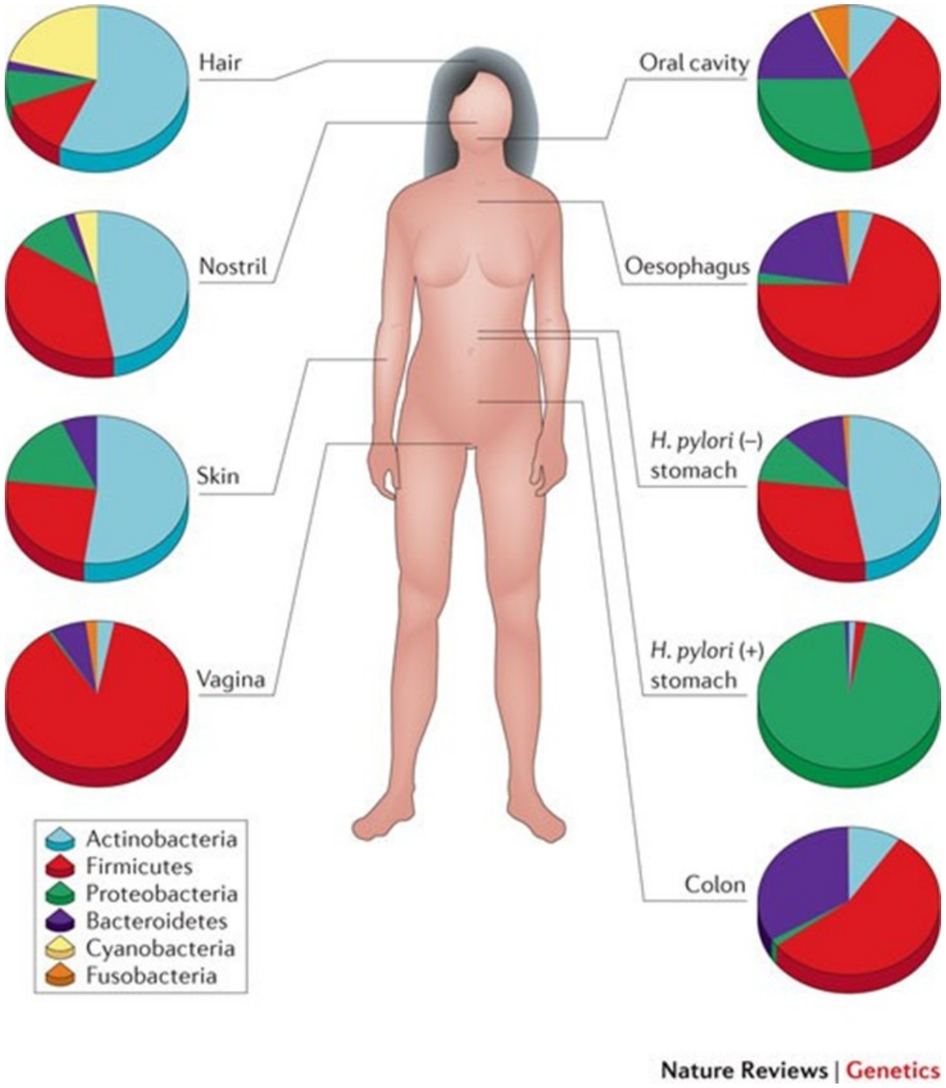


The importance of gut microbiome analysis – the role of micronutrients

Helena Šimurina, dr. med.,family medicine specialist

MICROBIOTA



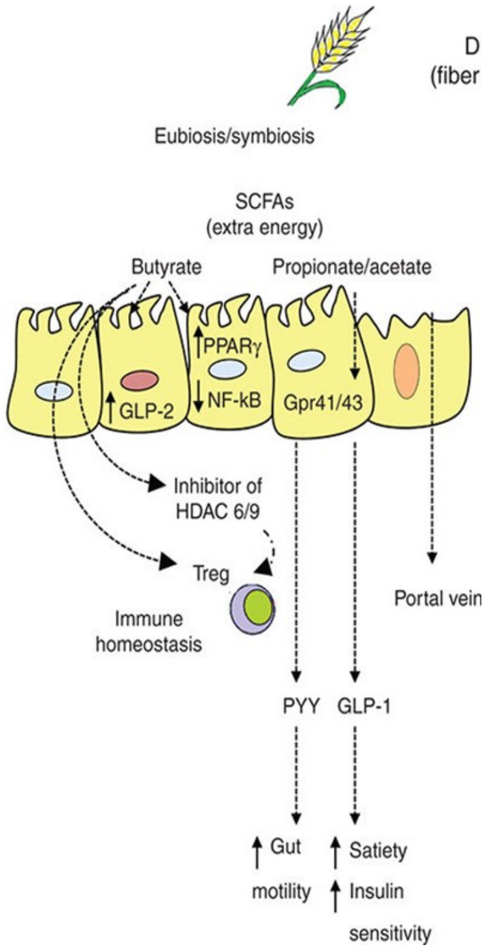
- It develops already in utero and is enriched and developed postnatally until the age of 3
- **3.8×10^{13} microorganisms in the gut – approximately the number of our own cells**
- > 3000 different species (new methods of detection)
- Everyone has a unique microbiota composition (1/3 of which is shared)
- Rural areas and traditional diets » richer microbiota
- Microbiota richness and diversity » stability and resilience against (short-term) stressors

