer-Veloo et al. 2000; Roy, Kien et al. 2006). Oligosaccharides are the most important factor for the intestinal flora and make up the third largest component after lactose in the human milk (Coppa, Bruni et al. 2004; Roy, Kien et al. 2006). 15-25% of the Oligosaccharides are acidic (Boehm, Jelinek et al. 2004). They are resistant to digestion, reach the colon, where the bacterial fermentation and the production of short chain fatty acids (SCFA) take place. This process promotes the growth of selective bacteria, especially the Bifidogenic flora (Coppa, Bruni et al. 2004; Roy, Kien et al. 2006).

SCFA:

- Are a substantial source of energy
- Have a trophic effect of monocytes
- Enhance the re-absorption of water and sodium

(Coppa, Bruni et al. 2004; Roy, Kien et al. 2006)

In preterm infants the flora is alike to that of formula-fed infants, especially in the first days of life. The Enterobacteria occur in higher amounts and the Bifidobacteria emerge later as in full-term formula fed infants (Magne, Suau et al. 2005; Roy, Kien et al. 2006). Healthy infants are colonized by Bifidobacterium longum, breve and lactis (Sakata, Tonooka et al. 2005; Isolauri, Kalliomaki et al. 2008), whereas disturbance in the composition of Bifidobacterium microbiota is associated with increased amount of Bifidobacterium adolescentis as well as Clostridia (Kalliomaki, Kirjavainen et al. 2001; Kirjavainen, Arvola et al. 2002; Isolauri, Kalliomaki et al. 2008).

1.1.1. Microbiota and Metabolic Functions

The gut microbiome has beneficial effects for its host. It is responsible for (1) maintaining homeostasis, (2) removing pathogens by competing about nutrients and epithelial sites, (3) producing antimicrobial compounds, (4) boosting gut barrier function (5) having immune-modulatory effects (Sheil, Shanahan et al. 2007), and in addition (6) is included in epithelial turnover, (7) gastrointestinal motility and (8) drug metabolism.
Furthermore the gut microbiome has metabolic functions like (8) synthesising micronutrients, (9) fermenting indigestible food substances, (10) is involved in the absorption of certain electrolytes and trace minerals and (11) responsibility for the break down of dietary toxins and carcinogens (DiBaise, Zhang et al. 2008) (12) protecting the epithelium against cell injury (13) involvement in regulating fat storage and (14) influencing angiogenesis in the intestine (Eckburg, Bik et al. 2005).

The affection of growth and differentiation of enterocytes and colonocytes is possible through the production of short chain fatty acids, generated by indigestible components which are fermented by the bacteria in the gut (DiBaise, Zhang et al. 2008). Bacteria have the ability to ferment indigestible substances and thereby extract energy. There is a syntrophic interaction between bacteria and Archaea. Bacteria generate due to the fermentation of short chain fatty acids, organic acids and gases like H_2 and CO_2. An accumulation of H_2 inhibits the NADH dehydrogenase and therefore energy extraction is impossible. For that reason Archaea have the potential to remove H_2 by utilizing it for methanogenesis (Samuel and Gordon 2006; DiBaise, Zhang et al. 2008).

1.1.2. Groups of Microbiota

The majority of bacteria in the gut are anaerobia. There is a differentiation between aerobic, anaerobic and microaerophil bacteria. For living conditions aerob bacteria need oxygen, whereas anaerob bacteria can survive without oxygen. Microaerophil represents that these bacteria grow in a milieu with reduced oxygen.
Furthermore there is a classification between obligate and transient microorganisms (Beckmann and Rüffer 2000). Beside the obligate flora, transient organisms occur,

sometimes in high amounts, and sometimes in such low amounts that they are not detectable (Zwielehner, Liszt et al. 2009). Obligate organisms are resistant and stable and have important functions in the gut while transient organisms are exogen organisms which pass the gut and do not have under healthy conditions a chance to damage the gut. Another differentiation is based on the metabolic activity of the microorganisms.
In addition, a classification between microorganisms with lipolytic activity, microorganisms with proteolytic activity and microorganisms with sacharolytic activity exists (Beckmann and Rüffer 2000).

Bacteria, Eukaryota and Archaea are the present life forms, although Bacteria clearly dominate (DiBaise, Zhang et al. 2008). Currently nine bacterial phyla are known to colonize the human gastrointestinal tract of which Firmicutes, Bacteroidetes and Actinobacteria are dominant (Backhed, Ley et al. 2005). The human gut microbiome is individually different and therefore it is very difficult to define an overall model (Rajilic-Stojanovic, Smidt et al. 2007). In general a high diversity is desirable (McCann 2000). Nonetheless 'core' species exist in the majority of humans (Dore 2007; Zwielehner, Liszt et al. 2009). In this paper, the focus will be centred on Lactobacilli, several Clostridia Clusters, Bacteroides and Bifidobacteria because these are the major bacteria which are most likely associated with dysfunctions of the gut.

1.1.2.1. Firmicutes

Gram positive bacteria of the Firmicutes phylum seem to have specific enzymatic enzymes which might play a crucial role in obesity (Turnbaugh, Hamady et al. 2009).
Patients with Morbus Crohn have a significant reduction of Firmicutes qualitatively as well as quantitatively in their faeces (Sokol, Lay et al. 2008). In patients with Ulcerative Colitis Firmicutes, the amount is as well reduced (Sokol, Lepage et al. 2006; Andoh, Sakata et al. 2007; Sokol, Lay et al. 2008).

1.1.2.1.1. Lactobacillus spp.:

Lactobacilli are mikroaerophil which means that they grow under oxygen reduced conditions. The major species in the gut are Lactobacillus acidophilus, Lactobacillus salvarius, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus cellobiosus.

Lactobacilli occur on plants, and are applied in the food industry as starter cultures. Lactobacilli belong to the obligate flora of the small and the large intestine, the mouth and the vagina. In healthy individuals constitutes the bacterial counts from 10^5 to 10^7 /g faeces (Beckmann and Rüffer 2000).

Lactobacilli produce antimicrobial agents and have therefore a positive impact on health. Furthermore they occupy receptors sites, compete about nutrients and about space (Rastall 2004) and as a result of SCFA generation, they acidify the milieu and inhibit thereby the colonisation of pathogens (Guarner 2005). The growth of Lactobacilli can be stimulated due to prebiotics like inulin and fructo-oligosaccharides (Van Loo 2004).

Lactobacilli seem to increase in their amount in obese individuals and therefore they might play an important role in weight gain (Armougom, Henry et al. 2009); due to their function in food conversion (Khan, Raoult et al. 2007). A reduced amount of Lactobacilli in IBD patients is observed as well. In patients with Ulcerative Colitis (UC) the number of Lactobacilli in the faeces seems to be reduced (Bullock, Booth et al. 2004; Sokol, Lay et al. 2008). In ulcerated tissues the number of Lactobacilli was reduced in comparison to non ulcerated tissues (Zhang, Liu et al. 2007; Armougom, Henry et al. 2009). Furthermore, in patients with diarrhea the amount of Lactobacilli seems to be significantly reduced (Sullivan, Edlund et al. 2001) .

1.1.2.1.2. Clostridium Cluster IV and XIV

Clostridial clusters IV [Clostridium leptum subgroup] and XIVa [Clostridium coccoides group] contain bacteria which are butyrate producers. Eubacterium, Roseburia, Faecalibacterium and Coprococcus are the main species (Pryde, Duncan et al. 2002). Clostridium Cluster IV influences beneficial functions like nutrient absorption, generation of short chain fatty acids and epithelial cell maturation and maintenance (Woodmansey 2007; Zwielehner, Liszt et al. 2009). Faecalibacterium prausnitzii is

part of the most abundant bacteria in the human faeces and belongs to Clostridia Cluster IV (Suau, Rochet et al. 2001).
E. rectale-C. coccoides group belongs to the clostridia cluster XIV. These are the main butyrate producing bacteria in the distal colon. Butyric acid is the product after the fermentation of carbohydrates and is used as an energy source for the epithelial cells. Through the reduction of these bacteria it might be possible that not as much energy can be harvested and therefore, this could positively influence weight loss.
Nevertheless, butyrate has beneficial effects such as better satiety. For that reason, other more complex mechanisms related to the fatty acid metabolism may be linked to Firmicutes and Clostridium clusters with obesity (Nadal, Santacruz et al. 2009).

Clostridia Cluster IV is significantly reduced in amount as well as diversity in the elderly in comparison to young individuals (Zwielehner, Liszt et al. 2009) especially Faecalibacterium prausnitzii (Bartosch, Fite et al. 2004). Patients with Crohn´s disease (CD) seem to have a decline in the amount of Clostridium Cluster IV in faeces (Scanlan, Shanahan et al. 2006). In addition the amount of Faecalibacterium prausnitzii was markedly reduced on the mucosal surfaces of CD patients (Bartosch, Fite et al. 2004; Frank, St Amand et al. 2007) On the contrary Clostridial Cluster XIVa is reduced in its amount in the mucosa of patients with UC (Swidsinski, Weber et al. 2005). In ulcerated tissue the number of Clostridial Cluster IV seems to be reduced in comparison to non ulcerated tissue (Zhang, Liu et al. 2007).

1.1.2.1.3. Clostridium Cluster II

Clostridium hystolyticum is part of the Clostridia Cluster II. This bacterium is highly proteolytic and produces acetate. Increased acetate amounts promote gut colonisation and stimulate lipid synthesis. Furthermore, Clostridium hystolyticum produces cytotoxic proteases which may have a pathogen effect for cells and tissue (Nadal, Santacruz et al. 2009).

1.1.2.1.4. Clostridium Cluster XI

C.lituseburense counts to the clostridia cluster XI. This cluster is heterogeneous and contains opportunistic pathogens such as Clostridium difficile. Clostridium difficile

and Clostridium hystolyticum may influence the potential virulence of the gut microbiota in obese patients (Nadal, Santacruz et al. 2009). Several studies concluded that Clostridium difficile occurs especially in children who develop atopic diseases (Bottcher, Nordin et al. 2000; Bjorksten, Sepp et al. 2001; Kalliomaki, Kirjavainen et al. 2001; Penders, Thijs et al. 2007; Sjogren, Jenmalm et al. 2009). In addition Clostridium difficile is very often the causative agent for antibiotic induced diarrhea (Bartlett 1987). Two toxins are produced by Clostridium difficile which are responsible for the mucosal damage and the inflammation in the colon (Hogenauer, Hammer et al. 1998).

1.1.2.2. Bacteroidetes

1.1.2.2.1. Bacteroides

Belong to the anaerob bacteria and are most important for colonial resistance because quantitatively they make up the largest amount. In the large intestine 10^8-10^{10} bacterial counts/g occur in healthy individuals (Beckmann and Rüffer 2000). Bacteroidetes are included in nutrient absorption, in the short chain fatty acid production and further in epithelial cell maturation and maintenance (Woodmansey 2007). Bacteroides produce propionate which inhibits lipid synthesis of acetate and this may enhance a lean phenotype (Nadal, Santacruz et al. 2009).

High variations between individuals occur in the species Bacteroidetes, even though Bacteroidetes thetaiotaomicron occurred in each human individual (Layton, McKay et al. 2006). Several studies pointed that a decline of Bacteroides and an increase of Firmicutes is associated with obesity. Much more astonishing was the conclusion that Bacteroidetes thetaiotaomicron seem to play an important factor in host adiposity (Ley, Turnbaugh et al. 2006; Samuel and Gordon 2006). Not less than 8% of its genome is dedicated for carbohydrate metabolism and functions in Bacteroidetes thetaiotaomicron (Xu, Bjursell et al. 2003). Bacteroides thetaiotaomicron is highly efficient in glycan metabolism whereby indigestible substances are metabolized and used as energy source (Comstock and Coyne 2003; DiBaise, Zhang et al. 2008).

The impact of Bacteroides in the development of atopic diseases is not clear until now. While Bjorksten et al. concluded that allergic infants had at the age of one year old a decreased colonisation with Bacteroides (Bjorksten, Sepp et al. 2001;

Songjinda, Nakayama et al. 2007); Songjinda et al. gathered that children who developed allergy by their second birthday had significant increased amounts of Bacteroidaceae in comparison to non-allergic subjects. These conflicting results may be caused by different sampling ages and also is a result due to sampling before and after weaning (Songjinda, Nakayama et al. 2007).

Bacteroides thetaiotaomicron regulates intracellular downstream of TLR signalling and NF-κB activation, and thereby reduces inflammation (Kelly, Campbell et al. 2004; Songjinda, Nakayama et al. 2007), Bacteroides fragilis stimulates increased induction of TH2 cells which leads to increased generation of inflammatory cytokines (Odamaki, Xiao et al. 2007; Songjinda, Nakayama et al. 2007). The amount of fecal bacteria, especially of the Bacteroides-Prevotella-Porphyromonas group, seems to be reduced during acute diarrhea in contrast to periods of a normal health state (Balamurugan, Janardhan et al. 2008).

1.1.2.3. Actinobacteria

1.1.2.3.1. Bifidobacterium spp.:

Bifidobacteria live under anaerob conditions. In the gut Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium bifidum are common bacteria. Mainly they occur in the large intestine and in small amounts in the small intestine. The amount in healthy individuals is between 10^8-10^{10} bacterial counts /g faces (Beckmann and Rüffer 2000).

Bifidobacteria seem to be the most crucial bacterium in terms of health (Gibson and Roberfroid 1995). At least 8% in the genome of Bifidobacterium longum are responsible for carbohydrate transport and metabolism functions (Xu, Bjursell et al. 2003). Furthermore, through acidification with SCFA, competing for nutrients, secretion of antimicrobial components and stimulation of the immune system the colonization of pathogens is inhibited (Guarner 2005). Several studies proved the effect of Bifidobacteria on enhanced barrier function of the gut epithelium (Zwielehner, Liszt et al. 2009).

The growth of Bifidobacteria can be enhanced with prebiotics like inulin or fructo-oligosaccharides (Kolida and Gibson 2007). Due to produced SCFA the growth of Bifidobacteria is stimulated (Roy, Kien et al. 2006).

Several studies have proven that the amount, as well as the diversity of Bifidobacteria is reduced in elderly in comparison to young individuals (Zwielehner, Liszt et al. 2009). The amount of Bifidobacteria in the epithelium seems to be much lower in UC patients than in healthy controls (Mylonaki, Rayment et al. 2005). Bifidobacteria might also have a protective role in development of atopic diseases. Nevertheless do we have to keep in mind, that different Bifidobacterium species have different functions (Apajalahti, Kettunen et al. 2003), and that allergic children have other Bifidobacteria species than healthy children (He, Ouwehand et al. 2001; Ouwehand, Isolauri et al. 2001; Penders, Stobberingh et al. 2007). While Bifidobacterium adolescentis occurs in higher amounts in allergic children, Bifidobacterium catenulatum and longum seem to be characteristic for healthy infants (He, Ouwehand et al. 2001; Ouwehand, Isolauri et al. 2001; Stsepetova, Sepp et al. 2007).

Generally the assumption exists that a low amount of Bifidobacteria in early childhood is associated with allergy development (Bjorksten, Naaber et al. 1999; Kalliomaki, Kirjavainen et al. 2001; Stsepetova, Sepp et al. 2007). In the case of diarrhea, the amount of Bifidobacteria seems to be reduced (Sullivan, Edlund et al. 2001).

1.1.2.4. Archaea

Methanobrevibacter smithii is hydrogen consuming methanogen and the most occurring phylotype of Archaea. Due to the Archaeal methanogenesis the fermentation of polysaccharides is facilitated because methanogenesis prevents the generation of hydrogen and other reaction end products. Thereby bacteria have the possibility to produce more acetate and butyrate, which seem to be energy sources for epithelial cells. For this reason, the assumption exists that Archaea especially that of Methanobreviacter smithii, might be an important factor in energy extraction and hence in gaining weight (DiBaise, Zhang et al. 2008).

1.1.3. Molecular Mechanisms in the Epithel

The normal intestinal immune--regulation

The mucosa associated immune system is an independent differentiated immunological compartment. The main function of the intestinal mucosa associated immune system of the gut (GALT) is to inhibit the invasion of infectious and toxic pathogens in the intestinal lumen. We find the most immune cells of the body in the mucosa associated immune system (Stange 2004).

Gut associated immune system

Unspecific factors:

- Acidity of the gastric juice
- Microbicidal function from digestive secrets
- Productive peristaltic
- Mucin secretion

(Beckmann and Rüffer 2000)

Specific factors:

There is a high density of immune cells in the submucosa of the gut. T-Lymphocytes are regulator- and effector-cells which induce the immunity by producing secretoric IgA. Due to the activity of T-Lymphocytes antigen components of the diet can not lead to a systemic immune response (oral tolerance). On the one hand, T-lymphocytes colonize as intraepithelial lymphocytes the mucosa of the gut and the lamina propria; and on the other hand the lymphoid tissue, the peyer´s patches.

In addition to B and T cells, macrophages and dendritic cells occur in the lamina propria. These cells are responsible for processing and subsequently presenting antigens. Furthermore they can provide lysosomale enzymes and set oxygen radicals free, which can remove tissue and eliminate microorganisms (Beckmann and Rüffer 2000).

1.1.4. Probiotics and Prebiotics generally

Some microbes in the gastrointestinal tract have beneficial functions such as producing nutrients, absorbing peptides and vitamins; on the contrary others produce toxins or compete with the host for common substrates (Torrallardona, Harris et al. 1996; Murry, Lewis et al. 1997). Administration with probiotics could prevent the overgrowth of pathogens an thereby have a possible impact on health (Drago, Gismondo et al. 1997).

Viable microbes can have an effect on health. However the composition of microbiota varies in each individual; therefore it is necessary to attune administration with probiotics for each individual. In doing so, it may be possible to improve health and prevent diseases. Microbiota ferment complex nutrients into simple sugars, short chain fatty acids and other nutrients; and thereby enable the absorption of these substances. The microbiota produces folic acid and the vitamins K, and B12 which are important in the bile acid metabolism and recirculation. The microbiota furthermore is essential in transforming carcinogens like N-nitroso compounds and heterocyclic amines. The activation of bioactive compounds is likewise possible (Mai and Draganov 2009).

Common probiotics are among Lactobacilli and Bifidobacteria, Streptococcus, Enterococcus, non-pathogenic E.coli and Saccharomyces boulardii (Shanahan 2001).

1.1.4.1. Probiotics Definition

The FAO and WHO defined probiotics in 2001 as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Bergonzelli, Blum et al. 2005).

In Europe, the International Life Science Institute (ILSI) defines a probiotic as “a viable microbial food supplement which beneficially influences the health of the host”. The efficacy and safety of probiotics for each strain and each product must be scientifically proven (Kalliomaki, Salminen et al. 2008).

1.1.4.2. Requirements on Probiotics

Certain criteria have to be fulfilled by bacteria before they can be established as a probiotic. Firstly, it must be of human origin and secondly, they must be generally regarded as safe (GRAS). A probiotic must have the ability to reach the gastrointestinal tract in adequate amounts so it can unfold its impact. Therefore it has to fulfil special criteria, such as, after the uptake it has to pass the stomach and the passage of the small intestine where thirdly it has to be resistant against bile and acid. How well the impact of a probiotic is, is fourthly dependent on the adherence to intestinal cells and fifthly on it persistence in the gut for some time. A colonisation of a probiotic is not required however persistence during the therapy is eligible (Dunne, O'Mahony et al. 2001; Sheil, Shanahan et al. 2007). Sixthly, it has to have the ability to produce antimicrobial substances, seventhly it must be antagonistic against pathogenic bacteria and finally it must have the ability to modulate the immune response (Sheil, Shanahan et al. 2007).

1.1.4.3. Beneficial effects of Probiotics

- Alleviation of lactose intolerance
- Reduction of gastrointestinal infections or even prevention of them
- Suppress cancer
- The concentrations of plasma cholesterol is reduced which leads to a decrease of the risk for heart disease
- Nutrients are better digested and therefore have a higher value

(Sheil, Shanahan et al. 2007)

1.1.4.4. Functions of Probiotics:

(1) Improve gut barrier function,
(2) Adhere on the mucosal surface
(3) Prevent the binding of pathogens
(4) Produce bacterocins
(5) Modulate inflammatory response
(6) Alleviate visceral hypersensitivity
(7) Ferment nutrients and thereby acidify the colon

(8) Support the stability of the colonic microbiota.

(Spiller 2008)

1.1.4.5. Prebiotics Definition

Prebiotics are defined as “nondigested food ingredients that artificially affect the host by selectively stimulating the growth or activity of one or a number of bacteria in the colon that can improve the host’s health” (Gibson and Roberfroid 1995).

The distinction of dietary fibers is between soluble and insoluble fibers. Soluble fibers are completely fermented by the bacteria whereas insoluble fibers are nearly entirely excreted with the stool and fermentation proceeds in just small amounts (Asp 1995; Wong, de Souza et al. 2006).

1.1.4.6. Functions of Prebiotics

Dietary fibers slow the absorbance rate and increase the stool bulk. Furthermore soluble fibers slow the transit, stimulate the growth of commensal bacteria and due to their fermentation, short chain fatty acids are generated which affect the cholesterol metabolism (Cummings and Englyst 1995), cause an increased amount of SCFA leads to stimulation of adipogenesis and an increased leptin production may be a result. Due to a decreased synthesis and secretion of very low density lipoproteins (VLDL), SCFA have a hypocholesterolemic effect (Roy, Kien et al. 2006).

1.1.4.7. Modern Molecular Findings

The action of probiotics is strain and species specific. To achieve appropriate effects the right choice of organisms is therefore important. Further essays about the mechanisms of probiotics are necessary to better understand the effects of probiotics. Until now investigators have found that probiotics alter gut microbial diversity, have an affect of intestinal barrier function, modulate the immune response and induce the development of T regulatory cells and dendritic cells. Until now the knowhow about the host microbe crosstalk which initiates the process is too small (Reiff and Kelly 2009).

1.1.4.7.1. Secretion of Bacteriocins

Hydrogen peroxide, organic acids, Diacetyl, Bacteriocins and Bacteriocins molecules are some of the products which are generated by probiotics and which have an antimicrobial effect in vitro (Ouwehand, Kirjavainen et al. 1999). Nevertheless, in vivo none of these products have been proven to have an impact on health (Rastall, Gibson et al. 2005).

Probiotics have antagonistic direct and indirect effects against pathogenic germs.

Direct:

One direct impact is the germ-killing lactic acid which is produced by Lactobacillus. Other microorganisms produce specific bacteriocines and other antimicrobial substances. Saccharomyces boullardii has the ability to destroy the toxins produced by Clostridium difficile due to its proteolytic activity.

E.coli Nissle 1917 produces two different antagonistic active microcines. These microcines destroy the pathogen and proinflammatory Enterobacteria. These bacteria are responsible for acute inflammatory attacks in inflammatory bowel diseases. Therefore it may have a positive impact on chronic inflammatory bowel disease.

The synthesis of bacteriocines is not automatic; it is administered by environmental conditions. The Quorum sensing is a cellular communication between bacteria where bacteria detect the population density. At a specific quantity of bacteria, the Quorum sensing causes the secretion of secondary metabolic products. Quorum sensing regulates alongside the bacteriocine production the production of biofilm, sporulation, symbioses and virulence. A further antimicrobial function is the occupation of probiotics of the mucus in the gut-epithelium so that pathogen germs do not have the possibility to colonise (Schulze, Sonnenborn et al. 2008).

Indirect:

Probiotics stimulate the mucin –gen expression and therefore enforce the amount of mucus which leads to a handicap for the interaction of pathogenic germs.

E.coli Nissle 1917 has also indirect functions. It secretes diffusible signal-substances which have an impact on Enterobacteria by reducing the permeability for invasive diarrhoea inducing bacteria. Furthermore probiotics stimulate the bodies' defensin-production. Defensins are antimicrobial peptides. In patients with chronic

inflammatory bowel disease, the Defensin production is reduced, which leads to the conclusion that probiotics may have a positive impact (Schulze, Sonnenborn et al. 2008).

1.1.4.7.2. Immunmodulatory Actions

Probiotics modulate the immunmodulatory actions either by altering the resident bacteria in the gut, or by enforcing the gut barrier function which is followed by a diminished potential for bacteria to activate the immune system. Moreover probiotics have an anti-inflammatory effect by stimulation of regulatory T-cells (Spiller 2008).
E.coli Nissle 1917, L.-casei-, L.-rhamnosus-, L.-plantarum-, L.-gasseri- and L.-acidophilus- strains possess significant immune-modulatory and anti-inflammatory functions. Lactobacilli allure macrophages and induce a modulation of the cytokine expression and the maturation of surface markers in dendritic cells (Schulze, Sonnenborn et al. 2008). In an experiment, healthy volunteers were treated with L.rhamnosus. Results showed that in peripheral blood mononuclear cells, an increase in IL-10 secretion and a decrease in TNF, IL-6 and interferon secretion appeared. Similar results arose in an experiment with atopic children after administration with L. rhamnosus. Other studies showed that VSL#3 increased a marker in Treg cells (Spiller 2008).
Administration with VSL#3 inhibited expression of inflammatory Th1 cells, reduced pro-inflammatory Il-12 generation and elevated anti-inflammatory IL10 in isolated dendritic cells of blood and intestine from humans. A Reduction of IL-10 in IL-10KO mice proceeded also because of treatment with VSL#3. Treatment with Bifidobacterium longum lowered the secretion of pro-inflammatory TNF-α and IL-8. Furthermore a colonic biopsy of inflamed mucosa in Ulcerative colitis patients showed that Bifidobacterium longum achieved a reduction of NF-κB positive lamina propria mononuclear cells. The probiotic LGG has the ability to reduce intestinal epithelial apoptosis in C57BLJ mice. The induction of IL-10 dependent T regulatory cells which expresses TGF-β is controlled by VSL#3 treatment in chemically induced IBD. Bifidobacterium infantis 35624 induced a Treg expression and hindered NF-κB activation by pathogens. Lactobacillus acidophilus also induces Treg cells and expresses TGF-β. Gut tolerance can be promoted by probiotics but further investigation of the mechanism are needed (Reiff and Kelly 2009).

1.1.4.7.3. Mucosal Adherence + Inhibition of Pathogenic Bacteria Adherence

A competition of nutrients and space between the different bacterial strains is present in the gut. Lactobacillus spp. has been shown to reduce the growth of the invasiveness and adherence of enterotoxigenic E.coli in the epithelial. B.longum and E.coli Nissle 1917 have shown similar effects (Spiller 2008). In vitro it's proven that probiotics can avoid the adhesion of pathogens such as E.coli, Salmonella enterica serotype Typhimuriium on the epithelial cells (Gill 2003). The supposed function behind this inhibition might be the competition of receptors, or the mucin production which is enforced due to probiotics (Mack, Michail et al. 1999; Lee and Puong 2002).

1.1.4.7.4. Enhanced Barrier Function of Epithelium

An experiment was conducted with IBD patients with pouchitis who stayed in remission because of antibiotic administration. Patients received VSL#3 which led to an increase of bacteria and a reduction of fungal diversity compared with the control group. An increase of mucosal barrier function leads to reduced translocation of bacteria and therefore the ability of pathogenic bacteria to attach to the gut mucosa is hindered. Probiotics may up-regulate expression of tight junctions like defensins, mucins or proteins. Barrier function is further up regulated by VSL#3 and Lactobacillus fermentum because of up-regulation of human β defensin 2. Administration with VSL#3 increased mucin gene expression and excretion in colonic epithelial cells. LGG leads to an increase in thickness of the mucosal mucus layer and a decrease of apoptotic cells in the gastric mucosa. L.acidophilus leads to an increased production of mucosal components and pro-inflammatory cytokines IL-8, IL-1β, and TNF-α in colonic epithelial cells. This up-regulation decreases the attachment capacities of E.coli O157:H7. Bifidobacterium lactis prevents an increase of epithelial permeability and protects tight junctions in Caco-2 cells. Administration with lactic acid bacteria decreased colonic permeability in IBS patients (Reiff and Kelly 2009).

There is a bacterial epithelial crosstalk between bacteria of the gut and the cells of the epithelium which enables the exchange of signal-substances. The cells of the epithelium are important for the infection defence in the gut by detecting microorganisms. These cells are part of the gut associated immune system. The cells

of the epithelium posses pattern recognition receptors which have the ability to identify typical microbial structures and interact with them. Pattern recognition receptors signal the interaction between cytoplasm-membrane and microorganisms in the inside of the epithelium cell, where henceforward through signal cascades in the cell nucleus the transcription of inflammatory genes initiates.
There upon a release of messengers takes place which attracts the immune cells residing behind the epithelium film. These immune cells can now act on the place of the inflammation and can fend the infection. Furthermore the epithelium cells contain intracellular agents NOD-receptors (Nucleotide Oligomerization Domain) which have an impact on the inflammation cascade (Schulze, Sonnenborn et al. 2008).

Studies demonstrated that an application of Lactobacilli, Bifidobacteria and E.coli Nissle 1917 strengthens or rather regenerate the mucosa barrier. Gastro-intestinal diseases like celiac disease or inflammatory bowel disease are among others characterized by a defective barrier of the gut (Schulze, Sonnenborn et al. 2008). Probiotics like VSL#3 and L.plantarum 299v do have a positive effect on barrier function (Spiller 2008).

1.1.4.7.5. Acidification of the Colon through Nutrient Fermentation

Probiotics have the potential to ferment unabsorbed polysaccharides and thereby generate short chain fatty acids. Lactic acid is one of them leading to acidification of the colon. The acidic milieu enables some bacteria to flourish while the growth of others is inhibited. Administration with L.plantarum leads to an ascent of acetic, propionic and butyric acid accompanied by an increase of Bifidobacteria spp. and Lactobacillus spp. and a decrease of Clostridia spp (Spiller 2008).

1.1.4.7.5.1. Short Chain Fatty Acid Production

Short chain fatty acids emerge out of bacterial fermentation in the colon. Substrates are polysaccharides, oligosaccharides, protein, peptides, and glycoprotein precursors. Short chain fatty acids are organic fatty acids which are made up out of 1 to 6 carbon atoms. The abundance of produced short chain fatty acids is in dependence of (1) the species and amount of microflora in the colon, (2) the concentration of substrat and (3) the gut transit time. Besides SCFA, H_2 and CO_2 are

the end products after fermentation. The absorption of SCFA proceeds in the colon and is connected to an easier sodium absorption and bicarbonate excretion. (Wong, de Souza et al. 2006). 60-70% of the energy needs of colonocytes are supplied by SCFA and 10% of energy needs of the body are covered. Through an acidification with SCFA Bifidobacteria, Lactobacilli can inhibit the growth of potential pathogens. Other effects of Bifidobacteria and Lactobacilli which prevent an increase of pathogenic bacteria are that they compete for nutrients and for colonization sites. Furthermore Bifidobacteria and Lactobacilli enhance the immune system and secrete antimicrobial molecules. Through the increase of these bacteria a reduction of potential pathogenic bacteria would be the conclusion (Roy, Kien et al. 2006).

