bio|vis DIAGNOSTIK

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External ID

Name	**Muster**	Date of Birth	**03.02.1964**	Order ID	**11626417**
First Name	**Muster**	Sex	Male	Order Date	20.11.2018
Sampling Date	18.11.2018 11:00	Validation Date	Thomas Gugerel	Findings Status	**Final Report**
Sample Material	FE	Validation on	27.11.2018	Findings Date	28.11.2018

Test	Result	Unit	Standard Range	Previous Result
Stool Diagnostics				
Moleculargenetic Microbiomeanalysis MIDI				
Stool Properties				
Colour	dark brown			FE NA) VISU
Consistency	mushy			FE NA) VISU
pH	**7,5**		5,8 - 6,5	FE NA) TESTS
Biodiversity				
Diversity	5,57		> 5,0	FE NA) MGSEQ

The bacterial diversity in the intestinal tract may vary considerably from person to person. Antibiotic therapies, infections, increasing age, unbalanced diets or smoking are causes of declining diversity.

Grad **5**

Bacteria Phyla (Distribution)				
Actinobacteria	1,5	%	1,0 - 5	FE NA) MGSEQ
Bacteroidetes	44,1	%	30 - 60	FE NA) MGSEQ
Firmicutes	48,8	%	30 - 60	FE NA) MGSEQ
Fusobacteria	0,0	%	0,0 - 1,0	FE NA) MGSEQ
Proteobacteria	**5,5**	%	1,5 - 5,0	FE NA) MGSEQ
Verrucomicrobia	**0,0**	%	1,5 - 5	FE NA) MGSEQ
Other	0,1	%		FE NA) MGSEQ
Ratio				
Firmicutes/Bacteroidetes	1,11	Quotient	< 1,5	FE NA) RECHN
Enterotype				
Bacteroides				FE NA) MGSEQ

Human intestinal microbiomes can be differentiated into three Enterotypes. Enterotypes are defined by dominant bacterial clusters with distinct metabolic properties.

Enterotyp **1**

Dysbiosis index

The dysbiosis index represents a measure of deviations within the microbiome. Depending on their relevance, all detected phyla, genera and species are considered.

NA) RECHN

0 5 10 15 20 25 30

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FE=Stuhl

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Test	Result	Unit	Standard Range	Previous Result
Bacteria Phyla - most important genera and species				
Actinobacteria				
Bifidobacteria	1,1 x 10^10	CFU/g faeces	> 5,0 x 10^9	FE NA) MGSEQ
Bifidobacterium longum	49	%		FE NA) MGSEQ
Bifidobacterium adolescentis	44	%		FE NA) MGSEQ
Equol producing bacteria	**3,9 x 10^9**	CFU/g faeces	> 5,0 x 10^9	FE NA) MGSEQ
Bacteroidetes				
Bacteroides	3,1 x 10^11	CFU/g faeces	> 1,5 x 10^11	FE NA) MGSEQ
Prevotella	**1,2 x 10^8**	CFU/g faeces	> 1,0 x 10^10	FE NA) MGSEQ
Firmicutes				
Butyrate producing bacteria				
Faecalibacterium prausnitzii	1,2 x 10^11	CFU/g faeces	> 5,0 x 10^10	FE NA) MGSEQ
Eubacterium rectale	**5,3 x 10^9**	CFU/g faeces	> 1,0 x 10^10	FE NA) MGSEQ
Eubacterium hallii	**3,3 x 10^9**	CFU/g faeces	> 5,0 x 10^9	FE NA) MGSEQ
Roseburia spp.	3,2 x 10^10	CFU/g faeces	> 2,0 x 10^10	FE NA) MGSEQ
Ruminococcus spp.	4,0 x 10^10	CFU/g faeces	> 3,0 x 10^10	FE NA) MGSEQ
Coprococcus	**1,1 x 10^10**	CFU/g faeces	> 2,0 x 10^10	FE NA) MGSEQ
Total bacterial count	3,0 x 10^11	CFU/g faeces	> 1,3 x 10^11	FE NA) MGSEQ
Clostridia				
Clostridia total bacterial count	1,5 x 10^9	CFU/g faeces	< 4,0 x 10^9	FE NA) MGSEQ
Clostridia cluster I	1,3 x 10^8	CFU/g faeces	< 2,0 x 10^9	FE NA) MGSEQ
Fusobacteria				
Fusobacterium spp.	< 1,0 x 10^6	CFU/g faeces	< 1,0 x 10^7	FE NA) MGSEQ
Verrucomicrobia				
Akkermansia muciniphila	**< 1,0 x 10^6**	CFU/g faeces	> 5,0 x 10^9	FE NA) MGSEQ
Proteobacteria				
Pathogenic or potentially pathogenic bacteria				
Haemophilus	**1,7 x 10^9**	CFU/g faeces	< 1,0 x 10^9	FE NA) MGSEQ
Acinetobacter	< 1,0 x 10^6	CFU/g faeces	< 1,0 x 10^6	FE NA) MGSEQ
Escherichia coli Biovare	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Proteus species	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Klebsiella species	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Enterobacter species	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Serratia species	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Hafnia species	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE A) KULTAZ
Morganella spp.	< 1,0 x 10^4	CFU/g faeces	< 1,0 x 10^4	FE NA) MIB
Histamin Developing Bacteria				
Histaminbildende Bakterien	**7,4 x 10^8**	CFU/g faeces	< 5,0 x 10^8	FE NA) MGSEQ
H2S production				
Sulphate reducing bacteria	1,4 x 10^8	CFU/g faeces	< 2,0 x 10^9	FE NA) MGSEQ

