

Promjene mikrobiote u postmenopauzi

Doc.dr.sc. Donatella Verbanac

Mihaela Perić, Hana Čipčić Paljetak, Mario Matijašić, Marina Panek,
Ana Kunović, Darija Vranešić i Željko Krznarić

Odjel za međustaničnu komunikaciju Centar za translacijska i klinička istraživanja Sveučilište u Zagrebu
Medicinski fakultet i Klinički bolnički centar Zagreb

Zagreb, 19-11-2016



Your Gut Has a
Mind of Its Own

The Second Brain

A Groundbreaking New Understanding
of Nervous Disorders of the
Stomach and Intestine

"Persuasive, impassioned . . . hopeful news [for those]
suffering from functional bowel disease."

— *New York Times Book Review*

Michael D. Gershon, M.D.

Mikrobiota – je naš organ

„The international *MetaHIT (Metagenomics of the Human Intestinal Tract)* project has published a gene catalogue of the human gut microbiome derived from 124 healthy, overweight and obese human adults, as well as inflammatory disease patients, from Denmark and Spain. The data provide the first insights into this *gene set - over 150 times larger than the human gene complement* - and permit the definition of both a minimal gut metagenome and a minimal gut bacterial genome.

Project Coordinator – Prof. S.D. Ehrlich

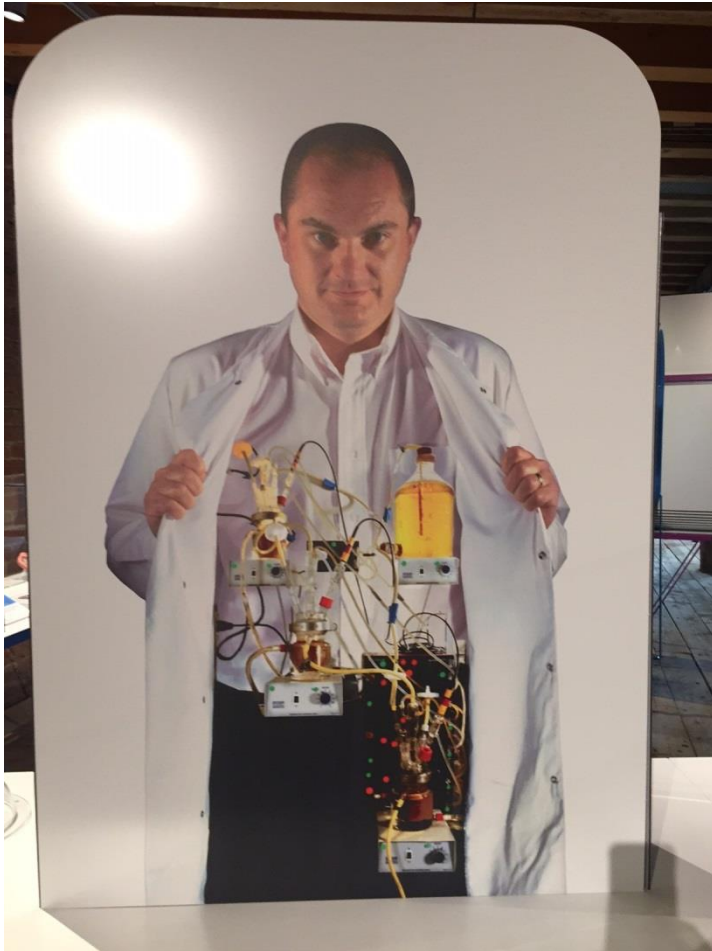
Nature, 2011

Mi smo zdravi kad smo sastavljeni od:

**10% ljudskih stanica i
90% bakterijskih stanica**



Simulacije probavnog trakta



Uloga mikrobiote

• Zaštitna

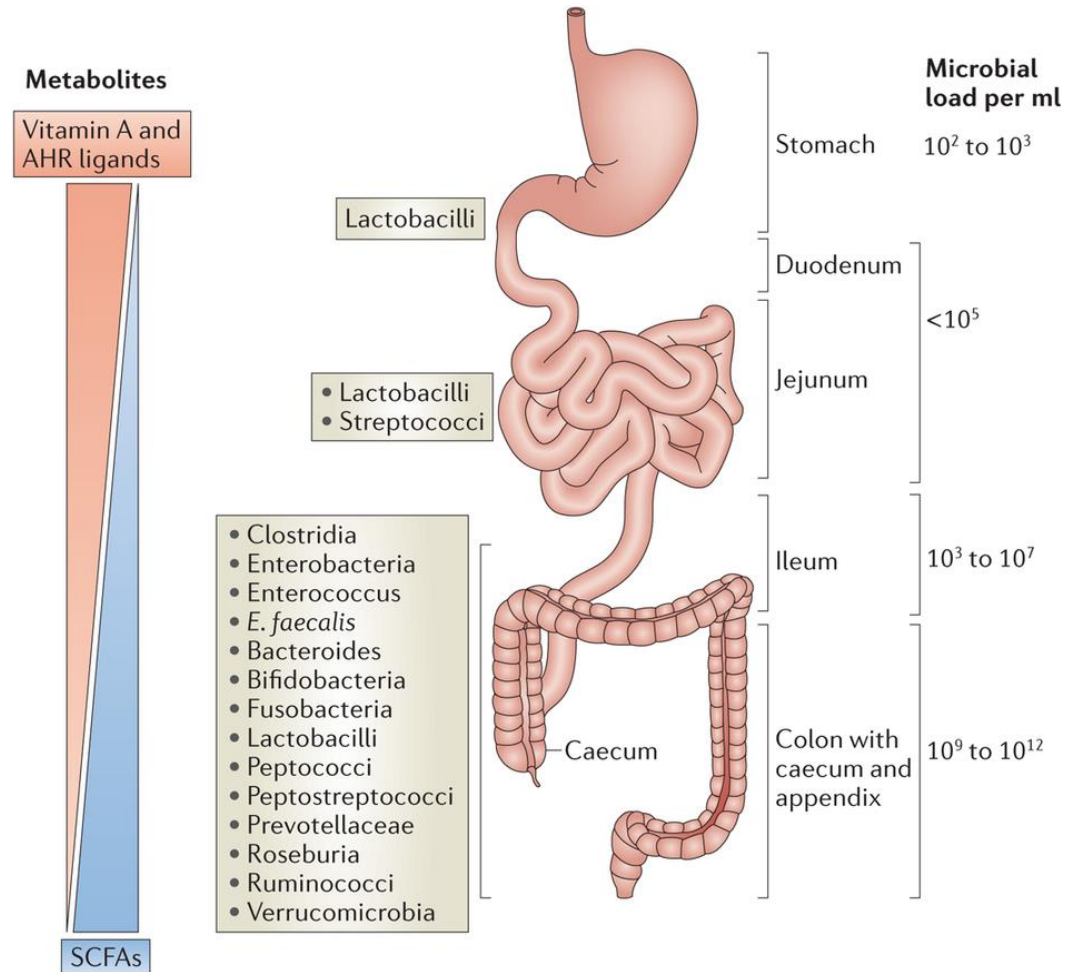
- Otklanja patogene bakterije
 - Kompeticija za hranjiva
 - Kompeticija za receptore
 - Lučenje antimikrobnih čimbenika

• Strukturna

- „Prirodna granica”
 - „Tight junctions” među stanicama epitela
- Razvoj imunog sustava
- **Sinteza IgA**


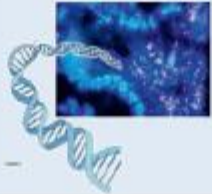


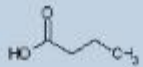
• Metabolička

- Sinteza kratko-lančanih MK
- Metabolizam UH iz hrane
- Sinteza vitamina K, folne kiseline i biotina
- Apsorpcija Fe^{2++}

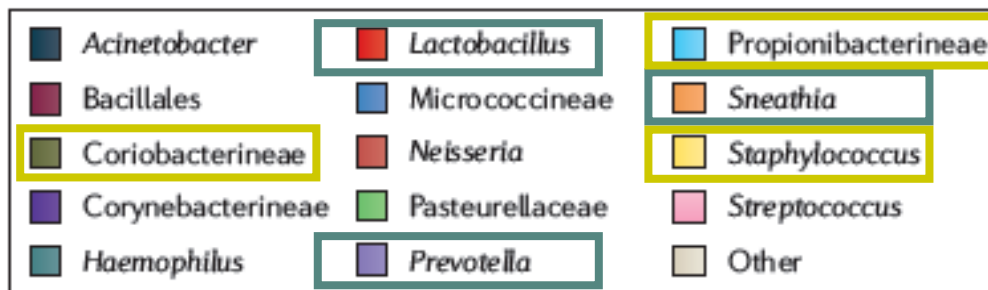
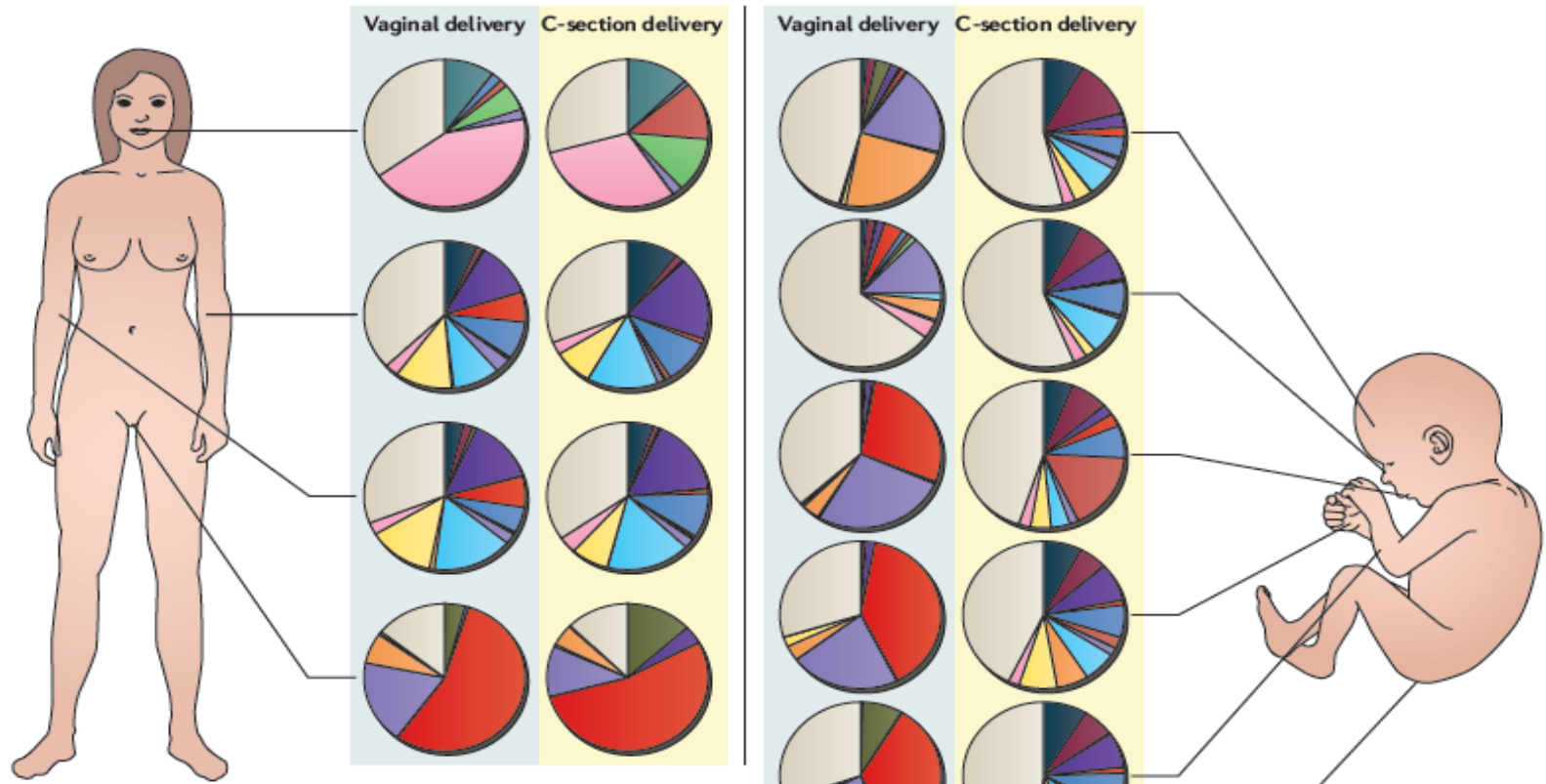