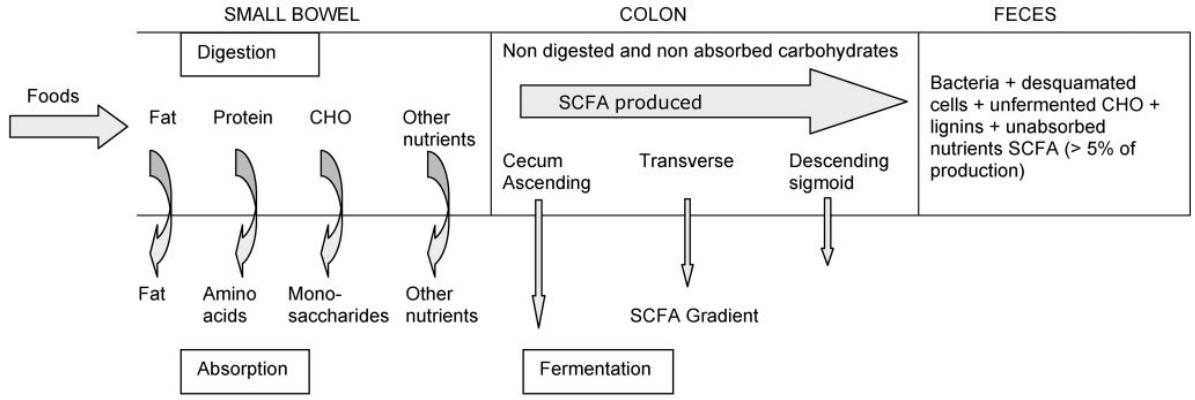


Figure IV. Overall scheme of nutrients and carbohydrates (CHO) in particular, in their transit through the GI tract, their fermentation to short chain fatty acids (SCFA), and their fecal excretion.
(Roy, Kien et al. 2006)

Butyrate, acetate and propionate are the main short chain fatty acids in the colon. The molar ratio of short chain fatty acid production is approximately 60:20:20 (Acetate: Propionate: Butyrate) (Cummings, Hill et al. 1979; Wong, de Souza et al. 2006). Usually the ratio is constant, nevertheless dietary changes can influence the production and absorption of short chain fatty acids (Wong, de Souza et al. 2006).

Acetate: is the main SCFA in the colon of humans and especially pectin is a major source (Kien, Schmitz-Brown et al. 2006; Roy, Kien et al. 2006). Acetate is fundamental for muscle heart and brain cells. Additionally it is the primary substrate for cholesterol generation. But the hypothesis exists, that it's substrate dependent, whether SCFA induces or prevents cholesterol generation. Lactulose for example

increased cholesterol synthesis whereas viscous fibers like psyllium are not as fermentable and reduce serum lipids (Wong, de Souza et al. 2006).

Propionate: In some studies a positive impact on cholesterol levels was assessed. In small amounts it has butyrate impact on proliferation and differentiation of colonocytes (Topping and Clifton 2001; Roy, Kien et al. 2006). Propionate can be an inhibitor of gluconeogenesis but also a substrate. The propionate metabolism in humans is less well understood. Polyfructans like Inulin are used for Type 2 diabetes mellitus and hyperlipidemia because they decrease the acetate: propionate ratio which leads to a cholesterol reduction. The absorption of propionate is easier than the absorption of acetate in the human colon(Wong, de Souza et al. 2006).

Butyrate: is the major colonocyte fuel. It induces proliferation and differentiation of cells. It is prevention for tumours because it induces growth arrest, differentiation and apoptosis in colorectal and polyp cell lines. Main sources are starch, oat and wheat bran (Kien, Schmitz-Brown et al. 2006; Roy, Kien et al. 2006). Butyrate is the energy source for colonocytes. 70-90% of butyrate is metabolized by colonocytes and it also plays an important role in proliferation and differentiation, and apoptosis of cells (Wong, de Souza et al. 2006). Furthermore it has an anti-inflammatory effect because it inhibits the activation of NF-κB and therefore reduces generation of proinflammatory cytokines. The use of butyrate is preferred compared to propionate and acetate (Pryde, Duncan et al. 2002).

<u>Functions of short chain fatty acids:</u>

(1) Are a nutrient supply for colonocytes
(2) Lead to a decline of the ph in the colon and intracellular which leads to changes in the gut microbiota, to a declined solubility of bile acids, to an increased mineral absorption especially calcium and decreased ammonia absorption.
(3) Modulate functions of ion transport
(4) Regulate proliferation, differentiation and gene expression
(Wong, de Souza et al. 2006)
(5) Through up-regulation of Toll like receptors they stimulate the bacterial-epithelial crosstalk (Forchielli and Walker 2005).
(6) Cytokines like Interleukin 10 and INF-γ are up-regulated (Roller, Rechkemmer et al. 2004).

(7) Increased production of polymeric IgA (Roller, Rechkemmer et al. 2004; Zapolska-Downar, Siennicka et al. 2004).
(8) Before prebiotics convert to SCFA they interfere the attachment of bacteria to microvillus glycoconjugates (Forchielli and Walker 2005).

(Roy, Kien et al. 2006)

1.1.5. Analytical Methods

Since the 1990s, culture independent methods which opened up new vistas are common. Nevertheless 70% of the gut microbiota remained unexplored due to the utilization of culture based techniques. The culture independent methods identify bacteria via the ribosomal RNA molecules (16s rRNA) which are small subunits of sequences. Variable and hypervariable regions compose of the 16s rRNA gene which enable designing probes or primers to detect the entire domain, a phylum, a group, a genus or just a single species of a bacterium (Sokol, Seksik et al. 2009).

The faeces are often used for analysis to assess the intestinal microbiota because it is easy to collect. Nevertheless we have to distinguish between mucosal microflora and faecal microflora but up until now the exact differences are not clear (Eckburg, Bik et al. 2005).
About 40-80% of microbiota which are detected under the microscope are impossible to culture at the moment; and even if they are cultivable, experience is necessary and the individual interpretation indeed differs (Tannock 2001; Guarner and Malagelada 2003; Penders, Stobberingh et al. 2007).
New techniques arose with whom, uncultivable species are detectable. The samples can be kept frozen for later analysis and furthermore anaerob handling and expertise is not necessary (Furrie 2006; Penders, Stobberingh et al. 2007).
Bacterial communities are analyzed with the aid of the small ribosomal sub-unit RNA because it contains hypervariable regions which contain the signature of phylogenetic groups and sometimes even species (Tannock 2002; Penders, Stobberingh et al. 2007). The sequencing of the 16S rRNA gene from clone libraries pointed that uncultivated species and novel microorganisms make up the largest amount of the gut microbiota (DiBaise, Zhang et al. 2008).

For studying gut microbiota the Fluorescent in situ hybridisation (FISH) and the Polymerase chain reaction (PCR) used together with a denaturing or temperature-gradient-gel-electrophoresis (DGGE/TGGE) are useful for studying the gut microbiota. The real time PCR is another molecular technique (Zoetendal, Collier et al. 2004; Penders, Stobberingh et al. 2007).

FISH is a technique, which is adequate for assessment of the spartial distribution of the bacteria in the intestine. Fluorescent oligonucleotide probes detect 16S ribosomal RNA sequences of intact bacterial cells (Tannock 1999). This technique is insensitive and the detection limit are 10^6 bacterial cells per gram (Penders, Stobberingh et al. 2007). PCR-TGGE/-DGGE is a further molecular method which is insensitive as well and unsuitable for analyses of large sample numbers. In addition, a quantitative assessment is not possible with this method because it generates a bacterial fingerprint of the composition in the faeces (Penders, Stobberingh et al. 2007). The bacteria DNA is extracted from faeces and the PCR amplifies the fragments of the 16s rRNA gene and afterwards the TGGE/DGGE separates the 16s molecular species. A polyacrylamide gel is needed in which the double stranded 16s fragments migrate until they are fully denaturized (Tannock 1999; Tannock 2002; Penders, Stobberingh et al. 2007). The quantitative real-time PCR is suitable for assessment of quantitative amounts of bacteria in the intestine. The amount of PCR products of DNA are amplified due to fluorescent oligonucleotide probes and the quantitative real-time PCR can determine the abundance of PCR products. The amplicon concentration is demonstrated by the fluorescent intensity which is emitted during the amplification. During amplification it cycles changes in amplicon concentration emerge and thereby an estimation of DNA/RNA target is possible (Mackay 2004; Zhang and Fang 2006; Penders, Stobberingh et al. 2007).

Ascending necessity for identification and quantification of small groups which gain in importance makes a more frequent sequencing function inevitable. These increased sequence requirements are covered by 454 sequencing (Petrosino, Highlander et al. 2009).

1.2. MICROBIOTA AND DISEASES

Several studies have demonstrated that not a specific pathogen but rather a broad spectrum of bacteria influences the induction of intestinal inflammation (Andoh and Fujiyama 2006). A series of studies indicate that microbiota influence the development of obesity, inflammatory bowel diseases, atopic diseases, diarrhea and constipation (Mai and Draganov 2009) which are precisely discussed in this work, but further exist.

1.2.1. Inflammatory Bowel Disease

It is a general term for a group of chronic inflammatory disorders including the main forms Ulcerative colitis (UC) and Morbus Crohn (CD). The precise aetiology is not established until now (Reiff and Kelly 2009). Two hypotheses exist, the first suggests that a dysregulation of the mucosal immune system is the primary cause which leads to immunologic responses to the normal microflora. The second declares that an alteration in the bacteria in the gut or a decreased epithelial barrier function is the cause for an excessive mucosal immune response against microbial antigens (Strober, Fuss et al. 2007). However it is certain that an abnormal response of the immune system is the cause for this disease. Determined is the abnormality by genetic, as well as environmental factors. The hygiene hypothesis, which suggests that a restricted exposure of microbes increase the susceptibility for autoimmune diseases, plays an important role indeed. The Toll-like-receptors on colonocytes respond abnormally to the commensal bacteria which lead to an activation of the immune system and of the T lymphocytes (Roy, Kien et al. 2006).

1.2.1.1. Etiology

It is certain that the pathologic process is almost certainly driven by an aberrant local immune response. There is the assumption that more than one factor triggers inflammatory bowel diseases (Baumgart and Sandborn 2007).

Genetic-factors

Some studies have shown and the fact that the illness occurs cumulative in first degree-relatives, it is therefore obvious that it is driven genetically (Baumgart and Sandborn 2007).

Immunological-factors:

Whenever it is possible for antigen constituents of the intestinal flora to infiltrate the mucosa barrier and there inducing a defence reaction arising by reason of already existing alterations of the immune system, it can lead to inflammatory reactions. Many studies confirm that there is an interaction between intestinal flora and the immune system (Baumgart and Sandborn 2007).

With inflammatory bowel disease, the largest immunological compartment is affected, the intestinal mucosa associated immune-system. It exist a disturbance of the intestinal immune-regulation. An imbalance between pro- and contra- inflamed cytokines leads to the inflammation (Stange 2004). Innate as well as adaptive immune systems play an important role in the pathogenesis of IBD.

The innate mucosal immune system:

In healthy individuals, the intestinal epithelium together with an overlying mucin layer acts as a strict barrier between luminal microenvironment and the GI mucosa. The transit across the epithelial barrier is regulated through tight junctions. For tolerance promotion antigens, which are present in the lumen, are taken up from M cells and dendritic cells and presented to lymphocytes in the Peyers patches. The pattern recognition receptors like Toll like receptors (TLR) and Nucleotide binding oligomerization domain (NOD) receptors are also part of the innate immune system. The pattern recognition receptors adhere to “pathogen-associated molecular patterns” (PAMPs) which are present on noneukaryotic organisms. Due to this adherence, NF-κB becomes activated inducing the expression of inflammatory mediators. Furthermore invading pathogens lead to a chemotatic accumulation of neutrophils within the GI mucosa. The neutrophils produce reactive oxygen controvert the invading pathogens (Arseneau, Tamagawa et al. 2007).

Defect of innate immune system in IBD:

Ulcerative colitis patients have higher amounts of neutrophils leading to an increased generation of reactive oxygen. Patients with Crohn’s disease carry mutations of pattern recognition receptors leading to an increase in intestinal permeability and an elevated invasion of pathogens. Polymorphisms in NOD2, MDR-1, OCTN, DLG5

and TLRs genes are associated with Crohn's pathogenesis. Polymorphisms in TLR4, TLR5 and TLR9 genes are associated with the CD pathogenesis. Consequently the TLR family is directly involved in the pathogenesis of Crohn's disease. The defects of the receptors lead on the one hand, to an impaired ability to recognize pathogen bacteria and induce appropriate immune responses; and furthermore on the other hand the tolerance towards the gut's commensal flora is disturbed. In addition, the intestinal epithelial barrier function is decreased in patients with Ulcerative Colitis and Morbus Crohn. An altered expression of tight junctional proteins controlling the epithelial paracellular permeability is also a cause for increased permeability. The "leaky gut" is disastrous for the immune cells in the intestinal mucosa. If this defect is cause for the pathogenesis of this illness, probiotics might be a therapeutic target because through this invasion of bacteria, the normally benign commensal strains are overwhelmed and inflammation can spread (Arseneau, Tamagawa et al. 2007).

Adaptive Immune system:

Specific antigen recognition and cell mediated immunity belong to the adaptive immune response. After Antigen presenting cells phagocytise the invading pathogens they present them to naïve CD4 T cells. Subsequently a differentiation of the T cells to regulators T-cells or effector T cells proceeds. In dependence of the phenotype of the Effector T helper cell (TH1, TH2, Th17), the expression of unique cytokine profile takes place. After the pathogen is removed, the T regulatory cells express regulatory cytokines like IL-10 and TGFβ and thereby suppress inflammation. The consequence is a restoration of the gut homeostasis (Arseneau, Tamagawa et al. 2007).

Defect in Adaptive Immune response in IBD:

Polarizing cytokines initiate an enormous increase in accumulation of T-cells in the intestinal mucosa leading to inflammation development in IBD patients. Down-regulation of apoptosis prevents the body's ability to clear the pathogenic T-cells and further aggravates the conditions in the intestinal mucosa. Originally the hypothesis that Crohn's disease is Th1 mediated and Ulcerative Colitis is Th2 mediated. In recent years several findings have led to the assumption that a strict Th1/Th2 paradigm is no longer adequate for defining the complexity of immune responses in IBD pathogenesis. Furthermore the newly detected phenotype of effector CD4 helper cells Th17 plays an important role in patients with IBD. Th17 expresses IL-17 and IL-22, especially occurring in patients with Crohn's disease promoting inflammation development. These findings lead to the indication that Th1, Th2 and Th17 play an

important role in the innate immune response in IBD pathogenesis (Arseneau, Tamagawa et al. 2007).

Environmental factors:

Environmental factors play an important part because in epidemiologic data it is evident that the incidence of IBD correlates with the industrial development. NSAR (non-steroidal anti-inflammatory drugs (Aspirin)) can lead to an illness attack because of their impact on the mucosal barrier. Smoking reduces the risk to contract Ulcerative Colitis but it increases the risk for Morbus Crohn (Baumgart and Sandborn 2007).

For quite some time, inflammatory bowel disease was seen as a psychosomatic disease. More recently the supposition exists that it is primarily an organic disease, which can have, as all chronic diseases, psychosomatic concomitants. The present model for further discussion supposes that environmental factors in individuals with a genetic burden can lead to a lapse of the immune system (Baumgart and Sandborn 2007).

Ulcerative Colitis and Morbus Crohn both belong to inflammatory bowel disease. Ulcerative colitis and Morbus Crohn differ mainly in the location and the nature of the inflammatory changes. While Morbus Crohn can affect the entire gastrointestinal tract from mouth to anus, Ulcerative Colitis is restricted to the colon and the rectum. Furthermore Crohn´s disease affects the entire bowel wall, whereas Ulcerative Colitis is restricted to the mucosa. In the first diagnoses, 10% of cases can not be determined as Morbus Crohn or Ulcerative Colitis (Baumgart and Sandborn 2007).

1.2.1.2. Questionnaire

As a result of the ascent in patients suffering from IBD, initiatives have arisen to help those identified to suffer from this disease. This following link, http://www.ced-check.at/cedcheck.shtml offers the possibility to check if one has or might have contracted inflammatory bowel disease. To find out, one must answer the questions with either yes or no, and if one answers yes to just one question, they are susceptible for IBD and should seek medical attention.

IBDIS-CED Check

Wenn auch nur eine der Fragen 1-6 mit einem JA beantwortet werden muss, gibt es einen Hinweis auf eine Chronisch Entzündliche Darmerkrankung. Bitte wenden sie sich in diesem Fall unverzüglich an Ihren Hausarzt!

1) Besteht/bestand länger als 4 Wochen Durchfall (= mehr als 3 flüssige Stühle pro Tag) oder wiederholte Episoden von Durchfällen? *Angabe nötig* →

☐ nein ☐ ja

2) Besteht/bestand länger als 4 Wochen Bauchschmerzen oder wiederholte Episoden von Bauchschmerzen? *Angabe nötig* →

☐ nein ☐ ja

3) Besteht/bestand regelmäßig oder wiederholt über mehr als 4 Wochen Blut im Stuhl? *Angabe nötig* →

☐ nein ☐ ja

4) Bestehen/bestanden nächtliche Bauchbeschwerden wie Bauchschmerz oder Durchfall? *Angabe nötig* →

☐ nein ☐ ja

5) Besteht/bestand regelmäßig oder wiederholt über mehr als 4 Wochen schmerzhafter Stuhldrang? *Angabe nötig* →

☐ nein ☐ ja

6) Bestehen/bestanden Fisteln oder Abszesse im Analbereich? *Angabe nötig* →

☐ nein ☐ ja

7) Besteht/bestand allgemeines Krankheitsgefühl, Schwäche oder Gewichtsverlust? *Angabe nötig →*

☐ nein ☐ ja

8) Bestehen/bestanden Beschwerden außerhalb des Magen-Darm-Traktes wie Gelenksschmerzen, Augenentzündungen oder spezifische Hautveränderungen (z.B. „Erythema nodosum“: Kennzeichnend dafür sind z.B. mehrere, unscharf begrenzte Flecken bzw. Knötchen unter der Haut, die leicht erhaben und sehr druckempfindlich sind). *Angabe nötig →*

☐ nein ☐ ja

9) Existiert in der Familie ein Hinweis auf Morbus Crohn oder Colitis ulcerosa? *Angabe nötig →*

☐ nein ☐ ja

10) Können andere Ursachen einer Durchfalls-Erkrankung ausgeschlossen werden? (z.B. Fernreisen, Infektionen, Nahrungsmittelunverträglichkeiten, Medikamenteneinnahme wie NSAR (Antirheumatika) oder Antibiotika, sexuelle Praktiken). *Angabe nötig →*

☐ nein ☐ ja

http://www.ced-check.at/cedcheck.shtml

1.2.1.3. General Nutritional Requirements

Genetic susceptibility, environmental factors and aberrant immune responses seem to play an important role in the development of IBD (Shanahan 2001). Environmental factors are the local micro environment as well as the nutritional environment (Bernstein and Shanahan 2008). New lifestyles can be a reason why IBD has increased in the past years. The diet is an important component which should be considered in treatment of the illness. The consumption of cow’s milk by children, food with high amounts of refined sugar and fat and the decline in consuming fiber, fruits and vegetables could be detrimental (Lucendo and De Rezende 2009).

Breastfeeding is known to have a protective factor. Children who were breastfed over a short period of time had an increased risk for IBD, compared with children who were fed for a longer time. Breastfeeding seems to be protective because it provides against gastrointestinal infections, enhances the development of the gastrointestinal mucosa and improves the immunological capacity in children. Breast feeding enables a delayed contact with cow's milk, infectious agents and other allergens. Mycobacterium avium paratuberculosis is an infectious agent, originating from infected cows and is therefore transmitted via cow's milk. This bacterium is supposed to play a role in the development of CD. Nevertheless the epidemiological support is too little (Lucendo and De Rezende 2009).
Although it's not really proven, several investigators have suggested the consumption of low-refined carbohydrates for CD patients. The fat type might also play a role in IBD. The vast increase of fast food consumption in recent years may be associated with the development of UC. Increased consumption of monounsaturated as well as polyunsaturated fatty acids increased the risk for UC. While n-3 polyunsaturated fatty acids (PUFAs) might have a protective factor due to their anti-inflammatory properties, n-6 PUFAs are supposed to be detrimental due to their pro-inflammatory properties (Lucendo and De Rezende 2009).

Patients who suffer from IBD might have an increased necessity for proteins and calories but it's not entirely clear because the question is not clarified whether these deficiencies are cause or consequence of the disease. In patients with CD, calorie-protein malnutrition is common, whereas in UC protein malnutrition is very often.
Inflammatory mediators like TNF-α, Interleukin-1 and Interleukin-6 are responsible for an increased catabolism and lead to anorexia. Iron, folic acid, zinc, selenium and vitamin B12 are often detrimentally reduced. Furthermore IBD patients have an increased risk for osteoporosis and osteopenie due to a vast depletion of bone mass. Alongside other factors like genetic factors, female gender, smoking, the consistent use of corticosteroids, age and type of IBD and hormonal factors, inflammatory cytokines like TNF-α stand in association with increased bone loss (Lucendo and De Rezende 2009).
A deficit of folic acid increases risk for colitis associated carcinogenesis. Deficiency occurs due to sulphasalazine or methotrexate. Folic acids as well as Vitamin B12 are essential in the metabolic route of homocysteine-methionine. Zinc is important for

healing wounds and a protective factor for cell damage caused by free radicals. Selenium is an important component in gluthatione-peroxidase. Oxidative stress fuels inflammation, therefore antioxidants like vitamin A,C, E and Selenium are important components for inhibiting inflammation (Lucendo and De Rezende 2009).

Nutrition is an important aspect for patients with IBD. First, IBD patients are a risk group for malnutrition, second, it's the primary therapy in the active phase of the disease and furthermore important in maintaining remission, and third, nutrients are risk factors which are involved in the aetiology of IBD.
Those who suffer from IBD are vulnerable for osteoporosis, malnutrition, micronutrient deficiency and growth and development in children could be negatively influenced. Thus nutritional care is very important (Hartman, Eliakim et al. 2009).
20-40% of CD outpatients have a significant weight loss due to their illness (Van Patter, Bargen et al. 1954; Lanfranchi, Brignola et al. 1984). In UC patients it is not as critical but during the active phase of the disease a nutrient deficiency can develop in a short period of time (Rocha, Santana et al. 2009).
Malnutrition is caused by a decreased food intake (due to intolerances), a malabsorption of nutrients, an increased intestinal loss, hypermetabolic state and the interaction between drugs and nutrients. Although analyses of IBD patients have shown that most patients in remission are in a good nutritional condition there are remarkable abnormalities in the body composition (Hartman, Eliakim et al. 2009). A decrease in muscle mass was observed in more than half of UC and CD patients, though they were not malnourished. In the active phase of CD parameter like BMI, arm muscle area and triceps as well as subscapular skin fold thickness values were remarkably decreased (Rocha, Santana et al. 2009). In addition, although the patients were in remission, CD patients had a significant decrease in plasma concentration as well as lower amounts of vitamins and minerals (Hartman, Eliakim et al. 2009).

1.2.1.3.1. Nutrition

Patients who suffer from IBD are recommended to consume a healthy well balanced diet. Approximately 2000 kcal per day should be taken up whereas 15% of nutritional energy should be composed of protein, 30% should be composed of fat and 55%

should be composed of carbohydrates. Meat should be consumed only 2-3 times a week and fish 1-2 times a week. The consumption of Omega-3-fatty acids should be elevated and 5 portions of fruits and vegetables per day are recommended (Kluthe, Dittrich et al. 2004).

Diet should be rich and varied including fruit, vegetables, meat, olive oil, and fish. During flares of the disease, the consumption of fibers might be restricted. Nevertheless there is no supporting data. Milk products are urgently necessary, due to their high calcium content. Just in those cases of lactose intolerance, one must substitute milk with fermented products such as yoghurt, cheese, calcium enriched soya products, etc. (Lucendo and De Rezende 2009). The consumption of citrus fruits, fruit juices and vegetables could have a preventive factor for IBD. Until now it's not clear whether the protective role comes from the fiber content in this edibles or if other micronutrients that might be the important components. Due to colonic fermentation of dietary fibers and other unabsorbable carbohydrate, short chain fatty acids like butyrate are generated. In UC patients, a deficiency of SCFA and a decreased oxidation rate was recognized (Lucendo and De Rezende 2009).

Edibles which are responsible for initiating intolerances in more than 5% of patients should be excluded in the diet. Generally patients should avoid:

- high fat diet especially fried and roasted food
- high fat or smoked meat, sausages or fish
- hard boiled eggs, mayonnaise and high fat scrambled eggs
- high fat dairy products
- high fat soups and sauces
- fresh bread, fat bakery products and rough wholemeal products
- preparations of fried potatoes
- vegetables which are flatulent or indigestive for example cabbage, sauerkraut, field garlic, onion, paprika, mushrooms, olives, cucumber, radish, dried legumes
- vegetables which are prepared with an high fat amount
- immature fruits, stone fruits, nuts, almonds, pistachio, avocados
- fat sweets

- alcohol in every form
- carbonated beverages
- iced beverages
- high amounts of hot spicery, onion and garlic powder

(Kluthe, Dittrich et al. 2004)

1.2.1.3.2. Parenteral Nutrition

Parenteral nutrition is not as common since investigators have found that enteral nutrition is as effective and causes lower costs and has fewer significant side effects. Exceptions are cases with severe malnutrition or support before or after a surgery in CD and UC (Hartman, Eliakim et al. 2009).

1.2.1.3.3. Enteral Nutrition

Enteral nutrition (EN) is not proven to be used as primary therapy in UC. Nevertheless supplementary enteral nutrition might be effective in patients with CD for maintaining remission. Several studies showed that enteral nutrition induced clinical remission, enhanced nutritional status, enhanced body composition, boosted mucosal healing, lowered the production of pro-inflammatory cytokine levels, and a decline of serum inflammatory markers was also observable in CD patients (Hartman, Eliakim et al. 2009).

Enteral nutrition has not shown to have advantages, in comparison to steroids, in the vast majority of CD patients. If patients have a steroid intolerance, refuse steroids or if patients have inflammatory stenosis of the small intestine, enteral nutrition could be an alternative for steroids. Patients who are undernourished might receive EN as well as steroids. However in children with active CD enteral nutrition plays a greater role. EN is as efficient as steroids in this group. A replacement of steroids with EN might be an opportunity to avoid the negative side effects of steroid's retarded growth and development of children (Smith 2008). Enteral nutrition should be recommended as primary treatment for all patients who suffer from CD and for patients with serious cases from UC. It's also possible to add it to medical treatment as a second treatment to achieve or maintain remission. Enteral feeding is advantageous in patients with CD if the small intestine is affected, whereas in patients with UC an

urgent necessity is not established. Enteral nutrition might modulate the immune system in the mucosa, regulating imbalances in the microbiota composition which are responsible for the inflammation (Lucendo and De Rezende 2009).

Mechanisms of Enteral Nutrition:

(1) Improved nutritional status
(2) Pro-inflammatory cytokines are down-regulated
(3) Anti-inflammatory actions
(4) Promotion of epithelial healing
(5) A decrease in gut permeability
(6) Decreased antigenic load to the gut
(7) Modification of the gut flora

(Hartman, Eliakim et al. 2009)

Variants of Enteral Nutrition

Elemental as well as polymeric formulas are variants for enteral nutrition. Elemental formula and polymeric formula are efficacious in the same way (Hartman, Eliakim et al. 2009). Polymeric diet contains nitrogen in the form of whole protein. Carbohydrates are contained in the form of hydrolysates of starch, and medium chain fatty acids provide the fat contingent. Fibers are also contained, although their effectiveness has not been proven until now. Elemental diet is composed of simple nutrient forms like amino acids, simple carbohydrates, fats, vitamins and minerals and are therefore easier in digestion (Smith 2008). Nonetheless the polymeric formula seems to have some advantages (Hartman, Eliakim et al. 2009). In a trial with children who suffered from CD polymeric formula was more adequately for gaining weight which is desirable for paediatric CD (Ludvigsson, Krantz et al. 2004). Polymeric formula is more palatable than elemental enteral nutrition and therefore more compliant. Furthermore it is cheaper and there seem to be no differences between polymeric and elemental diet in their efficiency (Smith 2008).

In enteral nutrition, the fat content is supposed to play an underlying role. Enrichment with bioactive peptides like glutamine, growth factors, butyrate omega-3 fatty acids and anitoxidants might have an anabolic or anti-inflammatory effect. However until now the advantages of such additions in elemental diet is not proven (Hartman, Eliakim et al. 2009).

Patients with CD have an increased gut permeability which allows increased uptake of antigens which might be a factor for enhanced inflammation. Elemental diet decreases gut permeability, thus it might have a positive effect. Components of the elemental diet are primary absorbed in proximal small bowel. The decreased digestion and peristaltic might play a role indeed (Smith 2008).

Exclusively Enteral Nutrition

Steroids and exclusively enteral nutrition (EEN) were compared in children with CD. EEN was found to be as effective as corticosteroids in inducing remission. EEN has in comparison to steroids the advantage that they have no side effects. Therefore they might be a preferred choice for first-line therapy in children with active CD. Johnson et al. declared that partial EN (PEN) in comparison to EEN was not as effective in children with CD for inducing remission (Hartman, Eliakim et al. 2009).

1.2.1.3.4. Probiotics

Due to the fact that the inflammation occurs in parts of the intestine in which the highest bacterial load occurs, probiotics might be appropriate for disease improvement. Furthermore it has been proven that an interruption of the faecal stream alleviates the disease symptoms. An increased mucosal permeability which leads to direct contact to bacteria is a further reason. Genetic susceptible individuals have a loss of immunological tolerance to commensal bacteria and this, in addition points out that treatment with probiotics might be helpful (Bergonzelli, Blum et al. 2005).

1.2.1.4. Ulcerative Colitis

Ulcerative colitis is a type of inflammatory bowel disease. The initiation of the disease is due to an unbalanced immune response to luminal antigens (Mitsuyama, Matsumoto et al. 2008).