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Test	Result	Unit	Standard Range	Previous Result
Immunogenicity / Mucus production				
Immunogenically effective bacteria				
Escherichia coli	6,0 x 10^7	CFU/g faeces	10^6 - 10^7	FE A) KULTAZ
Enterococcus species	2,0 x 10^6	CFU/g faeces	10^6 - 10^7	FE A) KULTAZ
Lactobacillus species	**< 1,0 x 10^4**	CFU/g faeces	10^5 - 10^7	FE A) KULTAZ
Mucin production / Mucosa barrier				
Akkermansia muciniphila	**< 1,0 x 10^6**	CFU/g faeces	> 5,0 x 10^9	FE NA) MGSEQ
Faecalibacterium prausnitzii	1,2 x 10^11	CFU/g faeces	> 5,0 x 10^10	FE NA) MGSEQ
Yeasts / Molds				
Candida albicans	< 1,0 x 10^3	CFU/g faeces	< 1,0 x 10^3	FE A) KULTAZ
Candida species	< 1,0 x 10^3	CFU/g faeces	< 1,0 x 10^3	FE A) KULTAZ
Geotrichum candidum	< 1,0 x 10^3	CFU/g faeces	< 1,0 x 10^3	FE A) KULTAZ
Moulds	negative		negative	FE A) KULTAZ
Parasites				
Giardia lamblia	negative		negative	FE NA) MOLEK
Entamoeba histolytica	negative		negative	FE NA) MOLEK
Cryptosporidium spp.	negative		negative	FE NA) MOLEK
Blastocystis hominis	negative		negative	FE NA) MOLEK
Dientamoeba fragilis	negative		negative	FE NA) MOLEK
Cyclospora cayetanensis	negative		negative	FE NA) MOLEK
Maldigestion, Malabsorption, MIS				
Digestive Residues				
Quantitative determination of fat	**3,60**	g/100g	< 3,5	FE NA) PHOT
Quantitative determination of nitrogen	0,60	g/100g	< 1,0	FE NA) PHOT
Quantitative determination of sugar	**2,60**	g/100g	< 2,5	FE NA) PHOT
Quantitative determination of water	78,50	g/100g	75 - 85	FE NA) PHOT
Determination of Maldigestion				
Pancreatic elastase	547,74	µg/g	> 200	FE A) ELISA
Bile acids in stool	negative		negative	FE NA) PETIKO
Detection of Malabsorption				
Calprotectin	**343,09**	mg/l	< 50	FE A) ELISA
Alpha1-Antitrypsin	**150,1**	mg/dl	< 27,5	FE A) ELISA
Special Request				
Secretory IgA	**>7500**	µg/ml	510 - 2040	FE A) ELISA
Leaky good: Zonulin, Histamine				
Zonulin	25,23	ng/ml	< 55	FE A) ELISA
Histamine in stool	**5132,7**	ng/ml	< 959	Fe A) ELISA

Changed reference range after modification and validation.

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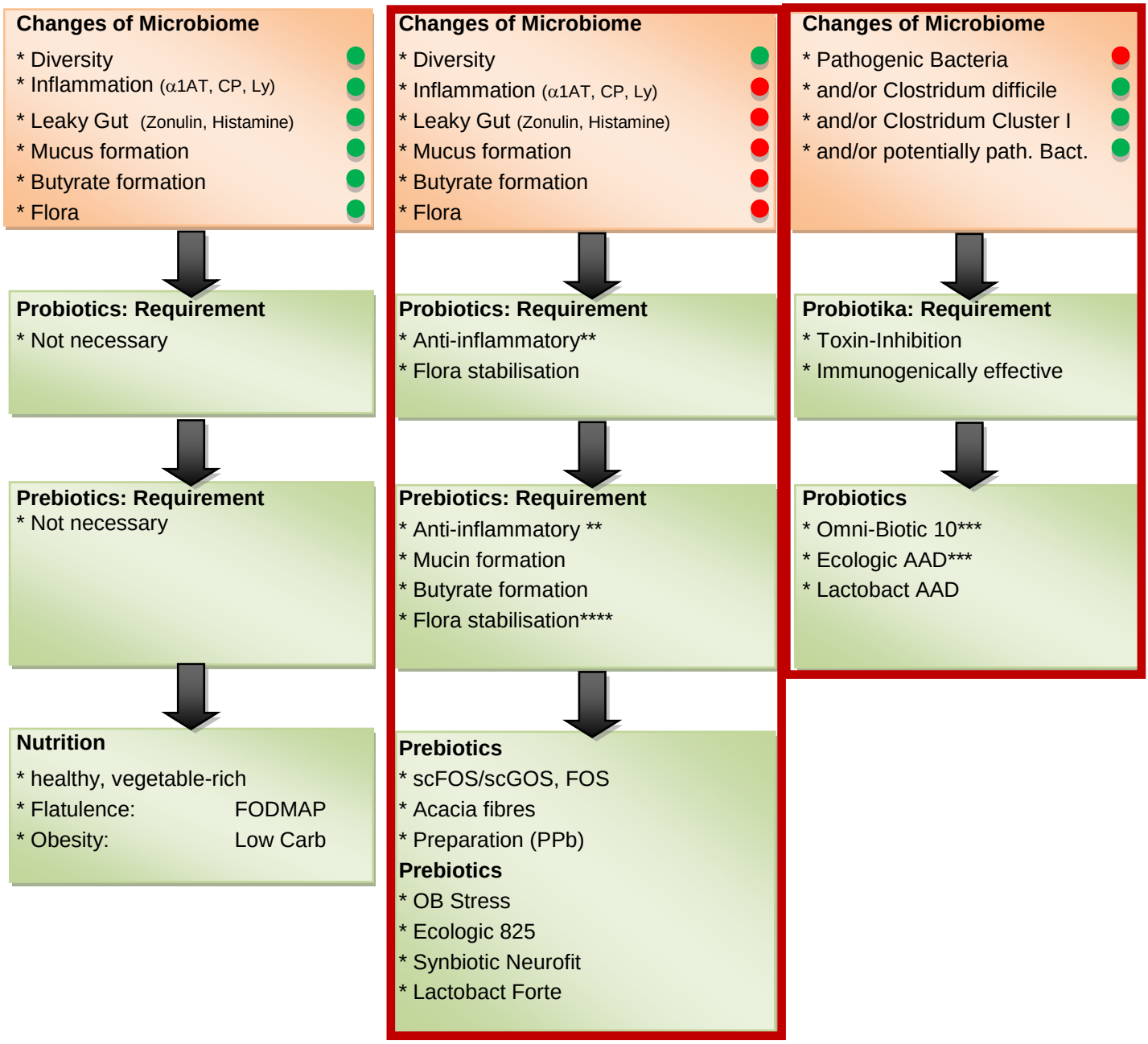
11626417	Muster	Muster	03.02.1964

Overview - Results and Therapy Options

Dysbiose-Index 19

pH	↑	milieu stabilizing probiotics *
Enterotype	1	check vitamin A. E, iron and calcium supply
Biodiversitiy	●	
Ratio Firmicutes/Bacteroidetes	●	
Equol producing bacteria	↓	
Butyrate producing bacteria	↓	prebiotics on the basis of resistant starch* or scFOS/scGOS*
Mucus production	↓	prebiotics (scFOS/scGOS)*
Mucosa intergrity	●	
Milieu stabilising bacteria	↓	milieu stabilizing probiotics*, prebiotics (scFOS/scGOS)*
Immunogenic bacteria	↓	immunogenic effective probiotics*
Clostridia - total bacteria count	●	
Clostridia cluster I	●	
Fusobakterien	●	
Histaminbildende Bakterien	↑	
H2S producing bacteria (SRB)	●	
Potentially Pathogenic Bacteria	↑	immunogenic effective / toxin inhibiting probiotics*
Candida (facultive pathogenic)	●	