Metode istraživanja mikrobiote

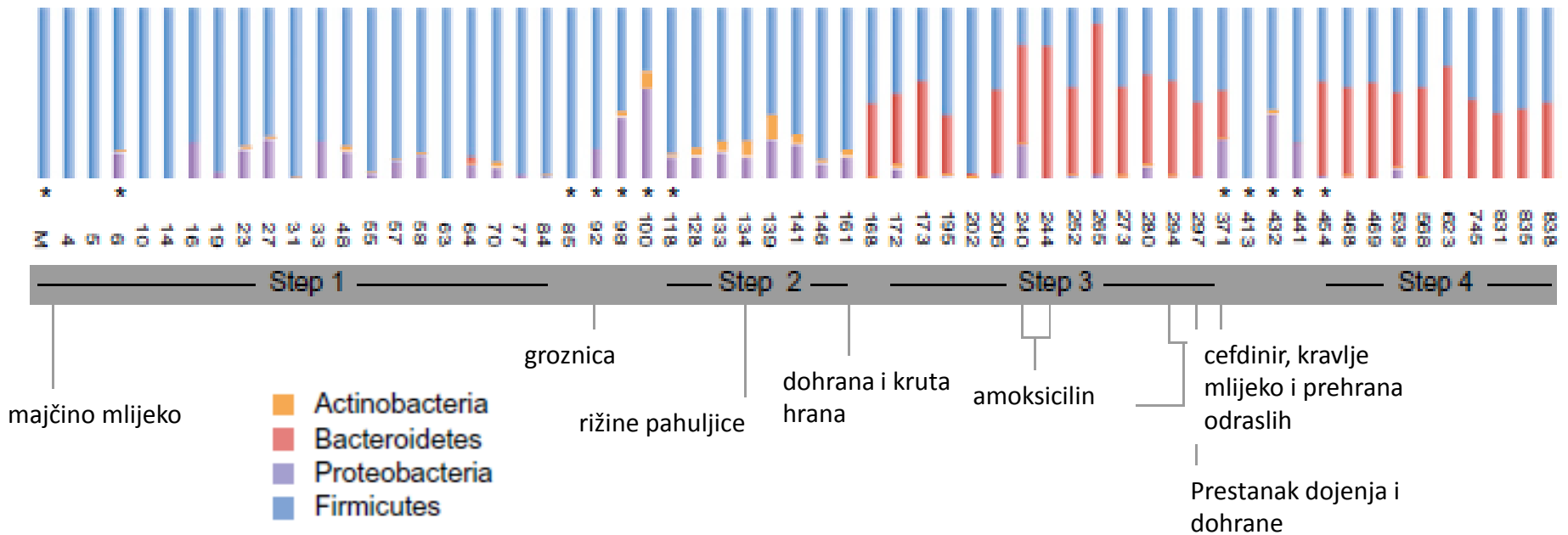
- **Filogenetska analiza**
 - Sekvenciranje gena za 16S rRNA
 - Sastav, relativna zastupljenost
- **Metagenomika**
 - Sekvenciranje genomske DNA
 - Analiza setova gena – funkcionalna metagenomika
 - Filogenetski odnosi
- **Metatranskriptomika**
 - Analiza cjelokupne mRNA, uvid u diferencijalnu ekspresiju gena
- **Metaproteomika**
 - Analiza ukupnih proteina – različita ekspresija ovisno o uvjetima
- **Metabolomika**
 - Profiliranje metabolizma domaćina i mikrobiote

Meta-omics		Molecule
Phylogeny		16S rDNA
Metagenomics		Chromosomal genomic DNA
Metatranscriptomics		Messenger RNA/ cDNA
Metaproteomics		Proteins/ Peptides
Metabolomics		Metabolites