1.2.1.4.1. Clinical Picture

Ulcerative Colitis is a chronic inflammation which is limited to the mucosa of the colon and the rectum. The initiation takes place in the rectum and in approximately half of the patients, it spreads in the proximal direction. The inflammation is limited to the mucosa and mainly the crypt epithelium is affected through abscesses. The mucosa is reddened and inflamed leading to bloody diarrhea and abdominal pain. Ulcerative colitis is a continuous inflammation which means that the inflamed alterations spread from the rectum in the oral direction and do not overleap healthy mucosa parts (Baumgart and Sandborn 2007).

The extension of the inflammation determines if it's a proctitis where just the rectum is affected, a proctosigmoiditis where the rectosigmoid colon is affected or a left-sided colitis where the descending colon is involved. A pancolitis means that the entire colon is affected. In such a case, enemas can not reach the entire damaged area (Renz-Polster and Krautzig 2008).

Symptoms:

The main symptom is bloody and mucous diarrhea. Furthermore abdominal pain and general symptoms like weight loss, nausea, absence of appetite and fever occurs. In the acute attack, 10-20 bloody and mucous bowel movements are common; tenesmus is also possible. The loss of blood and the inflammation can lead to anaemia and hypoproteinaemia. Extraintestinal Symptoms can also occur, but are not as common as in Morbus Crohn (Renz-Polster and Krautzig 2008).

The severity of the disease can differ and so in clinical practice they distinguish between mild, moderate, severe and fulminant activity. A mild activity is characterized by up to four bloody stools daily without toxicity, a moderate activity exists if there are four to six bloody stools daily with minimal toxicity, and a severe activity is characterized by more than six bloody stools daily accompanied by fever, tachycardia, anaemia and a raised erythrocyte sedimentation rate. Fulminant colitis is characterised by more than ten bloody stools daily accompanied by high fever, extensive bleeding, increased inflammation markers, and weight loss (Baumgart and Sandborn 2007).

Course:

An attack can last from 1 to 8 weeks. Between this time, patients have symptom free intervals which can last from months to years (Renz-Polster and Krautzig 2008).

1.2.1.4.2. Therapy

Quintessentially fort this disease is the remission and relapse period. Therefore the prior treatment are immunosuppressive drugs and anti-inflammatory drugs (Herias, Koninkx et al. 2005).

1.2.1.4.2.1. Medical Treatment

5-Aminosalicylic acid (mesalazine) is the primary therapy for patients with mild or moderate activity. If this treatment is not effective a therapy with oral corticosteroids like prednisone or budesonide is necessary. Patients who require frequent steroid therapy should change to treatment with azathioprine or mercaptopurine. If the treatment with one of the two is not effective Infliximab is recommended for therapy. Infliximab is a chimeric monoclonal antibody to TNF-α. Patients with severe or fulminant Ulcerative colitis receive only medical therapy as long as it's not toxic. The first line therapy is an Intravenous Corticosteroids treatment. If there is no response after 5 days, other medical alternatives are Infliximab, Ciclosporin and Tacrolismus. To maintain remission, oral mesalazine therapy is common. If this is not effective azathioprine or mercaptopurine and infliximab treatment is established (Baumgart and Sandborn 2007).

1.2.1.4.2.2. Surgical Treatment

Perforation, refractory rectal bleeding and toxic megacolon are urgent causes for surgery, especially when they are not responsive to medical treatment. Dysplasia, cancer, intolerance to long-term immuosuppression or ineffectiveness to medical treatment might also be a cause for a surgery. The most common technique is the total proctocolectomy. Complications after the surgery are pouchitis, high stool frequency, faecal inconsistence and reduced fertility. There is also the risk for a necessity of reoperation (Baumgart and Sandborn 2007).

1.2.1.4.3. Composition and Differences to Normal Microbiota

Bacteria are supposed to be involved in the development of colitis because studies with germ-free mice have shown that these rodents were not able to develop

disease. The number of Lactobacilli was reduced in the faeces in patients with active UC in comparison to those who were in remission. Nevertheless the total concentration of bacteria was increased, but on the contrary the biodiversity reduced. Enterobacteria were increased in particular E.coli B2 + D groups. The UC patients had higher amount of sulphate reducing-bacteria. These bacteria produce hydrogen sulphide whereby they inhibit butyrate oxidation which results in colonic lesions. The numbers of Bifidobacteria were lower in UC patients than in controls. The results of Bacteroidetes are controversial. As well as an increased amount, as an decreased amount was detected in several studies (Sokol, Lay et al. 2008). The antibody production against obligate anaerobes as well as the mucosal IgG against normal colonic microbiota is elevated in patients with Ulcerative colitis. There is the assumption that bacteria are involved in initiation as well as in maintenance of the disease. Fusobacteria, shigella, salmonella and yersinia have an association with ulcerative colitis. Facultative anaerobes are increased in the faeces of IBD patients. In a study with the healthy individuals and nine UC patients, investigators analyzed the bacteria of the mucosa in the rectal epithelium. They noticed that healthy individuals had a 30-fold higher level of Bifidobacteria than UC patients. Bifidobacterium angulatum and Bifidobacterium bifidum occurred in both groups, but in UC patients Bifidobacterium angulatum it was most prevalent, whereas in healthy individuals Bifidobacterium adolescentis dominated (Macfarlane, Furrie et al. 2005). In other studies a decreased Bifidobacterium content occurred as well but also in association with an increase of Bacteroides (Pathmakanthan, Thornley et al. 1999; Macfarlane, Furrie et al. 2005). Matsuda et al. has already concluded that Peptostreptococci only occurs on the mucosal surface of UC patients but not on healthy individuals (Matsuda, Fujiyama et al. 2000; Macfarlane, Furrie et al. 2005). Macfarlane et al. agrees with this result. Peptostreptococci and E.faecalis occurred in UC patients but not in healthy individuals. An analysis of the rectal epithelium with the microscope showed that the majority of the bacteria were growing in microcolonies. This might lead to higher concentrations of produced antigens or toxins (Macfarlane, Furrie et al. 2005).

In another trial, investigators compared the faeces of Ulcerative Colitis patients with that of healthy individuals. They diagnosed that patients with Ulcerative Colitis had lower counts of Firmicutes, especially the species Faecalibacterium prausnitzii was underrepresented in patients with active inflammatory bowel disease. Furthermore

patients with active colitis had lower counts of Bifidobacterium in comparison to healthy individuals. The conclusion of this study was that all patients with active colitis had a decrease of the Firmicutes/Bacteroidetes ratio. Yet investigators also pointed out that until now it is not clear whether the changed composition of bacteria in colitis patients is responsible for the inflammation, or if the change of bacteria is the consequence of the inflammation. Anyhow, the results allude, that the decrease of Firmicutes especially Faecalibacterium prausnitzii is involved in the development of inflammatory bowel disease (Sokol, Seksik et al. 2009).

Until now it is not clear if the changes of the gastrointestinal microbiota are primary or a secondary phenomenon. It could be possible that an alteration in motility and diet in patients with IBD leads to alterations of the colonic microenvironment and is reflected to the microbiota, or a change in microbiota is the primary phenomenon (DiBaise, Zhang et al. 2008).

Dominant bacteria appear to be relatively stable in each individual, no matter if the tissue is ulcerated or non-ulcerated. In contrast, the number of Lactobacilli and Clostridium leptum varied in the same individual in dependence if the tissue was ulcerated or not. The number of Lactobacilli in the mucosa is significantly decreased in patients with IBD. Lactobacilli have beneficial functions for the host and many studies have already shown that they can reduce the severity and maintain remission in this disease (Zhang, Liu et al. 2007).

In a study from Ott et al., investigators conducted biopsies of CD patients, UC patients and healthy individuals. In patients with Ulcerative Colitis the bacterial diversity was reduced by 30% compared with healthy controls. Especially a decline of anaerobic bacteria like Bacteroides species, Eubacterium species and Lactobacillus species were the result of the diseases (Ott, Musfeldt et al. 2004).

Characteristic features of this disease are an inflamed mucosa and a disturbed mucosal barrier. In the inflamed large intestine of the mucosa, there are much more bacteria than in healthy individuals. The inflammation of the mucosa leads to an invasion of microorganisms. In the large intestine of colitis patients are the apical surface; furthermore the mucosal epithelial cells colonised with microorganisms which is not prevalent in healthy individuals. The amount of inflammation-markers correlate negatively with the amount of bacteria in the mucosa (Schulze, Sonnenborn et al. 2008).

Author	UC patients	Healthy Individuals
(Sokol, Lay et al. 2008)	Bacterial load ↑ Biodiversity ↓ Lactobacilli ↓ Enterobacteria ↑ Sulphate reducing bacteria ↑	
(Macfarlane, Furrie et al. 2005)	Bifidobacterium angulatum predominant Peptostreptococci & E. faecalis only in UC patients	Bifidobacteria 30 fold ↑ Bifidobacterium adolescentis predominant No Peptostreptococci & E. faecalis
(Pathmakanthan, Thornley et al. 1999)	Bifidobacteria ↓ Bacteroides ↑	
(Matsuda, Fujiyama et al. 2000)	Peptostreptococci only in UC patients	No Peptostreptococci
(Sokol, Seksik et al. 2009)	Firmicutes:Bacteroides ratio ↓ Faecalibacterium prausnitzii ↓ Bifidobacteria ↓	
(Zhang, Liu et al. 2007)	Lactobacilli ↓	
(Ott, Musfeldt et al. 2004)	Bacterial diversity ↓ Bacteroides ↓ Eubacterium species ↓ Lactobacillus species ↓	

Table 1. Summarized results from studies investigating the differences of microbiota in UC patients.

1.2.1.4.4. Probiotics and Counselling

Patients in remission

Studies showed that an increased consumption of meat is detrimental for colitis patients. Therefore patients should reduce their meat consumption to once a week. Hydrogen sulphides are increased in the gut of colitis patients in comparison to healthy individuals. Therefore “red” meat, cheese, milk, fish, nuts, eggs and alcohol

should be reduced. These are precisely suppliers for hydrogen sulphide. Hydrogen sulphide acts against the positive functions of butyrate. Prebiotics have shown positive effects in the gut of colitis patients because patients have low amounts of butyrate in the gut in comparison to healthy individuals. Butyrate has anti-inflammatory effects and enhances the barrier function in the gut (Schiener, Reiterer et al. 2008).

Active phase

Generally speaking there is agreement that parenteral nutrition is only essential in cases of very aggressive forms of colitis. Enteral nutrition is recommended for patients who are malnourished. Apart form that it is recommended that patients should consume a low-fiber diet during the active phase (Schiener, Reiterer et al. 2008).

Probiotics

The pathogenesis of this disease might be associated with a disturbed microflora. “Aggressive” bacteria dominate in contrast to “protective” bacteria. With this account, possible disease alleviation might be the manipulation of the intestinal flora. The strategies appear promising, yet nevertheless further studies are needed to establish this administration (Andoh and Fujiyama 2006). Probiotics might be effective in treatment of UC patients, especially VSL#3, E.coli Nissle1917, Saccharomyces boulardii and Bifidobacterium-fermented milk have shown promising results (Mitsuyama, Matsumoto et al. 2008).

VSL#3

Administration of probiotics, especially VSL#3 can prevent or alleviate the symptoms of IBD. VSL#3 comprises of eight different lactic acid bacteria (Lactobacillus acidophilus, L.bulgaricus, L. casei, L. plantarum, Streptococcus thermophilus, Bifidobacterium breve, B. infantis, and B. longum). Unfortunately probiotics are more adequate for maintaining remission than having an effect in severe active forms of IBD (Reiff and Kelly 2009). VSL#3 was effective for maintaining remission in UC patients. From 20 patients, 15 (75%) remained in remission during a 12 month treatment (Venturi, Gionchetti et al. 1999). Patients who were administered with VSL#3 in uncontrolled pilot studies maintained in remission over 12 months (Andoh and Fujiyama 2006). In a trial with UC patients, patients who were allergic or intolerant to sulphasalazine and mesasalazine were treated with VSL#3 over 12

months. The impact of the faecal flora was assessed. Only after 20 days a significant increase of Lactobacilli, Bifidobacteria, and Streptococcus thermophilus occurred, which was held stable as long as the treatment lasted. After the treatment stopped, the composition of the faeces turned to basal levels within 15 days. 75% of the patients remained in remission during the trial (Gionchetti, Rizzello et al. 2006). An Italian study conducted a study with 90 patients with mild to moderate disease activity and treated them either with low-dose balsalazide and VSL#3, or medium dose balsalazide, or mesalazine. The trial lasted for 8 weeks and those patients who received the combination of balsalazide and VSL#3 gained remission in the shortest time of all of them, and furthermore the most form this group achieved remission (Tursi, Brandimarte et al. 2004). A further study showed that in 32 patients with active and severe UC, administration with VSL#3 induced remission in 56% only after 6 weeks. 25% had a response due to this treatment, and 9% had no response (Bibiloni, Fedorak et al. 2005).

Lactobacilli

Probiotics might have a beneficial effect by maintaining remission. Lactobacilli reuterii, Lactobacillus plantarum and Lactococcus lactis have been shown effective in mice studies (Macfarlane, Furrie et al. 2005). Lactobacillus rhamnosus GG lowered relapse time but did not decrease relapse rate (Reiff and Kelly 2009). Further results appeared that after an open labelled trial with active and mild UC patients, who were treated with Lactobacillus casei Shirota (LcS) for 8 weeks, a significantly better clinical activity index score occurred after treatment in comparison to the control group. Administration with LcS was more effective than the conventional therapy alone (Mitsuyama, Matsumoto et al. 2008). In a randomized open-label study, LGG was investigated at 187 patients in remission state. Investigators concluded that, LGG is equivalent with mesalamine and therefore adequate for therapy (Zocco, dal Verme et al. 2006).

Bifidobacteria

A trial with eighteen active colitis patients was conducted in which patients either received a symbiotic composed of B.longum and a prebiotic or a placebo for 4 weeks. In response to the symbiotic treatment, patients had significant reduction levels of inflammation markers (Macfarlane, Furrie et al. 2005). Alleviation of disease symptoms in UC patients was due to Bifidobacterium breve in a fermented milk product. In a Japanese study, 20 randomized patients received either the probiotic

combined with medical therapy or a placebo with medical therapy. Steroids were excluded during this trial. Results showed that the Bifidobacteria treatment was much more successful than the placebo administration (Kato, Mizuno et al. 2004). In another trial, patients who were in remission received for 2 months sulfasalazine and glucocorticoid either followed by administration with Bifidobacteria or followed by administration with placebo. During this time, only 20% of the probiotic group had a relapse whereas 93.3% of the placebo group relapsed. The inflammatory activity was further marked by a reduction in the Bifidobacteria group (Cui, Chen et al. 2004).

E.coli

In maintaining remission, E. coli Nissle 1917 is as effective as mesalazine (Gionchetti, Rizzello et al. 2006). Treatment with E.coli strain Nissle 1917 showed to be as effective as Mesalazine in maintaining remission in 120 UC patients (Kruis, Schutz et al. 1997). Because the first study population was too small, Kruis et al. conducted another study with 327 UC patients who for one year received either E.coli Nissle or mesalamine. One third of the patients discontinued, however the results showed that the remaining 222 patients that had E.coli Nissle administration had the same effect as mesalazine (Kruis, Fric et al. 2004). Rembacken confirmed these results due with the aid of 116 UC patients who received either mesalazine or a non-pathogenic E.coli strain (Rembacken, Snelling et al. 1999).

Saccharomyces boulardii

Is able to induce remission in 71% of patients which are affected by mild to moderate disease course (Gionchetti, Rizzello et al. 2006). In a pilot study the effect of S. boulardii was investigated in patients with active UC. Patients who were intolerant to steroids received S. boulardii for 4 weeks and 17 of 24 patients thereby achieved clinical and endoscopic remission (Guslandi, Giollo et al. 2003).

Prebiotics

The effects of SCFA in Ulcerative colitis are inconsistent.

Patients with ulcerative colitis have a lack of luminal short chain fatty acids because the uptake as well as the oxidation by colonocytes is blocked. Reduced Coenzyme A is responsible for the inhibited oxidation. Coenzyme A is reduced due to sulphur-containing compounds which are produced by the colonic microbiota. An extensive administration with SCFA could counteract against the lowered uptake and the reduced oxidation. In a study with Ulcerative colitis active and quiescent patients,

Roediger et al. found that the butyrate oxidation in these patients were lower than in controls (Roediger 1980; Wong, de Souza et al. 2006). In another experiment Harig et al. conducted a treatment with a SCFA solution for 2 to 6 weeks. This led to an improvement in endoscopic appearance, and patients with rectosigmoid colitis, with a nutrient deficiency, had a higher inflammation state (Harig, Soergel et al. 1989; Wong, de Souza et al. 2006). Yet in another study with the same SCFA solution, no endoscopic or histological changes appeared after 2 weeks (Guillemot, Colombel et al. 1991; Wong, de Souza et al. 2006). An explanation for these contradictory findings could be the amount of SCFA, the type of SCFA and the frequency and duration of administration (Wong, de Souza et al. 2006).

An inadequate energy supply of butyrate for colonocytes might be a detrimental factor for colitis. Until now it has not been clear in which amount butyrate would be the optimal concentration for treatment (Pryde, Duncan et al. 2002).

The capacity in colon cells to oxidise butyrate is lowered in patients with ulcerative colitis. Therefore the levels of butyrate in the faeces were higher in colitis patients than in controls. An induction of remission was possible through administration with SCFA enemas (McOrist, Abell et al. 2008). Fructooligosaccharides (FOS) are present in barley in high amounts. Consumption of barley reduced the clinical activity index. Unfortunately both studies which conducted this trial had a small number of patients and only one with such results (Kanauchi, Mitsuyama et al. 2003).

1.2.1.4.5. Modern Molecular Findings, Probiotics

In IL-10 knockout mice, it was observed that the mice developed colitis when they were raised under conventional conditions rather than germ-free conditions. Due to the administration of VSL#3, suppression of the mucosal TNF-α and IFN-γ production occurred and a normalization of barrier function and physiological transport was the consequence. Several reports document that VSL#3 has anti-inflammatory effects (Andoh and Fujiyama 2006). VSL#3 up regulates alkaline sphingomyelinase in the mucosa of the intestine and reduces inflammation (Reiff and Kelly 2009).

IL-6 plays a key role in intestinal inflammation. The Lactobacillus casei strain Shirota (LcS) inhibited the Interleukin-6 production in lipopolysaccharide-stimulated peripheral blood mononuclear cells which were isolated from UC patients

(Mitsuyama, Matsumoto et al. 2008). Under normal concentration IL-6 is important for host defence (Matsumoto, Hara et al. 2005).

In another experiment, investigators assessed the probiotic effect of LcS during the active phase and ten days after they induced dextran sodium sulphate (DSS) UC mice. DSS has been qualified for UC induction because it exhibits the characteristic symptoms of UC, it is capable of inducing acute as well as chronic UC and because the model is useful and reliable. The results demonstrated that LcS administration improved clinical parameters' in comparison to controls. Nevertheless the LcS treatment could not inhibit an induction of UC. Further observations included, that due to the UC induction, equal in the LcS group or the control group a dramatic increase of Enterobacteriaceae occurred (Herias, Koninkx et al. 2005). In IL-6 gene knockout mice, the DSS induced Colitis was less severe. LcS derive a polysaccharide-peptidoglycan complex which down-regulates the IL-6 production in lamina propria mononuclear cells (LPMCs) in murine chronic colitis as well as ileitis in SAMP1/Yit mice (Matsumoto, Hara et al. 2005). An important relevance is the awareness that the PSPG complex of L.casei, neither the PSPG complex of L.rhamnosus has an impact on the inhibition of IL-6. Only the PSPG complex of LcS inhibts the IL-6 production in LPS-stimulated colonic lamina propria mononuclear cells. Furthermore only the LcS has the ability to inhibit phosphorylation of NF-κB in lipopolysaccharide stimulated RAW cells. Investigators found that PSPG is composed of two PSPG components: PSPG-I and PSPG-II. But only PSPG-I had the ability to inhibit phosphorylation of NF-κB and IκB after the lipopolysaccharide stimulation in lamina propria mononuclear cells (Matsumoto, Hara et al. 2009).

The inflammation markers seem to be reduced due to administration of Bifidobacteria (Cui, Chen et al. 2004; Macfarlane, Furrie et al. 2005).

1.2.1.5. Morbus Crohn

1.2.1.5.1. Clinical Picture

The disease develops in genetic susceptible individuals due to an aberrant immune response, which is directed against luminal antigens such as dietary factors or commensal bacteria (Andoh and Fujiyama 2006). The increased cytokine production leads to inflammation and further to tissue damage. CD4+ T lymphocytes play a major role because the mucosa of CD patients is heavily infiltrated in contrast to healthy individuals. Furthermore CD+4 T lymphocytes are resistant against apoptosis. In Crohn's disease Th1 cells are predominant in the inflamed gut (Fantini, Monteleone et al. 2007).
Morbus Crohn is a discontinuous inflammation and in contrast to Ulcerative colitis it can potentially affect the entire gastrointestinal tract from mouth to anus (Baumgart and Sandborn 2007). The affection starts in the terminal ileum and extends in the course of time. In one third of individuals, simply the small intestine, especially the ileum (Iletitis), is affected; in one third the terminal small intestine and the large intestine is affected, and in one third of individuals only the colon is affected. In rare cases the proximal small intestine, the stomach or the mouth are also affected (Renz-Polster and Krautzig 2008).

Because of the variable affection, clinical symptoms are much more diverse than in Ulcerative colitis. Like in Ulcerative colitis general symptoms and abdominal pain as well as diarrhea are predominant. Extra intestinal symptoms occur especially in young patients and in 50% of all Crohn-patients (Renz-Polster and Krautzig 2008).

- Joint manifestations are common especially two different forms of arthritis: The nondeforming arthritis of the large joints and the central arthritis also called ankylosing spondylitis which can only occur in patients who are HLA-B27-positive.
- Skin manifestations: Frequently are Aphtous ulcers of the mouth and clubbed fingers. Erythema nodosum, and Pyoderma gangrenosum rarely occurs.
- Liver and bile: Common is a fatty degeneration of the liverparenchym and Pericholangitis. Slcerosing Cholangitis with an increasing obstruction of the

biliary tract is more common in patients with Ulcerative Colitis than in Crohn-patients and can lead to biliary cirrhosis.

- Eyes Iritis, Episkleritis, Uveitis
- Nephrolithiasis and gallstones

(Renz-Polster and Krautzig 2008)

In contrast to Ulcerative Colitis it is a discontinuous inflammation and the remission is not free of symptoms (Renz-Polster and Krautzig 2008).

The cause for the disease is not clear until now. Genetic factors, a disturbance of the immune system as well as environmental factors might play a role in disease initiation. Intestinal or extraintestinal bacterial infections could initiate intestinal inflammation. Although some microorganisms are supposable, results of a single microorganism as the cause are controversial. In susceptible individuals the intestinal inflammation could be driven by pathogens by a disruption of the mucosal barrier. An increased uptake of luminal antigens is the consequence. Mimicry of self-antigens and modulation of NF-κB or other transcription factors lead to an activation of the mucosal immune system and are further causes for the inflammation. Enhanced mucosal permeability can be the primary (genetically predisposed) or the secondary effect of inflammation. The secondary effect might occur after contact with pathogenic bacteria or as a consequence of the inflammation. Increased mucosal permeability leads to a loss of tolerance to the non-pathogenic resident enteric bacteria. Lesions are especially in regions where the highest number of luminal bacteria exists. Alongside the importance of the enteric flora, which is responsible for the full expression of the disease, the manner in which the enteric immune system recognizes bacterial antigens is of particularly remarkable. Polymorphisms in the NOD2 gene leads to an ineffective immune response to bacterial components and a defect in the clearance of bacteria in the human epithelia (Sheil, Shanahan et al. 2007).

1.2.1.5.2. Therapy

The goal is to induce remission initially followed by the administration of maintenance medications to prevent a relapse of the disease. It is very important to find a way for an initial remission and keep the remission afterwards (Renz-Polster and Krautzig 2008).

It is not possible to cure patients who suffer from Morbus Crohn. In contrast, patients with Ulcerative Colitis have the possibility to carry out a colectomy which would cure the illness. However, it is possible that after the operation a pouchitis would appear. The conservative therapy which is alike in both diseases is based on anti-inflammatory, immunosuppressed and immunomodulating drugs. A dietary-therapy does sometimes help, especially in the acute attack (Renz-Polster and Krautzig 2008).

1.2.1.5.2.1. Medical Treatment

The treatment is dependent on the disease location. Induction of remission in patients with mild ileocaceal or colonic disease is possible with sulfasalazine or mesalazine (ASA) but not effective for maintaining remission. If this treatment as first-line therapy is not effective, prednisolone therapy is the second possibility. The treatment with infliximab is effective if none of the two other drugs induce remission. Aziathioprine and mercaptopurine have a slow onset of action and are therefore not adequate for induction. However for maintenance they are ideal. Infliximab can be added to treatment with the immunosuppressive aziathioprine or mercaptopurine (Baumgart and Sandborn 2007).

1.2.1.5.2.2. Surgery Treatment

Surgical therapy is necessary if an intensive medical management in patients do not resort. A surgery does not cure patients with Crohn's disease, yet it is often necessary for inducing remission. A part, or a complete bowel obstruction, internal fistulas, enterovesical fistulas and enterocutaneous fistulas are reasons for an urgent surgery (Baumgart and Sandborn 2007).

1.2.1.5.3. Composition and Differences to Normal Microbiota

The faecal microbiota is stable in healthy humans considered individually, whereas in patients with Crohn's disease a temporal instability is predominant. Studies showed that bacterial composition varied between remission and active disease (Seksik, Rigottier-Gois et al. 2003; Sokol, Lay et al. 2008). Furthermore the bacterial biodiversity is marked by a reduction. Firmicutes are reduced, in particular Faecalibacterium prausnitzii which is part of the Clostridium leptum group (Sokol, Lay et al. 2008). Giaffer et al. noted that E.coli is significantly increased in the faeces in patients with CD (Giaffer, Holdsworth et al. 1991; Sokol, Lay et al. 2008). Enterobacterial populations were found in each faeces of CD patients but in none of a healthy human (Seksik, Rigottier-Gois et al. 2003; Sokol, Lay et al. 2008). Analysis of the mucosa indicate that the bacterial load is increased, but the diversity is significantly reduced (Sokol, Lay et al. 2008). In other studies a distortion of the composition of Firmicutes has been previously described in IBD patients. The biodiversity in IBD patients was restricted in a trial which was conducted by Manichanh et al., mainly the C. leptum group. They also detected an association between Crohn's disease and a decline of C. leptum group (Manichanh, Rigottier-Gois et al. 2006; Sokol, Seksik et al. 2009). Faecalibacterium prausnitzii, which is part of the C. leptum group, was particularly reduced (Maidak, Cole et al. 2001; Martinez-Medina, Aldeguer et al. 2006; Sokol, Seksik et al. 2009). Many bacteria which are part of the Clostridium leptum group are butyrate producing bacteria. Butyrate possesses anti-inflammatory and immunmodulatory properties and is a major source of energy for colonocytes. Therefore butyrate might have a protective role for the mucosa (Segain, Raingeard de la Bletiere et al. 2000; Klampfer, Huang et al. 2003; Sokol, Seksik et al. 2009). A deficiency of the Firmicutes bacteria therefore might be a cause for disease development (Sokol, Seksik et al. 2009).

Ott et al. investigated the microflora of the mucosa from patients with Crohn's disease. The bacterial diversity was 50% reduced compared to that of healthy individuals. Especially a decline of anaerobic bacteria like Bacteroides species, Eubacterium species and Lactobacillus species were the result of the diseases (Ott, Musfeldt et al. 2004). Investigators have already determined a decrease in the amount and biodiversity of Firmicutes in patients with Crohn's disease. Especially Faecalibacterium prausnitzii which is a subgroup of C.leptum is substantially

reduced. Crohn's disease is caused by a defect of the immune system which is supposed to be triggered by a change in the composition of the intestinal microbiota. In a trial the mucosa associated microbiota of patients with Crohn's disease was investigated after surgical resection and following 6 months thereafter. The results demonstrated that, patients who had lower counts of Faecalibacterium prausnitzii after surgery were predisposed for recurrence 6 months after surgery. That led to the conclusion that the lower abundance of Faecalibacterium prausnitzii was associated with the recurrence of the disease. The anti-inflammatory effect of Faecalibacterium prausnitzii might be the cause for patients who stayed in remission. Administration with Faecalibacterium prausnitzii could be a strategy in the treatment of CD patients (Sokol, Pigneur et al. 2008).

Author	CD Patients	Healthy Individuals
(Sokol, Lay et al. 2008)	Bacterial diversity ↓ Firmicutes ↓ Faecalibacterium prausnitzii ↓	
(Giaffer, Holdsworth et al. 1991)	E.coli ↑	
(Seksik, Rigottier-Gois et al. 2003)	Enterobacteria occurred only in the faeces of CD patients	No Enterobacteria
(Manichanh, Rigottier-Gois et al. 2006)	Bacterial diversity ↓ C. leptum ↓	
(Maidak, Cole et al. 2001)	Faecalibacterium prausnitzii ↓	
(Martinez-Medina, Aldeguer et al. 2006)	Faecalibacterium prausnitzii ↓	
(Ott, Musfeldt et al. 2004)	Bacterial diversity 50% ↓ Bacteroides ↓ Eubacterium ↓ Lactobacilli ↓	
(Sokol, Pigneur et al. 2008)	Faecalibacterium prausnitzii ↓	

Table 2. Summarized results from studies investigating the differences of microbiota in CD patients.

1.2.1.5.4. Probiotics and Counselling

Nutrition during the active phase in patient's with Morbus Crohn

In addition to the normal diet, enteral nutrition is suitable for the acute phase. This enteral nutrition enables compensation of the deficiencies of vitamins, minerals and energy. If the patient is in worse condition, an exclusively enteral nutrition is recommended which should supply the needs of daily energy. Is the patient in the worst condition with its inflammation probably only parenteral nutrition may be helpful. In this case the entire gastrointestinal tract is circumvented due to an access over the blood system. The parenteral nutrition should last 2-4 weeks. General recommendations during the acute phase are a high consumption of beverages especially water and tea which can be enriched with salt to balance the loss of electrolytes. Small meals are recommended therefore 6-7 times over the day. Diet should not be fat free, but the amount of fat should be reduced. Enough consumption of proteins should also be assured. Lactose is very often not tolerated from patients during the active stadium and therefore yoghurts and products without lactose should be consumed. In the acute phase patients are recommended to avoid dietary fibers. Thereby resorption takes place in the upper part of the small bowel whereby the inflamed part is tranquilized (Schiener, Reiterer et al. 2008).