- **150x more genes than the human genome**

ROLES OF THE GUT MICROBIOTA

- **PROTECTION FROM PATHOGENS AND GUT BARRIER INTEGRITY**
  - competition
  - bactericidal activity
  - maintenance of the barrier and intestinal cell's villi
  - stimulation of repair
  - maintaince of mucus layer

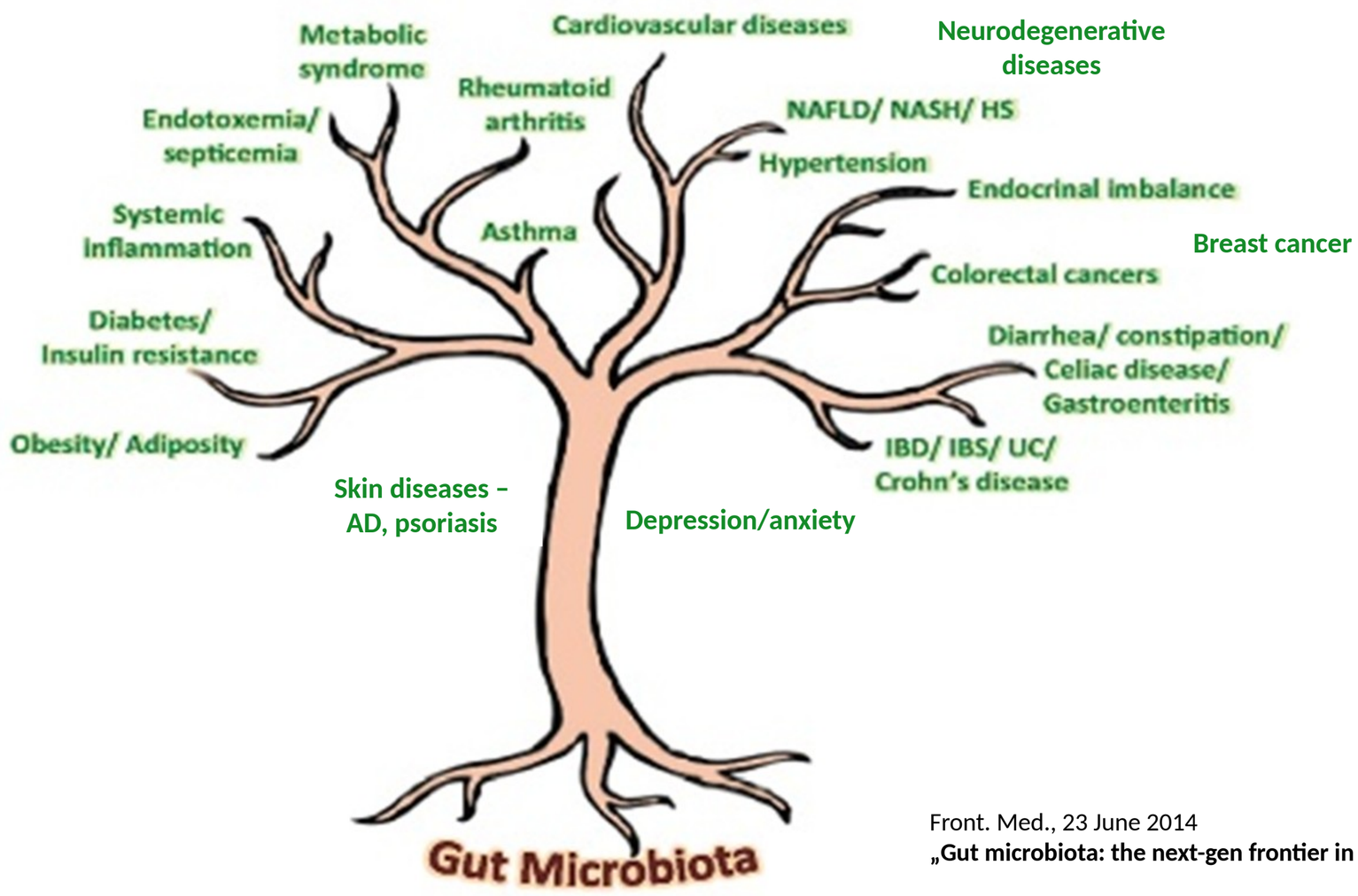
- **IMMUNITY**
  - maturation of the immune system
  - teaching tolerance to commensal bacteria and food
  - stimulation and modulation of the immune system
  - promotion of IgA production
  - anti-inflammatory effect



- **METABOLISM**
  - breakdown of polysaccharides and polyphenols, proteolysis
  - production of SCFA
  - synthesis of vitamins (K, B1, B6, B9, B12..)
  - regulation of fat and glucose metabolism and storage
  - bile acids metabolism and gut-liver axis
  - drug and hormone metabolism
  - bacterial metabolites entry into the systemic circulation

- **GUT-BRAIN COMMUNICATION**
  - bidirectional communication
  - involved in maturation of the HPA axis
  - production of neurotransmitters,
  - involved in HPA regulation, mood, sleep, stress response
  - dysbiosis – neuroinflammation