Usvajanje mikrobiote – novorođenčad



Razvoj mikrobiote u najranijoj dobi



Čimbenici koji moduliraju crijevnu mikrobiotu kod novorođenčadi

POZITIVNI

vaginalni porod
 terminski porod
 dojenje
 izlaganje mikroorganizmima

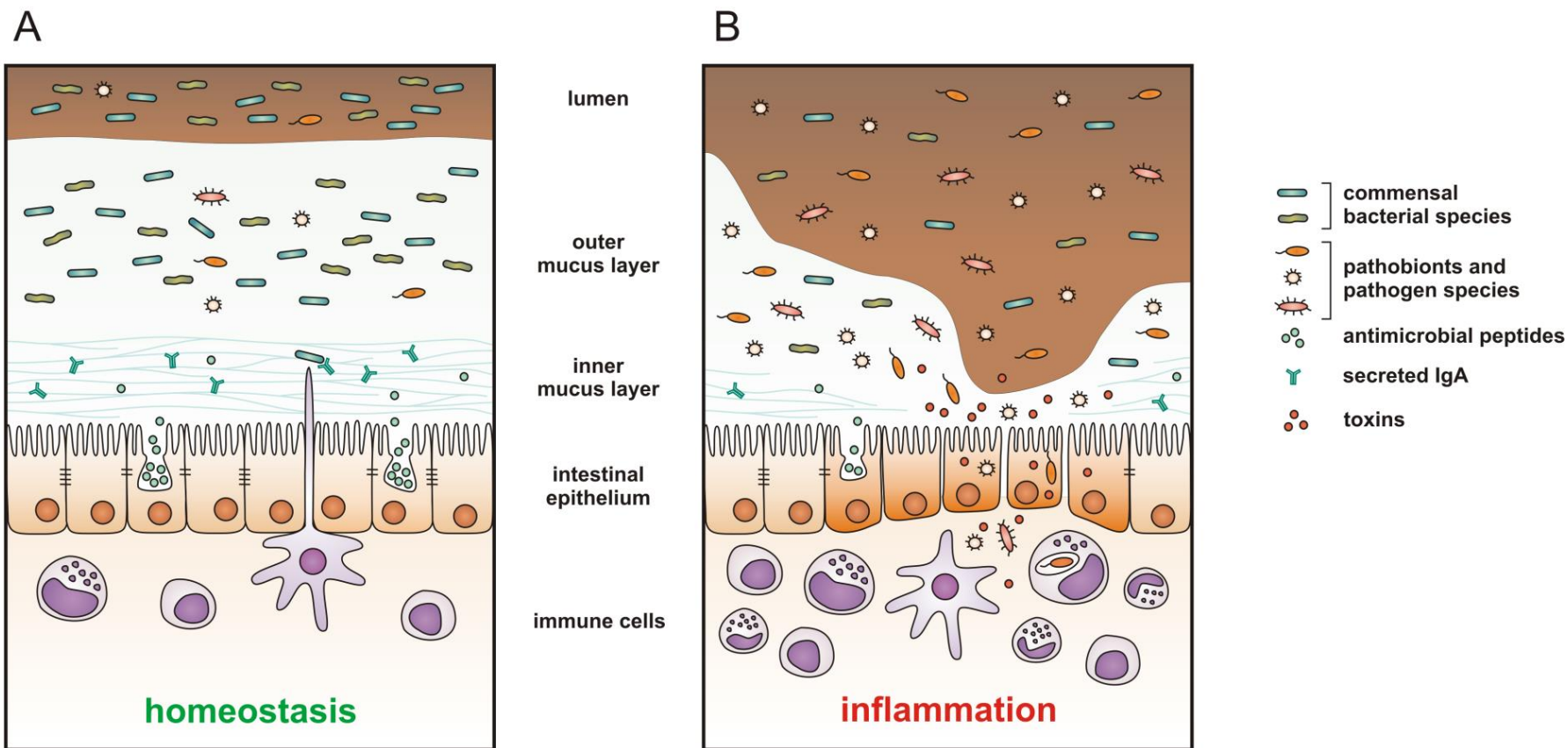
NEGATIVNI

porod na carski rez
 terapija antibioticima
 dohrana
 prijevremeni porod

INTERVENCIJE

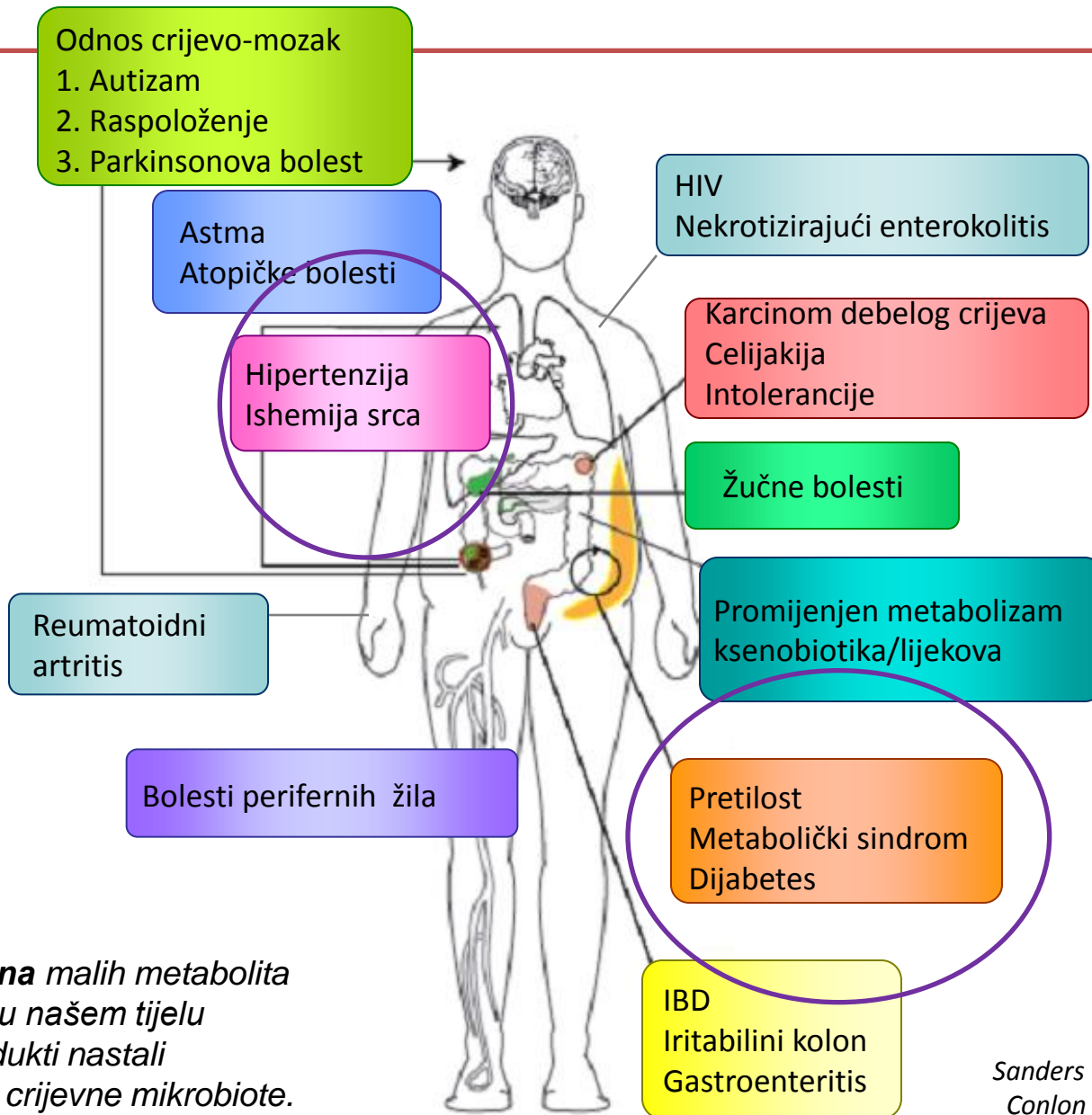