Therapy options with prebiotics and probiotics in overview (11626417)

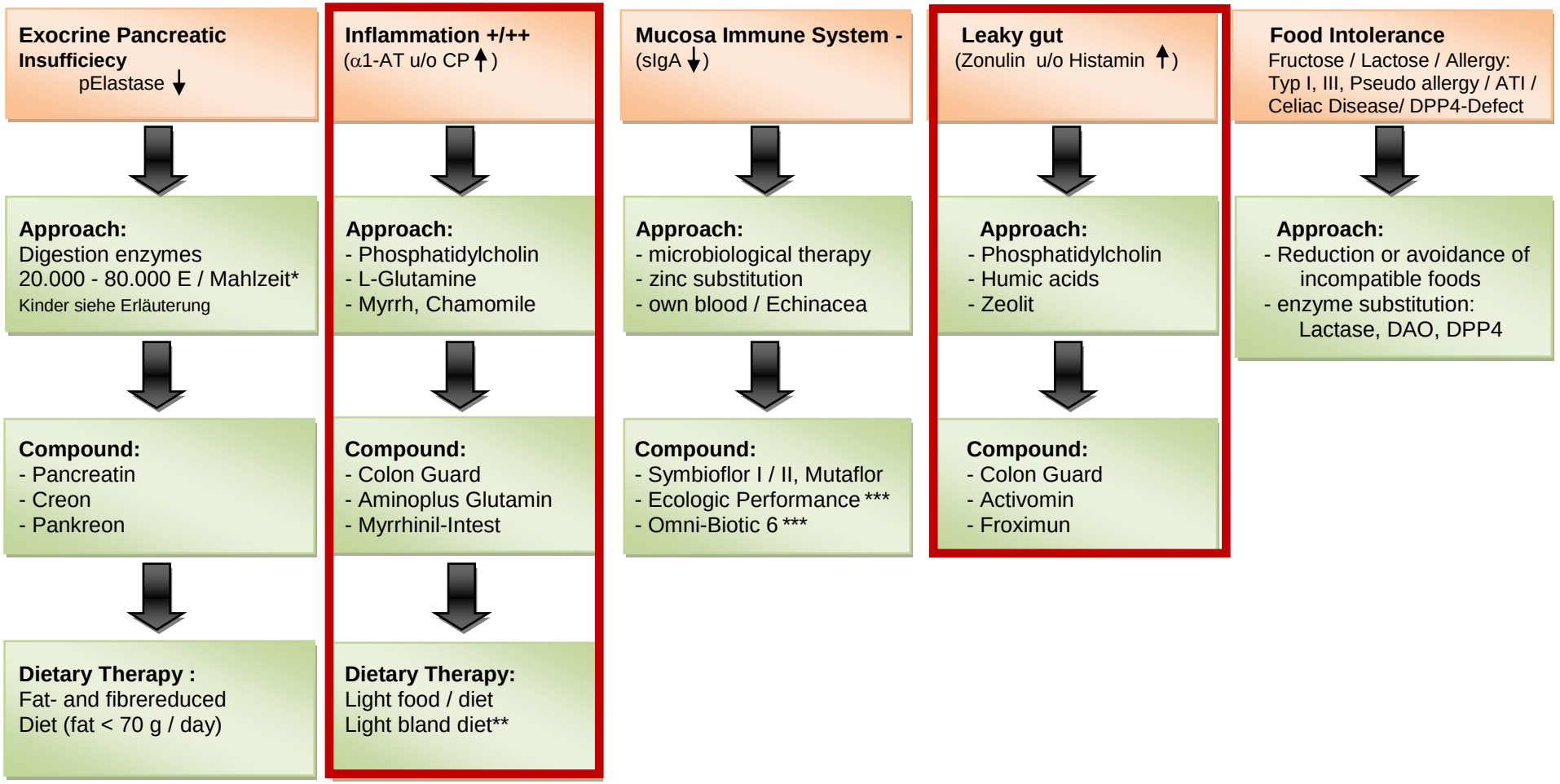


* age related: Omni-Biotic 60+ active

** age adapted: Lactobact 60plus

*** in combination with other probiotics

Therapy options based on results of pElastase, inflammation marker, sIgA and / or zonulin / histamine



* **Dosage** depending on fat content in stool, for **children** age and weight related dosages apply.
In case of slightly reduced pElastase values but normal fatty residues: possible administration of vegetable enzyme mixtures (e. g. Digest, Full Spectrum, Combizym).

** in case of α1-antitrypsin values > 100 mg / dl and / or calprotectin > 150 mg / l *** MIS-activating probiotics (alternatively see table „probiotics acc. to effects“)

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Introduction

The **intestinal microbiome** (entirety of all bacteria living in the intestinal tract) has considerable influence on health or illness of humans. It modulates the immune defence, supplies the organism with vitamins (vitamin B1, B2, B6, B12, and K), participates in the digestion of food components, supplies intestinal epithelia with energy via developing short-chain fatty acids and stimulates intestinal peristalsis. The microbiome also plays an important role in the scope of xenobiotic detoxification. Shifts within the microbiome are causally relevant factors for diseases like adiposity, non-alcoholic fatty liver disease, diabetes, coronary heart disease or cancer. After the composition of the human intestinal microbiome was studied in more detail, alterations can be detected and counteracted with well-aimed measures.

Result Evaluation

With the help of the **molecular-genetic stool analysis,** the intestinal microbiome was analysed in order to assess the composition and to determine possible shifts. The evaluation yielded the following **results**:

Evaluation of Stool Consistency, Color and pH-Value

General viewing of the stool sample showed **mushy consistency**. Healthy stool should be mushy and formed. Liquid or slurry stool indicates accelerated, doughy or solid stool samples delayed intestinal passage.

The color of the analysed stool sample was dark brown. The **pH-value** was **above normal range** at 7,5.

Evaluation of the Intestinal Diversity

More important than individual bacteria species or types is the interaction of the bacteria present in the microbiome. Manifold tasks of the intestinal flora require adequate **diversity**. The intestinal diversity of humans may vary considerably.

In the microbiome of healthy people one finds **300 to 500 bacteria species**, in sick persons there are often a lot less. Causes for reduced diversity are manifold. They are for example repeated **antibiotic therapies, infections,** increasing **age, unbalanced diet** or **smoking**.