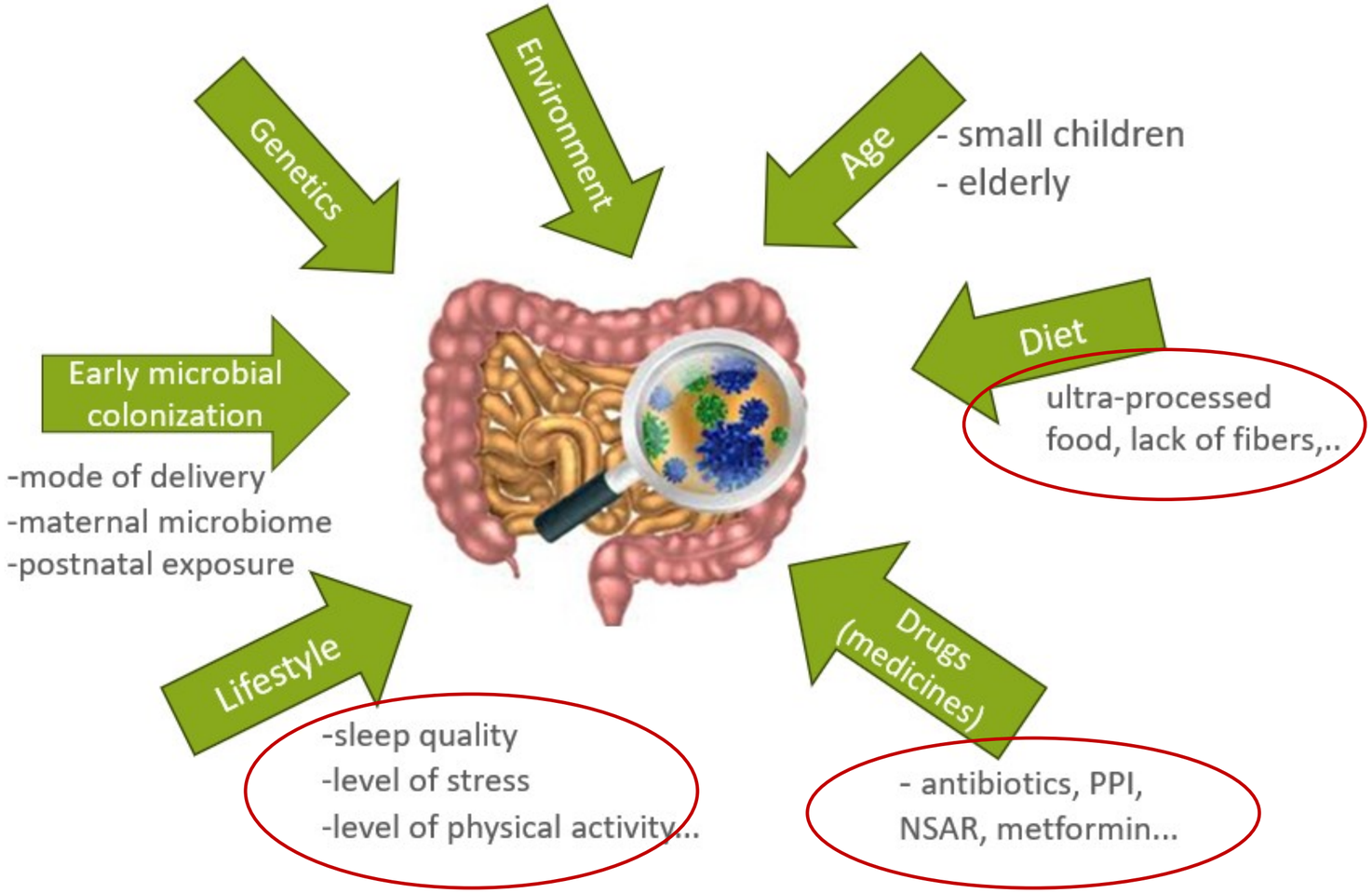
ASSOCIATION WITH VARIOUS PATHOLOGIES



Front. Med., 23 June 2014

„Gut microbiota: the next-gen frontier in preventive and therapeutic medicine?"

WHAT INFLUENCES MICROBIOTA



- **Diet** is the fastest way to alter the microbiome
- by consuming sufficient fiber, prebiotics... we support a healthy microbiome
- The **role of micronutrients**

- **Antibiotics** – greatly alter the composition of the microbiome, recovery takes months

- At the population level, **PPI drugs** have a more pronounced negative effect on the microbiome than antibiotics - PPI medications alter pH and disrupt the microbiome diversity and balance

- **NSAR** - suppresses prostaglandins, which are essential for maintaining the gut lining » resulting in "leaky gut" and mucosal inflammation; cause decrease in butyrate producing bacteria and increase in Gram-negative bacteria

Elevated cortisol causes a decrease in the abundance of SCFA producers, while pro-inflammatory species increase.

MICRONUTRIENTS, PHYTONUTRIENTS AND MICROBIOTA

	Microbial diversity	anti-inflammatory bacteria	Supporting intestinal epithelial barrier /regulation of tight junction proteins	pro-inflammatory bacteria
VITAMIN D	++	++	++	
VITAMIN C	++	++		↓↓
VITAMIN E	++	++	++	↓↓
VITAMIN A	++	++	++	↓↓
VITAMINS B	+	++		
ZINC	++		++	
resveratrol	++	++		↓↓
luteolin	++	++	++	↓↓

in excess causes dysbiosis and inflammation

Zeb F, Osaili T, Hashim M, et al. **Effect of Vitamin D Supplementation on Human Gut Microbiota: A Systematic Review of Randomized Controlled Trials**. *Nutr Rev*. 2026;84(6):1157-1166.

Cantorna MT, Snyder L, Arora J. **Vitamin A and vitamin D regulate the microbial complexity, barrier function, and the mucosal immune responses to ensure intestinal homeostasis**. *Crit Rev Biochem Mol Biol*. 2019;54(2):184-192.