Patients in remission

In the remission, it is of high importance to compensate the developed deficiencies during the active phase. In the symptom free interval a diet which is rich of dietary fibers is crucial. After the active phase the gradual return to solid food has to take place slowly. First the enteral diet changes from fiber poor to fiber rich. After a start of tea, followed by white bread a transfer to gruels made of wholemeal, boiled rice, noodles, potatoes, and boiled vegetables; afterwards fruits in forms of compote, fatless poultry, fish and meat. The amount of fat should increase very slowly and in the last step cheese and snag can be added. Patients very often have individual intolerances, where a nutrition diary may help to detect individual intolerances. Nevertheless these intolerances are transient and after a few weeks most edibles are again compatible. Coffee should be avoided because it activates the peristaltic and therefore encourages diarrhea (Schiener, Reiterer et al. 2008).

Probiotics

The current existing trials are promising that probiotics may have a therapeutic effect. Nevertheless larger controlled trials are needed before probiotics can be integrated in routine medical treatment (Sheil, Shanahan et al. 2007). General probiotic administration is not as successful in patients with Crohn's disease like it is in patients with Ulcerative colitis, but a combination of VSL#3 and Saccharomyces boulardii have had a therapeutic effect (Reiff and Kelly 2009). Up until now results from several studies have not been consistent, especially that of the dose and the type of probiotics strains differing between the trials (Park and Floch 2007).

Saccharomyces boulardii

In a trial with CD patients after combined treatment with S.boulardii and mesalazine a clinical relapse occurred in 6.25% of patients, whereas in patients with only mesalazine treatment relapse occurred in 37.5% of patients (Guslandi, Mezzi et al. 2000). Saccharomyces boulardii can improve but can not normalize the leaky gut in patients suffering from Crohn's disease (Garcia Vilela, De Lourdes De Abreu Ferrari et al. 2008; Reiff and Kelly 2009).

Lactobacilli

In a study from Malin et al. treatment with Lactobacillus GG increased gut IgA levels, which could promote the gut immunological barrier (Malin, Suomalainen et al. 1996). Admittedly in a large study with paediatric patients who were administered with LGG combined with medical treatment had no improvement in comparison to a placebo group combined with medical treatment (Bousvaros, Guandalini et al. 2005). Administration with L. rhamnosus GG added to Prednisone and immunmodulatory drugs improved the condition of 4 children with mild to moderate disease activity already after 1 week and lasted throughout the study (6 months). In 3 of the patients a reduction of the steroid dose was possible and an improved gut barrier function was recognized (Gupta, Andrew et al. 2000). Nonetheless several trials exist which could not prove the beneficial effects of Lactobacilli in Crohn's disease. In a trial with 11 patients with active Crohn's disease, patients were either administered with L. rhamnosus GG (LGG) or a placebo for 6 months. Before the beginning of the trial a reduction of the steroid amount was performed and antibiotics were applied. L. rhamnosus had not the ability neither to induce remission nor to maintain remission (Schultz, Timmer et al. 2004). Nearly similar results appeared with a study population who were in the stage after surgery. Administration with LGG for one year after

surgery showed no differences to the placebo group neither in symptomatic nor endoscopic recurrence (Prantera, Scribano et al. 2002). The study form Marteau at al. showed similar results with Lactobacillus johnsonii which had no effect in preventing postoperative recurrence in comparison to the control group (Marteau, Lemann et al. 2006).

VSL#3

The effect of VSL#3 in combination with an antibiotic has also been investigated. VSL#3 is composed of Lactobacillus plantarum, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus delbrueckii spp. bulgaricus, Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium longum and Streptococcus salivarius thermophilus (Sheil, Shanahan et al. 2007). Forty CD patients who previously had a surgery received either rifaximin for 3 months, followed by VSL#3 for 9 months or mesalazine for 12 months. 20% of those who were in the probiotic group had a relapse, whereas a relapse occurred in 40% of the CD patients who were part of the mesalazine group (Campieri, Rizzello et al. 2000).

E.coli Nissle1917

Although it was a small pilot study and therefore the results were not statistically significant, treatment with E.coli Nissle1917 reduced the relapse rate in comparison to the placebo group. From 12 patients in the E.coli group, 33% had a relapse whereas from the 11 patients in the control group 63% were affected by a relapse (Malchow 1997).

In summary these results show that up until now results are inconsistent and just treatment S. boulardii showed to have effectiveness in several studies (Park and Floch 2007).

1.2.1.5.5. Modern Molecular Findings, Probiotics

Thus far, none of the available studies have found a beneficial effect of probiotics for patients with Crohn's disease. Lactobacillus rhamnosus GG (LGG) had no positive impact either in reduction of severity of recurrent lesions, nor in inhibiting endoscopic recurrence (Prantera, Scribano et al. 2002; Reiff and Kelly 2009). Saccharomyces boulardii is at the moment the only probiotic which has a positive impact for patients

with Morbus Crohn. It improved the leaky gut but it could not normalize it (Garcia Vilela, De Lourdes De Abreu Ferrari et al. 2008; Reiff and Kelly 2009).
So far it is not known which probiotic strain, which dose, which mode of administration is suitable and how long a therapy should be conducted (Sheil, Shanahan et al. 2007).

1.2.2. Obesity

Obesity and its associated metabolic disorders have become a major public health issue worldwide. Obesity is seen ass an illness with multicourse factors. Alongside environmental factors, genetic predisposition and individual behaviour, investigators have found that epigenetic factors indeed play a role (Campion, Milagro et al. 2009).
A positive energy balance is seen as the chief cause for obesity, but the nutrition in early childhood also seems to have significance in this illness. Formula or formula mixed fed infants seem to be a reason for increased susceptibility for obesity, whereas breastfeeding seems to be a protective factor (Nadal, Santacruz et al. 2009). Also the birth process is determining; a natural birth should be preferred to a caesarean section (Kalliomaki, Collado et al. 2008).

1.2.2.1. Clinical Picture

A positive energy balance which leads to a state of chronic, low-grade inflammation with an abnormal cytokine production and acute-phase inflammatory protein production is characteristic of obesity (Nadal, Santacruz et al. 2009).
Many obesity related disorders increase type 2 diabetes mellitus, cardiovascular disease, pulmonary hypertension, obstructive sleep apnoea, gastrooesophageal reflux disease and musculoskeletal disorders, just to name only a few. Obesity is also the cause for psychosocial problems, and a variety of cancers. An increased risk of mortality is also associated with obesity (DiBaise, Zhang et al. 2008).

1.2.2.2. Therapy

The primary goal of obesity- therapy is a stable weight reduction which is possible due to negative energy balance. Therefore a change in diet behaviour as well as in exercise behaviour is essential. A continuous weight reduction over a long period is preferable, compared to fast weight reduction. Due to a decreased food intake it is important to consume nutrient rich products to assure an adequate supply of essential nutrients. Besides the change in diet there is also the alternative of additional administration with different medicaments to decrease weight, such as thyroid hormones, anoretica, lipase inhibitors, fat-subsitutes or diuretica. These drugs can support weight reduction, although a change in behaviour is inevitable. Psychotherapy can support individuals as well in their weight reduction. There is also the possibility for an operative intervention but this should be seen as the last resort and is only advisable in patients with severe obesity (Elmadfa and Leitzmann 2004).

1.2.2.3. Composition and Differences to Normal Microbiota

1.2.2.3.1. Studies with mice

In obese mice the percentage of Bacteroidetes is lower than in lean mice, whereas the Firmicutes amounts are in obese mice higher than in lean mice. Due to weight loss Ley et al. recognized an increase in Bacteroidetes and a decline of Firmicutes (Ley, Backhed et al. 2005). Ley et al. analyzed the 16SRNA sequences from cecal microbiota of genetically obese (ob/ob) mice, their lean ob/+ and +/+ siblings and their ob/+ mothers. All received the same polysaccharide-rich diet. Remarkable was the fact that, ob/ob mice had 50% fewer Bacteroidetes and correspondingly more Firmicutes than their lean littermates. In addition they discovered a link between kinship and distal gut microbial diversity, but the differences in obese mice existed independent of kinship and sex (Ley, Backhed et al. 2005; DiBaise, Zhang et al. 2008). Turnbaugh et al. wanted to figure out how the gene content in the gut microbiota contributed to obesity. They analyzed the distal gut microbiota in obese and lean mice. In obese mice the ratio of Firmicutes to Bacteroidetes were increased compared to their lean littermates. The amounts of Archaea were also higher in obese mice compared to the lean ob/+ and +/+ littermates. The microbiota of obese mice included genes which encoded enzymes that broke down otherwise indigestible

dietary polysaccharides. In the faeces they found more end products of fermentation and fewer calories, which lead to the assumption that the gut microbiota lead to a facilitation of calorie extraction from indigested food. The major end products butyrate and acetate were increased. This was a logical conclusion because Firmicutes are butyrate producer (Turnbaugh, Ley et al. 2006; DiBaise, Zhang et al. 2008).

Finally they wanted to show that the gut microbiota is the determining factor and so they transferred the gut microbiota of either obese or lean mice to gnotobiotic lean mice. The results were that after two weeks, gnotobiotic mice which received the gut microbiota from obese mice, extracted more calories from food and also showed a significant higher fat gain than the mice with the lean microbiota. These findings lead to the conclusion, that gut microbiota indeed plays an important role in the pathogenesis of obesity (Turnbaugh, Ley et al. 2006; DiBaise, Zhang et al. 2008).

The interaction between bacteria and Archaea in the gut may also be an important factor in increased energy extraction. As a result of the Archaeal methanogenesis the production of hydrogen and other reaction products is inhibited. Acetate and butyrate produced by the bacteria in the gut are not oxidized and the amount of the production can increase if the Archaea help to remove hydrogen and formate. Acetate and butyrate are important carbon sources for the epithelium cells in the colon. Thus this syntrophism leads to an increased energy extraction from indigestible polysaccharides and might be a factor in obesity regulation (DiBaise, Zhang et al. 2008).

Bacteroides thetaiotaomicron is highly efficient in glycan metabolism, metabolizes indigestible sugars and therefore harvests additional energy (Comstock and Coyne 2003; DiBaise, Zhang et al. 2008). Methanobrevibacter smithii makes up 10% of the anaerob microbiota in the human colon of healthy individuals (Macfarlane and Macfarlane 1997; DiBaise, Zhang et al. 2008). Consequently Samuel and Gordon conducted an experiment with mice in which they colonized the gut of these mice with common colonic bacteria either with Bacteroides thetaiotaomicron or Methanobrevibacter smithii or both. The increase of adiposity after colonization with bacteria and Archaea was higher than colonization with just one of the two. In addition the investigators discovered that M. smithii influenced the metabolism of B. thetaiotaomicron by influencing it to consume mainly fructose containing polysaccharides breaking down in several substances, but also formate which is an important energy source for M. smithii. This detection leads to the hypothesis that the

synergism between microorganisms indeed plays an important role in energy homeostasis. M. smithii might be a therapeutic target for obese patients, however further studies are needed (Samuel and Gordon 2006; DiBaise, Zhang et al. 2008).

In a series of experiments Backhed et al. reasoned that young conventionally reared mice have a 40% higher body fat content and 47% gonadal fat content than germ free mice, although the germ free mice consumed more food than the conventional mice. After these findings they transplanted the distal gut microbiota from the conventional mice into the gnotobiotic mice. In conclusion the gnotobiotic mice had a 60% increase of body fat within two weeks even though the food consumption and the energy expenditure were stable. These findings lead to supporting the assumption that the composition of the microbiota has an impact on the energy extraction from the diet. Besides the increase in body fat, insulin resistance, adipocyte hypertrophy and increased levels of circulating leptin and glucose appeared (Backhed, Ding et al. 2004; DiBaise, Zhang et al. 2008).

In another experiment Bäckhed et al elucidated the fat storage of fatty acids into adipocytes with the help of germ-free mice (gnotobiotic). A comparison between conventionally raised mice and the gnotobiotic mice was conducted. The conventional mice had 42% more body fat than the germfree mice although the food intake was 29% less than the food intake of the germ free mice. After these findings the authors conventionalized some germfree mice. After 14 days an increase of 57% in body fat, a 61% increase of epidemical fat and a 7% decrease of lean body mass became apparent. The serum triglycerides did not change (Backhed, Manchester et al. 2007).

Bacteroides and Clostridia were the most common species in the mouse cecum and therefore investigators choose B. thetaiotaomicron for the conventionalization for over 2 weeks. B. thetaiotaomicron is rich of glycosylhydrolases and therefore responsible for degradation of plant polysaccharides. A significant increase in total body fat was the consequence. The oxygen consumption was an indicator for the metabolic rate and was therefore measured. The metabolic rate was 27% lower in the leaner germfree mice in comparison to the 14-day conventionalized mice. The results were unexpected for the authors and therefore they measured the tricarboxylic acid cycle and assessed that a significant increase in citrate, ketoglutarate and malate was distinctive for the tissues of conventionalized mice in comparison to the germ free mice. Curiously the conventionalized and the germfree

mice had the same stores of energy compounds. Investigators assume that conventionalized mice have an inefficient metabolism and in conclusion an increased body fat storage. Beside this, conventionalized mice have a higher insulin resistance and evaluated circulating leptin and glucose levels. Shortly after the germ-free mice became conventionalized, an increase in adipocyte size appeared, but not an increase in adipocyte number. After the first conventionalization of germ-free mice, the lipoprotein lipase activity increased about 122% in epidemical fat pads and 99% ascent in heart tissue. Increased triglyceride levels were the consequence due to an increased lipoprotein lipase activity which led to elevated fatty acid uptake. Angiopoietin-like protein-4 (Fasting induced adipocyte factor = Fiaf) was high in germ-free mice and suppressed lipoprotein lipase, whereas after conventionalization a suppression of Fiaf by the gut microbiota occurred. This Fiaf suppression led to increased lipoprotein lipase activity (Backhed, Ding et al. 2004; DiBaise, Zhang et al. 2008).

1.2.2.3.2. Studies with humans

Ley et al. analyzed the fecal gut microbiota continuously in 12 obese patients during a weight loss programme for the duration of one year. In this experiment one group received a fat restricted diet and another group consumed a carbohydrate-restricted low calorie diet. Before the start of the administered diet all of the obese patients had higher levels of Firmicutes and lower levels of Bacteroidetes than the lean control group. After the weight loss the amount of Bacteroidetes increased while the levels of Firmicutes decreased; these levels correlated with the percentage of weight loss and not with the chances in dietary caloric content. Before the weight loss Bacteroidetes made up 3% of the gut bacteria and 15% after the weight loss. Until now it is not known why obese people have more Firmicutes. Firmicutes have a vast diversity and therefore may be able to have a more efficient energy extraction which contributes to an easier fat storage (Ley, Turnbaugh et al. 2006; DiBaise, Zhang et al. 2008).
In another experiment taken by French investigators, results presented that obese patients had a bacterial profile poor in Bacteroidetes and rich in Lactobacillus. The obese/lean ratio of Methanobrevibacter smithii accounted 1.72. The increased amount of Lactobacillus in obese patients supports the assumption that Lactobacillus plays a role in weight gain. Interestingly, they also elevated the bacterial composition

of anorexic patients. These patients had the highest amounts of Methanobrevibacter smithii, whereas all the other bacterial profiles where similar to those of the lean patients. An explanation could be that M. smithii lead to a superior exploitation of energy which is the result of a very low caloric diet. Another explanation by the authors was that Methanobrevibacter could be related to constipation, which is a common phenomenon in anorexia nervosa patients (Armougom, Henry et al. 2009).

In a study conducted in 2008 the investigators sought to elucidate if there was an association between the gut microbiota in early childhood and weight development in the following years. This study was based on a previous study from 2001. 25 obese and overweight children and 24 normal weight children were selected and the BMI of these children at the age of 7 was determined. The microbiota of the faeces in the 6 and 12 month was analyzed. The results showed that normal weight children had significantly more Bifidobacteria than the obese children. The contents of S. aureus were numerously lower in normal weight children than in obese children (Kalliomaki, Collado et al. 2008).

In a study taken in 2009 the composition of the gut microbiota was analyzed in 9 individuals. Three of them had normal weight, three were morbidly obese and 3 had a gastric bypass. The faeces were analyzed in the group with the gastric bypass 8 and 15 months after the surgery. In this time one of these three individuals stopped losing weight. In the overall analysis of the gut microbiota, the investigators detected 6 main bacterial phyla. Firmicutes and Bacteroidetes made up the major content. Beside of these two, Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia occurred. The results showed that obese patients had increased levels of Archaea, in contrast to the normal weight individuals, who contained no Archaea and just one of the three gastric bypass patients had small amounts. Although the Bacteroidetes number was not significantly higher in obese patients, Prevotellaceae, a subgroup of Bacteroidetes was significantly increased in obese patients. Prevotellaceae is a group which has members who are carbohydrate- and protein-fermenting and acetate and H_2 producers. The conclusion of this study was that the gut of obese patients contained increased amounts of H_2 producing bacteria particularly the Prevotellacea family and some members of the Firmicutes (Zhang, DiBaise et al. 2009). Admittedly, this study could not support the results from Ley et al. In the study from Ley et al. obese patients had lower amounts of Bacteroidetes

and proportionally more Firmicutes than lean individuals (Ley, Turnbaugh et al. 2006).

In a study from Nadal et al. investigators wanted to assess the effects of calorie restriction and exercise on the faecal microbiota and the immunoglobulin-coating bacteria in obese/overweight adolescents. Within 10 weeks the participants were instructed to have a calorie restricted diet as well as perform increased physical activity. After the 10 weeks the patients were divided into groups depending on their weight loss. Group A, which lost more than 4kg, and group B with those whose weight loss was below 2.5kg. Within participants of group A the weight reduction resulted in a significant decrease of Gram positive Clostridium hystolyticum, Clostridium lituseburense and E. rectale-C. coccoides proportions. The decrease correlated with the loss of body weight. In contrast the Gram negative Bacteroides-Prevotella levels significantly increased. Lactobacillus-Enterococcus proportions, Enterobacteriaceae, E. coli, and Roseburia groups increased but not in high amounts. The decrease of Bifidobacteria and sulphate reducing bacteria was not significant and therefore not relevant. In group B, in which the weight loss was low, none of the bacteria showed significant differences. Nevertheless the biochemical parameters changed in group B. Serum glucose was slightly changed and serum HDL-cholesterol values were higher than before the intervention. After the intervention Group A had a significant reduction in fecal energy content in contrast to group B. Some biochemical parameters changed in group A after the intervention. A significant decline of serum glucose and total cholesterol concentration was the consequence after the intervention. This decline correlated with changes in the enteric group proportions as well as with changes in total gram negative bacteria. LDL cholesterol was reduced after the intervention but not significantly (Nadal, Santacruz et al. 2009).

IgA coating bacteria are an indicator for low grade inflammation. Before the intervention IgA coating bacteria where higher than after the 10 weeks. The drop of IgA coating bacteria was associated with decrease of E.rectale-C. coccoides and C. hystolyticum. The explanation for the correlation between these bacteria might be that the reduction of this butyrate producing bacteria leads smaller butyrate levels. In conclusion the immune cells have a reduced energy amount and can therefore produce less IgA –producing cells and in succession the IgA concentration falls. For

that reason considerations that weight loss depends on the diet and the gut microbiota must not be excluded (Nadal, Santacruz et al. 2009).

Author	Obese
(Ley, Backhed et al. 2005)	Bacteroides ↓
	Firmicutes ↑
(Turnbaugh, Ley et al. 2006)	Firmicutes: Bacteroides ratio ↑
(Armougom, Henry et al. 2009)	Lactobacilli ↑
(Kalliomaki, Collado et al. 2008)	Bifidobacteria ↓
(Zhang, DiBaise et al. 2009)	Archaea and Prevotellaceae ↑
(Nadal, Santacruz et al. 2009)	After Weight reduction:
	Clostridium hystolyticum ↓
	Clostridium lituseburense ↓
	E. rectale-C.coccoides ↓
	Bacteroides ↑
	Lactobacilli ↑
	Enterobacteriaceae ↑
	E.coli ↑
	Roseburia ↑

Table 3. Summarized results from studies investigating the differences of microbiota in obese individuals.

1.2.2.3.3. Modern Molecular Findings, Mechanisms of Microbiota

The microbiota comprises a number of interrelate mechanisms for energy regulation.

(1) The first mechanism is the fermentation of indigestible dietary polysaccharides to absorbable forms.

(2) The second mechanism is the intestinal absorption of monosaccharides and short chain fatty acids with their subsequent conversion to fat within the liver.

(3) Finally the third mechanism is the regulation of host genes that promote deposition of fat in the lipocytes.

(DiBaise, Zhang et al. 2008)

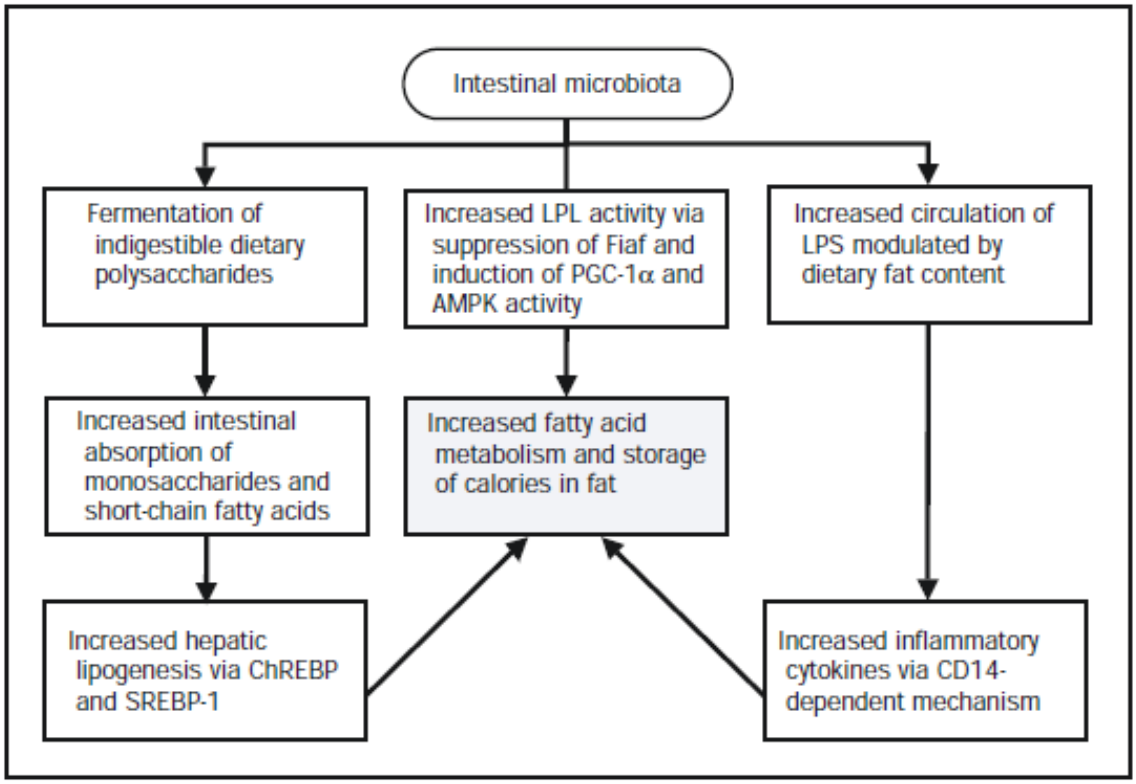


Figure V. Mechanisms by which the intestinal microbiota may contribute to obesity.
AMPK = adenosine monophosphate-activated protein kinase; ChREBP = carbohydrate response element binding protein; Fiaf = fasting induced adipocyte factor; LPL = lipoprotein lipase; LPS = lipopolysaccharide; PGC-1α = peroxisome proliferator-activated receptor γ coactivator 1α; SREBP-1 = sterol response element-binding protein type 1.
(DiBaise, Zhang et al. 2008)

Metabolic efficiency and energy recovery is individually different and therefore a possible explanation why some obese patients do not seem to overeat. The gut microbiota, can on the one hand, influence energy harvest from dietary substances and on the other hand affect genes that regulate the energy expenditure and storage (DiBaise, Zhang et al. 2008).

ARCHAEA

H_2 is an end product of bacterial fermentation, which by accumulation reduces the efficiency of processing dietary polysaccharides (Stams 1994; Gill, Pop et al. 2006). Methanogenic-Archaea oxidizes H_2. An increased amount of methanogenic-Archaea leads to an easier fermentation of plant polysaccharides and dietary fibers of H_2 producing fermenters, such as Prevotellaceae and in conclusion to an increased SCFA production. The SCFA are taken up through the epithelium of the human intestine which leads to the assumption that there is an association between Archaea and increased energy harvest (Zhang, DiBaise et al. 2009).

FIAF

Investigators have found that the gut microbes suppress the intestinal fasting induced adipocyte factor (Fiaf) which is also known as angiopoetin-like protein 4. Fiaf is a lipoprotein lipase inhibitor (LPL). Through suppression of Fiaf the activity of the Lipoprotein Lipase increases and in succession influences the import and storage of triglyceride-derived fatty acids in adipocytes. In a study with mice the investigators induced a Fiaf suppression which led to an increased lipoprotein lipase activity and resulted in elevated fat storage (Backhed, Ding et al. 2004).

In a study from Backhed et al. germ-free mice who consumed a Western-style, high fat, sugar rich diet were investigated. These mice were protected from obesity by two mechanisms:

1. Because of the increased Fiaf levels the production of peroxisome proliferator-activated receptor-γ coactivator was induced. Peroxisome proliferator-activated receptor-γ coactivator is known to elevate the gen expression which encodes regulators of mitochondrial fatty acid oxidation.
2. Furthermore a protection from obesity by an increased activity of the enzyme adenosine monophosphate-activated protein kinase. This enzyme controls cellular energy status.

(Backhed, Manchester et al. 2007; DiBaise, Zhang et al. 2008)

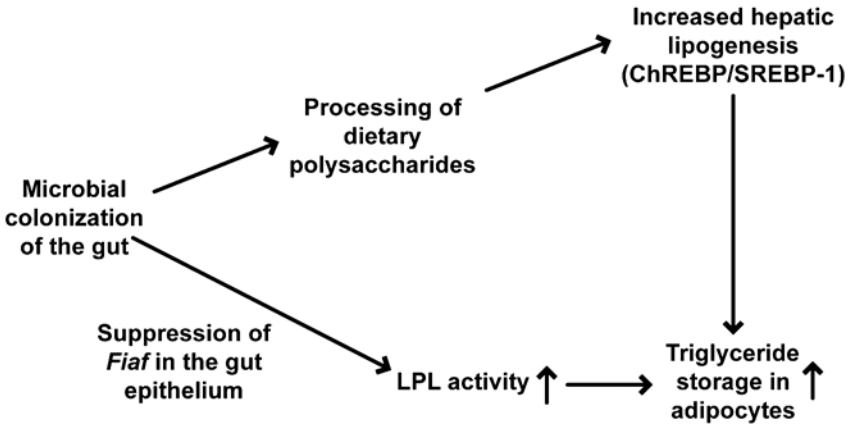


Figure VI. Schematic view of how the gut microbiota effects host fat storage.
The microbiota act through Fiaf to coordinate increased hepatic lipogenesis with increased lipoprotein lipase (LPL) activity in adipocytes, thereby promoting the storage of calories from the diet into Fiaf. (Backhed, Ding et al. 2004)

Cani et al. described a mechanism linking microbiota with obesity (Cani, Amar et al. 2007; DiBaise, Zhang et al. 2008). This mechanism is based on the fact that obesity and insulin resistance is associated with low-grade chronic systemic inflammation (Wellen and Hotamisligil 2005; DiBaise, Zhang et al. 2008). The investigators assume that gram negative bacteria which reside in the gut provide lipopolysaccharides (LPS). The LPS act as a triggering factor linking inflammation to high fat diet –induced metabolic syndrome. They conducted various experiments with mice receiving a high-fat diet, which led to increasing levels of endotoxemia, and in a row a reduction of predominant bacterial population in the gut. Gram positive as well as gram negative bacteria were reduced, but favouring an elevation in gram negative to gram positive ratio. The chronic metabolic endotoxemia induces obesity, insulin resistance and diabetes (Cani, Amar et al. 2007; DiBaise, Zhang et al. 2008). In an experiment from Wright et al. CD14 mutant mice received a high-fat diet, showing that the metabolic endotoxemia induced the expression of inflammatory cytokines due to a CD14 dependent mechanism. The LPS bind at the surface of the innate immune cells, leading to a secretion of pro-inflammatory cytokines by CD14 (Wright, Ramos et al. 1990; DiBaise, Zhang et al. 2008). Due to human studies, it exists the assumption that the tone of insulin sensitivity is set by LPS/CD14 system and that this system furthermore regulates the onset of obesity and diabetes. Humans received the antibiotic polymyxin B which targets gram negative organisms and therefore reduces LPS expression and hepatic steatosis (Pappo, Becovier et al. 1991; DiBaise, Zhang et al. 2008). A study from 2006 came to the finding that patients with type 2 diabetes had higher LPS levels than the control group without diabetes (Creely, McTernan et al. 2007; DiBaise, Zhang et al. 2008).

Another assumption would be that a high fat diet is the cause for changes in gut microbiota and not conversely, that the gut microbiota is the cause for gaining weight. Host phenotype, genotype, immune function and diet impair the composition of the gut microbiome. In an experiment investigators consulted knockout mice with the phenotype RELMβ to elucidate the importance of these factors. At the beginning of the study the knockout mice and wild mice were fed with a normal chow diet and were therefore lean. After switching to a high-fat diet the wild mice had an increased weight gain, whereas the knockout mice remained lean. Due to the switch to high fat diet changes in the microbiota compositions occurred. A decrease of Bacteroidetes

and an increase of Firmicutes and Proteobacteria appeared in both genotypes. This showed that a high-fat diet leads to alterations in microbiota and not weight gain (Hildebrandt, Hoffmann et al. 2009).

1.2.2.4. Probiotics and Counselling

1.2.2.4.1. Prebiotics

The colonic bacteria ferments prebiotic agents like fructo oligosaccharide and thus modulates the growth of colonic bacteria. The prebiotics are not digested in the upper gastrointestinal tract and have several functions in the gut (Gibson, Beatty et al. 1995; Roberfroid 2002; DiBaise, Zhang et al. 2008). Recently two studies were conducted in which rats received a standard (Cani, Dewever et al. 2004) or a high fat (Cani, Joly et al. 2006) diet with addition of oligofructose (DiBaise, Zhang et al. 2008). The findings of this study were controversial to previous studies because the oligofructose caused a reduced energy intake and consumption, and protected against weight gain. The specific modulation of the gut microbiota as well as other physiological effects of fibers like the increased satiety because of slower gastric emptying might be explanations for these contradictions. A single-blind cross-over study over two weeks with 10 healthy individuals with normal weight found that the satiety after breakfast and dinner was increased because of oligofructose and therefore led to a 5% reduced energy uptake in contrast to the placebo group (Cani, Joly et al. 2006; DiBaise, Zhang et al. 2008). Cani et al. noticed in a recent study that an increase of oligofructose led to an ascent of Bifidobacterial contents in high-fat-diet mice. The amount of Bifidobacteria correlates negatively with endotoxemia. Furthermore Bifidobacteria positively influences the glucose tolerance, glucose induced insulin secretion and normalizes the inflammatory tone. Therefore prebiotic supplementation might be a relevant target in the therapy of obese patients, but further studies are needed (Cani, Neyrinck et al. 2007; DiBaise, Zhang et al. 2008).