Research revealed that numerous diseases come along with reduced diversity and thus presumably promote disease manifestation. Very often reduced diversity is found in patients suffering from **adiposity, fatty liver** (NAF), **diabetes type 2, Alzheimer disease, chronic inflammatory bowel disease, intestinal cancer** or **irritable colon syndrome**. Due to decreasing diversity the intestinal microbiome no longer grants adequate protection against endogenic infections. Obese patients with reduced diversity tend to gain more weight, respond worse to diets and there are often already indications of fat metabolism disorders or insulin resistance. In patients suffering from chronic inflammatory bowel disease (CIBD) reduced diversity promotes recurrence and chronicity. Research data are also available for the irritable bowel syndrome, the manifestation of which is promoted by reduced diversity.

Result

The analysis indicates **adequate biodiversity**.

Frequency Scale of the Most Important Bacteria Phyla

The colon is populated by bacteria, which reach a total density of approximately 10^{11}-10^{12} bacterial cells/ml colon content. This dense community of bacteria consists mainly of three or four large bacteria phyla: **Bacteroidetes, Firmicutes, Actinobacteria** and **Proteobacteria**. Other phyla (Verrucomicrobia, Fusobacteria) show smaller shares.

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In most cases 30-60 % of the microbiota are Bacteroidetes. The Firmicutes have the same share and mainly consist of Lachnospiraceae and Ruminococcaceae families. Actinobacteria have significantly lower bacteria counts. Mainly Bifidobacteria make up the Actinobacteria phylum. In the microbiome of healthy people Proteobacteria have a share of 1.5-5%, which can, however, after repeated antibiotic therapies or in case of inflammatory bowel diseases, increase significantly.

Result

The distribution of the bacteria-phyla shows an increase of:

- Proteobacteria

The distribution of the bacteria-phyla shows a reduction of:

- Verrucomicrobia

Determination of the Firmicutes / Bacteroidetes Ratio

Patients suffering from **irritable bowel syndrome** or **obesity** often show a high share of Firmicutes.

Obesity increases the risk of diseases like e.g. diabetes, coronary heart disease and cancer. It influences life expectancy and quality of life. In studies, the influence of the microbiome on the development of overweight was evaluated. **Firmicutes** have been shown to be capable of fermenting **complex, indigestible carbohydrates** to produce short-chain fatty acids (SCFA) which are absorbed through the intestinal mucosa and serve as additional energy sourced to the host (19, 20). Due to the fermentation of carbohydrates by firmicutes **10-12 %** more **energy** is available (21).

Bacteroidetes are not able to utilize complex carbohydrates. If firmicutes dominate bacteroides in the microbiome one speaks of an increased **firmicutes-bacteroidetes-ratio** which may promote gaining weight.

In case of patients suffering from irritable colon syndrome increased firmicutes-bacteroidetes-ratios often come along with meteorism or flatulence.

Result

The microbiome analysis showed a balanced ratio of firmicutes compared to bacteroidetes. The firmicutes-bacteroidetes-ratio is **within normal range.**

Determination of the Enterotype

Recent research showed that the human microbiome can be assigned to **three main groups-** so-called enterotypes. Intestinal bacteria develop – depending on the enterotype – stable, clearly different clusters with typical metabolic properties (9). **Enterotype 1** is characterized by high **bacteroides counts** and **enterotype 2** by strong **Prevotella** population. **Enterotype 3** is only found rarely – in hardly more than 5 % of the analysis. This type shows strong **Ruminococcus** flora.

The described enterotypes show significantly differing **metabolic performance.** The bacteroides dominated flora (enterotype 1) is optimally adjusted to the utilisation of **fat, fatty acids, protein and amino acids**. **Carbohydrates**, however, are metabolized significantly worse than by Prevotella dominated flora (enterotype 2), which in turn cannot metabolize fat and protein adequately.

The enterotypes also influence the absorption of minerals like **sodium, potassium, calcium** (11) or **iron.** Enterotypes are independent of sex or age and remain stable for years. Via **long-term change of diet** and taking **prebiotics** they can be influenced (12, 13 and positively effects human sustenance and health.

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Result

The microbiome analysis indicates **enterotype 1** with dominating **bacteroides flora** and clearly less present Prevotella and Ruminococcus sp.

A bacteroides dominated flora is specialized in energy generation from **oligosaccharides**, **animal proteins** and **saturated fatty acids.** Enterotype 1 is therefore mainly only found in persons, who regularly eat meat. Bacteroides only rarely dominate in vegetarians and fruit and vegetable enthusiasts. Bacteroides species are on one hand able to **synthesize vitamins** (biotin, riboflavin (B2), pantothenic acid (B5), folic acid (B9) and vitamin C); on the other hand the enterotype also influences intestinal **nutrient absorption.** The latter is significantly lower than in Prevotella dominated enterotype 2.

Actinobacteria

Bifido bacteria are gram-positive anaerobic rod-shaped bacteria, which utilize starch, but mainly oligosaccharides. Mostly acetic and lactic acid are developed.

By developing short-chained fatty acids and related pH-value reduction in the intestinal lumen bifido bacteria do not only counteract proliferation of pathogenic bacteria (colonisation resistance), they also have anti-inflammatory effects.

Result

In case of Mr. / Ms. Muster the bifido bacteria count is **within the norm.** The most common representative in the microbiome is B. longum. The second common species was B. adolescentis. A strong bifido bacteria flora protects against endogenous infections and has an anti-inflammatory effect.

Equol producing genera and species

Equol is a metabolite with strong binding affinity to estrogen receptors, which is formed by intestinal microbiota from isoflavones, ie secondary plant substances.

Recent studies suggest that the ability to bacterially produce equol from daidzin or daidzein is associated with **reduced menopausal symptoms** and a **reduced risk of chronic disease** (Birru et al., 2016, Davinelli et al., 2017; Yoshikata et al., 2016). However, the bacterial formation of Equol is strongly differing interindividually and only about 20-30 % of the population of Western cultures, compared to 50-60 % of Asian populations are capable of forming Equol (Setchell and Clerici, 2010).

According to recent research, almost exclusively species from the family Coriobacteriaceae from the Phylum of Actinobacteria are able to form Equol. Particularly important species are **Adlercreutzia spp.**, **Eggerthella lenta** and **Slackia spp.** (Rafii, 2015).

Result

Not enough equol-producing bacteria were found.

Bacteroidetes

Results

Bacteroides is the most common genus in the microbiome of many people. In case of Mr. / Ms. Muster 31 % are of these genus, which equals a bacteria count of 3,1 x 10^11 CFU / g Stool.