MICRONUTRIENTS AND MICROBIOTA

IRON

- 10% to 20% of ingested iron is absorbed in the upper small intestine – unabsorbed iron to passes into the colon where it acts as a nutrient for luminal bacteria
- lower-dose or alternate-day dosing regimens maximizes absorption, or combine it with prebiotics and probiotic to minimize adverse microbial shift
- **iron in the colon alters the gut microbiota - promotes the growth of Proteobacteria** (*Escherichia coli*, *Salmonella*, *Shigella..)*
- **Iron overload in the lumen suppresses health-promoting, SCFA producing bacteria**,specifically *Bifidobact.* spp., *Lactobac.* spp., *Akkermansia*, *Faecalibacterium*, *Roseburia*

Rusu IG, Suharoschi R, Vodnar DC, et al. **Iron Supplementation Influence on the Gut Microbiota and Probiotic Intake Effect in Iron Deficiency-**A Literature-Based Review. *Nutrients*. 2020;12(7):1993. Published 2020 Jul 4.

Puga AM, Samaniego-Vaesken ML, Montero-Bravo A, Ruperto M, Partearroyo T, Varela-Moreiras G. **Iron Supplementation at the Crossroads of Nutrition and Gut Microbiota: The State of the Art.** *Nutrients*. 2022;14(9):1926.

MICROBIOME ANALYSIS

TESTING HISTORY

- **Culture-Dependent Techniques:** Involve growing microorganisms on agar plates or in liquid media using Gram stains and biochemical tests. *Limitation:* Over 99% of gut and environmental microbes are unculturable in a lab

- **Sanger Sequencing:** Used historically for targeted sequencing of isolated strains or specific marker genes. *Limitation:* Low throughput and unable to process complex, mixed communities directly

- **16S rRNA sequencing:** uses PCR to target specific bacterial/archaeal marker genes, yielding lower-cost, genus-level taxonomy for bacteria only. Does not measure functional genes. Tools can statistically *conclude* what metabolic paths might be active based on taxonomy, but this misses novel or strain-specific functions

- Shotgun metagenomic sequencing:

- ❖ sequences all genomic DNA in a sample to provide multi-kingdom (bacteria, archaea, viruses, fungi) species- and strain-level resolution
- ❖ sequences functional genes – insight in enzyme production and metabolic pathways
- ❖ direct functional profiling

Bars-Cortina D, Ramon E, Rius-Sansalvador B, et al. **Comparison between 16S rRNA and shotgun sequencing in colorectal cancer, advanced colorectal lesions, and healthy human gut microbiota.** BMC Genomics. 2024;25(1):730. Published 2024 Jul 29. doi:10.1186/s12864-024-10621-7

MICROBIOME ANALYSIS - SHOTGUN METAGENOMIC SEQUENCING

Wellmicro® Gut Test Plus

- Uses high-throughput **Shotgun Metagenomic Sequencing** via the Illumina NovaSeq 6000Dx platform
- Detection of commensal, oportunistic and enteropathogenic bacterial species, parasites, fungi, and viruses
- **Use of in-house database** (derived from public databases) for identification and analysis
- **provides insight into the degree of dysbiosis**, abundance and ratio of genera, species and strains; comparison to healthy controls
- **analysis of microbiome metabolic potential**
  (potential for producing of SCFAs, B-group and K vitamin, production of H_2S and methane, polyphenol metabolism and metabolism of bile acids….)
- Analysis of GABA and typtophan metabolism (indol, IAA, IPA, serotonin)
- Production of pyruvate and fumarate (sports performance)
- Activity of beta glucuronidase and sulfatase enzyme (metabolism of drugs and hormones)