probiotici od rođenja
vaginalni brisevi

Poremećaj u ravnoteži crijevne mikrobiote – disbioza



Kronična upala - vodi do nastanka kroničnih metaboličkih bolesti

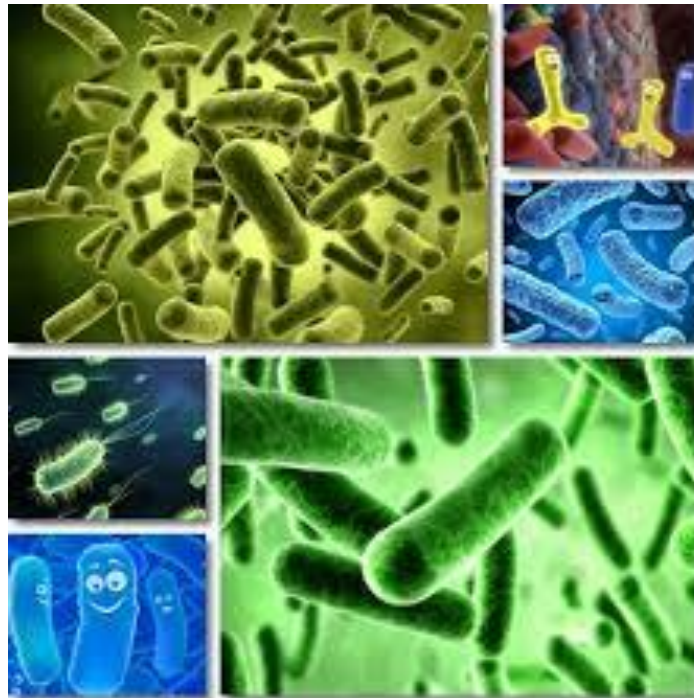
Bolesti povezane s promjenom mikrobiote



Približno **trećina** malih metabolita koji cirkuliraju u našem tijelu izravni su produkti nastali pod utjecajem crijevne mikrobiote.