1.2.2.4.2. Probiotics

Lactobacillus rhamnosus PL60 promises antiobese effects according to the study from Lee et al. This bacterium generates conjugated linoleic acid in diet-induced obese mice. Animal studies detected that beside other positive health functions,

linoleic acid reduces body fat. In an 8 week experiment in which mice received L. rhamnosus PL60 a weight reduction occurred without reducing the energy intake of the mice (Lee, Park et al. 2006; DiBaise, Zhang et al. 2008). In further studies the explanations for these effects were investigated. The anitobesity effects can be related to the apoptosis and messenger RNA expression in the white adipose tissue. How relevant L. rhamnosus for energy reduction in humans is doubtful because L rhamnosus reduces the cell numbers of adipose tissue but not the cell size. In adults the cells numbers are constant and just the size can change after weight gain. It is not evident if L. rhamnosus would be a therapeutic target in obesity treatment (DiBaise, Zhang et al. 2008).

A study with 122 obese humans aggravated the fact that L. rhamnosus is not adequate for weight reduction. In the randomized controlled trial the patients were treated with 3.4g conjugated linoleic acid or placebo for one year and it had no effect (Larsen, Toubro et al. 2006; DiBaise, Zhang et al. 2008).

In another experiment from Sonnenburg et al, germfree mice received B. thetaiotaomicron and Bifidobacterium longum which led to an increase in the polysaccharide range targeted for degradation. Similar results appeared in a probiotic administration with Lactobacillus casei (Sonnenburg, Chen et al. 2006; DiBaise, Zhang et al. 2008). Martin et al. found that a probiotic administration resulted in distinct changes of the microbiome accompanied by alterations of the metabolism in the tissue concerning energy, lipid and amino acid metabolism (DiBaise, Zhang et al. 2008; Martin, Wang et al. 2008).

Many questions have to be clarified before it is possible to determine if differences in the gut may lead to different weight in humans. It has to be determined if the alterations in the gut are the cause or the result for obesity. Also hormones and other signals and their influence on the composition of the microbiota must be regarded. Besides the understanding of the relative proportions of Firmicutes, Bacteroidetes and Archaea in mice and humans have to be elucidated. The environmental as well as the genetic factors of each individual must also be regarded, but also the surface-adherent differences of the microbiota in the mucosa must be elucidated for obese and person who already lost weight (DiBaise, Zhang et al. 2008).

1.2.2.4.3. Modern Molecular Findings, Probiotics

Obese persons have another composition of the microflora than lean persons. Gnotobiotic lean mice received the gut flora of obese mice and an increase in weight was the succession. Lactobacillus gasseri, Lactobacillus reuteri, Lactobacillus acidophilus appear to have a cholesterol reducing function by assimilation of cholesterol. L.gasseri inhibits the rear resorption of bile acids in the terminal ileum and this leads to an increased excretion of acid steroids with the stool.

Undoubtedly the reduction of lipids is only proven in animal experiments and needs further investigation, especially on humans (Schulze, Sonnenborn et al. 2008).

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1.2.3. Atopic Disease

In westernized countries the frequency of atopic diseases increased in the last years. Especially eczema, food allergy, hay fever and asthma are common disorders. Because it is clear that not only the genetic make up is the cause, environmental factors must indeed play an important role (Asher and Weiland 1998; Nowak, Suppli Ulrik et al. 2004; Penders, Stobberingh et al. 2007). Strachan's observations were that, children with more siblings as well as children growing up in rural regions are not as susceptible for atopic diseases like children from urban regions and children with fewer siblings (Strachan 1989; Strachan 2000; Karmaus and Botezan 2002; Penders, Stobberingh et al. 2007). Hospital deliveries, caesarean sections, smaller family size, the use of antibiotics, good hygiene, and differences in the maternal diet lead to changed exposure of bacteria for children (Brandtzaeg 2002; Gore, Munro et al. 2008). A protection of allergies might be the infections of gastrointestinal pathogens (Matricardi, Rosmini et al. 2000; Wang, Karlsson et al. 2008).

The bacterial colonisation is decelerated in infants from industrialized countries in comparison to children living in developing countries (Adlerberth, Carlsson et al. 1991; Adlerberth, Strachan et al. 2007). Hence their immune system is less stimulated (Shroff, Meslin et al. 1995; Adlerberth, Strachan et al. 2007). Neonates who developed allergies seem to have an altered colonisation of their microbiota (Bjorksten 2004; Prescott and Bjorksten 2007).

1.2.3.1. Clinical Picture

Asthma bronchiale, atopic dermatitis, allergic rhinitis and conjunctivitis as well as Urticaria and IgE-mediated food and medication allergy belong to the generic term atopic diseases (Renz-Polster and Krautzig 2008).

Atopic diseases are chronic inflammatory disorders. The cause is an aberrant immune response against common harmless environmental antigens (allergens) in susceptible individuals. More precisely alterations in T-helper 2 cells are the cause (Romagnani 2006; Penders, Stobberingh et al. 2007). These T helper cells type 2 produce proinflammatory cytokines like IL-4, IL-5 and IL-13 which trigger IgE production and eosinophila. Under “normal” conditions the Th1, Th3 and T regulatory cells counteract due to their production of other cytokines, which is initiation is partially triggered by the intestinal microbiota (Rautava, Kalliomaki et al. 2005; Isolauri, Kalliomaki et al. 2008).

Atopic dermatitis

Is one of the most frequent allergic skin diseases and the incidence is ascending (Renz-Polster and Krautzig 2008). It is a pruritic chronically relapsing inflammatory skin disease which is the portal for other sensitizations (Hanifin 2002; Isolauri, Kalliomaki et al. 2008).

Urticaria

Is mostly idiopathic but can also be an IgE-mediated reaction due to environmental factors like coldness, compression, heat, cholinergic or adrenergic stimulation. It is characterized by pruritus, erythem and quaddels on the skin (Renz-Polster and Krautzig 2008).

Asthma bronchiale

It’s the most common chronic inflammation disease in humans. The most triggering factors are house dust mites, mildew spores, allergens from cats and pollen. It is

characterized by acute dyspnoea and obstruction of the lower air passages. The obstruction is caused by mucus swelling due to inflammation, bronchia construction as well as dyscrinie (Renz-Polster and Krautzig 2008).

Allergic rhinitis

Manifestation incidences in childhood, although with increasing age in most cases the symptoms alleviate. Major causes for the symptoms are pollen. Half of the persons with allergic cold, develop asthma bronchiale after 8 years (Müller 2006).

Allergic rhinoconjunctivitis

Pruritus and sneezing, watery rhinorrhea, obstructed nose, light sensibility and swollen lids are the main symptoms. General symptoms are headache, fatigue, exhaustion, increased thirst and minimized productivity (Müller 2006).

Food allergy

Can arise in each state of age, but occur in most cases in the first two months of life. The absence of an intact barrier function in the gastrointestinal tract is the cause. Cow milk allergens are the important agents in development (Müller 2006).

1.2.3.2. Therapy

The most important issue which should always be kept in mind is allergen elimination. In the case of a medication allergy it is easier than in the case of a food allergy or house dust mites. Hence a medical prophylaxis is necessary in many cases. If medical prophylaxis does not avoid the occurrence of symptoms the treatment with antihistaminic drugs is helpful to dampen the first reaction. Due to glucocorticoides the following inflammatory reactions can be stopped (Renz-Polster and Krautzig 2008).

1.2.3.3. Composition and Differences to Normal Microbiota

While Bifidobacteria and Lactobacilli seem to be reduced in individuals who are susceptible for atopic diseases, Clostridia, Enterobacteriaceae and Staphylococci might be the cause for the development of the disease (Penders, Stobberingh et al. 2007). In children who have developed allergy within their first two years of life, Bjorksten et al. noticed a decreased colonisation of Enterococci, Bifidobacteria and Bacteroides, whereas the amount of Staphylococcus aureus was increased in faeces

(Bjorksten, Naaber et al. 1999; Bjorksten, Sepp et al. 2001; Songjinda, Nakayama et al. 2007). He et al. found out that the composition of Bifidobacteria differs between allergic and non-allergic children. While allergic children had high amounts of Bifidobacterium adolescentis similarly to adults, non-allergic children had increased amounts of Bifidobacterium bifidum (He, Ouwehand et al. 2001; Shreiner, Huffnagle et al. 2008). Clostridium difficile seems to play an important role in atopic infants. In recurrent wheezy infants IgG levels, which are characteristic for Clostridium difficile were significantly increased in comparison to non-wheezy infants (Woodcock, Moradi et al. 2002; Shreiner, Huffnagle et al. 2008).
Fact is, that due to different study designs, differences of the investigated microbes, different methods and different outcomes it is difficult to compare the studies (Penders, Stobberingh et al. 2007). Furthermore it is until now not clear if the alternated microbiota in early childhood persists in preschool age (Sepp, Julge et al. 2005; Stsepetova, Sepp et al. 2007).

Wang et al. investigated the 1 week old faeces of 15 remarkable atopic and 20 healthy children from a previous study project, namely ALLERGYFLORA. Within this project investigators assessed the bacterial colonisation of 318 Swedish, British and Italian infants, but could not find significant differences in colonisation between infants with atopic eczema or without atopic eczema during the first 18 months of life (Adlerberth, Strachan et al. 2007; Wang, Karlsson et al. 2008). Nevertheless Wang et al. found that that the diversity was significantly lower in children who developed atopy later, in comparison to children who stayed healthy during their first 18 months of life. The authors came to the conclusion that a high diversity of microbiota might be a preventive factor for the development of atopic diseases (Wang, Karlsson et al. 2008).

If the gut microbiota is less diverse, special species have the chance to enforce which might have detrimental consequences for development of allergic diseases. Clostridium difficile is an example. Pender et al. concluded that an increased amount of Clostridium difficile occurred especially in children who developed atopic diseases (Penders, Thijs et al. 2007). Sjögren et al. did not come to such results, in contrary the opposite was the case. Children who developed allergic diseases were not colonized with Clostridium difficile during their first 2 months of life (Sjogren,

Jenmalm et al. 2009). However several other studies exist which concluded that increased appearance of Clostridia was accompanied by lower diversity of other bacteria which resulted in allergies (Bottcher, Nordin et al. 2000; Bjorksten, Sepp et al. 2001; Kalliomaki, Kirjavainen et al. 2001; Sjogren, Jenmalm et al. 2009).

Kalliomaki et al. monitored 76 high-risk infants during their first year of life. Children in which atopy developed had a distinct bacterial fatty acid profile in their faeces at the age of 3 weeks. Furthermore atopic infants had lower numbers of Bifidobacteria and an increased amount of Clostridia (Kalliomaki, Kirjavainen et al. 2001; Penders, Stobberingh et al. 2007).
A further study had partially contrary results. The number of Clostridia was decreased in infants with eczema in comparison to healthy subjects. It must be pointed out that this study quantified Clostridium lituseburense subgroup and Clostridium histolyticum subgroup whereas the previous study just included Clostridium hystolyticum subgroup. Anyhow both studies have in common a lower amount of Bifidobacteria in infants with eczema. The amount of lactic acid bacteria was higher than in healthy subjects (Mah, Bjorksten et al. 2006; Penders, Stobberingh et al. 2007).

In a study which was part of the National Asthma Campaign Manchester Asthma and Allergy Study (^{NAC}MAAS) the intestinal microbiota of patients with recurrent wheeze was compared with that of healthy individuals. No differences were detected in the faecal composition of healthy subjects (Murray, Tannock et al. 2005; Penders, Stobberingh et al. 2007). A further study which was part of ^{NAC}MAAS investigated 1-year-old sensitized wheezy infants. Results showed that they had significant increased serum IgG levels against C. difficile in comparison to healthy infants (Woodcock, Moradi et al. 2002; Penders, Stobberingh et al. 2007).

Another study concluded that patients with allergic rhinitis were more often IgG-seropositive to intestinal bacterial pathogens like Clostridium difficile compared to healthy participants (Linneberg, Ostergaard et al. 2003; Penders, Stobberingh et al. 2007).

Faecal samples of 957 infants were investigated in the KOALA Birth Cohort study. The infants were monitored for the development of atopic symptoms. Colonisation with C. difficile was associated with a higher susceptibility for developing eczema and colonisation with E.coli was associated with eczema as well, even though in a concentration dependent manner (Penders, Stobberingh et al. 2007; Penders, Thijs et al. 2007).

In a cross sectional study with two year old Estonian as well as Swedish children, the colonisation of bacteria in the gut was assessed of healthy and allergic children. The amount of Lactobacilli was decreased in allergic children. Furthermore the amount of Staphylococcus aureus was increased in allergic children from Sweden and Coliforms were increased in allergic children from Estonia (Bjorksten, Naaber et al. 1999; Penders, Stobberingh et al. 2007). Because investigators could not figure out if the allergy was first, or the changed composition of bacteria in the intestine, they conducted a further study with Swedish and Estonian children, in whom they collected faecal samples during the first year of life, and the children were clinically monitored until the age of two. Results showed that infants who developed allergy had a diminished amount of Bifidobacteria in the first year of life in comparison to healthy children (Bjorksten, Sepp et al. 2001; Penders, Stobberingh et al. 2007). Additionally the allergic infants had at the age of one year a decreased colonisation with Bacteroides (Bjorksten, Sepp et al. 2001; Songjinda, Nakayama et al. 2007). This result is contradictory to a pilot study from Songjinda et al. in which faecal samples of allergic and non-allergic infants were collected in their first 5 postnatal days and at the age of 1 and 2 months. Children who developed allergy by their second birthday had significant increased amounts of Bacteroidaceae in comparison to non-allergic subjects. These conflicting results may be caused by different sampling ages and also result due to sampling before and after weaning (Songjinda, Nakayama et al. 2007).

The impact of Bacteroides is not clear until now. While Bacteroides thetaiotaomicron regulates intracellular downstream of TLR signalling and NF-κB activation, and thereby reduces inflammation (Kelly, Campbell et al. 2004; Songjinda, Nakayama et al. 2007), Bacteroides fragilis stimulates increased induction of TH2 cells leading to increased generation of inflammatory cytokines (Odamaki, Xiao et al. 2007; Songjinda, Nakayama et al. 2007)

The association between intestinal microbiota and Immunoglobulin E were investigated in 5 year old Estonian children in another study. Children who had specific IgE antibodies to food and/or inhalant allergens had higher numbers of Clostridia. In addition the relative share of Bifidobacteria was decreased in allergic children in comparison to healthy infants (Sepp, Julge et al. 2005; Penders, Stobberingh et al. 2007).

A study from Japan compared the composition of bacteria in atopic dermatitis patients with healthy individuals. Bifidobacteria were reduced in atopic dermatitis patients and furthermore Staphylococci amounts were increased in atopic dermatitis patients (Watanabe, Narisawa et al. 2003; Penders, Stobberingh et al. 2007).

Forty Estonian children were selected from a former larger study in which 213 children were investigated. From these 40 children, 20 had an allergy and 20 had no signs of allergy during their first year of life. Of interest was the fact that allergic children had an earlier substitution from breast milk with cow's milk in comparison to healthy infants. Due to the results of the study investigators concluded, that the diversity of bacteria in general was lower in allergic children compared to the control group. There were no differences in the amount of Bifidobacteria between the two groups. Nevertheless the species of Bifidobacteria varied between the two groups. Bifidobacterium adolescentis occurred primarily in the faeces of allergic children, Bifidobacterium catenulatum and Bifidobacterium pseudocatenulatum were more frequent in non-allergic children (Stsepetova, Sepp et al. 2007). These results are equal to the results from He et al and Ouwehand et al. (He, Ouwehand et al. 2001; Ouwehand, Isolauri et al. 2001; Stsepetova, Sepp et al. 2007). By isolating B. adolescentis strains from allergic infants, investigators discovered that this bacterium do not adhere well on the human mucus cell wall, which might be an association to the aberrant immune response (He, Morita et al. 2002; Stsepetova, Sepp et al. 2007). Furthermore this bacterium species induces proinflammatory cytokines like IL-6, IL-12, and tumornecrosis factor-α, but can not activate regulatory cytokines like IL-10 (Kramer, Sutherland et al. 1995; He, Morita et al. 2002; Stsepetova, Sepp et al. 2007). In contrast B. bifidum, B. longum and B. catenulatum/pseudocatenulatum trigger the production of IL-10 (Young, Simon et al. 2004; Stsepetova, Sepp et al. 2007).

In a study from Gore et al. infants at the age of 3 and 6 months were investigated. Infants had either eczema or were healthy. Results showed no significant differences of Bifidobacteria in eczema patients in comparison to the control group. Bifidobacterium bifidum occurred especially in the faeces of breast fed infants whereas Bifidobacterium pseudocatenulatum was significantly increased in the faeces of non-breast fed infants. In infants with eczema Bifidobacterium pseudocatenulatum occurred more often than in healthy infants. Nevertheless the prevalence of the amount of Bifidobacterium bifidum significantly did not differ between the both groups (Gore, Munro et al. 2008).
In a study from Young et al. the Bifidobacterial composition between infants from Ghana, United Kingdom and New Zealand were compared. Bifidobacterium infantis was present in all infants from Ghana, whereas none of the children from the United Kingdom or New Zealand harboured these species. The infants from the United Kingdom and New Zealand had 1 or more of the species B. bifidum, B. longum, B. adolescentis, B. pseudocatenulatum. The prevalence for atopic diseases is in Ghana is in contrast to the other countries very low. The authors concluded, that, B. infantis might play an important role because B infantis is in contrast to other species of Bifidobacterium not induced in the Th2 driven immune response, in contrast to B. bifidum, B. longum, B. adolescentis and B. pseudocatenulatum (Young, Simon et al. 2004; Gore, Munro et al. 2008).

In a Finnish case control study the microbiota in the faeces was compared between seven allergic breast fed infants with that of six healthy breast-fed infants. Results demonstrated that Bifidobacterium adolescentis occurred more often in allergic children, whereas Bifidobacterium bifidum was more common in healthy infants (Ouwehand, Isolauri et al. 2001; Penders, Stobberingh et al. 2007).

The faeces of 47 Swedish children were investigated at 1 week, 1 month and or 2 months of age and the children were followed until they reached their fifth birthday. 16 developed allergy during the first 5 five years, whereas 31 stayed healthy the entire study period. Results showed the fecal samples from healthy individuals had higher amounts of Bifidobacterium adolescentis and Lactobacilli I group (Lactobacillus rhamnosus, L. casei, L. paracasei) than children who developed atopic disease. The increased amount of B. adolescentis in early childhood in healthy

individuals is a contrary result to He et al. who concluded that Bifidobacterium adolescentis occurred more often in allergic children (He, Ouwehand et al. 2001; Sjogren, Jenmalm et al. 2009). Explanation of the authors might be the time window which means, that just very early in life bacteria have an impact on the development of the immune system (Garn and Renz 2007; Sjogren, Jenmalm et al. 2009).

A further study from Japan investigated faecal samples from 11 adult atopic dermatitis patients and 14 healthy individuals. The amount of Enterobacteriaceae was significantly increased in AD patients in comparison to healthy subjects (Matsumoto, Ohishi et al. 2004; Penders, Stobberingh et al. 2007).

Allergic children seem to have increased amounts of Coliforms and Staphylococci, whereas the numbers of Bacteroides are lowered (Bjorksten, Naaber et al. 1999; Sandin, Braback et al. 2009).

Short chain fatty acids (SCFAs)

SCFAs are products generated by the gut microbiota and are therefore also suitable to assess the composition of bacteria in the gut.

Propionic, i-butyric, butyric, i-valeric and valeric acid were higher in non allergic one year old children compared to allergic. I-caproic acid and acetic acid were higher in allergic children (Bottcher, Nordin et al. 2000; Sandin, Braback et al. 2009). I-caproic acid is characteristic for the presence of Clostridium difficile (Bottcher, Nordin et al. 2000; Shreiner, Huffnagle et al. 2008).

In a study from Sandin et al. the faeces of 1 year old children were investigated. Those children who developed allergy by their fourth birthday had increased levels of acetic acid but decreased amounts of i-butyric, i-valeric and valeric acids (Sandin, Braback et al. 2009). A decline in acetic and propionic acids and an elevation of acids with more carbon atoms results due to the switch to an anaerobic flora (Norin, Midtvedt et al. 2004). Hence the results from Sandin et al. show, that children with allergic diseases, seem to have a reduced microbial diversity (Sandin, Braback et al. 2009).

Author	Allergic infants	Non-Allergic Infants
(He, Ouwehand et al. 2001)	Bifidobacterium adolescentis ↑	Bifidobacterium bifidum ↓
(Stsepetova, Sepp et al. 2007)	Bifidobacterium adolescentis ↑	Bifidobacterium pseudocatenulatum ↑
(Sjogren, Jenmalm et al. 2009)		Bifidobacterium adolescentis ↑
(Ouwehand, Isolauri et al. 2001)	Bifidobacterium adolescentis ↑ Clostridium difficile ↓	Bifidobacterium bifidum ↑
(Gore, Munro et al. 2008)	Bifidobacterium pseudocatenulatum ↑	
(Young, Simon et al. 2004)	Bifidobacterium infantis ↑	
(Matsumoto, Ohishi et al. 2004)	Enterobacteriaceae ↑	
(Bjorksten, Naaber et al. 1999)	Coliforms ↑ Staphylococci ↑ Enterococci ↓ Bifidobacteria ↓	Bacteroides ↓
(Bjorksten, Sepp et al. 2001)	Bacteroides ↓ Clostridia ↑ Enterococci ↓ Bifidobacteria ↓	
(Songjinda, Nakayama et al. 2007)	Bacteroides ↑	
(Bottcher, Nordin et al. 2000)	Clostridia ↑	
(Kalliomaki, Kirjavainen et al. 2001)	Clostridia ↑ Bifidobacteria ↓	
(Mah, Bjorksten et al. 2006)	Clostridia ↓ Bifidobacteria ↑	
(Penders, Thijs et al. 2007)	Clostridium difficile ↑	
(Woodcock, Moradi et al. 2002)	IgG levels against Clostridium difficile ↑	
(Linneberg, Ostergaard et al. 2003)	IgG levels against Clostridium difficile ↑	
(Sepp, Julge et al. 2005)	Clostridia ↑ Bifidobacteria ↓	
(Watanabe, Narisawa et al. 2003)	Staphylococci ↑ Bifidobacteria ↓	

Table 4. Summarized results from studies investigating the differences of microbiota in atopic children.

1.2.3.4. Probiotics and Counselling

Nutrition

Diet may have an impact on allergy development. Dietary fats might be responsible for increased leukotriene and prostaglandin generation, leading to elevated likelihood for allergies (Shreiner, Huffnagle et al. 2008). Dietary trans-fatty-acids were positively associated with childhood asthma, allergic rhinoconjunctivitis and atopic eczema in children (Weiland, von Mutius et al. 1999; Shreiner, Huffnagle et al. 2008). In a further study, increased fat intake in adult men showed increased susceptibility for asthma (Strom, Janzon et al. 1996; Shreiner, Huffnagle et al. 2008).

But the type of fat seems to indeed play a role. Polyunsaturated fatty acids affect the integrity of the intestinal epithelium. They regulate the tight junction permeability (Usami, Muraki et al. 2001). Additionally the growth and adhesion of probiotics to mucus and epithelial cells is alleviated (Kankaanpaa, Salminen et al. 2001; Isolauri, Kalliomaki et al. 2008). The immune function is among others regulated by dietary long-chain polyunsaturated fatty acids. Especially n-3 polyunsaturated fatty acids seem to have anti-inflammatory properties, due to their function to inhibit the release of arachidonic acid. Arachidonic acid is released by membrane phospholipids and produces pro-inflammatory eicosanoids (Whelan 1996; Isolauri, Kalliomaki et al. 2008). Nevertheless in several studies consumption of fish or supplementation of fish oil had no effect on asthma or atopic eczema (Woods, Thien et al. 2002; van Gool, Zeegers et al. 2004; Isolauri, Kalliomaki et al. 2008).

Vitamin A has a crucial role indeed. It inhibits IgE production in mouse peripheral blood mononuclear cells in case of allergic disease (Worm, Herz et al. 2001; Isolauri, Kalliomaki et al. 2008). Furthermore it suppresses generation of Interferon-γ, IL-4 and increases mucosal IgA, which protects the mucosal surface (Albers, Bol et al. 2003). The activation of arachidonic acid cascade can also be changed due to Vitamin A supplementation inhibiting prostaglandin E_2 generation in vitro (Halevy and Sklan 1987; Isolauri, Kalliomaki et al. 2008).

A study from Italy came to the conclusion that high consumption of vegetables might be preventive for asthma (La Vecchia, Decarli et al. 1998; Shreiner, Huffnagle et al. 2008). In antioxidant supplementation different results occurred. One study from Martindale et al. concluded that, Vitamin C supplementation in prenatal women increased the atopic risk for infants whereas Vitamin E supplementation lowered the

risk (Martindale, McNeill et al. 2005; Shreiner, Huffnagle et al. 2008). Nevertheless a separate study identified increased Vitamin C in breast milk as a preventive agent for atopic diseases in infants. Notably was that Vitamin C as part of the diet occurred in breast milk in opposition to Vitamin C as a supplement (Dubos 1971; Fanaro, Chierici et al. 2003; Shreiner, Huffnagle et al. 2008).
Laitinen et al. assessed the impact on diet and the effect of prenatal probiotic administration until the fourth year of life. Children with Atopic eczema who had prenatal probiotic administration and additionally consumed higher amounts of retinol, calcium, zinc and protein and lesser amount of ascorbic acid were less susceptible for atopic eczema development (Laitinen, Kalliomaki et al. 2005).

Probiotics

The treatment with probiotics seems to have only an impact in early infancy when the immune responses are still developing. Hence, in studies with older individuals with whom sensitization is already established, probiotics do not seem to have an effect. Due to the fact, that one probiotic strain is not sufficient to alter the diverse composition of bacteria in the gut, investigators assume prebiotics might be beneficial to stimulate the growth of Bifidobacterium and Lactobacilli (Prescott and Bjorksten 2007). Nevertheless alterations in the gut microbiota due to probiotics reduced the risk for atopic disease (Mai and Draganov 2009).
At the moment contradictory results exist. While Kalliomaki et al. and Kukkonen et al. concluded that probiotic administration at the age of 2 years has a preventive effect for eczema, Taylor et al. and Kopp et al. gathered that probiotic administration had no impact (Kalliomaki, Salminen et al. 2001; Kukkonen, Savilahti et al. 2007; Taylor, Dunstan et al. 2007; Kopp, Hennemuth et al. 2008; Niers, Martin et al. 2009).

In several studies the supplementation of specific probiotics alludes to have a positive effect. Lactobacillus GG and Bifidobacterium lactis Bb-12 showed to reduce allergic disorders in atopic children. They may influence the development, the microbial crosstalk as well as the composition of microbiota (Mattila-Sandholm, Blum et al. 1999; Isolauri, Arvola et al. 2000; Kirjavainen, Arvola et al. 2002; Gueimonde, Sakata et al. 2006; Stsepetova, Sepp et al. 2007).
In a broader trial, infants with atopic dermatitis and a possible cow milk allergy received for one month either hydrolyzed whey formula with Lactobacillus GG or one

without this probiotic strain. Tumor necrosis factor-α decreased and additionally an alleviation of the clinical course proceeded (Majamaa and Isolauri 1997; Isolauri, Kalliomaki et al. 2008). A separate investigation conducted an experiment with infants who manifested atopic eczema during exclusive breastfeeding. Administration over 6 months with Lactobacillus GG or Bifidobacterium lactis was simultaneous with hydrolyzed whey formula feeding. A control group without a probiotic strain and only hydrolyzed whey formula feeding indeed existed. The probiotic group had a reduction of soluble CD4 concentration in serum and a decline of eosinophilic protein X in urine after 2 months with probiotic administration (Isolauri, Arvola et al. 2000; Isolauri, Kalliomaki et al. 2008).

Lahtinen et al. conducted a study with 122 mothers who either received Lactobacillus rhamnosus GG or placebo during pregnancy. Results demonstrated that the infants at 90 days of age from the mothers receiving the probiotic strain had a higher colonisation with Bifidobacterium longum group in comparison to controls. The investigators concluded that an administration with probiotics in late state of pregnancy can have a positive impact on the development of the intestinal microbiota in infants. Remarkable was the observation that in the probiotic group it did not matter if infants were born by vaginal delivery or caesarean section. In contradiction to the placebo group in whom, the type of delivery did make a difference. In this group caesarean section infants had increased amounts of Bifidobacterium adolescentis and decreased amounts of B. longum (Lahtinen, Boyle et al. 2009).

Niers et al. assessed probiotic administration in pregnant mothers followed by administration of neonates in their first 12 months of life, who were a risk group for allergic diseases. Bifidobacterium bifidum W23, Bifidobacterium infantis W52 and Lactobacillus lactis W58 made up the probiotic supplement. Results showed that the neonates who received probiotics were higher colonized with Lc. Lactis during their first 3 months of life. Additionally the probiotic group had a decline in IL-5 generation in vitro. Although the amounts of Bifidobacterium spp. were increased in all participants, qualitative differences occurred between the two groups. While Bifidobacterium bifidum occurred in all probiotic infants, just half of the placebo group possessed this strain. The appearance of B. bifidum in half of the placebo group is explained by the authors due to lactation in these children wherefore they were colonized with this bacterium as well. In general it was observable that the infants

who received probiotics had a significant risk reduction of parenteral eczema during the first 3 months of life. The effect continued until the age of 2 years but it decreased with age. The authors concluded that prenatal probiotic treatment might be necessary to avoid the development of atopic eczema (Niers, Martin et al. 2009).

Administration with B. catenulatum/pseudocatenulatum may certainly have a relieving effect in allergic diseases, if they are used as probiotics (Stsepetova, Sepp et al. 2007).