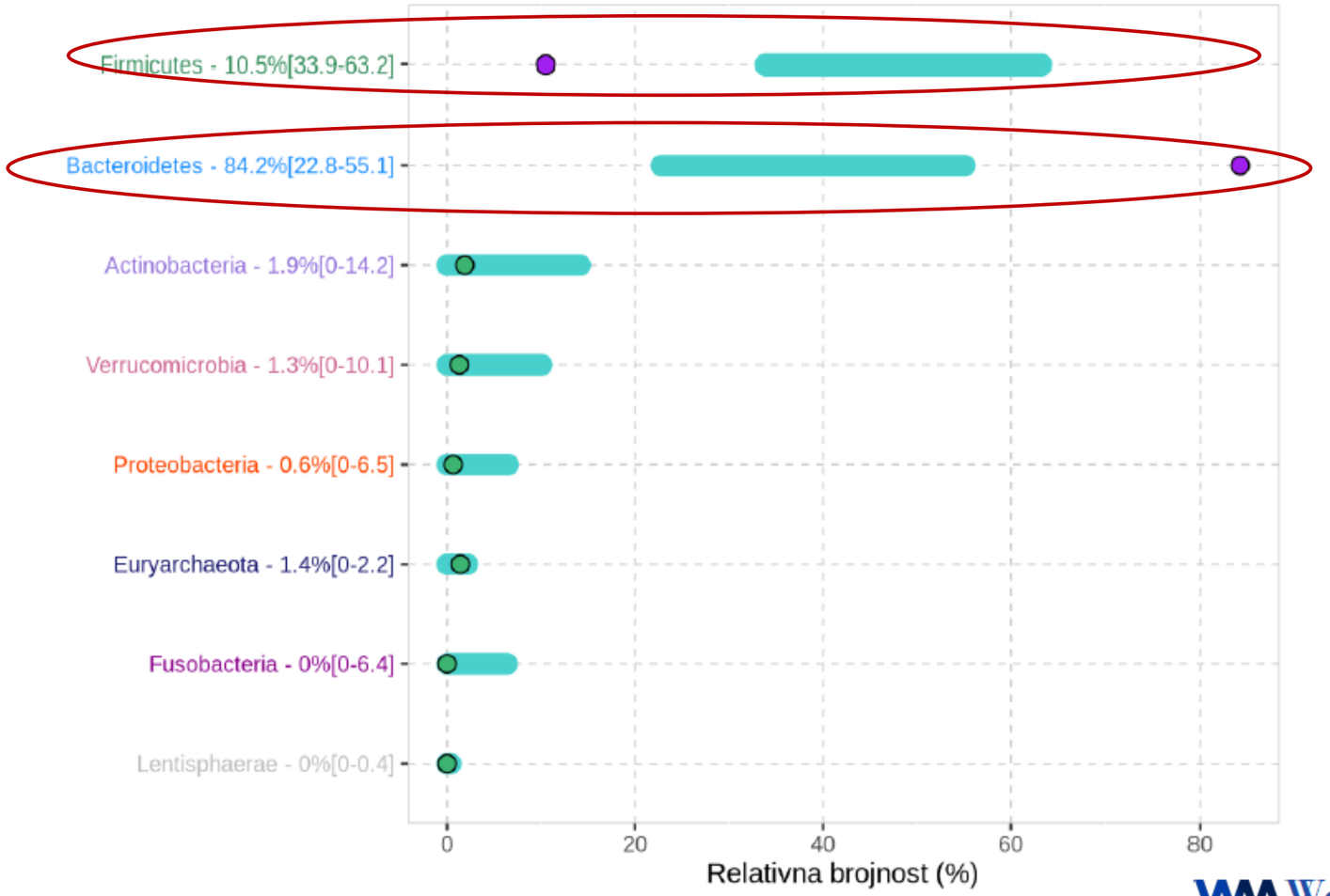
The influence of the microbiome on bodily functional indices
(immunity, lipid and carbohydrate metabolism, cardiovascular health, skin health, gut-brain axis, gut-liver axis…)

PERSONALIZED RECOMMENDATIONS

- nutrition – specific food
- probiotics/prebiotics,
- micronutrition, phytonutrients