Also high **prevotella** bacteria counts can be reached, especially in case of vegetarians. But here it is with 1,2 x 10^8 CFU / g stool **below normal range**.

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Firmicutes

A. Development of Butyrate and Short-Chain Fatty Acids by Firmicutes

Carbohydrate fermentation in the colon leads to the development of short-chain fatty acids (SCFA) (37) and gases (H_2, CO_2, methane). SFCA detectable in stool samples are mainly **formic acid, acetic acid, propionic acid** and **butyric acid**. Dietary changes lead to altered production rates of short-chain fatty acids. **Low-carb diets** lead to butyrate development reduction to one quarter (38) while **prebiotic agents** or **increased fibre consumption** lead to butyrate and propionate increases (39), the acetate levels decrease.

Short-chain fatty acids have positive influence on health. They stimulate intestinal motility and reduce inflammatory reactions by binding with GPR receptors (GPR 41 / GPR 43).
Butyrate is the most important **energy source** for colonocytes; it has an anti-inflammatory effect (40, 41, 42), protects against cell degeneration and also has **preventive influence** in regard to colorectal carcinoma.

Propionate
is metabolized in the liver, **acetate** in peripheral tissue. It is a precursor of cholesterol metabolism and lipid development. By giving prebiotics a shift of the fermentation products – from acetate to butyrate - may therefore be an advantage and lead to reduction of the **cholesterol level** (43).

Higher **SFCA concentrations** in the intestinal tract may increase mineral consumption like for example calcium (44). Therefore alterations of the intestinal microbiota after giving **FOS** come along with an increase of calcium absorption and improvement of the bone situation.

Mainly **firmicutes** develop butyrate. Among firmicutes mostly **Eubacterium rectale**, **Roseburia species** and **Ruminococcus sp.** are potent butyrate developers. The strongest butyrate developer, however, is **Faecalibacterium prausnitzii –** also a firmicute **-** which in contrast to the other listed butyrate developers cannot utilize starch. As butyrate is quickly absorbed via the intestinal mucosa, measurements in stool only provide unreliable results. Important information about butyrate development can be obtained with the aid of quantitative analyses of butyrate developing bacteria.

Result

The molecular-genetic microbiome analysis on butyrate-forming bacteria showed **deficits in several important butyrate formers**.

The **total bacteria count** of the butyrate formers however was within the norm.

Due to deficits in several important butyrate formers, a **non-optimal butyrate formation** should be considered despite the inconspicuous total bacteria count.
E. hallii is a bacterium that can convert acetate to butyrate. The butyrate source is not available, or only to a limited extent, when the number of microorganisms is low. A butyrate deficiency can result.

B. Evaluation of the Clostridia Flora (Total Bacteria Count, Toxin Development)

Clostridia belong to the group of firmicutes. They are obligatory anaerobic bacteria and develop spores. Pathogens belong to the clostridia species, but also apathogenic, useful bacteria, which have an immune modulating effect and lead to an increase of IL-10. Mainly Clostridium botulinum, Clostridium tetani or Clostridium difficile belong to the group of pathogenic representatives. In regard to their favoured energy sources clostridia can be assigned to two groups: **proteolytic** and **saccharolytic species**.

Proteolytic clostridia utilize protein and amino acids. Saccharolytic species on the other hand ferment carbohydrates, starch or fibres. During this process butyrate, acetone, butanol, CO_2 and hydrogen are developed. Dominance of proteolytic species often indicates so-called **"putrescence dyspepsia",** which frequently comes along with increased pH-values in stool. If the pH-value is – in spite of high counts of proteolytic spe

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cies – within the norm or reduced, this is most often caused by accelerated intestinal passage. High clostridia counts may also come along with "**fermentative dyspepsia**". In this case, however, they are saccharolytic species.

Some clostridia groups – so-called **Cluster I-Clostridia** contain **toxin developing species**, like for example C. perfringens, C. sporogenes or C. histolyticum. Cluster I clostridia are often found in diseases of the autistic spectrum disorders and are not rarely the cause of **autism associated intestinal** and frequently also **extra-intestinal complaints.**

Result

The microbiome analysis of Mr. / Ms. Muster showed **inconspicuous clostridia counts**.

Toxin developing clostridia (Cluster I) could also not be detected during sequencing. But only the most important representatives C. perfringens, C. sporogenes und C. histolyticum are considered.

Proteobacteria

Like microbiome analyses show there is decreasing digestive performance in older age, which often leads to an increase of Enterobacteriaceae (**Escherichia coli**, **Klebsiella**, **Enterobacter**, **Proteus)** or pasteurellaceae (e.g. **haemophilus**). There are also alterations of the obligatory anaerobic flora. Increases of **clostridia** are suspicious. **Bifido bacteria** and **lactobacilli** on the other hand reduce.

The described alterations can also be caused by other factors. Reapplied **antibiotic therapies** lead to increasing enterobacteria, enterococci and clostridia counts as well as to significantly decreasing bifido bacteria. (62). Similar can be observed in case of **chronic inflammatory bowel diseases or irritable colon syndromes** (63, 64).

Determination of Pathogenic or Potentially Pathogenic Bacteria

Result

The following pathogens were found in the microbiome:

- Haemophilus

Haemophilus
Haemophilus species are facultative, anaerobic, gram-negative bacteria, which live on the human mucosa and might cause diseases.
Haemophilus influenzae mainly lives on the mucosa of the upper respiratory tract (nose, pharynx, and windpipe) and causes local inflammatory diseases (epiglottis, bronchitis, pneumonia, meningitis). Encapsulated H. influenzae is obligatory pathogenic. Uncased strains are only pathogenic under certain circumstances. Haemophilus parainfluenzae mainly occurs as pathogen in case of endocarditis.

Histamine-forming bacteria

The stool sample detected bacterial species capable of converting histidine to histamine. Histamine is a messenger substance for inflammatory reactions, stimulates smooth muscle contraction and inhibits certain cells of the immune system. It is normally degraded by the enzyme diamine oxidase (DAO).

Damage of the Intestinal Mucosa due to Hydrogen Sulphide Development (H_2S)

Hydrogen sulphide is a toxic metabolic product, which – in case of higher concentrations – leads to damage of intestinal epithelia and such promotes the occurrence of cellular atypia. H_2S is developed in the colon by **sulphate reducing bacteria** – mainly by **Bilophila wadsworthii**, **Desulfomonas pigra** and **Desulfovibrio piger** (46, 47). Meat is an important source of sulphur, which promotes the growth of sulphate reducing

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bacteria (48). The **cancer promoting potential** of hydrogen sulphide is based on the development of **free radicals** (oxidative stress) and up-regulation of **cyclooxygenase-2** activity in the epithelia cells (49).