In another trial children with moderate to severe eczema were either treated with Lactobacillus fermentum VRI-033 PCC or with a placebo. Results showed a significant decline of eczema scores in the probiotic group (Weston, Halbert et al. 2005; Isolauri, Kalliomaki et al. 2008). Administration with probiotics pre- and postnatally over 6 months in 156 children who were susceptible for atopic disease led to a reduction of atopic eczema in later infancy, in comparison to a control group (Kalliomaki, Salminen et al. 2001; Kalliomaki, Salminen et al. 2003; Isolauri, Kalliomaki et al. 2008).

A study with nineteen adults between 18 and 45 years of age who suffered from allergic rhinitis was conducted. For two weeks the participants received either a probiotic drink comprising of Lactobacillus casei Shirota or a placebo. Results demonstrated a significant decline of allergy related cytokines like IL-5, IL-6 and IFN-γ generation in the probiotic group. Additionally the IgE levels decreased whereas the IgG levels increased in the probiotic group. Therefore investigators concluded that a supplementation with probiotics might be beneficial in alleviating the symptoms of allergic rhinitis (Ivory, Chambers et al. 2008).

1.2.3.5. Modern Molecular Findings, Probiotics

Studies pointed out that the most important risk reduction for eczema development should be implemented in the first year of life (Niers, Martin et al. 2009). Perinatal probiotic administration influences the bacterial colonisation and has therefore an impact on the development of the infant's immune system. By the impact on dendritic cells, the T-cell differentiation is affected leading to generation of T regulatory cells

(Smits, Engering et al. 2005; Niers, Martin et al. 2009). Probiotic supplementation led in a human study in vitro to an increased generation of IL-10, which is a regulatory cytokine (Lammers, Brigidi et al. 2003; Prescott and Bjorksten 2007).

L. paracasei has impact on the development of CD4(+) T cells which produce TGF-β and IL-10, while it inhibits the secretion of cytokines from Th1 and Th2 cells (von der Weid, Bulliard et al. 2001; Penders, Stobberingh et al. 2007). Lactobacillus reuteri and Lactobacillus casei foster the monocyte-derived dendritic cells, whereby the Treg cells are enhanced to increase the IL-10 generation (Smits, Engering et al. 2005; Penders, Stobberingh et al. 2007). Nevertheless not all Lactobacilli affect dendritic cells which are capable for the production of T regulatory cells. L. plantarum appeared not to be capable (Smits, Engering et al. 2005; Prescott and Bjorksten 2007).

Bifidobacterium adolescentis fosters the production of TNF-α, IL-6, and IL-12 which are pro-inflammatory cytokines, but it can not induce the production of IL-10 which would have a regulatory function (Kramer, Sutherland et al. 1995; He, Morita et al. 2002; Stsepetova, Sepp et al. 2007).

B.bifidum, B.longum, and B.catenulatum/pseudocatenulatum produce the regulatory cytokine IL-10 (Young, Simon et al. 2004; Stsepetova, Sepp et al. 2007).

In peripheral blood mononuclear cells Bifidobacterium genomic DNA induced the IL-10 secretion in vitro (Lammers, Brigidi et al. 2003; Penders, Stobberingh et al. 2007)

IgA production in the intestine is influenced by the microbiota as well. The B cells and effector T cells which generate IgA, mature in the mucosa of the gut. After maturation they migrate to the distal mucosa sites in the respiratory tract. This is an example of how the gut microbiota impacts Th1 responses and IgA generation (Vancikova, Lodinova-Zadnikova et al. 2003; Pohjavuori, Viljanen et al. 2004; Prescott, Dunstan et al. 2005).

Pathway	Documented effects in human beings and/or animals	Proposed mechanism of immunomodulation
Local effects		
Effects on mucosal barrier	Repair and maintenance of intestinal barrier and tight junctions Increased mucous production	Reduced permeability and reduced systemic penetration of allergens/antigens[7]
Enterocytes	Reduced cell signaling via nuclear factor-κB local inflammation[6] Increased production of TGF-β and prostaglandin E2, which promote tolerogenic DCs[9]	Reduced local inflammation/promotion of tolerogenic conditions
Innate mucosal recognition (TLR)	Anti-inflammatory effects of probiotics mediated by TLR9 [8] Possible changes in TLR2 *in vitro*[37]	Inhibition of T_H2 allergic responses: mechanisms unclear TLR2/4 agonists shown to reduce inflammation in murine lung
DCs	Increased activity of DCs in human gut[10]	Promotes tolerogenic DC (IL-10 production)
Effector T cells	T_H1 skewed responses observed[12]	Inhibition of T_H2 differentiation? Effects of T-cell trafficking?
Treg	$CD4^+CD28^+$ T cells associated with oral tolerance T-cell–producing IL-10 and TGF-β associated with oral tolerance Probiotics: Increased TGF-β (T_H3) Treg[13]	TGF-β produced locally (including by enterocytes) promotes tolerogenic DC, local IgA, and Treg activity
B cells and antibodies	Colonization: increased lymphoid tissue Probiotics: increased local IgA production	Promotes tolerogenic microenvironment (as above); IgA may reduce systemic antigen load
Systemic effects		
Monocytes	Probiotics: increased circulating monocytes[19,20]	Mechanisms not known
T cells	Probiotics: increased T_H1 differentiation	Secondary to effects on T cells trafficking through gut?
B cells/IgA	Probiotics: increased IgA production in other tissues (respiratory tract)[18]	Secondary to effects on B cells trafficking through gut?
Stem cells	Probiotics: increased circulating bone marrow derived $CD34^+$ stem cells[21]	Mechanisms not known

Treg, Regulatory T cells.

Figure VII. Immunologic pathways affected by probiotics.
(Prescott and Bjorksten 2007)

The allergic response is caused by T-helper type 2 (Th2) lymphocytes which are responsible for the generation of IL-4, IL-5, and IL-6. These cytokines foster the IgE production (Foley and Hamid 2006; Ivory, Chambers et al. 2008).
Due to early probiotic administration, an increased colonisation with Lactobacillus lactis was observed, which was accompanied by a decline in cytokine production. In blood cultures at 3 months of age a lowered IL-5 and IL-13 generation in vitro was observed. Additionally the eczema incidence was reduced. At the same time it did not have an impact of high-risk children for atopic eczema (Niers, Martin et al. 2009).

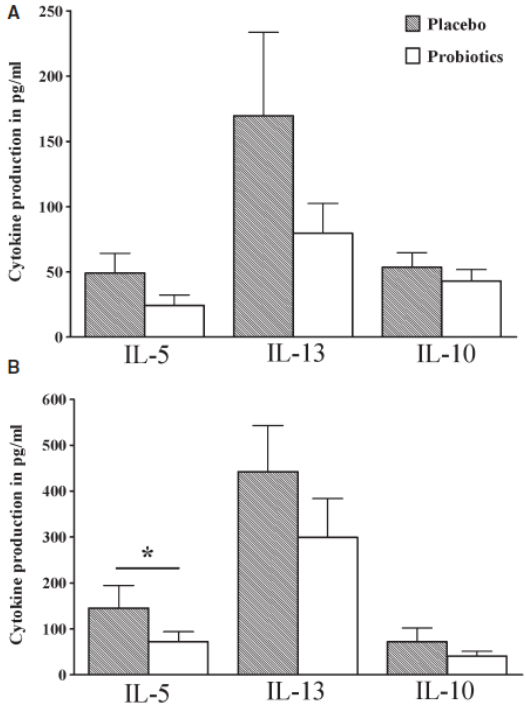


Figure VIII. Production of cytokines in whole blood cultures at age 3 months.
Whole blood cultures were left unstimulated (medium) or stimulated with anti CD2/anti CD28 monoclonal antibody, or Phytohemgglutinin (PHA). Supernatants were collected at 48 h and 72 h and cytokines were measured by a multiplex assay (Luminex). (A) Anti CD2/CD28 48 h; (B) Anti CD2/CD28 72 h. Data represent mean ± SEM. * $P < 0.05$.
(Niers, Martin et al. 2009)

IL-4, IL-5, IL-13 promote IgE production and are generated by Th2 cells (Rautava, Kalliomaki et al. 2005). In a study from Ivory et al. treatment with Lactobacillus casei Shirota led to a decline in the production of IL-5, IL-6 and IFN-γ. The IgE amounts were higher in the placebo group in comparison to the probiotic group whereas, the IgG amounts were lower (Ivory, Chambers et al. 2008). Lactobacillus rhamnosus strain GG suppresses lymphocyte proliferation by generation of proteases and lowers the generation of IL-4 (Sutas, Hurme et al. 1996; Sutas, Soppi et al. 1996; Isolauri, Kalliomaki et al. 2008). Lactobacillus casei inhibited the IgE responses in a murin model of food allergy (Shida, Takahashi et al. 2002; Isolauri, Kalliomaki et al. 2008). An in vitro study with blood mononuclear cells demonstrated that Bacteroides fragilis is responsible for an increased Th2 production, which produced IL-6 and a lowered Th1 production, which is responsible for IFN-γ and IL-12 generation in comparison with Bifidobacteria (Odamaki, Xiao et al. 2007; Songjinda, Nakayama et al. 2007).

The Lipopolysaccharides from Enterobacteriaceae have proinflammatory effects via the Toll like receptor 4 pathway. Bacteroides fragilis has this function indeed, however it is 100-1000 fold lower (Mancuso, Midiri et al. 2005; Songjinda, Nakayama et al. 2007). Bacteroides thetaiotaomicron reduces inflammation by down regulation of TLR signalling and NF-κβ induction (Kelly, Campbell et al. 2004; Songjinda, Nakayama et al. 2007).

1.2.4. Diarrhea and Constipation

1.2.4.1. Clinical Picture

Viral infections, parasites or bacterial toxins can lead to gastroenteritis which is characterized by diarrhea (Wilson 2005). Without treatment it can have life-threatening consequences in malnourished or ill individuals (Alam and Ashraf 2003).

1.2.4.1.1. Travellers Diarrhea

Diarrhea is the most common health problem for travellers. 20-70% of the travelling population are affected. Of course several factors influence its susceptibility and the travel destination is certainly crucial (Castelli, Saleri et al. 2006).
In most cases E.coli is the common agent for the infection. In addition other bacterial agents like Campylobacter jejuni, Salmonella spp., Shigella spp., Aeromonas hydrophila, Plesiomonas shigelloides and non choleric vibrios are determinative in diarrhea development. Viruses can definitely have an impact such as, Rotaviruses, Calciviruses and Enteroviruses. Protozoa can also be detrimental (Castelli, Saleri et al. 2006).

1.2.4.1.2. Antibiotic-Associated Diarrhea

5-30% of patients have to face an antibiotic associated diarrhea which lasts up to two months (Wistrom, Norrby et al. 2001).Treatment with antibiotics seems to disrupt the ecological balance which can be the cause for diarrhea (Nord, Bergan et al. 1986). Furthermore it leads to overgrowth of one species and an antibiotic resistance of pathogens or commensal bacteria is indeed possible (Lepage, Seksik et al. 2005).

1.2.4.1.3. Infants Diarrhea

The UNICEF has reported that diarrhea accounts for 1.5 million deaths of children annualy, under the age of 5 (UNICEF 2009).

1.2.4.1.4. Diarrhea in Elderly

Elderly people are a risk group because they are hospitalized twice as frequent as younger people, have to stay twice as long and have double the treatment with drugs in comparison to younger individuals (Bartosch, Fite et al. 2004). Antibiotic treatment is more frequent and is therefore the chief cause for alterations of the intestinal microbiota (Sullivan, Edlund et al. 2001).

1.2.4.1.5. Constipation

Constipation is very common in modern societies and especially elderly are frequently affected. Constipation is more prevalent in elderly who live in nursing homes and hospitals in comparison to elderly living in the community. 80% of residents in nursing homes suffer from constipation. Changes in food habits, the lack of exercise and the loss of privacy or personality are some factors which contribute to the development of constipation (An, Baek et al.). The treatment of medication is a further reason for the development of constipation (An, Baek et al.; Frank, Schmier et al. 2002). The large intestine is affected. Constipation is characterized by low defecation frequency, irregular stool expulsion, painful and strained defecation, hard and dry stool consistency, passing of abnormally small stools and a feeling of incomplete rectal defecation (An, Baek et al.; Bosaeus 2004).

1.2.4.2. Therapy

1.2.4.2.1. Travellers Diarrhea

In healthy individuals it is self limiting, whereas in risk groups, like young children, elderly, and ill persons it can be more difficult. Hospitalization or at least bed rest might be necessary (Ericsson 2003; Castelli, Saleri et al. 2006).

1.2.4.2.1.1. Preventative treatment with Antibiotics

Treatment with antibiotics showed in several studies a preventive effect. Several studies identified antibiotics like neomycin (Kean 1986), doxycycline (Sack, Kaminsky et al. 1978), trimethoprim-sulfamethoxazole (DuPont, Evans et al. 1982), and fluoroquinolones (Parry, Howard et al. 1994) as preventive drugs for traveller's diarrhea (Castelli, Saleri et al. 2006). Du Pont et al. conducted a trial with US students travelling to Mexico receiving rifaximin. Results showed that 72% were protected against traveller's diarrhea (DuPont, Jiang et al. 2005; Castelli, Saleri et al. 2006). The efficacy of the treatment depends on the type of antibiotic as well as the area of destination (Castelli, Saleri et al. 2006).

However this kind of prevention is not innocuous. Antibiotics have several adverse effects, only the antibiotic rifaximin seems to be well tolerated with little adverse effects (Ericsson and DuPont 2005; Castelli, Saleri et al. 2006). Nevertheless, due to the fact that traveller's diarrhea is a self-limiting disease it is disputable if antibiotic treatment as prevention is reasonable (Castelli, Saleri et al. 2006).

1.2.4.2.1.2. Treatment of Diarrhea

If someone suffers from diarrhea, rehydration should be the first attempt to alleviate the symptoms. Symptomatic therapy and antimicrobial therapy are further possibilities for treatment.

Rehydration

Rehydration is an important issue if someone suffers from diarrhea. Due to the restoration of hydric and electrolytic balance, possible complications might be avoided. Oral rehydration solutions contain glucose, and thereby facilitate the absorption of sodium, water and bicarbonates. The rehydration is also important to reconstitute the milieu which is acidified due to diarrhea (Castelli, Saleri et al. 2006).

WHO/UNICEF oral rehydration solution		Home-made solution	
Water	1 liter	Water	1 liter
Glucose (anhydrous)	20 g	Sucrose	5 spoons of ordinary sugar
Sodium chloride	3.5 g	Sodium chloride	1 coffee-spoon of ordinary salt
Sodium bicarbonate *or*	2.5 g	Potassium	fruit juices
Trisodium citrate	2.9 g		
Potassium chloride	1.5 g		

Figure IX. Composition of WHO/UNICEF and home-made oral rehydration solutions.
(Scarpignato and Rampal 1995; Castelli, Saleri et al. 2006)

Symptomatic Therapy:

Symptomatic therapy is needed to reduce the number of unformed stools. Most common medications are bismuth subsalicylate preparation and loperamide, which have an antisecretory effect (Scarpignato and Rampal 1995).

Antibiotic Therapy:

While mild cases of traveller's diarrhea should only be treated with rehydration or eventually a symptomatic therapy, severe cases might need antibiotic treatment. Nevertheless it should be considered that antibiotics have adverse effects and thereby should be used carefully. Trimethoprim-Sulfamethoxazole seems to be the first choice, followed by Doxycycline. Fluoroquinolones and Azithromycin are a further antibiotic choice. In uncomplicated infectious diarrhea, rifaximin seems to be the best treatment due to it's poor absorption ability (Castelli, Saleri et al. 2006).

1.2.4.2.2. Treatment of Antibiotic-Associated Diarrhea

The standard treatment for Clostridium difficile infection is Vancomycin or Metronidazole. The response rate for Metrondiazole is declining and therefore alternative therapies might be a major interest in health care systems (McFarland, Beneda et al. 2007).

1.2.4.2.3. Treatment of Constipation

Treatment with laxatives is the common therapy for patients suffering from constipation. This treatment is well established and safe, however it is not helpful for all patients and therefore it is necessary to establish other possibilities (Chmielewska and Szajewska; Bongers, Benninga et al. 2009).

1.2.4.3. Composition and Differences to Normal Microbiota

In healthy individuals the human gut microbiota protects the gastrointestinal tract from enteric infections (Sekirov and Finlay 2009).

1.2.4.3.1. Antibiotic Associated Diarrhea

Sekirov et al conducted a trial in which antibiotic treatment led to a significant increase of the inflammatory response to Salmonella enterica infection (Sekirov and Finlay 2009). The chief cause for antibiotic-induced diarrhea (AAD) is Clostridium difficile which is responsible for 10-25% of all cases of AAD and for nearly all cases of pseudomembranous colitis (Bartlett 1987). Clostridium difficile is known as a causative agent of AAD since 1978 (Bartlett, Moon et al. 1978). Two toxins are produced by Clostridium difficile, which are responsible for the mucosal damage and inflammation in the colon (Hogenauer, Hammer et al. 1998).
Further bacteria which might be involved in antibiotic-associated diarrhea are C.perfringens, Staphylococcus aureus, Candida spp., Klebsiella oxytoca and Salmonella spp. (Hogenauer, Hammer et al. 1998).

1.2.4.3.2. Infants Diarrhea

Alterations in the composition of gut microbiota appear during diarrhea. The amount of fecal bacteria especially of Bacteroides-Prevotella-Porphyromonas group, E. rectale, L. acidophilus and F. prausnitzii group are reduced during acute diarrhea in contrast to periods of a normal health state (Balamurugan, Janardhan et al. 2008).

1.2.4.3.3. Diarrhea in Elderly

Especially Bifidobacteria, Lactobacilli and Bacteroides are crucially reduced which enables pathogens to enforce (Sullivan, Edlund et al. 2001).

1.2.4.3.4. Constipation

Patients suffering from constipation have a different composition of microbiota in comparison to healthy individuals (Chmielewska and Szajewska; Salminen and Salminen 1997; Zoppi, Cinquetti et al. 1998). Different species of Clostridia and Bifidobacteria and Enterobacteriaceae seem to be increased in comparison to healthy individuals (Chmielewska and Szajewska; Picard, Fioramonti et al. 2005).

1.2.4.4. Probiotics and Counselling

1.2.4.4.1. Alimentary Precautions in Traveller Diarrhea

Due to changed food consumption during travel time humans are confronted, mostly with bacterial agents which they are not used to, and therefore the mucosal immunity is lacking, which then leads to altered bacterial colonization, in addition to inflammation and finally to diarrhea. Hence the general advisement exists: “boil it, cook it, peel it, or forget it”. Fresh vegetables and fruits should be washed and peeled, raw and rare cooked food, like meat and fish should be avoided as well as dietary products and drinking tap water should be boiled for 3-5 minutes before drinking (Castelli and Carosi 1995; Castelli, Saleri et al. 2006).

1.2.4.4.2. Probiotics

In order to assess the specific functions of probiotics in the gastrointestinal tract, a better regime in defined clinical situations is necessary. Furthermore a mix of different probiotic strains with improved designed functions are urgently needed to reach a clinical success (Madsen 2008).
Several strains possess the qualification to produce proteins which either degrade the toxins generated by Clostridium difficile or increase the immune response to Clostridium difficile toxins A and B (Kekkonen, Lummela et al. 2008).

1.2.4.4.2.1. Probiotics in Diarrhea

Lactobacillus rhamnosus was adequate for treating rotaviral diarrhea in a double blind study. Treatment with Lactobacillus rhamnosus reduced the morbidity due to shortening the time of dehydration and hence the time of intravenous rehydration was diminished (Goossens, Jonkers et al. 2003).
Probiotics are suitable as a prophylaxis for travelling diarrhea. A Meta-analysis from Mc FARLAND (2007) summarised 12 studies with a total of 4709 probands about probiotic supplementation. It reasoned that probiotics can significantly reduce the risk to contract diarrhea by about 15%. Lactobacillus acidophilus combined with Bifidobacterium bifidum or Saccharomyces boulardii, indicate the best effects for a prophylaxis (Schulze, Sonnenborn et al. 2008).

Yet currently conflicting results in studies exist (Katelaris, Salam et al. 1995) and a positive impact has just been proven by Saccharomyces boulardii (D'Souza, Rajkumar et al. 2002) and Lactobacilus GG (Marteau, de Vrese et al. 2001; Castelli, Saleri et al. 2006). Although probiotics are safe, the data is not sufficient enough to use them as a solely preventive therapy (Castelli, Saleri et al. 2006).

1.2.4.4.2.2. Probiotics in Infant's Diarrhea

A prevention study in a child care centre in Israel was accessed in order to assess the impact of Bifidobacterium lactis or Lactobacillus reuteri strain in comparison to a placebo in diarrhea. Due to the probiotic treatment, the amount of days with diarrhea was shortened although only Lactobacillus reuteri showed significant effects (Bergonzelli, Blum et al. 2005; Weizman, Asli et al. 2005). Lactic acid bacteria even when belonging to the same species have different impacts on diarrhea. While L. rhamnosus GG reduced the duration of diarrhea and the quantity of watery stools, L. rhamnosus strain Lactophilus had no impact (Majamaa, Isolauri et al. 1995; Bergonzelli, Blum et al. 2005). In a study of 230 hospitalized Bangladeshi children, due to acute gastroenteritis, administration with L.paracasei NCC2461 was assessed. The treatment had no effect on the rota-virus associated diarrhea but in children with non-rotavirus diarrhea the duration time was reduced. Furthermore the stool output became reduced and stool frequency and oral rehydration solution could be reduced indeed (Bergonzelli, Blum et al. 2005; Sarker, Sultana et al. 2005). In Allgeria the consumption of yoghurt containing L.delbrueckii and S. thermophilus significantly reduced the duration of diarrhea and reduced the number of watery stools in comparison to children which were fed with milk formula (Boudraa, Touhami et al. 1990; Boudraa, Benbouabdellah et al. 2001; Bergonzelli, Blum et al. 2005). Another study with malnourished Indian children could not reach such positive results (Bhatnagar, Singh et al. 1998). Lactobacillus GG seems to have an impact in European children while in children from developing countries it seems to have no effect (Bergonzelli, Blum et al. 2005). Separate studies with Finnish infants (Isolauri, Juntunen et al. 1991; Kaila, Isolauri et al. 1992) as well as a study with Italian infants (Guarino, Canani et al. 1997) and a further study with an increased Euopean population (Guandalini, Pensabene et al. 2000) showed a positive effect of

Lactobacillus GG, by shortening the duration time of diarrhea as well as reducing the number of diarrheal stools (Bergonzelli, Blum et al. 2005).
In studies with children from developing countries no positive impact could be achieved with probiotic administration (Raza, Graham et al. 1995; Costa-Ribeiro, Ribeiro et al. 2003; Salazar-Lindo, Miranda-Langschwager et al. 2004; Bergonzelli, Blum et al. 2005).

Prevention trials

Lactobacillus GG was administered to 80 children from Poland, which led to a significant reduction of incidence rate in diarrhea but the treatment had no impact on the duration of diarrhea (Szajewska, Kotowska et al. 2001; Bergonzelli, Blum et al. 2005). In comparison to this study, a study from Peru with 200 children found no positive impact due to Lactobacillus GG treatment (Oberhelman, Gilman et al. 1999; Bergonzelli, Blum et al. 2005). Similar results gained the investigators from a finish study with 570 children in whom the treatment with L.rhamnosus GG had no effect on diarrhea (Hatakka, Savilahti et al. 2001; Bergonzelli, Blum et al. 2005).

The above mentioned studies showed conflicting results. While in European countries Lactobacillus seems to have a positive impact on diarrhea, this is not observable in developing countries. While in developing countries bacterial pathogens seem to play an important role, rotavirus is crucial for diarrhea development in industrialized countries. On the one hand this could be an explanation why in the studies above, probiotic treatment did not have an impact in developing countries. Otherwise in the studies, the L.paracasei treatment was only efficient in non-rotavirus diarrhea which led to the conclusion by Bergonzelli et al. that a combination of L.paracasei NCC2461 and L.rhamnosus GG might be helpful against viral and bacterial diarrhea (Bergonzelli, Blum et al. 2005).

1.2.4.4.2.3. Probiotics and Prebiotics in Constipation

Probiotics

A trial was implemented on elderly from a nursing home suffering from chronic constipation. Participants received lactic acid bacteria, more precisely Lactobacillus acidophilus, Pediococcus pentosaceus and Bifidobacterium longum SPM1205 twice a day for 2 weeks. Fecal samples were collected before the trial and after the trial.

Results showed an increase of defecation and amount of stool defecation occurred due to probiotic administration. Nevertheless, the changes were not significant. Investigators concluded that probiotics might lead to an alleviation of symptoms and might therefore be used as additional treatment (An, Baek et al.). B.lactis DN-173010 improved the colonic transit in a healthy population (Picard, Fioramonti et al. 2005) as well as in patients suffering from constipation (Chmielewska and Szajewska; Agrawal, Houghton et al. 2008). Möllenbrick and Bruckschen conducted a study with 70 constipated patients. Patients received either E.coli Nissle 1917 or a placebo. Investigators found that the treatment led to a significant improvement in tolerance and effectiveness in the probiotic group in comparison to the placebo group (Chmielewska and Szajewska; Mollenbrink and Bruckschen 1994).

In another study 140 adults suffering from constipation received two probiotics strains, namely E.coli Nissle 1917 and L. casei Shirota. The probiotic administration significantly improved stool frequency and consistency (Chmielewska and Szajewska; Banaszkiewicz and Szajewska 2005).

Koebnick et al. conducted a study with 70 patients suffering from constipation. The probiotic group were treated with a beverage enriched with L. casei Shirota, the control group received a placebo beverage. Results demonstrated a significant improvement in severity, stool consistency, occurrence of hard stools and frequency of defecation (Chmielewska and Szajewska; Koebnick, Wagner et al. 2003).

Yang et al. accomplished a study over a two week time frame with women suffering from constipation. Participants received either a fermented milk product enriched with B. lactis DN-173 010, S. thermophilus and L. bulgaricus (experimental group) or acidified milk which contained non-living bacteria (control group). The experimental group had in comparison to the control group a higher stool frequency, only after one week. The defecation conditions were significantly improved as well as the stool consistency in the experimental group (Chmielewska and Szajewska; Yang, He et al. 2008).

Ouwehand et al. concluded that a combined administration with L.rhamnosus and Propionibacterium freudenreichii increased the defecation frequency (Chmielewska and Szajewska; Ouwehand, Lagstrom et al. 2002).

In a trial from Lee et al. constipated patients and healthy subjects received either Streptococcus faecium combined with Bacillus subtilis, or a placebo. The trial lasted 2 weeks. Results indicated that due to probiotic treatment an improved defecation

and a better bowel preparation occurred in the probiotic group in comparison to the placebo group (Lee, Kim et al. 2009).

Prebiotics

Lactulose and Sorbitol are besides others, nondigestible disaccharides and have a laxative effect. Furthermore they are regarded as safe and enhance the growth of the Bifidoflora. In elderly suffering from constipation administration with Galacto-Oligosaccharides, led to an alleviation of constipation (Kaur and Gupta 2002; Roy, Kien et al. 2006). In general, prebiotics seem to have a beneficial impact in constipation therapy. Prebiotics increased stool frequency in healthy individuals (Hond, Geypens et al. 2000; Roy, Kien et al. 2006). Acetate and propionate seem to have impact on colonic blood flow. Due to short chain fatty acids the contractility of the colon is certainly increased in rats. Short chain fatty acids seem to have an impact on the upper gut musculature, enhance nutrient absorption by their impact on blood flow and fasten transit through the colon (Roy, Kien et al. 2006).

1.2.4.5. Modern Molecular Findings, Probiotics, Prebiotics and Counselling

1.2.4.5.1. Probiotics

Probiotics have beneficial effects on the modulation of intestinal barrier function which is formed by intestinal epithelial cells and intercellular junctions. Treatment with E.coli Nissle 1917 fostered gene and protein expression of the tight junction molecule ZO-1 (Ukena, Singh et al. 2007).

Lipopolysaccharides (LPS) are a triggering factor for inflammation. Via the toll like receptor 4 pathway, they induce TNF-α, IL-1 and IL-6 which are pro-inflammatory cytokines (Peluso, Fina et al. 2007). In an animal trial investigators came to the result that Bifidobacteria seem to improve the barrier function of the gut mucosa. Due to the increase of Bifidobacteria the translocation of bacterial LPS could be reduced (Riedel, Foata et al. 2006). L.paracasei subsp. Paracasei B21060 blocked the T cell growth and might therefore be beneficial in modulating immune response (Peluso, Fina et al. 2007).

L.reureri produces a bacteriocin, namely reuterin which is effective against yeast, fungi, protozoa and viruses (Spinler, Taweechotipatr et al. 2008).

Probiotics have a positive impact for patients suffering from constipation because they lower the pH in the colon which leads to an increased production of short chain fatty acids by bacteria. Additionally a lower pH fosters the peristalsis in the colon (Salminen and Salminen 1997) and thereby decreases colonic transit time (Chmielewska and Szajewska).

B. animalis DN-173 010 has a positive impact on transit time and is therefore appropriate as additional therapy for patients suffering from constipation. However it is just as effective in living form and not heat-treated (Chmielewska and Szajewska; Bouvier, Meance et al. 2001).

1.2.4.5.2. Prebiotics

Patients suffering from diarrhea seem to reach a symptom-alleviation by prebiotic administration. Due to the short chain fatty acid generation out of prebiotics, the absorption of Na^+ is fostered. Oral rehydration solutions should be supplemented with Oligosaccharides. Short chain fatty acids increase the growth and differentation of certain bacteria, furthermore they up-regulate Toll like receptors and thereby influence the epithelial crosstalk (Forchielli and Walker 2005; Roy, Kien et al. 2006), additionally they stimulate production of cytokines like IL-10 and INF-γ, and foster the generation of polymeric IgA (Roller, Rechkemmer et al. 2004; Zapolska-Downar, Siennicka et al. 2004; Roy, Kien et al. 2006). Nevertheless no meaningful studies have been conducted with prebiotics and their impact on diarrhea until now (Roy, Kien et al. 2006).

During enteral tube feeding, diarrhea is very common (Euler, Mitchell et al. 2005; Roy, Kien et al. 2006). Colonic atrophy and a deficit of short chain fatty acids might be the cause for frequent diarrhea. The short chain fatty acid deficit leads to a decline of anaerobes and to an increase of aerobes, which could have detrimental effects (Schneider, Le Gall et al. 2000; Schneider, Girard-Pipau et al. 2005; Roy, Kien et al. 2006). Therefore enrichment of enteral formula with Fructooligosaccharides increased Bifidobacteria and reduced Lactobacilli and of course a 2-fold increase of SCFA concentrations was notable (Whelan, Judd et al. 2005; Roy, Kien et al. 2006). Enrichment of tube feeding with pectin led to a decrease in liquid stools and the formula was better tolerated (Zimmaro, Rolandelli et al. 1989; Roy, Kien et al. 2006).