Probiotici

- živi mikroorganizmi s pozitivnim utjecajem na ravnotežu u crijevu
 - *Lactobacillus*, *Bifidobacterium*, *Bacillus*
- Mijenjaju metabolizam:
 - Lipida, ugljikohidrata, kolesterola, makro i mikro nutrijenata, te reguliraju tjelesnu masu



Prebiotici

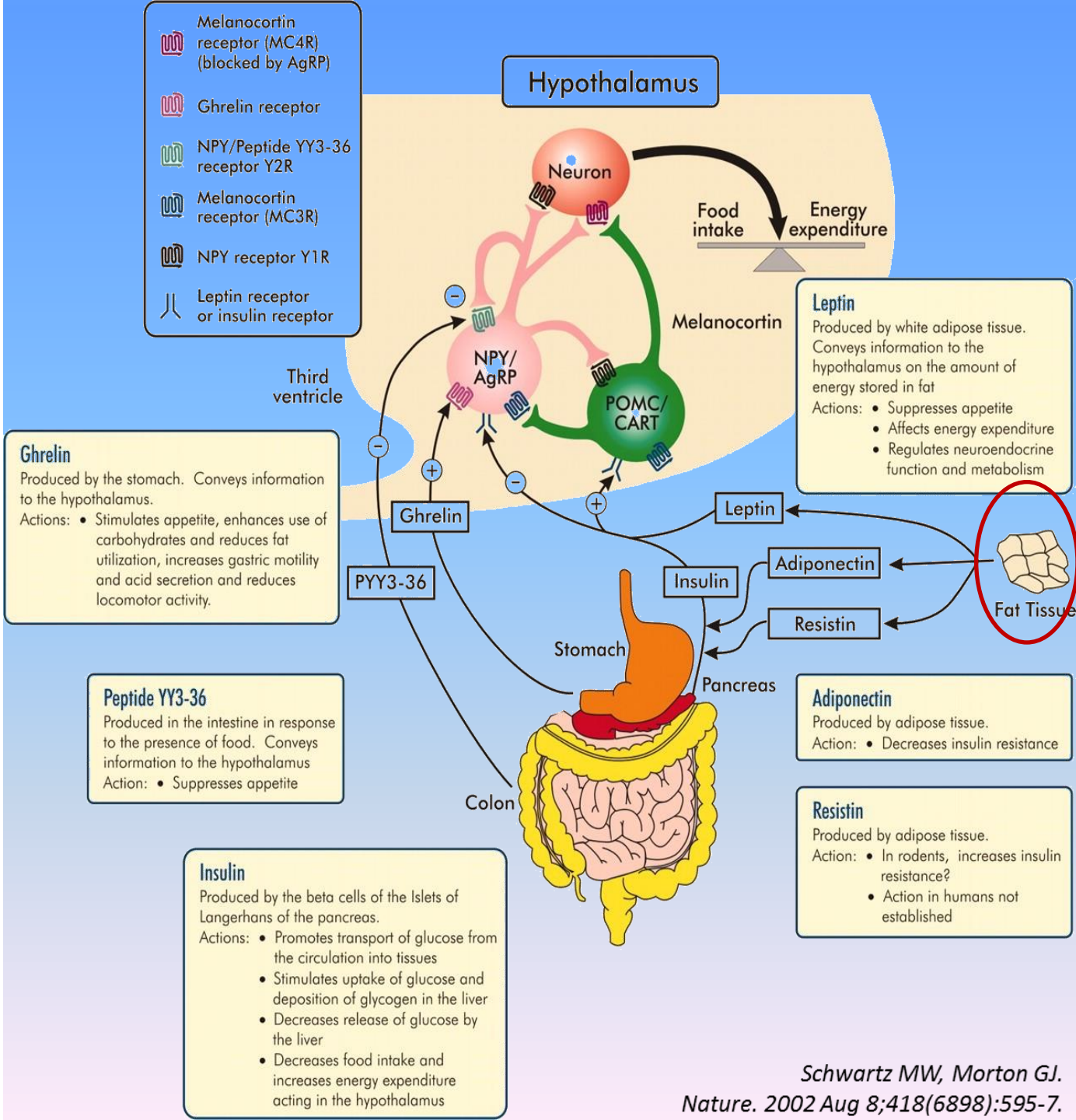
- Nerazgradive sastavnice hrane - potiču rast i/ili aktivnost povoljnih crijevnih bakterija
 - Polioli – ksilitol, sorbitol, manitol
 - Disaharidi – **laktuloza**, laktitol
 - Oligosaharidi – rafinoza iz soje, **oligofruktoze**, **galakto-oligosaharidi**,
 - Ostali nerazgradivi poli- i oligosaharidi – palatinoza, laktosaharoza
 - Namirnice/jela: kuhani krumpir; svježi voćni sokovi iz bobičastog voća
 - Polisaharidi – **inulin**, škrob otporan na glikolitičke enzime
 - Oligosaharidi majčinog mlijeka
- Sinbiotici – sinergističke mješavine probiotika i prebiotika



Novije istraživanja

SPOLNI HORMONI I MENOPAUZA SPECIFIČNI ASPEKTI MIKROBIOTE

Naš metabolizam pod utjecajem je središnjeg živčanog sustava, ali i...



Promjena sastava mikrobiote u pretilosti i posljedičnim bolestima

❑ Istraživanja su pokazala da se udio bakterija u crijevu mijenja promjenom životnog stila:

- Povećanje *Bacteroidetes* i smanjenje *Firmicutes* mršavljenjem (Nadal, 2008)
- Kod pretilo djece više *Firmicutes* (Kalliomaki 2008)
- Smanjenje *Firmicutes* nakon barijatričke kirurgije i mršavljenja (Zhang, 2009), te povećanje *Bacteroides/Prevotella* (Furet, 2010)