Gut bacteria can also develop N-nitroso compounds. Their quantity increases in case of high-protein diets, especially if a lot a meat is consumed (51). Cooking meat produces heterocyclic amines, which can be transformed to cancer promoting intermediate products (50).

Results

In the scope of sequencing no increased Bilophila wadsworthia, Desulfomonas pigra or Desulfovibrio piger bacteria counts could be determined. This indicates **minor H_2S development.**

Bacteria with an Immunogenic Effect

E. coli and enterococci have an **immunogenic effect** and are in interaction with other bacteria mainly responsible for the **immune modulating effect of the microbiota**.
And at last **lactobacilli** together with enterococci are the main representatives of the small intestine flora. Furthermore they have an **immunogenic effect**, are **anti-inflammatory** and **stabilize the milieu.** They are able to develop substances similar to antibiotics (**bacteriocins**), which counteract proliferation of endogenic pathogens.

E.coli, enterococci and **lactobacilli** were the major pillars of intestinal flora analysis; therefore they are also taken into consideration in this context.

Result

We determined reduced **lactobacilli** counts in the microbiome of Mr. / Ms. Muster. Enterococci were within normal range.

Reduced bacteria counts often indicate non-physiological flora condition in the terminal ileum. Frequently such microbiome alterations are found in patients suffering from **neurodermatitis**, **food allergies** or **intolerances**.

Mucin Development and Mucosa Barrier

In healthy colons a layer of mucosa mucus (**mucin layer)** protects the epithelia cells (45). If the mucin layer is damaged or not sufficient mucin is developed, pathogens, pollutants or allergens can come into direct contact with the mucosa and lead to inflammations. Mucin development and mucosa barrier are therefore closely connected with each other. The maintenance of intact mucosa barriers protects against bacterial translocation (LPS) and thus against inflammations. Bacteria like **A. muciniphila** significantly participate in maintaining the mucin layer. They produce mediator substances, which trigger goblet cells to develop mucosal mucus.

Result

Reduced Akkermansia muciniphila counts in the microbiome of Mr. / Ms. Muster indicate **insufficient mucin development.**

The **Faecalibacterium prausnitzii count** in stool was **normal.**

Mycological Stool Analysis

No yeasts could be found in the stool sample of Mr. / Ms. Muster.

Laboratory-Id N^{o}.	**11626417**
Received	**20.11.2018**
Report	**06.12.2018**
Last Name	**Muster**
First Name	**Muster**
Date of Birth	**03.02.1964**
Index	BOSC

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Determination of Parasites or Parasitic Enteritis Pathogens

There was no indication of amoebae, cryptosporidium, cyclospora, lamblia and blastocysts in stool.

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Supplementary Parameters

Determination of Digestive Disorders

The stool sample of Mr. / Ms. Muster showed **slightly increased fat residues**. There is no indication of pronounced **nutritional errors** or **digestive disorders**.

Determination of Maldigestion

Digestive function of the pancreas
Pancreatic elastase 1 correlates closely to the digestive function of the exocrine pancreas. The value obtained for patient Mr. / Ms. Muster speaks for an adequate function of the organ.

Bile Acids in Stool

The concentration of bile acids was within normal range. Loss of bile acid as cause of maldigestion can therefore be excluded. There is no ileum dysfunction.

Determination of Malabsorption

Mucosa Integrity and Permeability
The **increased calprotectin values** argue in favour of inflammatory mucosa alterations, which may lead to impairment of metabolic foods product and micronutrient resorption (malabsorption). **Increased alpha-1-antrypsin values** often come along with increased intestinal mucosa permeability.

Mucosa Immunity

Mucosa Integrity and Permeability
The increased sIgA concentration in stool indicates active defence reactions of the intestinal mucosa. This may be caused e.g. by inflammatory or allergic processes.

Histamine in Stool

The histamine value in stool is increased.
Causes may be food allergies, pseudo-allergies or chronic stress, which – via mast cell degranulation – lead to **increased mucosa permeability**.
In case of clinical manifestation of food allergies the immunologically mediated inflammatory reaction is at first only reduced to mucosa and sub-mucosa. The mucosa of the gastro-intestinal tract (GIT) has a surface of about 300 m² and can thus bind considerable amounts of specific IgE without significant increase of allergen-specific IgE antibodies in serum. In this case basic diagnostics (Prick Test, total IgE, allergen specific IgE) can often not provide precise results, but the histamine value in stool is increased.

Zonulin

Zonulin is a protein which regulates the number of molecule **transmissions.** Zonulin controls a whole cascade of processes, which influence the **tight junctions**. High levels come along with increased intestinal permeability. Low levels argue in favour of stable and dense mucosa condition. Increased mucosa permeability may come along with inflammatory mucosa reactions and induces sensitizations. Therefore increased Zonulin values are found in patients suffering from celiac disease, diabetes mellitus or numerous autoimmune diseases.
The zonulin value was within **normal range**.

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Therapeutic Approaches

The results of the microbiome analysis require therapeutic approaches, which protect the microflora against negative consequences or ease existing complaints by supporting the microflora.

Successful therapies, however, also take basics into consideration, which practicably apply for everyone and often already lead to significant improvement of ailments. These basic therapies are based on decade-long experiences. They are listed in short form below and can be found under www.biovis.de.

Basics for healthy intestines:

Diet Healthy diets consist of a plentiful breakfast, a main meal at lunch and a modest dinner. It should be varied and diverse.

Giving Psyllium seed husks (dosage 1-2 tablespoons) should lead to 1 – 2 formed stools per day. They are tolerated well and may also be given in case of obstipation or diarrhoea.

Wheat Avoid or significantly reduce wheat. Wheat is often not tolerated well, even if there is no evidence of intolerance. This is caused by amylase-trypsin inhibitors (ATI), which inhibit digestive enzymes and promote mucosa irritations.

Sugar Radical reduction of sugar consumption (maximum 1g/day)

Chewing Thoroughly chewing and salivating of food is the first step to healthy digestion and nutrient absorption. Chewing 30-40 times leads to optimal preparation of food for intestinal processes.

Exercise Adequate moderate exercise

Relaxation Keep adequate resting phases

Detoxification Drink enough (2-3 l water / unsweetened herbal teas) – this provides for improved intestinal passage and excretion of foreign matters. Possibly drainage of toxic substances via zeolite and/ or humic acids may be sensible.