2. DISCUSSION

The aim of this work was the assessment of the impact of probiotic administration in patients suffering from genetic and molecular dispositions.
Several studies in which the compositions of the gut microbiota in diverse dispositions were assessed are discussed in this work. Furthermore the influence of probiotic administration was evaluated in these studies.
In recent years genetic and molecular dispositions increased, especially in westernized countries. On this account additional therapies are more than ever needed to get this vast increase of dispositions under control.
The hygiene hypothesis declares that the increase of atopic diseases is associated with the reduced exposure of microbes in early infancy. This is due to environmental changes in industrialized countries. Sanitation and living conditions are improved and vaccination and antimicrobial therapy have their impact as well. Additionally the changed microbiota might be associated with the vast increase of obesity (Isolauri, Kalliomaki et al. 2009).
Many questions have to be clarified before it is possible to determine if differences in the gut may lead to different weight in humans. It has to be determined if the alterations in the gut are the cause or the result for obesity. Also hormones and other signals and their influence on the composition of the microbiota must be regarded. Besides the understanding of the relative proportions of Firmicutes, Bacteroidetes and Archaea in mice and humans have to be elucidated. The environmental as well as the genetic factors of each individual must also be regarded, but also the surface-adherent differences of the microbiota in the mucosa must be elucidated for obese and person who already lost weight (DiBaise, Zhang et al. 2008).
The character and specifity of counselling concerning inflammatory bowel diseases has changed due to increased microbiological knowledge. Due to the fact that the knowledge is permanently developing, it is difficult to assess in which direction it will lead.
At the moment the core microbiome attracts increased interest leading to intensive discussions. The consequences of the microbiome in association with the disorders are still waiting to be investigated. Investigations of the microbiome may lead to better therapeutic treatment of single disorders.

3. ABSTRACT

The composition of microbiota seems to differ between healthy individuals and patients suffering from genetic and molecular disorders. Several studies have already been conducted to assess whether the changed microbiota may be the cause or the consequence of the disorders. Until now it has not been proven that the alternated microflora compositions are determining in disease development. Nevertheless it has been proven that environmental, genetic and as well as immunological factors influence the development of several disorders such as, allergies, obesity, inflammatory bowel disease, diarrhea and constipation, of which will be discussed in detail in this work. Several studies have already identified differences between microbiota in healthy individuals in comparison to patients suffering from disorders. The disorders have in common, that the bacterial diversity appears to be reduced while the bacterial load seems to be increased.

In patients with Inflammatory bowel disease the amount of Firmicutes particularly Lactobacilli, Bifidobacteria and Faecalibacterium prausnitzii is lowered while the amount of E.coli has increased in those patients.

In contrary to obese humans the Firmicutes concentration is increased, while the Bacteroidetes abundance is diminished. Some bacteria seem to extract more calories out of edibles compared to others, and are therefore supposed to be decisive in obesity development. The phyla Archaea seem to be indeed crucial, particularly Methanobrevibacter smithii. The combined occurrence of Methanobrevibacter smithii and Bacteroides thetaiotaomicron influence the energy extraction out of edibles.

Allergy development is affected as well by the bacterial colonisation. Children who develop an allergy appear to have an increased colonisation with Clostridia, Enterobacteria and Staphylococcus, whereas Bifidobacteria and Lactobacilli are lowered in their amount in early childhood.

Patients suffering from diarrhea or constipation have an alternated microbiota as well. Bifidobacteria, Lactobacilli and Bacteroides seem to be crucially reduced whereby potential pathogen bacteria can be enforced and consequently damage the gut.

Nutritional recommendations for specified diseases based on modern molecular findings are provided in this work. Due to the alternated microbiota, administration with probiotics might be a safe and inexpensive therapeutic target to alleviate the symptoms. Especially administration with Lactobacilli and Bifidobacteria seems to have a beneficial impact. Nevertheless there are several studies with conflicting results and therefore further studies are needed to aggravate this assumption.

4. ZUSAMMENFASSUNG

Die Zusammensetzung der Mikrobiota scheint sich bei Personen mit genetischen und molekularen Erkrankungen individuell zu unterscheiden. Es wurden bereits verschiedene Studien durchgeführt, um festzustellen, ob die veränderte Mikrobiota-Zusammensetzung die Ursache oder die Folge der Erkrankungen waren.

Bisher konnte noch nicht bestätigt werden, dass die veränderte Zusammensetzung der Mikroflora für den Krankheitsverlauf entscheidend ist. Trotz allem ist bekannt, dass umweltbedingte, genetische, sowie immunologische Faktoren Einfluss auf den Krankheitsverlauf unterschiedlichster Erkrankungen wie Allergien, Adipositas, chronisch entzündliche Darmerkrankungen, Diarrhö und Obstipation haben. Diese Erkrankungen werden in der folgenden Arbeit genauer untersucht. Durch diverse Studien konnten bereits Unterschiede in der mikrobiellen Zusammensetzung bei gesunden im Vergleich zu kranken Individuen festgestellt werden. Alle Erkrankungen haben gemein, dass die bakterielle Diversität signifikant reduziert ist, wohingegen die bakteriellen Konzentrationen deutlich erhöht sind.

Patienten, die an chronisch entzündlichen Darmerkrankungen leiden, weisen eine Reduktion an Firmicutes auf, vor allem Laktobazillen, Bifidobakterien, und Faecalibacterium prausnitzii sind vermindert, während E.coli-Gehalte bei diesen Patienten erhöht sind.

Im Gegensatz dazu weisen adipöse Patienten erhöhte Firmicutes-Gehalte auf, während die Menge an Bacteroides reduziert ist. Manche Bakterien scheinen mehr Kalorien aus Lebensmitteln zu extrahieren als andere, weshalb sie von großer Bedeutung für die Adipositas-Entwicklung sind. Des Weiteren scheint das Phylum Archaea, vor allem Methanobrevibacter smithii, eine bedeutende Rolle zu spielen. Gemeinsames Vorkommen von Methanobrevibacter smithii und Bacteroides thetaiotaomicron beeinflusst die Energieextraktion aus Lebensmitteln.

Die Entwicklung allergischer Erkrankungen steht ebenfalls in Zusammenhang mit der bakteriellen Kolonisation des Darmes. Kinder, die eine Allergie entwickeln, weisen eine erhöhte Kolonisation von Clostridien, Enterobakterien und Staphylococcus auf, während die Menge an Bifidobakterien und Lactobacillen in der frühen Kindheit reduziert ist.

Patienten, die von Diarrhö oder Obstipation betroffen sind, weisen ebenfalls eine veränderte Mikrobiota-Zusammensetzung auf. Bifidobakterien, Lactobacillen und Bacteroides scheinen deutlich reduziert zu sein, wodurch potentiell pathogene Bakterien vermehrt auftreten und damit den Darm schädigen können.

Ernährungsempfehlungen für diese speziellen Krankheiten basieren auf modernen molekularen Erkenntnissen und werden in dieser Arbeit behandelt. Aufgrund der veränderten Mikrobiota könnte eine Behandlung mit Probiotika ein sicheres und billiges therapeutisches Ziel sein, um die Symptome zu mildern. Vor allem Laktobazillen und Bifidobakterien haben in diesem Zusammenhang besonders positive Auswirkungen. Allerdings gibt es viele Studien mit widersprüchlichen Ergebnissen, weshalb weiterer Forschungsbedarf auf diesem Gebiet gegeben ist.

5. REFERENCES

Achtman, M. and M. Wagner (2008). "Microbial diversity and the genetic nature of microbial species." Nat Rev Microbiol **6**(6): 431-40.

Adlerberth, I., B. Carlsson, et al. (1991). "Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital-delivered infants." Acta Paediatr Scand **80**(6-7): 602-10.

Adlerberth, I., D. P. Strachan, et al. (2007). "Gut microbiota and development of atopic eczema in 3 European birth cohorts." J Allergy Clin Immunol **120**(2): 343-50.

Agrawal, A., L. A. Houghton, et al. (2008). "Clinical trial: the effects of a fermented milk product containing Bifidobacterium lactis DN-173-010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation." Aliment Pharmacol Ther.

Alam, N. H. and H. Ashraf (2003). "Treatment of infectious diarrhea in children." Paediatr Drugs **5**(3): 151-65.

Albers, R., M. Bol, et al. (2003). "Effects of supplementation with vitamins A, C, and E, selenium, and zinc on immune function in a murine sensitization model." Nutrition **19**(11-12): 940-6.

An, H. M., E. H. Baek, et al. "Efficacy of Lactic Acid Bacteria (LAB) supplement in management of constipation among nursing home residents." Nutr J **9**(1): 5.

Andoh, A. and Y. Fujiyama (2006). "Therapeutic approaches targeting intestinal microflora in inflammatory bowel disease." World J Gastroenterol **12**(28): 4452-60.

Andoh, A., S. Sakata, et al. (2007). "Terminal restriction fragment length polymorphism analysis of the diversity of fecal microbiota in patients with ulcerative colitis." Inflamm Bowel Dis **13**(8): 955-62.

Apajalahti, J. H., A. Kettunen, et al. (2003). "Selective plating underestimates abundance and shows differential recovery of bifidobacterial species from human feces." Appl Environ Microbiol **69**(9): 5731-5.

Armougom, F., M. Henry, et al. (2009). "Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients." PLoS One **4**(9): e7125.

Arseneau, K. O., H. Tamagawa, et al. (2007). "Innate and adaptive immune responses related to IBD pathogenesis." Curr Gastroenterol Rep **9**(6): 508-12.

Asher, M. I. and S. K. Weiland (1998). "The International Study of Asthma and Allergies in Childhood (ISAAC). ISAAC Steering Committee." Clin Exp Allergy **28 Suppl 5**: 52-66; discussion 90-1.

Asp, N. G. (1995). "Classification and methodology of food carbohydrates as related to nutritional effects." Am J Clin Nutr **61**(4 Suppl): 930S-937S.

Backhed, F., H. Ding, et al. (2004). "The gut microbiota as an environmental factor that regulates fat storage." Proc Natl Acad Sci U S A **101**(44): 15718-23.

Backhed, F., R. E. Ley, et al. (2005). "Host-bacterial mutualism in the human intestine." Science **307**(5717): 1915-20.

Backhed, F., J. K. Manchester, et al. (2007). "Mechanisms underlying the resistance to diet-induced obesity in germ-free mice." Proc Natl Acad Sci U S A **104**(3): 979-84.

Balamurugan, R., H. P. Janardhan, et al. (2008). "Molecular studies of fecal anaerobic commensal bacteria in acute diarrhea in children." J Pediatr Gastroenterol Nutr **46**(5): 514-9.

Banaszkiewicz, A. and H. Szajewska (2005). "Probiotics in the treatment of constipation: A systematic review of randomized controlled trials." Pediatria Współczesna **7**: 9-14.

Bartlett, C. L. (1987). "Efficacy of different surveillance systems in detecting hospital-acquired infections." Chemioterapia **6**(3): 152-5.

Bartlett, J. G., N. Moon, et al. (1978). "Role of Clostridium difficile in antibiotic-associated pseudomembranous colitis." Gastroenterology **75**(5): 778-82.

Bartosch, S., A. Fite, et al. (2004). "Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota." Appl Environ Microbiol **70**(6): 3575-81.

Baumgart, D. and W. Sandborn (2007). Inflammatory bowel disease. Lancet.

Beckmann, G. and A. Rüffer (2000). Mikroökologie des Darms. Hannover, Schlütersche GmbH & Co. KG

Bergonzelli, G. E., S. Blum, et al. (2005). "Probiotics as a treatment strategy for gastrointestinal diseases?" Digestion **72**(1): 57-68.

Bernstein, C. N. and F. Shanahan (2008). "Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases." Gut **57**(9): 1185-91.

Bhatnagar, S., K. D. Singh, et al. (1998). "Efficacy of milk versus yogurt offered as part of a mixed diet in acute noncholera diarrhea among malnourished children." J Pediatr **132**(6): 999-1003.

Bibiloni, R., R. N. Fedorak, et al. (2005). "VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis." Am J Gastroenterol **100**(7): 1539-46.

Bjorksten, B. (2004). "Effects of intestinal microflora and the environment on the development of asthma and allergy." Springer Semin Immunopathol **25**(3-4): 257-70.

Bjorksten, B., P. Naaber, et al. (1999). "The intestinal microflora in allergic Estonian and Swedish 2-year-old children." Clin Exp Allergy **29**(3): 342-6.

Bjorksten, B., E. Sepp, et al. (2001). "Allergy development and the intestinal microflora during the first year of life." J Allergy Clin Immunol **108**(4): 516-20.

Boehm, G., J. Jelinek, et al. (2004). "Prebiotics in infant formulas." J Clin Gastroenterol **38**(6 Suppl): S76-9.

Bongers, M. E., M. A. Benninga, et al. (2009). "Health-related quality of life in young adults with symptoms of constipation continuing from childhood into adulthood." Health Qual Life Outcomes **7**: 20.

Bosaeus, I. (2004). "Fibre effects on intestinal functions (diarrhea, constipation and irritable bowel syndrome)." Clin Nutr Suppliments **1**: 33-38.

Bottcher, M. F., E. K. Nordin, et al. (2000). "Microflora-associated characteristics in faeces from allergic and nonallergic infants." Clin Exp Allergy **30**(11): 1590-6.

Boudraa, G., M. Benbouabdellah, et al. (2001). "Effect of feeding yogurt versus milk in children with acute diarrhea and carbohydrate malabsorption." J Pediatr Gastroenterol Nutr **33**(3): 307-13.

Boudraa, G., M. Touhami, et al. (1990). "Effect of feeding yogurt versus milk in children with persistent diarrhea." J Pediatr Gastroenterol Nutr **11**(4): 509-12.

Bousvaros, A., S. Guandalini, et al. (2005). "A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease." Inflamm Bowel Dis **11**(9): 833-9.

Bouvier, M., S. Meance, et al. (2001). "Effects of consumption of a milk fermented by the probiotic Bifidobacterium animalis DN 173 010 on colonic transit time in healthy humans." Biosci Microflora **20**: 43-48.

Brandtzaeg, P. E. (2002). "Current understanding of gastrointestinal immunoregulation and its relation to food allergy." Ann N Y Acad Sci **964**: 13-45.

Bullock, N. R., J. C. Booth, et al. (2004). "Comparative composition of bacteria in the human intestinal microflora during remission and active ulcerative colitis." Curr Issues Intest Microbiol **5**(2): 59-64.

Campieri, M., F. Rizzello, et al. (2000). "Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn's disease: a randomized controlled study versus mesalamine." Gastroenterology **118**: A781.

Campion, J., F. I. Milagro, et al. (2009). "Individuality and epigenetics in obesity." Obes Rev **10**(4): 383-92.

Cani, P. D., J. Amar, et al. (2007). "Metabolic endotoxemia initiates obesity and insulin resistance." Diabetes **56**(7): 1761-72.

Cani, P. D., C. Dewever, et al. (2004). "Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats." Br J Nutr **92**(3): 521-6.

Cani, P. D., E. Joly, et al. (2006). "Oligofructose promotes satiety in healthy human: a pilot study." Eur J Clin Nutr **60**(5): 567-72.

Cani, P. D., A. M. Neyrinck, et al. (2007). "Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia." Diabetologia **50**(11): 2374-83.

Castelli, F. and G. Carosi (1995). "Epidemiology of traveler's diarrhea." Chemotherapy **41 Suppl 1**: 20-32.

Castelli, F., N. Saleri, et al. (2006). "Prevention and treatment of traveler's diarrhea. Focus on antimicrobial agents." Digestion **73 Suppl 1**: 109-18.

Chmielewska, A. and H. Szajewska "Systematic review of randomised controlled trials: probiotics for functional constipation." World J Gastroenterol **16**(1): 69-75.

Comstock, L. E. and M. J. Coyne (2003). "Bacteroides thetaiotaomicron: a dynamic, niche-adapted human symbiont." Bioessays **25**(10): 926-9.

Coppa, G. V., S. Bruni, et al. (2004). "The first prebiotics in humans: human milk oligosaccharides." J Clin Gastroenterol **38**(6 Suppl): S80-3.

Costa-Ribeiro, H., T. C. Ribeiro, et al. (2003). "Limitations of probiotic therapy in acute, severe dehydrating diarrhea." J Pediatr Gastroenterol Nutr **36**(1): 112-5.

Creely, S. J., P. G. McTernan, et al. (2007). "Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes." Am J Physiol Endocrinol Metab **292**(3): E740-7.

Cui, H. H., C. L. Chen, et al. (2004). "Effects of probiotic on intestinal mucosa of patients with ulcerative colitis." World J Gastroenterol **10**(10): 1521-5.

Cummings, J. H. and H. N. Englyst (1995). "Gastrointestinal effects of food carbohydrate." Am J Clin Nutr **61**(4 Suppl): 938S-945S.

Cummings, J. H., M. J. Hill, et al. (1979). "The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine." Am J Clin Nutr **32**(10): 2094-101.

D'Souza, A. L., C. Rajkumar, et al. (2002). "Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis." BMJ **324**(7350): 1361.

DiBaise, J. K., H. Zhang, et al. (2008). "Gut microbiota and its possible relationship with obesity." Mayo Clin Proc **83**(4): 460-9.

Dore, J. (2007). Gut microbiota in health and disease. Report of the 2nd International Workshop in Amsterdam, April**:** 26-28.

Drago, L., M. R. Gismondo, et al. (1997). "Inhibition of in vitro growth of enteropathogens by new Lactobacillus isolates of human intestinal origin." FEMS Microbiol Lett **153**(2): 455-63.

Dubos, R. (1971). Man Adapting. Yale University Press. New Haven.

Dunne, C., L. O'Mahony, et al. (2001). "In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings." Am J Clin Nutr **73**(2 Suppl): 386S-392S.

DuPont, H. L., D. G. Evans, et al. (1982). "Prevention of travelers' diarrhea with trimethoprim-sulfamethoxazole." Rev Infect Dis **4**(2): 533-9.

DuPont, H. L., Z. D. Jiang, et al. (2005). "A randomized, double-blind, placebo-controlled trial of rifaximin to prevent travelers' diarrhea." Ann Intern Med **142**(10): 805-12.

Eckburg, P. B., E. M. Bik, et al. (2005). "Diversity of the human intestinal microbial flora." Science **308**(5728): 1635-8.

Elmadfa, I. and C. Leitzmann (2004). Ernährung des Menschen. Wien, Eugen Ulmer Stuttgart.

Ericsson, C. D. (2003). "Travellers' diarrhoea." Int J Antimicrob Agents **21**(2): 116-24.

Ericsson, C. D. and H. L. DuPont (2005). "Rifaximin in the treatment of infectious diarrhea." Chemotherapy **51 Suppl 1**: 73-80.

Euler, A. R., D. K. Mitchell, et al. (2005). "Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk." J Pediatr Gastroenterol Nutr **40**(2): 157-64.

Fanaro, S., R. Chierici, et al. (2003). "Intestinal microflora in early infancy: composition and development." Acta Paediatr Suppl **91**(441): 48-55.

Fantini, M. C., G. Monteleone, et al. (2007). "New players in the cytokine orchestra of inflammatory bowel disease." Inflamm Bowel Dis **13**(11): 1419-23.

Foley, S. and Q. Hamid (2006). "Inflammatory patterns in allergic rhinitis." Clin Exp Allergy Rev **6**: 91-5.

Forchielli, M. L. and W. A. Walker (2005). "The role of gut-associated lymphoid tissues and mucosal defence." Br J Nutr **93 Suppl 1**: S41-8.

Frank, D. N., A. L. St Amand, et al. (2007). "Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases." Proc Natl Acad Sci U S A **104**(34): 13780-5.

Frank, L., J. Schmier, et al. (2002). "Time and economic cost of constipation care in nursing homes." J Am Med Dir Assoc **3**(4): 215-23.

Furrie, E. (2006). "A molecular revolution in the study of intestinal microflora." Gut **55**(2): 141-3.

Garcia Vilela, E., M. De Lourdes De Abreu Ferrari, et al. (2008). "Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission." Scand J Gastroenterol **43**(7): 842-8.

Garn, H. and H. Renz (2007). "Epidemiological and immunological evidence for the hygiene hypothesis." Immunobiology **212**(6): 441-52.

Giaffer, M. H., C. D. Holdsworth, et al. (1991). "The assessment of faecal flora in patients with inflammatory bowel disease by a simplified bacteriological technique." J Med Microbiol **35**(4): 238-43.

Gibson, G. R., E. R. Beatty, et al. (1995). "Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin." Gastroenterology **108**(4): 975-82.

Gibson, G. R. and M. B. Roberfroid (1995). "Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics." J Nutr **125**(6): 1401-12.

Gill, H. S. (2003). "Probiotics to enhance anti-infective defences in the gastrointestinal tract." Best Pract Res Clin Gastroenterol **17**(5): 755-73.

Gill, S. R., M. Pop, et al. (2006). "Metagenomic analysis of the human distal gut microbiome." Science **312**(5778): 1355-9.

Gionchetti, P., F. Rizzello, et al. (2006). "Antibiotics and probiotics in treatment of inflammatory bowel disease." World J Gastroenterol **12**(21): 3306-13.

Goossens, D., D. Jonkers, et al. (2003). "Probiotics in gastroenterology: indications and future perspectives." Scand J Gastroenterol Suppl(239): 15-23.

Gore, C., K. Munro, et al. (2008). "Bifidobacterium pseudocatenulatum is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants." J Allergy Clin Immunol **121**(1): 135-40.

Guandalini, S., L. Pensabene, et al. (2000). "Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial." J Pediatr Gastroenterol Nutr **30**(1): 54-60.

Guarino, A., R. B. Canani, et al. (1997). "Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea." J Pediatr Gastroenterol Nutr **25**(5): 516-9.

Guarner, F. (2005). "Inulin and oligofructose: impact on intestinal diseases and disorders." Br J Nutr **93 Suppl 1**: S61-5.

Guarner, F. and J. R. Malagelada (2003). "Gut flora in health and disease." Lancet **361**(9356): 512-9.

Gueimonde, M., S. Sakata, et al. (2006). "Effect of maternal consumption of lactobacillus GG on transfer and establishment of fecal bifidobacterial microbiota in neonates." J Pediatr Gastroenterol Nutr **42**(2): 166-70.

Guillemot, F., J. F. Colombel, et al. (1991). "Treatment of diversion colitis by short-chain fatty acids. Prospective and double-blind study." Dis Colon Rectum **34**(10): 861-4.

Gupta, P., H. Andrew, et al. (2000). "Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study." J Pediatr Gastroenterol Nutr **31**(4): 453-7.

Guslandi, M., P. Giollo, et al. (2003). "A pilot trial of Saccharomyces boulardii in ulcerative colitis." Eur J Gastroenterol Hepatol **15**(6): 697-8.

Guslandi, M., G. Mezzi, et al. (2000). "Saccharomyces boulardii in maintenance treatment of Crohn's disease." Dig Dis Sci **45**(7): 1462-4.

Halevy, O. and D. Sklan (1987). "Inhibition of arachidonic acid oxidation by beta-carotene, retinol and alpha-tocopherol." Biochim Biophys Acta **918**(3): 304-7.

Hanifin, J. M. (2002). "Atopiform dermatitis: do we need another confusing name for atopic dermatitis?" Br J Dermatol **147**(3): 430-2.

Harig, J. M., K. H. Soergel, et al. (1989). "Treatment of diversion colitis with short-chain-fatty acid irrigation." N Engl J Med **320**(1): 23-8.

Harmsen, H. J., A. C. Wildeboer-Veloo, et al. (2000). "Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods." J Pediatr Gastroenterol Nutr **30**(1): 61-7.

Hartman, C., R. Eliakim, et al. (2009). "Nutritional status and nutritional therapy in inflammatory bowel diseases." World J Gastroenterol **15**(21): 2570-8.

Hatakka, K., E. Savilahti, et al. (2001). "Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial." BMJ **322**(7298): 1327.

He, F., H. Morita, et al. (2002). "Intestinal Bifidobacterium species induce varying cytokine production." J Allergy Clin Immunol **109**(6): 1035-6.

He, F., A. C. Ouwehand, et al. (2001). "Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants." FEMS Immunol Med Microbiol **30**(1): 43-7.

Herias, M. V., J. F. Koninkx, et al. (2005). "Probiotic effects of Lactobacillus casei on DSS-induced ulcerative colitis in mice." Int J Food Microbiol **103**(2): 143-55.

Hildebrandt, M. A., C. Hoffmann, et al. (2009). "High-fat diet determines the composition of the murine gut microbiome independently of obesity." Gastroenterology **137**(5): 1716-24 e1-2.

Hogenauer, C., H. F. Hammer, et al. (1998). "Mechanisms and management of antibiotic-associated diarrhea." Clin Infect Dis **27**(4): 702-10.

Hond, E., B. Geypens, et al. (2000). "Effect of high performance chicory inulin on constipation." Nutr Res **20**: 731-736.

Isolauri, E., T. Arvola, et al. (2000). "Probiotics in the management of atopic eczema." Clin Exp Allergy **30**(11): 1604-10.

Isolauri, E., M. Juntunen, et al. (1991). "A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children." Pediatrics **88**(1): 90-7.

Isolauri, E., M. Kalliomaki, et al. (2008). "Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease." Curr Pharm Des **14**(14): 1368-75.

Isolauri, E., M. Kalliomaki, et al. (2009). "Obesity - extending the hygiene hypothesis." Nestle Nutr Workshop Ser Pediatr Program **64**: 75-85; discussion 85-9, 251-7.

Ivory, K., S. J. Chambers, et al. (2008). "Oral delivery of Lactobacillus casei Shirota modifies allergen-induced immune responses in allergic rhinitis." Clin Exp Allergy **38**(8): 1282-9.

Kaila, M., E. Isolauri, et al. (1992). "Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain." Pediatr Res **32**(2): 141-4.

Kalliomaki, M., M. C. Collado, et al. (2008). "Early differences in fecal microbiota composition in children may predict overweight." Am J Clin Nutr **87**(3): 534-8.

Kalliomaki, M., P. Kirjavainen, et al. (2001). "Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing." J Allergy Clin Immunol **107**(1): 129-34.

Kalliomaki, M., S. Salminen, et al. (2001). "Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial." Lancet **357**(9262): 1076-9.

Kalliomaki, M., S. Salminen, et al. (2008). "Positive interactions with the microbiota: probiotics." Adv Exp Med Biol **635**: 57-66.

Kalliomaki, M., S. Salminen, et al. (2003). "Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial." Lancet **361**(9372): 1869-71.

Kanauchi, O., K. Mitsuyama, et al. (2003). "Modification of intestinal flora in the treatment of inflammatory bowel disease." Curr Pharm Des **9**(4): 333-46.

Kankaanpaa, P. E., S. J. Salminen, et al. (2001). "The influence of polyunsaturated fatty acids on probiotic growth and adhesion." FEMS Microbiol Lett **194**(2): 149-53.

Karmaus, W. and C. Botezan (2002). "Does a higher number of siblings protect against the development of allergy and asthma? A review." J Epidemiol Community Health **56**(3): 209-17.

Katelaris, P. H., I. Salam, et al. (1995). "Lactobacilli to prevent traveler's diarrhea?" N Engl J Med **333**(20): 1360-1.

Kato, K., S. Mizuno, et al. (2004). "Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis." Aliment Pharmacol Ther **20**(10): 1133-41.

Kaur, N. and A. K. Gupta (2002). "Applications of inulin and oligofructose in health and nutrition." J Biosci **27**(7): 703-14.

Kean, B. H. (1986). "Travelers' diarrhea: an overview." Rev Infect Dis **8 Suppl 2**: S111-6.

Kekkonen, R. A., N. Lummela, et al. (2008). "Probiotic intervention has strain-specific anti-inflammatory effects in healthy adults." World J Gastroenterol **14**(13): 2029-36.

Kelly, D., J. I. Campbell, et al. (2004). "Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA." Nat Immunol **5**(1): 104-12.

Khan, M., D. Raoult, et al. (2007). "Growth-promoting effects of single-dose intragastrically administered probiotics in chickens." Br Poult Sci **48**(6): 732-5.

Kien, C. L., M. Schmitz-Brown, et al. (2006). "Increased colonic luminal synthesis of butyric acid is associated with lowered colonic cell proliferation in piglets." J Nutr **136**(1): 64-9.

Kirjavainen, P. V., T. Arvola, et al. (2002). "Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning?" Gut **51**(1): 51-5.

Klampfer, L., J. Huang, et al. (2003). "Inhibition of interferon gamma signaling by the short chain fatty acid butyrate." Mol Cancer Res **1**(11): 855-62.

Kluthe, R., A. Dittrich, et al. (2004). "Das Rationalisierungsschema 2004 des Bundesverbandes Deutscher Ernährungsmediziner (BDEM) e.V., der Deutschen Adipositas Gesellschaft e.V., der Deutschen Akademie für

Ernährungsmedizin (DAEM) e.V., der Deutschen Gesellschaft für Ernährung (DGE) e.V., der Deutschen Gesellschaft für Ernährungsmedizin (DGEM) e.V., des Verbandes der Diätsassistenten - Deutscher Bundesverband (VDD) e.V. und des Verbandes der Diplom-Oecotrophologen (VDO_E) e.V." Aktuelle Ernaehrung Med 2004 **29**: 245-253.

Koebnick, C., I. Wagner, et al. (2003). "Probiotic beverage containing Lactobacillus casei Shirota improves gastrointestinal symptoms in patients with chronic constipation." Can J Gastroenterol **17**(11): 655-9.

Kolida, S. and G. R. Gibson (2007). "Prebiotic capacity of inulin-type fructans." J Nutr **137**(11 Suppl): 2503S-2506S.

Kopp, M. V., I. Hennemuth, et al. (2008). "Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation." Pediatrics **121**(4): e850-6.

Kramer, D. R., R. M. Sutherland, et al. (1995). "Cytokine mediated effects in mucosal immunity." Immunol Cell Biol **73**(5): 389-96.

Kruis, W., P. Fric, et al. (2004). "Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine." Gut **53**(11): 1617-23.

Kruis, W., E. Schutz, et al. (1997). "Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis." Aliment Pharmacol Ther **11**(5): 853-8.

Kukkonen, K., E. Savilahti, et al. (2007). "Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial." J Allergy Clin Immunol **119**(1): 192-8.

La Vecchia, C., A. Decarli, et al. (1998). "Vegetable consumption and risk of chronic disease." Epidemiology **9**(2): 208-10.

Lahtinen, S. J., R. J. Boyle, et al. (2009). "Prenatal probiotic administration can influence Bifidobacterium microbiota development in infants at high risk of allergy." J Allergy Clin Immunol **123**(2): 499-501.