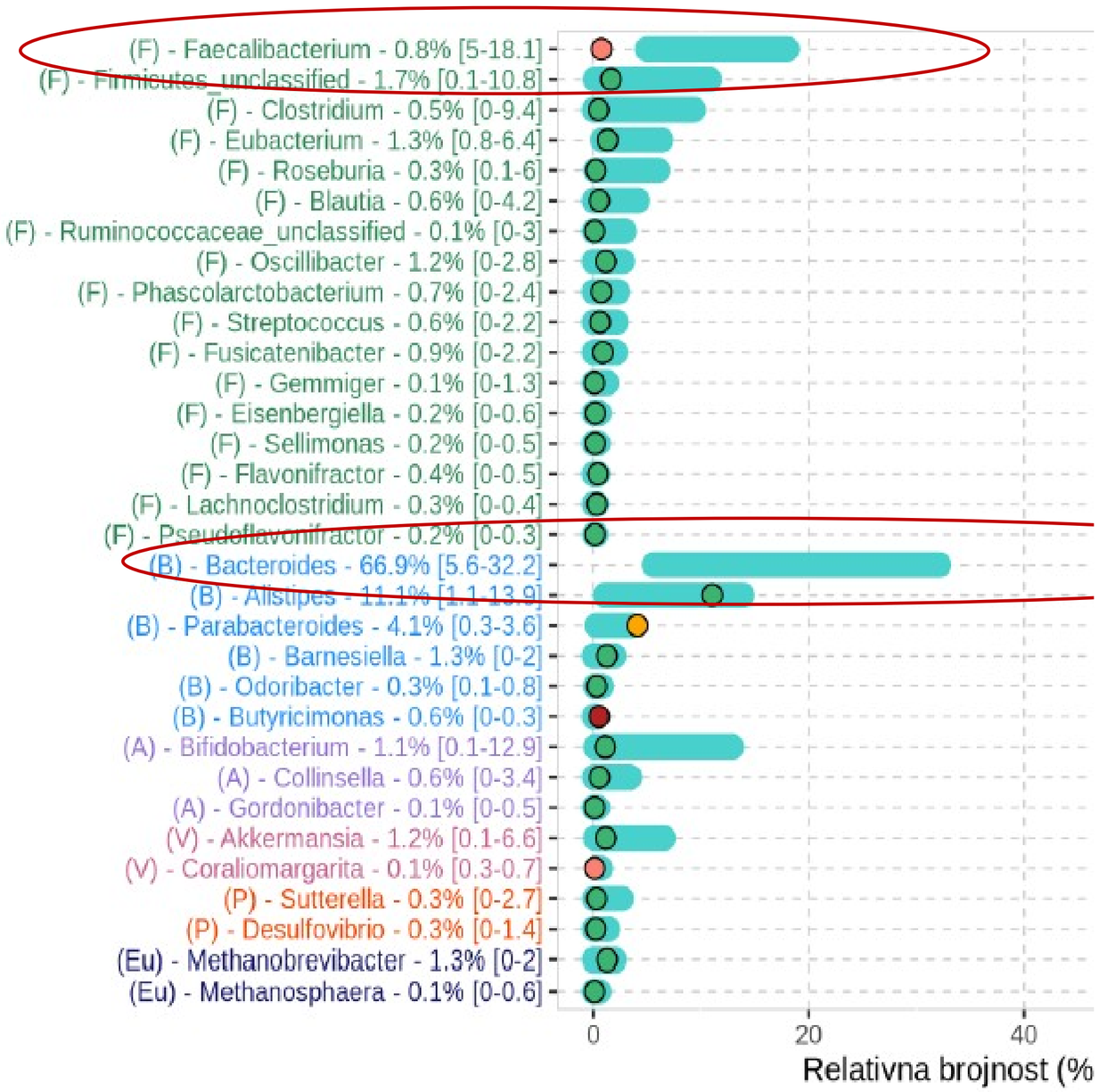
EXAMPLE OF MICROBIOME ANALYSIS

- Woman, 54 years old, perimenopause,
- weight 62 kg, height 172 cm
- Irregular diet, forgets to eat, mostly only one concrete meal a day, insufficient protein and fiber intake,
- Does not exercise,sedentary lifestyle, has had knee problems since youth
- Non-smoker, consumes alcohol on a weekly basis
- No food restrictions
- Suffers from significant constipation (8/10) on a daily basis, bloating (6/10) on a weekly basis, and abdominal pain (6/10) on a weekly basis.
- Experiences anxiety (6/10) on a weekly basis and depression (7/10) on a monthly basis.
- Has frequent urinary tract infections, osteoarthritis (hands, knees), and fatigue



Wellmicro®

JESENSKA AKADEMIJA SUPLEMENTACIJE U MEDIC Zagreb



Metabolizam	Nedostatak⇐ ⇒Višak
Acetat	1
Butirat	-4
Propionat	4
Sukcinat	1
Laktat	-4
GABA	4
Histamin	-4
Indol	4
Indol 3 octena kiselina (IAA)	-4
3 – Indolpropionska kiselina (IPA)	-4
Triptamin	-4
Serotonin	2

Metabolizam	Nedostatak⇐ ⇒Višak
Trimetilamin (TMA)	0
Polifenoli	2
Vitamini gr. B	1
Vitamini K2	4
Razgradnja glutena	0
Mukoliza	0
Proteoliza	0
Lipopolisaharid (LPS)	0
Sekundarne žučne kiseline	2
Etanol	3
Vodikov sulfid (H_2S)	1
Metan (CH_4)	0

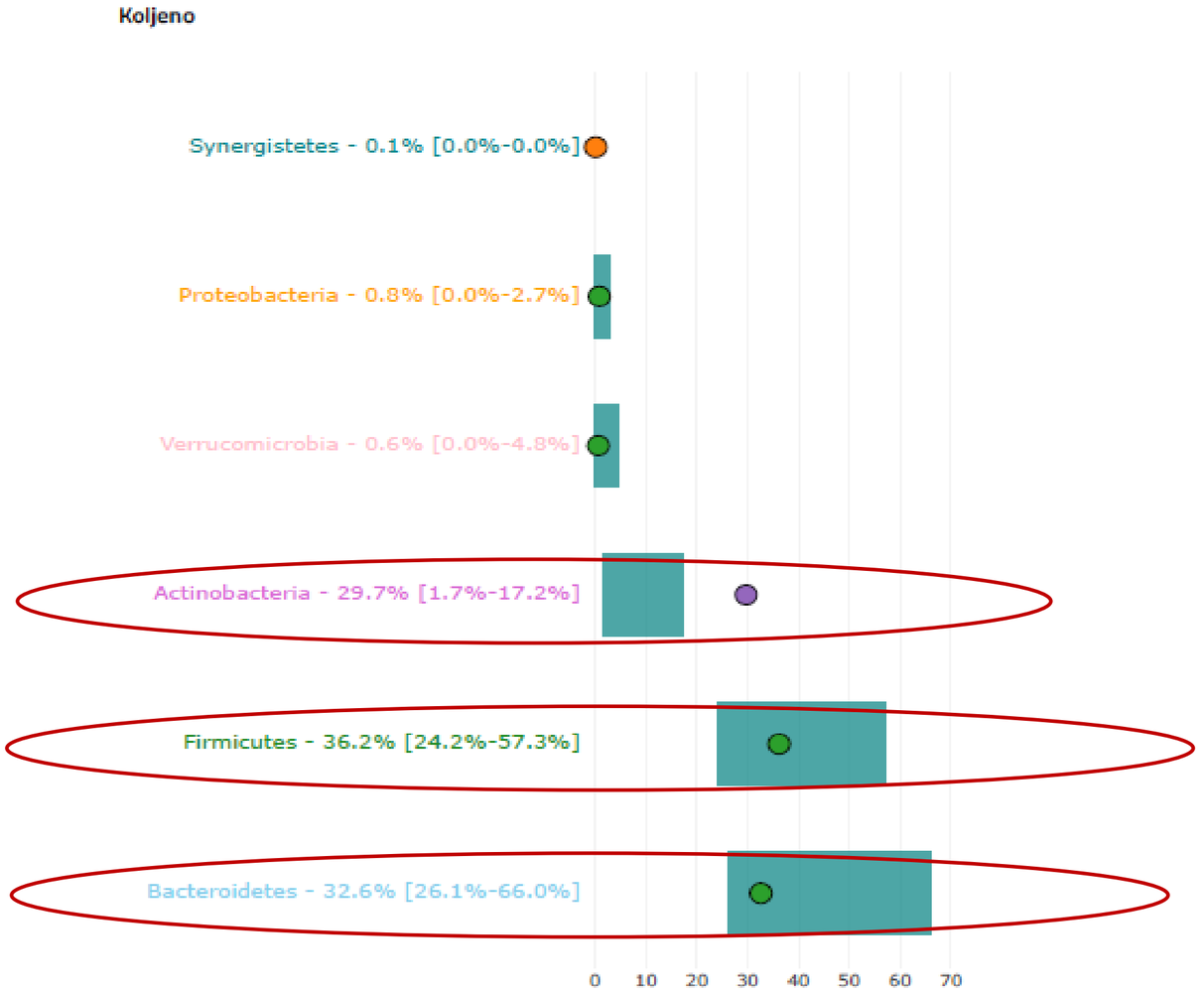
Indeks	Izmijenjeno⇦ ⇨Optimalno
Imunološka homeostaza	2
Homeostaza sluznice	4
Homeostaza glukoze	2
Metabolizam lipida	5
Protuupalno djelovanje	4
Antimikrobno djelovanje	5
Os crijeva-mozak	2
Intestinalno-kardiocirkulacijska osovina	4
Osovina crijevo-jetra	3
Os crijevo-koža	2
Cirkadijalni ritam	4