Substitution Consumption high-value herbal oils (e.g. linseed oil) and/or fish, possibly curcumin or aloe vera, which have an anti-inflammatory effect respectively promote butyrate development.

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Independent of the cause of marker increases, one should first try to reach healing of the mucosa reactions with the aid of anti-inflammatory measures. According to recent studies with CED patients treatment with **phosphatidylcholine** (lecithin) (e. g. Colon Guard®) seems to be very successful. Colon Guard® stabilizes and solidifies the mucosal mucus and thus leads to the development of an effective mucosa barrier. Also amino acids (glutamine, arginine, lysine and methionine) promote wound healing and mucosa reconstruction (e. g. Aminoplus® immun). Mainly **glutamine** - as nutrient of the intestinal epithelia cells – counteracts the 'leaky gut syndrome'; it can also be given alone (e. g. Aminoplus® Glutamin, Colon Guard®, and Adamin G®).

Diversity

The microbiome analysis indicates **adequate biodiversity**.

Please make sure to keep a **balanced diet** to provide for the maintenance of the microbiome diversity. An antibiotic therapy should always be accompanied by taking **probiotics.** They not only counteract proliferation of resistant pathogens, but also further reduction of bacteria diversity. Please keep in mind that also **smoking, aging, imbalanced high-fat diets** ("Western Diet") or diseases coming along with inflammatory mucosa irritations ("**low grade inflammation**") or medication (NSAR) lead to a biodiversity reduction. Therefore therapies should always start here and fight against causal factors.

Enterotype

The patient has **enterotype 1** dominated by strong bacteroides flora. Bacteroides species are able to synthesize vitamins (biotin, riboflavin, pantothenic acid, folic acid and vitamin C), but intestinal **nutrient resorption** of enterotype 1 – with the exception of some B-vitamins (B1, B2, B3) – is significantly **worse** than that of Prevotella dominated enterotype 2.

Consequence:
Enterotype 1 patients should therefore make sure their **micronutrient supply** is **adequate.** This before all applies for:

- **Vitamin A**
- **Vitamin E**
- **Iron**
- **Calcium**

Individual prebiotic or probiotic therapies

Prebiotics
Prebiotics can promote diversity and achieve targeted changes in the composition and metabolism of the gut microbiota. Prebiotics consist of hard-to-digest carbohydrates, such as **resistant starches**, which lead to the proliferation of firmicutes and some bifidobacteria. **Oligosaccharides** such as XOS, AXOS, FOS, GOS or acacia fibers also show a bifidogenic effect. They too lead to an increase in butyrate formers. In addition, Faecalibacterium prausnitzii or Akkermansia muciniphila can be propagated via FOS / GOS or acacia fibers, resulting in a stabilization of the mucus layer and the membrane barrier.

Probiotics
Probiotics are selected, living microorganisms that positively affect the environment in the intestine. Above all, strains of bifidobacteria and lactobacilli, but also E. coli, and enterococci are used. Whereas in the past it used to work predominantly with **individual strains**, it is now known that combinations of several potentiating probiotic strains can achieve significantly stronger effects. **Modern multispecies probiotics** can stimulate the mucosal immune system or have an immunomodulating effect. Depending on the selection and composition of the strains used, probiotics can stabilize the mucosal barrier in the intestine by stabilizing mast cell membranes and counteract a leaky gut. Modern multispecies probiotics have an anti-inflammatory effect and lead to a significant reduction of proinflammatory cytokines.

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Pre- and probiotics should be used as specifically as possible in order to achieve an optimal effect. The selection is based on the following criteria:

- Patient age
- Complaint image
- Diversity
- Mikrobiota changes
- Butyrate and mucin formation
- Existing pathogenic / potential-pathogenic germs
- Existing facultatively pathogenic yeasts
- Inflammatory mucosal changes
- Leaky Gut (disturbed mucous membrane barrier)
- Mucosal immune system
- Incompatibilities / intolerances
- Overweight or underweight

Nutritional forms, such as **FODMAP** or **low carb** have an impact on diversity and microbiota composition. Therefore, they are also taken into account in the following compilations.

Pre- and probiotics should be used as **specifically** as possible in order to achieve an **optimal effect**. The following tables allow you to determine suitable pre- and probiotics according to fixed criteria. If prebiotics can easily be restricted to the naming of active substances, this is practically impossible with probiotics, since even the same named bacterial species can vary greatly in their abilities. Even if products are named for these reasons, a claim for completeness cannot be guaranteed due to the large number of products offered. However, attempts were made above all to include probiotics which can substantiate the indication and efficacy with studies. If the listing is based only on similar parent compositions or indications by the manufacturer, this is marked in color. For further explanations, please refer to the tables.
Mr. / Ms. Muster does **not have enough equol-producing bacteria** and is therefore not capable of converting soy into bioactive secondary plant material.

Equol can be substituted in the absence of equol-producing bacteria. 10-40 mg per day are recommended for the reduction of **menopausal symptoms**. To protect against osteoporosis or complications of a metabolic syndrome, daily dosages of 10 mg are used.

If **histamine-producing bacteria** in the stool are detected, it is advisable to clarify the presence of **histamine intolerance (HIT)** via further laboratory diagnostics. This includes, in addition to a determination of diamine oxidase (DAO) in blood serum, especially a detection of histamine in the stool. If a HIT is present, existing symptoms can be significantly influenced by nutritional therapy, orthomolecular measures and possibly the administration of enzymes.

Dietetic Treatment

The microbiome composition is significantly influenced by the diet. Long-term change of diets alter the bacteria-phyla distribution (e.g. of firmicutes or bacteroidetes) as well as the bacteria count of those bacteria species, which are important for intestinal health.

Please note:
The recommended dietetic treatment may lead to flatulence in the beginning. If this is the case starch or oligo-saccharide containing foods have to be increased gradually.

Inflammatory mucosa irritations require dietetic measures in the sense of an **easily digestible bland diet**, which in spite of disordered resorption provides for sufficient resorption of food cleavage products and micronutrients.

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To promote growth of anti-inflammatory or mucosa stabilizing intestinal bacteria, foods containing fermentable carbohydrates (**oligosaccharides**) are favourable, like for example chicory, black salsify, radicchio, endive, asparagus, broccoli, sugar peas and sugar beet syrup. Also onion and garlic plants are good suppliers. In individual cases flatulence may occur. For this reason the individual tolerance should be tested with small amounts of the respective foods.

Increased Histamine Values in Stool – Dietary Therapy
Mainly in case of intolerance of biogenic amines a low-histamine diet is indicated. The following should be considered.