Laitinen, K., M. Kalliomaki, et al. (2005). "Evaluation of diet and growth in children with and without atopic eczema: follow-up study from birth to 4 years." Br J Nutr **94**(4): 565-74.

Lammers, K. M., P. Brigidi, et al. (2003). "Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells." FEMS Immunol Med Microbiol **38**(2): 165-72.

Lanfranchi, G. A., C. Brignola, et al. (1984). "Assessment of nutritional status in Crohn's disease in remission or low activity." Hepatogastroenterology **31**(3): 129-32.

Larsen, T. M., S. Toubro, et al. (2006). "Conjugated linoleic acid supplementation for 1 y does not prevent weight or body fat regain." Am J Clin Nutr **83**(3): 606-12.

Layton, A., L. McKay, et al. (2006). "Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water." Appl Environ Microbiol **72**(6): 4214-24.

Lee, H., Y. H. Kim, et al. (2009). "A Feasibility Study of Probiotics Pretreatment as a Bowel Preparation for Colonoscopy in Constipated Patients." Dig Dis Sci.

Lee, H. Y., J. H. Park, et al. (2006). "Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice." Biochim Biophys Acta **1761**(7): 736-44.

Lee, Y. K. and K. Y. Puong (2002). "Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate." Br J Nutr **88 Suppl 1**: S101-8.

Lepage, P., P. Seksik, et al. (2005). "Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD." Inflamm Bowel Dis **11**(5): 473-80.

Ley, R. E., F. Backhed, et al. (2005). "Obesity alters gut microbial ecology." Proc Natl Acad Sci U S A **102**(31): 11070-5.

Ley, R. E., P. J. Turnbaugh, et al. (2006). "Microbial ecology: human gut microbes associated with obesity." Nature **444**(7122): 1022-3.

Linneberg, A., C. Ostergaard, et al. (2003). "IgG antibodies against microorganisms and atopic disease in Danish adults: the Copenhagen Allergy Study." J Allergy Clin Immunol **111**(4): 847-53.

Lucendo, A. J. and L. C. De Rezende (2009). "Importance of nutrition in inflammatory bowel disease." World J Gastroenterol **15**(17): 2081-8.

Ludvigsson, J. F., M. Krantz, et al. (2004). "Elemental versus polymeric enteral nutrition in paediatric Crohn's disease: a multicentre randomized controlled trial." Acta Paediatr **93**(3): 327-35.

Macfarlane, G. T. and S. Macfarlane (1997). "Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria." Scand J Gastroenterol Suppl **222**: 3-9.

Macfarlane, S., E. Furrie, et al. (2005). "Mucosal bacteria in ulcerative colitis." Br J Nutr **93 Suppl 1**: S67-72.

Mack, D. R., S. Michail, et al. (1999). "Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression." Am J Physiol **276**(4 Pt 1): G941-50.

Mackay, I. M. (2004). "Real-time PCR in the microbiology laboratory." Clin Microbiol Infect **10**(3): 190-212.

Madigan, M. and J. Martinko (2006). Biology of microorganisms. San Francisco, Pearson.

Madsen, K. (2008). "Probiotics in critically ill patients." J Clin Gastroenterol **42 Suppl 3 Pt 1**: S116-8.

Magne, F., A. Suau, et al. (2005). "Fecal microbial community in preterm infants." J Pediatr Gastroenterol Nutr **41**(4): 386-92.

Mah, K. W., B. Bjorksten, et al. (2006). "Distinct pattern of commensal gut microbiota in toddlers with eczema." Int Arch Allergy Immunol **140**(2): 157-63.

Mai, V. and P. V. Draganov (2009). "Recent advances and remaining gaps in our knowledge of associations between gut microbiota and human health." World J Gastroenterol **15**(1): 81-5.

Maidak, B. L., J. R. Cole, et al. (2001). "The RDP-II (Ribosomal Database Project)." Nucleic Acids Res **29**(1): 173-4.

Majamaa, H. and E. Isolauri (1997). "Probiotics: a novel approach in the management of food allergy." J Allergy Clin Immunol **99**(2): 179-85.

Majamaa, H., E. Isolauri, et al. (1995). "Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis." J Pediatr Gastroenterol Nutr **20**(3): 333-8.

Malchow, H. A. (1997). "Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease?" J Clin Gastroenterol **25**(4): 653-8.

Malin, M., H. Suomalainen, et al. (1996). "Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG." Ann Nutr Metab **40**(3): 137-45.

Mancuso, G., A. Midiri, et al. (2005). "Bacteroides fragilis-derived lipopolysaccharide produces cell activation and lethal toxicity via toll-like receptor 4." Infect Immun **73**(9): 5620-7.

Manichanh, C., L. Rigottier-Gois, et al. (2006). "Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach." Gut **55**(2): 205-11.

Marteau, P., M. Lemann, et al. (2006). "Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial." Gut **55**(6): 842-7.

Marteau, P. R., M. de Vrese, et al. (2001). "Protection from gastrointestinal diseases with the use of probiotics." Am J Clin Nutr **73**(2 Suppl): 430S-436S.

Martin, F. P., Y. Wang, et al. (2008). "Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model." Mol Syst Biol **4**: 157.

Martindale, S., G. McNeill, et al. (2005). "Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life." Am J Respir Crit Care Med **171**(2): 121-8.

Martinez-Medina, M., X. Aldeguer, et al. (2006). "Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis." Inflamm Bowel Dis **12**(12): 1136-45.

Matricardi, P. M., F. Rosmini, et al. (2000). "Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study." BMJ **320**(7232): 412-7.

Matsuda, H., Y. Fujiyama, et al. (2000). "Characterization of antibody responses against rectal mucosa-associated bacterial flora in patients with ulcerative colitis." J Gastroenterol Hepatol **15**(1): 61-8.

Matsumoto, M., H. Ohishi, et al. (2004). "Faecal microbiota and secretory immunoglobulin A levels in adult patients with atopic dermatitis." Microbial Ecol Health Dis **16**: 13-17.

Matsumoto, S., T. Hara, et al. (2005). "Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells." Clin Exp Immunol **140**(3): 417-26.

Matsumoto, S., T. Hara, et al. (2009). "A component of polysaccharide peptidoglycan complex on Lactobacillus induced an improvement of murine model of inflammatory bowel disease and colitis-associated cancer." Immunology **128**(1 Suppl): e170-80.

Mattila-Sandholm, T., S. Blum, et al. (1999). "Probiotics: towardss demonstrating efficacy " Trends Food Sci Technol **10**: 393-399.

McCann, K. S. (2000). "The diversity-stability debate." Nature **405**(6783): 228-33.

McFarland, L. V., H. W. Beneda, et al. (2007). "Implications of the changing face of Clostridium difficile disease for health care practitioners." Am J Infect Control **35**(4): 237-53.

McOrist, A. L., G. C. Abell, et al. (2008). "Bacterial population dynamics and faecal short-chain fatty acid (SCFA) concentrations in healthy humans." Br J Nutr **100**(1): 138-46.

Mitsuyama, K., S. Matsumoto, et al. (2008). "Beneficial Effect of Lactobacillus casei in Ulcerative Colitis: A Pilot Study." Journal of Clinical Biochemistry and Nutrition **43**: 78-81.

Mollenbrink, M. and E. Bruckschen (1994). "[Treatment of chronic constipation with physiologic Escherichia coli bacteria. Results of a clinical study of the effectiveness and tolerance of microbiological therapy with the E. coli Nissle 1917 strain (Mutaflor)]." Med Klin (Munich) **89**(11): 587-93.

Müller, A. (2006). Allergologie. München, Urban & Fischer Verlag.

Murray, C. S., G. W. Tannock, et al. (2005). "Fecal microbiota in sensitized wheezy and non-sensitized non-wheezy children: a nested case-control study." Clin Exp Allergy **35**(6): 741-5.

Murry, A. C., R. D. Lewis, et al. (1997). "The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration, and bone mineral density of nursery pigs." J Anim Sci **75**(5): 1284-91.

Mylonaki, M., N. B. Rayment, et al. (2005). "Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease." Inflamm Bowel Dis **11**(5): 481-7.

Nadal, I., A. Santacruz, et al. (2009). "Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents." Int J Obes (Lond) **33**(7): 758-67.

Niers, L., R. Martin, et al. (2009). "The effects of selected probiotic strains on the development of eczema (the PandA study)." Allergy **64**(9): 1349-58.

Nord, C. E., T. Bergan, et al. (1986). "Impact of azlocillin on the colon microflora." Scand J Infect Dis **18**(2): 163-6.

Norin, E., T. Midtvedt, et al. (2004). "Development of faecal short-chain fatty acid pattern during the first year of life in Estonian and Swedish infants." Microbial Ecol Health Dis **16**: 8-12.

Nowak, D., C. Suppli Ulrik, et al. (2004). "Asthma and atopy: has peak prevalence been reached?" Eur Respir J **23**(3): 359-60.

Oberhelman, R. A., R. H. Gilman, et al. (1999). "A placebo-controlled trial of Lactobacillus GG to prevent diarrhea in undernourished Peruvian children." J Pediatr **134**(1): 15-20.

Odamaki, T., J. Z. Xiao, et al. (2007). "Fluctuation of fecal microbiota in individuals with Japanese cedar pollinosis during the pollen season and influence of probiotic intake." J Investig Allergol Clin Immunol **17**(2): 92-100.

Ott, S. J., M. Musfeldt, et al. (2004). "Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease." Gut **53**(5): 685-93.

Ouwehand, A. C., E. Isolauri, et al. (2001). "Differences in Bifidobacterium flora composition in allergic and healthy infants." J Allergy Clin Immunol **108**(1): 144-5.

Ouwehand, A. C., P. V. Kirjavainen, et al. (1999). "Probiotics: mechanisms and established effects." International Dairy Journal **9**: 43-52.

Ouwehand, A. C., H. Lagstrom, et al. (2002). "Effect of probiotics on constipation, fecal azoreductase activity and fecal mucin content in the elderly." Ann Nutr Metab **46**(3-4): 159-62.

Pappo, I., H. Becovier, et al. (1991). "Polymyxin B reduces cecal flora, TNF production and hepatic steatosis during total parenteral nutrition in the rat." J Surg Res **51**(2): 106-12.

Park, J. and M. H. Floch (2007). "Prebiotics, probiotics, and dietary fiber in gastrointestinal disease." Gastroenterol Clin North Am **36**(1): 47-63, v.

Parry, H., A. J. Howard, et al. (1994). "The prophylaxis of travellers' diarrhoea; a double blind placebo controlled trial of ciprofloxacin during a Himalayan expedition." J Infect **28**(3): 337-8.

Pathmakanthan, S., J. Thornley, et al. (1999). "Mucosally associated bacterial flora of the human colon: quantitative and species specific differences between normal and inflammed colonic biopsies." Microbial Ecol Health Dis **11**: 169-174.

Peluso, I., D. Fina, et al. (2007). "Lactobacillus paracasei subsp. paracasei B21060 suppresses human T-cell proliferation." Infect Immun **75**(4): 1730-7.

Penders, J., E. E. Stobberingh, et al. (2007). "The role of the intestinal microbiota in the development of atopic disorders." Allergy **62**(11): 1223-36.

Penders, J., C. Thijs, et al. (2007). "Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study." Gut **56**(5): 661-7.

Petrosino, J. F., S. Highlander, et al. (2009). "Metagenomic pyrosequencing and microbial identification." Clin Chem **55**(5): 856-66.

Picard, C., J. Fioramonti, et al. (2005). "Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits." Aliment Pharmacol Ther **22**(6): 495-512.

Pohjavuori, E., M. Viljanen, et al. (2004). "Lactobacillus GG effect in increasing IFN-gamma production in infants with cow's milk allergy." J Allergy Clin Immunol **114**(1): 131-6.

Prantera, C., M. L. Scribano, et al. (2002). "Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG." Gut **51**(3): 405-9.

Prescott, S. L. and B. Bjorksten (2007). "Probiotics for the prevention or treatment of allergic diseases." J Allergy Clin Immunol **120**(2): 255-62.

Prescott, S. L., J. A. Dunstan, et al. (2005). "Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis." Clin Exp Allergy **35**(12): 1557-64.

Pryde, S. E., S. H. Duncan, et al. (2002). "The microbiology of butyrate formation in the human colon." FEMS Microbiol Lett **217**(2): 133-9.

Qin, J., R. Li, et al. "A human gut microbial gene catalogue established by metagenomic sequencing." Nature **464**(7285): 59-65.

Rajilic-Stojanovic, M., H. Smidt, et al. (2007). "Diversity of the human gastrointestinal tract microbiota revisited." Environ Microbiol **9**(9): 2125-36.

Rastall, R. A. (2004). "Bacteria in the gut: friends and foes and how to alter the balance." J Nutr **134**(8 Suppl): 2022S-2026S.

Rastall, R. A., G. R. Gibson, et al. (2005). "Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications." FEMS Microbiol Ecol **52**(2): 145-52.

Rautava, S., M. Kalliomaki, et al. (2005). "New therapeutic strategy for combating the increasing burden of allergic disease: Probiotics-A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group report." J Allergy Clin Immunol **116**(1): 31-7.

Raza, S., S. M. Graham, et al. (1995). "Lactobacillus GG promotes recovery from acute nonbloody diarrhea in Pakistan." Pediatr Infect Dis J **14**(2): 107-11.

Reiff, C. and D. Kelly (2009). "Inflammatory bowel disease, gut bacteria and probiotic therapy." Int J Med Microbiol.

Rembacken, B. J., A. M. Snelling, et al. (1999). "Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial." Lancet **354**(9179): 635-9.

Renz-Polster, H. and S. Krautzig (2008). Basislehrbuch Innere Medizin. München, Urban und Fischer.

Riedel, C. U., F. Foata, et al. (2006). "Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation." World J Gastroenterol **12**(23): 3729-35.

Riesenfeld, C. S., P. D. Schloss, et al. (2004). "Metagenomics: genomic analysis of microbial communities." Annu Rev Genet **38**: 525-52.

Roberfroid, M. B. (2002). "Functional foods: concepts and application to inulin and oligofructose." Br J Nutr **87 Suppl 2**: S139-43.

Rocha, R., G. O. Santana, et al. (2009). "Analysis of fat and muscle mass in patients with inflammatory bowel disease during remission and active phase." Br J Nutr **101**(5): 676-9.

Roediger, W. E. (1980). "The colonic epithelium in ulcerative colitis: an energy-deficiency disease?" Lancet **2**(8197): 712-5.

Roller, M., G. Rechkemmer, et al. (2004). "Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and

Bifidobacterium lactis modulates intestinal immune functions in rats." J Nutr **134**(1): 153-6.

Romagnani, S. (2006). "Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders?" Allergy **61**(1): 3-14.

Roy, C. C., C. L. Kien, et al. (2006). "Short-chain fatty acids: ready for prime time?" Nutr Clin Pract **21**(4): 351-66.

Sack, D. A., D. C. Kaminsky, et al. (1978). "Prophylactic doxycycline for travelers' diarrhea. Results of a prospective double-blind study of Peace Corps volunteers in Kenya." N Engl J Med **298**(14): 758-63.

Sakata, S., T. Tonooka, et al. (2005). "Culture-independent analysis of fecal microbiota in infants, with special reference to Bifidobacterium species." FEMS Microbiol Lett **243**(2): 417-23.

Salazar-Lindo, E., P. Miranda-Langschwager, et al. (2004). "Lactobacillus casei strain GG in the treatment of infants with acute watery diarrhea: a randomized, double-blind, placebo controlled clinical trial [ISRCTN67363048]." BMC Pediatr **4**: 18.

Salminen, S. and E. Salminen (1997). "Lactulose, lactic acid bacteria, intestinal microecology and mucosal protection." Scand J Gastroenterol Suppl **222**: 45-8.

Samuel, B. S. and J. I. Gordon (2006). "A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism." Proc Natl Acad Sci U S A **103**(26): 10011-6.

Sandin, A., L. Braback, et al. (2009). "Faecal short chain fatty acid pattern and allergy in early childhood." Acta Paediatr **98**(5): 823-7.

Sarker, S. A., S. Sultana, et al. (2005). "Lactobacillus paracasei strain ST11 has no effect on rotavirus but ameliorates the outcome of nonrotavirus diarrhea in children from Bangladesh." Pediatrics **116**(2): e221-8.

Scanlan, P. D., F. Shanahan, et al. (2006). "Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease." J Clin Microbiol **44**(11): 3980-8.

Scarpignato, C. and P. Rampal (1995). "Prevention and treatment of traveler's diarrhea: a clinical pharmacological approach." Chemotherapy **41 Suppl 1**: 48-81.

Schiener, F., B. Reiterer, et al. (2008). CED und Ernährung. Crohnicle ÖMCCV. **1:** 1-96.

Schneider, S. M., F. Girard-Pipau, et al. (2005). "Effects of Saccharomyces boulardii on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition." World J Gastroenterol **11**(39): 6165-9.

Schneider, S. M., P. Le Gall, et al. (2000). "Total artificial nutrition is associated with major changes in the fecal flora." Eur J Nutr **39**(6): 248-55.

Schultz, M., A. Timmer, et al. (2004). "Lactobacillus GG in inducing and maintaining remission of Crohn's disease." BMC Gastroenterol **4**: 5.

Schulze, J., U. Sonnenborn, et al. (2008). Probiotika. Stuttgart, Hippokrates Verlag.

Segain, J. P., D. Raingeard de la Bletiere, et al. (2000). "Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease." Gut **47**(3): 397-403.

Sekirov, I. and B. B. Finlay (2009). "The role of the intestinal microbiota in enteric infection." J Physiol **587**(Pt 17): 4159-67.

Seksik, P., L. Rigottier-Gois, et al. (2003). "Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon." Gut **52**(2): 237-42.

Sepp, E., K. Julge, et al. (2005). "Intestinal microbiota and immunoglobulin E responses in 5-year-old Estonian children." Clin Exp Allergy **35**(9): 1141-6.

Shanahan, F. (2001). "Inflammatory bowel disease: immunodiagnostics, immunotherapeutics, and ecotherapeutics." Gastroenterology **120**(3): 622-35.

Sheil, B., F. Shanahan, et al. (2007). "Probiotic effects on inflammatory bowel disease." J Nutr **137**(3 Suppl 2): 819S-24S.

Shida, K., R. Takahashi, et al. (2002). "Lactobacillus casei strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model." Clin Exp Allergy **32**(4): 563-70.

Shreiner, A., G. B. Huffnagle, et al. (2008). "The "Microflora Hypothesis" of allergic disease." Adv Exp Med Biol **635**: 113-34.

Shroff, K. E., K. Meslin, et al. (1995). "Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut." Infect Immun **63**(10): 3904-13.

Sjogren, Y. M., M. C. Jenmalm, et al. (2009). "Altered early infant gut microbiota in children developing allergy up to 5 years of age." Clin Exp Allergy **39**(4): 518-26.

Smith, P. A. (2008). "Nutritional therapy for active Crohn's disease." World J Gastroenterol **14**(27): 4420-3.

Smits, H. H., A. Engering, et al. (2005). "Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin." J Allergy Clin Immunol **115**(6): 1260-7.

Sokol, H., C. Lay, et al. (2008). "Analysis of bacterial bowel communities of IBD patients: what has it revealed?" Inflamm Bowel Dis **14**(6): 858-67.

Sokol, H., P. Lepage, et al. (2006). "Temperature gradient gel electrophoresis of fecal 16S rRNA reveals active Escherichia coli in the microbiota of patients with ulcerative colitis." J Clin Microbiol **44**(9): 3172-7.

Sokol, H., B. Pigneur, et al. (2008). "Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients." Proc Natl Acad Sci U S A **105**(43): 16731-6.

Sokol, H., P. Seksik, et al. (2009). "Low counts of Faecalibacterium prausnitzii in colitis microbiota." Inflamm Bowel Dis **15**(8): 1183-9.

Songjinda, P., J. Nakayama, et al. (2007). "Differences in developing intestinal microbiota between allergic and non-allergic infants: a pilot study in Japan." Biosci Biotechnol Biochem **71**(9): 2338-42.

Sonnenburg, J. L., C. T. Chen, et al. (2006). "Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host." PLoS Biol **4**(12): e413.

Spiller, R. (2008). "Review article: probiotics and prebiotics in irritable bowel syndrome." Aliment Pharmacol Ther **28**(4): 385-96.

Spinler, J. K., M. Taweechotipatr, et al. (2008). "Human-derived probiotic Lactobacillus reuteri demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens." Anaerobe **14**(3): 166-71.

Stams, A. J. (1994). "Metabolic interactions between anaerobic bacteria in methanogenic environments." Antonie Van Leeuwenhoek **66**(1-3): 271-94.

Stange, E. F. (2004). Colitis Ulcerosa-Morbus Crohn. . Bremen, UNI-MED Verlag AG.

Strachan, D. P. (1989). "Hay fever, hygiene, and household size." BMJ **299**(6710): 1259-60.

Strachan, D. P. (2000). "Family size, infection and atopy: the first decade of the "hygiene hypothesis"." Thorax **55 Suppl 1**: S2-10.

Strober, W., I. Fuss, et al. (2007). "The fundamental basis of inflammatory bowel disease." J Clin Invest **117**(3): 514-21.

Strom, K., L. Janzon, et al. (1996). "Asthma but not smoking-related airflow limitation is associated with a high fat diet in men: results from the population study "Men born in 1914", Malmo, Sweden." Monaldi Arch Chest Dis **51**(1): 16-21.

Stsepetova, J., E. Sepp, et al. (2007). "Molecularly assessed shifts of Bifidobacterium ssp. and less diverse microbial communities are characteristic of 5-year-old allergic children." FEMS Immunol Med Microbiol **51**(2): 260-9.

Suau, A., V. Rochet, et al. (2001). "Fusobacterium prausnitzii and related species represent a dominant group within the human fecal flora." Syst Appl Microbiol **24**(1): 139-45.

Sullivan, A., C. Edlund, et al. (2001). "Effect of antimicrobial agents on the ecological balance of human microflora." Lancet Infect Dis **1**(2): 101-14.

Sutas, Y., M. Hurme, et al. (1996). "Down-regulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with Lactobacillus GG-derived enzymes." Scand J Immunol **43**(6): 687-9.

Sutas, Y., E. Soppi, et al. (1996). "Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with Lactobacillus casei GG-derived enzymes." J Allergy Clin Immunol **98**(1): 216-24.

Swidsinski, A., J. Weber, et al. (2005). "Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease." J Clin Microbiol **43**(7): 3380-9.

Szajewska, H., M. Kotowska, et al. (2001). "Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants." J Pediatr **138**(3): 361-5.

Tannock, G. W. (1999). "Analysis of the intestinal microflora: a renaissance." Antonie Van Leeuwenhoek **76**(1-4): 265-78.

Tannock, G. W. (2001). "Molecular assessment of intestinal microflora." Am J Clin Nutr **73**(2 Suppl): 410S-414S.

Tannock, G. W. (2002). "Molecular methods for exploring the intestinal ecosystem." Br J Nutr **87 Suppl 2**: S199-201.

Taylor, A. L., J. A. Dunstan, et al. (2007). "Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial." J Allergy Clin Immunol **119**(1): 184-91.

Topping, D. L. and P. M. Clifton (2001). "Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides." Physiol Rev **81**(3): 1031-64.

Torrallardona, D., C. I. Harris, et al. (1996). "Microbial amino acid synthesis and utilization in rats: incorporation of 15N from 15NH4Cl into lysine in the tissues of germ-free and conventional rats." Br J Nutr **76**(5): 689-700.

Tringe, S. G. and E. M. Rubin (2005). "Metagenomics: DNA sequencing of environmental samples." Nat Rev Genet **6**(11): 805-14.

Turnbaugh, P. J., M. Hamady, et al. (2009). "A core gut microbiome in obese and lean twins." Nature **457**(7228): 480-4.

Turnbaugh, P. J., R. E. Ley, et al. (2007). "The human microbiome project." Nature **449**(7164): 804-10.

Turnbaugh, P. J., R. E. Ley, et al. (2006). "An obesity-associated gut microbiome with increased capacity for energy harvest." Nature **444**(7122): 1027-31.

Tursi, A., G. Brandimarte, et al. (2004). "Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis." Med Sci Monit **10**(11): PI126-31.

Ukena, S. N., A. Singh, et al. (2007). "Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity." PLoS One **2**(12): e1308.

UNICEF (2009). "Diarrhea causes 1.5 million infant deaths a year." UN. Manila Bulletin Publishing Corporation.

Usami, M., K. Muraki, et al. (2001). "Effect of eicosapentaenoic acid (EPA) on tight junction permeability in intestinal monolayer cells." Clin Nutr **20**(4): 351-9.

van Gool, C. J., M. P. Zeegers, et al. (2004). "Oral essential fatty acid supplementation in atopic dermatitis-a meta-analysis of placebo-controlled trials." Br J Dermatol **150**(4): 728-40.

Van Loo, J. (2004). "The specificity of the interaction with intestinal bacterial fermentation by prebiotics determines their physiological efficacy." Nutr Res Rev **17**(1): 89-98.

Van Patter, W. N., J. A. Bargen, et al. (1954). "Regional enteritis." Gastroenterology **26**(3): 347-450.

Vancikova, Z., R. Lodinova-Zadnikova, et al. (2003). "The early postnatal development of salivary antibody and immunoglobulin response in children orally colonized with a nonpathogenic, probiotic strain of E. coli." Folia Microbiol (Praha) **48**(2): 281-7.

Venturi, A., P. Gionchetti, et al. (1999). "Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis." Aliment Pharmacol Ther **13**(8): 1103-8.

von der Weid, T., C. Bulliard, et al. (2001). "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 **8**(4): 695-701.

von Mering, C., P. Hugenholtz, et al. (2007). "Quantitative phylogenetic assessment of microbial communities in diverse environments." Science **315**(5815): 1126-30.

Wang, M., C. Karlsson, et al. (2008). "Reduced diversity in the early fecal microbiota of infants with atopic eczema." J Allergy Clin Immunol **121**(1): 129-34.

Watanabe, S., Y. Narisawa, et al. (2003). "Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects." J Allergy Clin Immunol **111**(3): 587-91.

Weiland, S. K., E. von Mutius, et al. (1999). "Intake of trans fatty acids and prevalence of childhood asthma and allergies in Europe. ISAAC Steering Committee." Lancet **353**(9169): 2040-1.

Weizman, Z., G. Asli, et al. (2005). "Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents." Pediatrics **115**(1): 5-9.

Wellen, K. E. and G. S. Hotamisligil (2005). "Inflammation, stress, and diabetes." J Clin Invest **115**(5): 1111-9.

Weston, S., A. Halbert, et al. (2005). "Effects of probiotics on atopic dermatitis: a randomised controlled trial." Arch Dis Child **90**(9): 892-7.

Whelan, J. (1996). "Antagonistic effects of dietary arachidonic acid and n-3 polyunsaturated fatty acids." J Nutr **126**(4 Suppl): 1086S-91S.

Whelan, K., P. A. Judd, et al. (2005). "Fructooligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid

concentrations caused by standard enteral formula in healthy humans." J Nutr **135**(8): 1896-902.

Wilson, M. E. (2005). "Diarrhea in nontravelers: risk and etiology." Clin Infect Dis **41 Suppl 8**: S541-6.

Wistrom, J., S. R. Norrby, et al. (2001). "Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study." J Antimicrob Chemother **47**(1): 43-50.

Wong, J. M., R. de Souza, et al. (2006). "Colonic health: fermentation and short chain fatty acids." J Clin Gastroenterol **40**(3): 235-43.

Woodcock, A., M. Moradi, et al. (2002). "Clostridium difficile, atopy and wheeze during the first year of life." Pediatr Allergy Immunol **13**(5): 357-60.

Woodmansey, E. J. (2007). "Intestinal bacteria and ageing." J Appl Microbiol **102**(5): 1178-86.

Woods, R. K., F. C. Thien, et al. (2002). "Dietary marine fatty acids (fish oil) for asthma in adults and children." Cochrane Database Syst Rev(3): CD001283.

Worm, M., U. Herz, et al. (2001). "Effects of retinoids on in vitro and in vivo IgE production." Int Arch Allergy Immunol **124**(1-3): 233-6.

Wright, S. D., R. A. Ramos, et al. (1990). "CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein." Science **249**(4975): 1431-3.

Xu, J., M. K. Bjursell, et al. (2003). "A genomic view of the human-Bacteroides thetaiotaomicron symbiosis." Science **299**(5615): 2074-6.

Yang, Y. X., M. He, et al. (2008). "Effect of a fermented milk containing Bifidobacterium lactis DN-173010 on Chinese constipated women." World J Gastroenterol **14**(40): 6237-43.

Young, S. L., M. A. Simon, et al. (2004). "Bifidobacterial species differentially affect expression of cell surface markers and cytokines of dendritic cells harvested from cord blood." Clin Diagn Lab Immunol **11**(4): 686-90.

Zapolska-Downar, D., A. Siennicka, et al. (2004). "Butyrate inhibits cytokine-induced VCAM-1 and ICAM-1 expression in cultured endothelial cells: the role of NF-kappaB and PPARalpha." J Nutr Biochem **15**(4): 220-8.

Zhang, H., J. K. DiBaise, et al. (2009). "Human gut microbiota in obesity and after gastric bypass." Proc Natl Acad Sci U S A **106**(7): 2365-70.

Zhang, M., B. Liu, et al. (2007). "Structural shifts of mucosa-associated lactobacilli and Clostridium leptum subgroup in patients with ulcerative colitis." J Clin Microbiol **45**(2): 496-500.

Zhang, T. and H. H. Fang (2006). "Applications of real-time polymerase chain reaction for quantification of microorganisms in environmental samples." Appl Microbiol Biotechnol **70**(3): 281-9.

Zimmaro, D. M., R. H. Rolandelli, et al. (1989). "Isotonic tube feeding formula induces liquid stool in normal subjects: reversal by pectin." JPEN J Parenter Enteral Nutr **13**(2): 117-23.

Zocco, M. A., L. Z. dal Verme, et al. (2006). "Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis." Aliment Pharmacol Ther **23**(11): 1567-74.

Zoetendal, E. G., C. T. Collier, et al. (2004). "Molecular ecological analysis of the gastrointestinal microbiota: a review." J Nutr **134**(2): 465-72.

Zoppi, G., M. Cinquetti, et al. (1998). "The intestinal ecosystem in chronic functional constipation." Acta Paediatr **87**(8): 836-41.

Zwielehner, J., K. Liszt, et al. (2009). "Combined PCR-DGGE fingerprinting and quantitative-PCR indicates shifts in fecal population sizes and diversity of Bacteroides, bifidobacteria and Clostridium cluster IV in institutionalized elderly." Exp Gerontol **44**(6-7): 440-6.

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