Protuupalno djelovanje	*Ruminococcus, Faecalibacterium, Parabacteroides, Bacteroides, Lactobacillus*	Avokado, bijeli jogurt, Borovnica, cimet, Crni ribizl, Grejp, Komorač , kupina, Kupina, naranca s crvenim mesom, Pu Erh čaj, rezistentni škrob tip 2, Tamarinda	arbutin, Astragalus resveratrol, ekstrakt sjemenki grejp, FOS, gorka dinja (Momordica charantia), gospina trava, hesperidin, inulin, kitozan, kofein, Korijen čička, korijen sladića, luteolin, melatonin, resveratrol, Retinoična kiselina, rezistentni škrob, taninska kiselina, vitamin B 3 , vitamin B1, vitamin B12, Vitamin B6, Vitamin B7, Vitamin C, Vitamin D, vražja kandža	*Bacillus subtilis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium butyricum, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus kefiri (NON KEFIR), Lactobacillus plantarum, Lactobacillus r hamnosus GG, Lactobacillus reuteri, Streptococcus Cajrmophilus*
Antimikrobno djelovanje	*Ruminococcus, Parabacteroides, Bacteroides, Lactobacillus*	Avokado, bijeli jogurt, cimet, Crni ribizl, Grejp, Komorač , kupina, Kupina, naranca s crvenim mesom, Pu Erh čaj, rezistentni škrob tip 2, Tamarinda	arbutin, ekstrakt sjemenki grejp, FOS, gorka dinja (Momordica charantia), gospina trava, hesperidin, inulin, kitozan, kofein, Korijen čička, korijen sladića, luteolin, melatonin, resveratrol, Retinoična kiselina, rezistentni škrob, taninska kiselina, vitamin B 3 , vitamin B1, vitamin B12, Vitamin B6, Vitamin B7, Vitamin C, Vitamin D, vražja kandža	*Bacillus subtilis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium butyricum, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus kefiri (NON KEFIR), Lactobacillus plantarum, Lactobacillus r hamnosus GG, Lactobacillus reuteri, Streptococcus Cajrmophilus*
Os crijeva-mozak	*Ruminococcus, Parabacteroides, Bacteroides, Lactobacillus*	Avokado, bijeli jogurt, cimet, Crni ribizl, Grejp, Komorač , kupina, Kupina, naranca s crvenim mesom, Pu Erh čaj, rezistentni škrob tip 2, Tamarinda	arbutin, ekstrakt sjemenki grejp, FOS, gorka dinja (Momordica charantia), gospina trava, hesperidin, inulin, kitozan, kofein, Korijen čička, korijen sladića, luteolin, melatonin, resveratrol, Retinoična kiselina, rezistentni škrob, taninska kiselina, vitamin B 3 , vitamin B1, vitamin B12, Vitamin B6, Vitamin B7, Vitamin C, Vitamin D, vražja kandža	*Bacillus subtilis, Bifidobacterium lactis, Bifidobacterium longum, Clostridium butyricum, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus kefiri (NON KEFIR), Lactobacillus plantarum, Lactobacillus r hamnosus GG, Lactobacillus reuteri, Streptococcus*