- Only eat very fresh fish respectively totally avoid fish or fish products
- Do not eat hard cheese, raw sausage or raw ham
- All foods produced with the addition of micro-organisms should be consumed carefully (beer, sauerkraut etc.
- The same applies for alcoholic drinks especially wine.

Attention: Biogenic amines are **resistant against heat and cold** and cannot be destroyed by freezing, cooking, baking or heating in the microwave.

Attachment:

Occurrence and content of biogenic amines in selected foods

Biogenic Amine	Food	Amine Content [mg/kg]
Histamine	tuna fish	0.1 – 13000
	sardine	110 – 1500
	sauerkraut	6 – 200
	spinach	38
	tomatoes	22
	salami	0.1 – 279
	Westphalian ham	38.2 – 159
	red wine	0.6 – 3.8
	Emmental cheese	0.1 – 555
	Harzer cheese	390
	Gouda	29.5 – 180

With kind regards

Your Biovis-Diagnostik

Prebiotics	Butyrate formation	Anti-inflammatory	Fp and/or Am	Bifidogenic effects	F/B-Ratio	LI	FM	Flatulence*	Diversity
RS	**+**	(+)	**-**	(+)[1)]	**+**	yes	yes	**40**	**+**
PPb	**+**	**+**	**+**	**+**	**+**	yes	yes	**60**	**+**
scFOS/scGOS	**+**	**+**	**+**	**+ +**	(+)	**no**	**no**	**100**	**+**
FOS	**+**	**+**	**+**	**+**	(+)	yes	**no**	**100**	**+**
Inulin	**+**	**+**	**+**	(+)[2)]	(+)	yes	**no**	**100**	**+**
Acacia fibres	**+**	**+**	**+**	**+**	**- -**	yes	yes	**20**	**+**
XOS / AXOS	**+**	**+**	**-**	**+**	?	yes	yes	**50**	**+**
Butyrate	**+**	**+**	**-**	**-**	+/-	yes	yes	**10**	+/-
FODMAP	**-**	-	**- -**	**- -**	**- -**	yes	yes	**- -**	**- -**
Low Carb	**-**	-	+/- [3)]	+/- [3)]	**- -** [3)]	yes	yes	**- -**	**- -**

Note:

***** Relative occurrence of flatulence compared to FOS/GOS (100 %)

+ Promoting effect | **-** no detectable or only very little effect | **+/-** no influence | **- -** reduction | **yes** compatible | **not** necessarily compatible, gradually increase dosage (start: 1 g / day)

[1)] Decomposition of RS by B. breve and B. adolescentis (Aliment Pharmacol Ther 2015; 42:158-179); [2)] depending on phenotype, incomplete decomposition of inulin (Appl Environ Microbiol 2009; 75:454-461); [3)] Decreasing numbers of bacteria such as A. muciniphila (Clin Nutr Experiment 2016; 6: 39-58), F. prausnitzii- and Bifidobacteria are described with a protein- and fat-rich low-carb-diet. (Proc Nutr Soc 2015; 74: 23 – 36). Low Carb diets can contain between 25 and 250 g carbohydrates per day.

RS: Resistant Starch
PPb: „Pro Prebioma“ (combination of several prebiotic substances)
FOS/GOS: **F**ructo-/**G**alacto**o**ligo**s**accharides: short chain variants (**sc**FOS / **sc**GOS) show significantly better compatibility
XOS/AXOS: **X**ylo-, **A**rabino**x**ylo**o**ligo**s**accharides: Butyrate formation mainly through bifidogenic effect („Cross-Feeding“)
FODMAP: **F**ermentable **O**ligo-, **D**i-, **M**onosaccharides and **P**olyols“ (Polyols: polyvalent alcohols)
Fp / Am: Reproduction of Faecalibacterium prausnitzii / Akkermansia muciniphila
F/B-Ratio: Firmicutes-Bacteroidetes-Ratio
LI: Compatibility for people with lactose intolerance
FM: Compatibility for people with fructose malabsorption
Diversity: Diversity promoting effect

Probiotics Indications	OB Panda Ec. Panda OF Start Lb.Junior[2)] AB Start	OB 6[4)] Sb. Basis Lb. omni FOS OF Plus pb pur	OB 60+ active Sb. Vital Lb. 60 plus OF Senior	OB 10[4)] Ec. AAD[4)] pb protect Lb. AAD AB Akut	OB Stress rp. Ec. 825 Sb. Neurofit Lb. Forte[1)]	OB Power Ec. Perform.	OB Hetox Ec. Barrier[5)]	OB Hetox light Ec. Barrier Ec. Sense	OB Flora plus+ OF Fem
Babies	+ + +			week 1 - 4	week 5 - 12				
Children	+ + +[2)]	*/+ +[4]				*	*	*	
Adults		+ + +	+	+ +	+ +	+ +	+ +	+ +	+ +
Seniors		+	+ + +	+ +	+ +	+ +	+ +	+ +	+ +
Antibiotics				+ + +					
Lack of Butyrate					+ + +	+ +			
C. albicans	+	+ +		+ +					+ +
C. krusei /glabrata		+		+					+ + +
Diversity low	+ +[3)]				+ +		+ +	+	
Inflammation					+ + + +[1)]	+ +	+ +	+ +	
Flora (pH +)		+ + +	+ + +		+	+			
MIS-Activity - [6)]	+ +	+ + +	+ +	+ +	+	+ + +	+ +	+	
Lack of Mucin									+ +
Leaky Gut	+ +[3)]				+ + +	+ + +	+ + +	+ + + +	
PO / PPO		+		+ + + +	+ +			+	
SRB		+ + +	+ +		+				

Notes:

+ + + / + + + + Method of choice | **+ +** appropriate | **+** slight effect detectable | ***** from 8 years on

[1)] Lb. Forte: Indication: inflammatory mucosa reactions, CED (Interval); [2)] from 2nd year of life on; [3)] detected for OB Panda and Ec. Panda;
[4)] OB 6, OB 10 AAD, Ec. AAD also for children from 2nd year of life on, until 3 years half of dosage; [5)] Ec. Barrier double dosage; [6)] see introductory paragraph

OB: Omni-Biotic | Ec.: Ecologic | Sb.: Synbiotic | Lb.: Lactobact | OF: Orthica Flora / Orthiflor | pb: Probiotik | AB: Arctibiotic

MIS: Mucosal immune system | PO / PPO: pathogens / potentially pathogenic bacteria | SRB: sulfate-reducing bacteria

Important:

Information based on scientific studies or on indication statements of manufacturers. Due to the large quantity of probiotics available, there is no claim for completeness.
Black: based on study | **Violet**: manufacturer's specification