❑ Mikrobiom posjeduje **nižu raznolikost**, primjeri

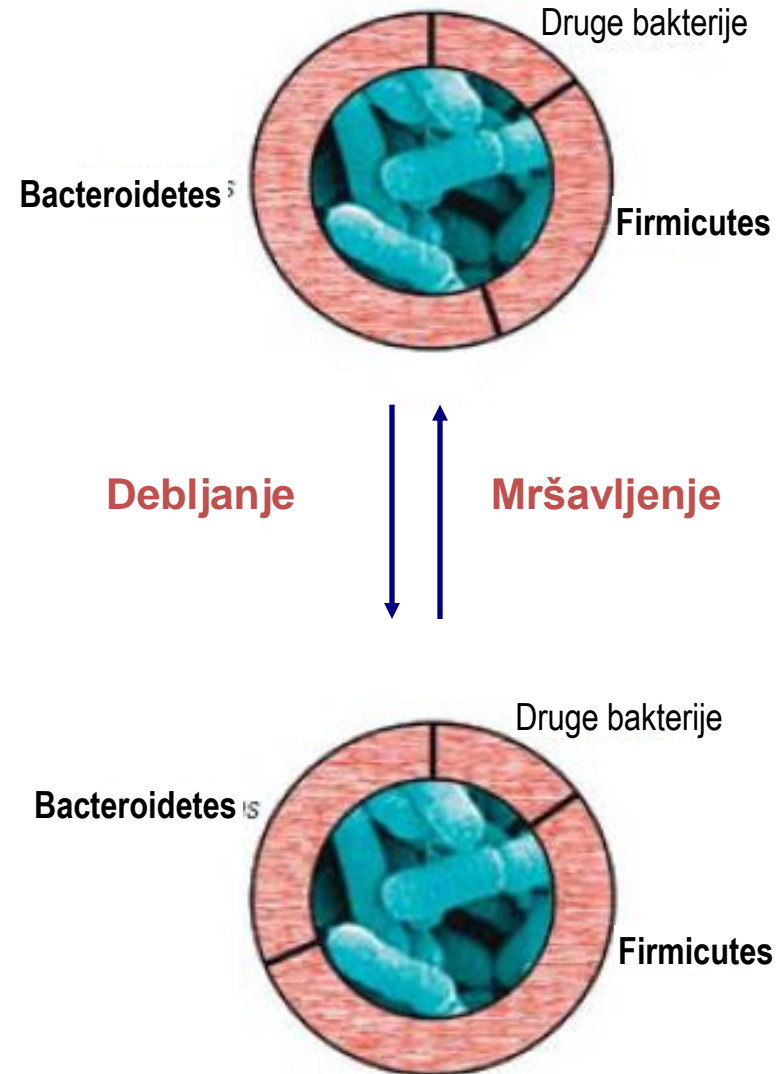
• **Pretilost (Greenblum, 2012)**

• Upalne bolesti crijeva (Ananthakrishnan, 2015)

• **Hipertenzija (Yang, 2015)**

❑ Omjer *Firmicutes* vs. *Bacteroidetes* (*F/B ratio*)

• Potencijalni biomarker patoloških promjena (Sanz, 2014)



Estradiol i prehrana vs. mikrobiota crijeva u OVX mišica

16 OVX mišica C57BL6

❖ 8 OVX (50 ug 17 β estadiol)

□ 8 OVX (uljna otopina - kontrola)

Ex

Uravnotežena dijeta

Dijeta bogata mastima

Dan 0

Dan 10

Dan 35

← Praćena tjelesna masa i skupljani su uzorci fecesa →

Rezultat:

8 OVX (uljna otopina - kontrola)

❖ Tjelesna masa **uvećana 30%**

❖ Firmicutes : Bacteroidetes



8 OVX (50 ug 17 β estadiol)

❖ Tjelesna masa održana

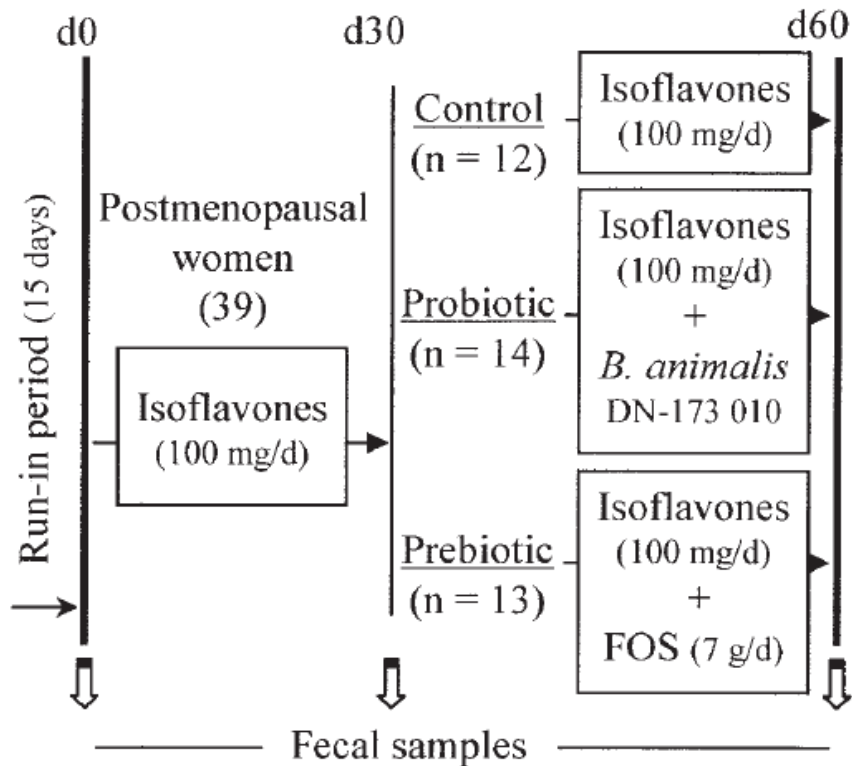
❖ Firmicutes: Bacteroidetes

STABILAN

Isoflavones and Functional Foods Alter the Dominant Intestinal Microbiota in Postmenopausal Women¹