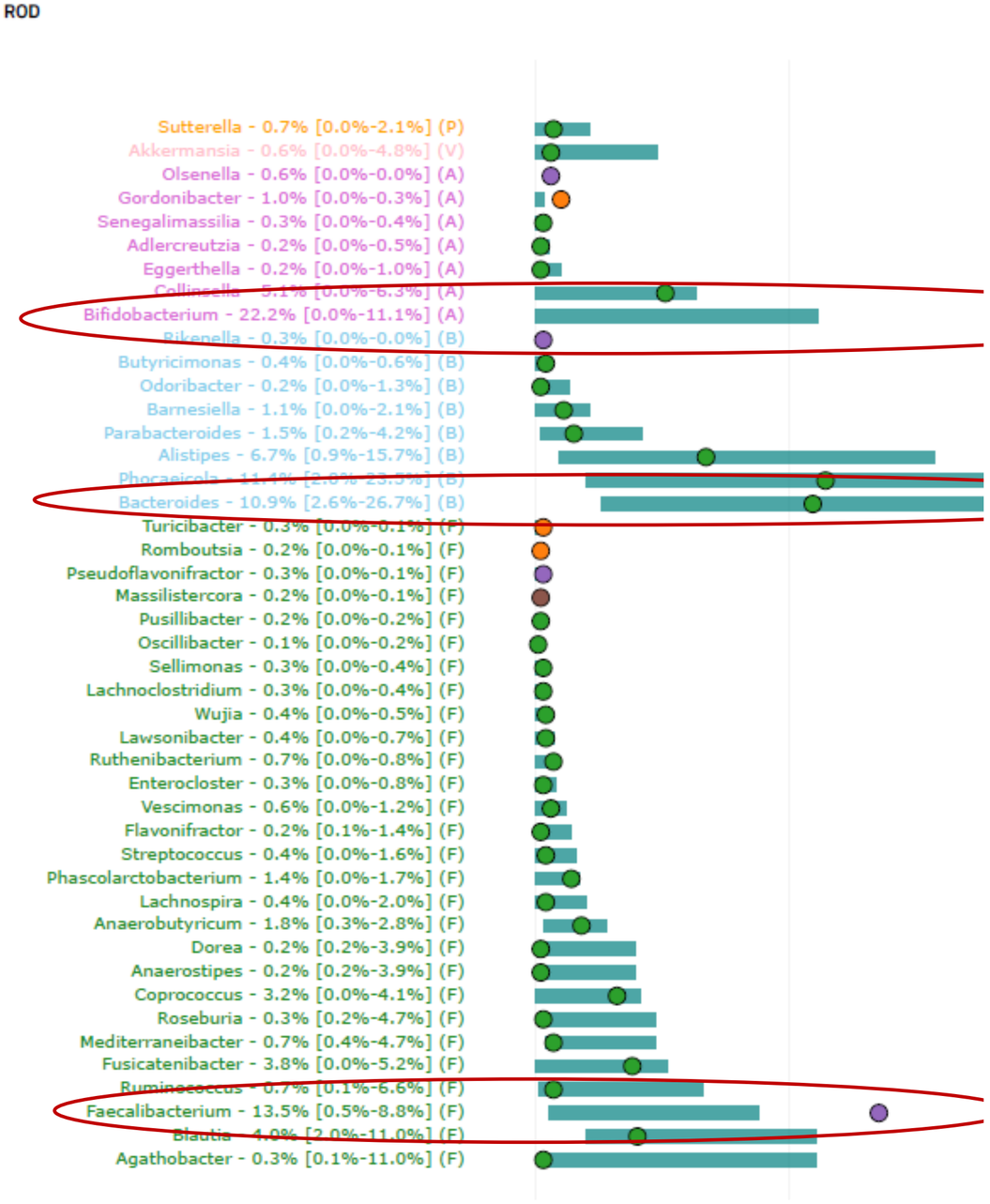
CONTROL MICROBIOME ANALYSIS AFTER 6 MONTHS

- Female, 54 years old, perimenopause,
- Body weight 62 kg, height 172 cm

- Adopted a routine of regular, healthy meals (breakfast, lunch, dinner)
- Pays attention to the intake of fiber, protein, and healthy fats
- Took probiotic strains based on Wellmicro recommendations for 3 months
- Multivitamin, glutamine



Wellmicro®



Metabolit	Vrijednost
Acetat	2.8
Butirat	2
Propionat	-1.6
Sukcinat	-1
Laktat	2.9
GABA	2.9
Serotonin	2.9
Histamin	0.7
Indol	0.3
Indol-3-octena kiselina (IAA)	0.6
Indol-3-propionska kiselina (IPA)	0.6
Triptamin	0.5
Trimetilamin (TMA)	-0.1
Putrescin	-1

Metabolit	Vrijednost
Kadaverin	-1
Spermidin	-1.2
Polifenoli	0.4
Vitamini skupine B	2.1
Vitamin K2	-0.4
Lipopolisaharid (LPS)	0
Sekundarne žučne kiseline	0.8
Etanol	0
Sumporovodik	0.1
Metan	-0.2
Fumarat	0.3
Piruvat	-0.2
Beta-glukuronidaza	-0.6
Sulfataza	-0.9

Indeks	Izmijenjeno⇦ ⇨Optimalno
Imunološka homeostaza	2
Homeostaza sluznice	4
Homeostaza glukoze	2
Metabolizam lipida	5
Protuupalno djelovanje	4
Antimikrobno djelovanje	5
Os crijeva-mozak	2
Intestinalno-kardiocirkulacijska osovina	4
Osovina crijevo-jetra	3
Os crijevo-koža	2
Cirkadijalni ritam	4

6 mjeseci intervencije

PRIMARNE FUNKCIJE	Vrijednost
Crijevna barijera	5.8
Imunosna homeostaza	5.2

- manje problema sa konstipacijom i nadutošću
- više energije
- manje bezvoljnosti i anksioznosti

SEKUNDARNE FUNKCIJE	Vrijednost
Cirkadijalni ritam	5
Crijevno-endokrina os	3.7
Crijevno-jetrena os	6.6
Crijevno-kardiovaskularna os	6.6
Crijevno-kožna os	6.8
Crijevno-mišićna os	6.8
Crijevno-moždana os	3.9
Homeostaza lipida	6.7
Homeostaza ugljikohidrata	5.6

ZAHVALJUJEM NA PAŽNJI!