Thomas Clavel,[†] Matteo Fallani,^{*} Patricia Lepage,^{*} Florence Levenez,^{*} Jacinthe Mathey,^{**} Violaine Rochet,^{*} Michèle Sérézat,^{*} Malène Sutren,^{*} Gemma Henderson,[†] Catherine Bennetau-Pelissero,[‡] Françoise Tondu,^{††} Michael Blaut,[†] Joël Doré,^{*2} and Véronique Coxam^{**}

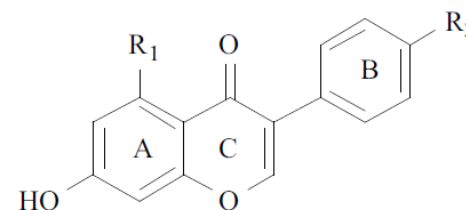
^{*}Institut National de la Recherche Agronomique, Unité d'Ecologie et de Physiologie du Système Digestif, Jouy-en-Josas, France; [†]German Institute of Human Nutrition Potsdam-Rehbruecke, Department of Gastrointestinal Microbiology, Nuthetal, Germany; ^{**}Groupe Ostéoporose, U3M, INRA Theix, Saint Genès-Champanelle, France; [‡]Unité Micronutriments, Reproduction, Santé, ENITA de Bordeaux, Gradignan, France; and ^{††}Danone Vitapole, Nutrition and Health Research, Palaiseau, France



- Prva studija te vrste provedena na ljudima
- Suplementacije hrane s **hormonski djelujućim tvarima (izoflavonima) i funkcionalnom hranom**
- Promjene u različitosti i sastavu dominantnih bakterija u crijevima.



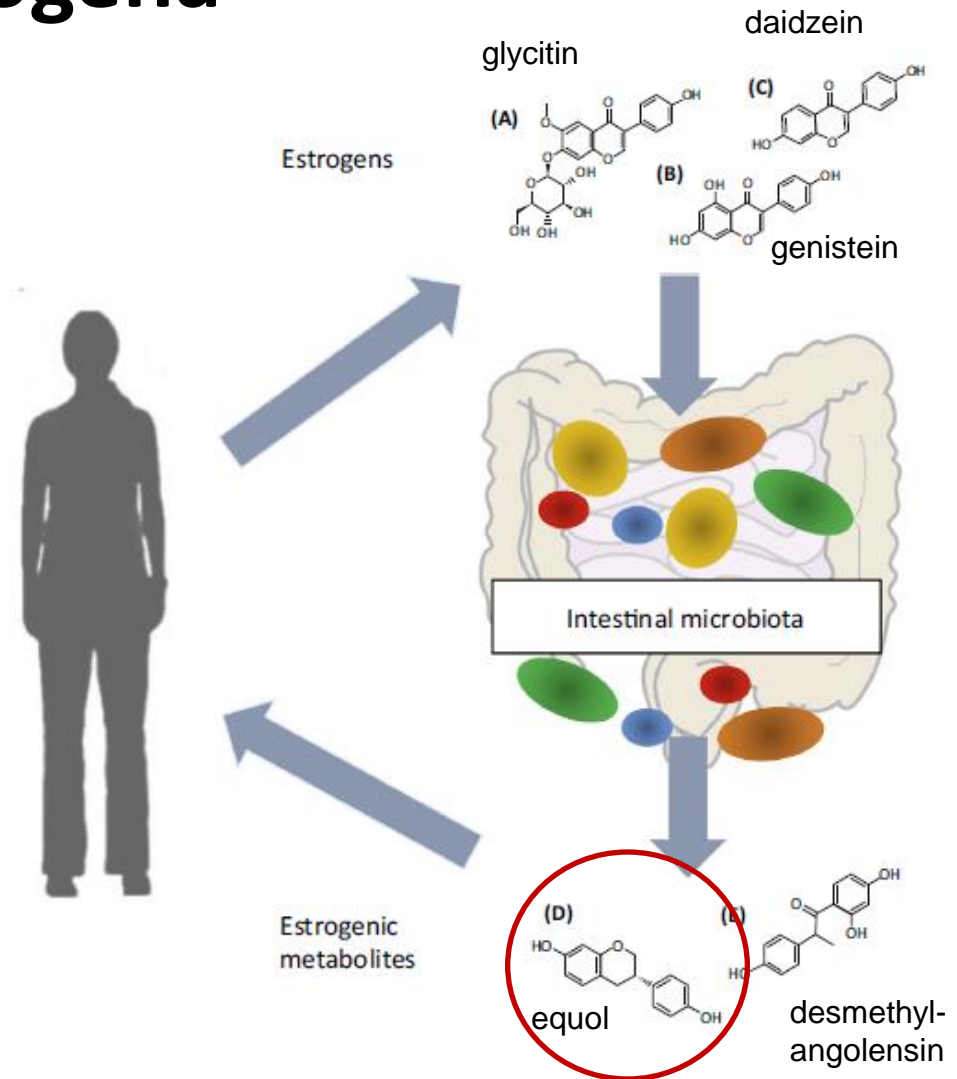
Izoflavoni soje



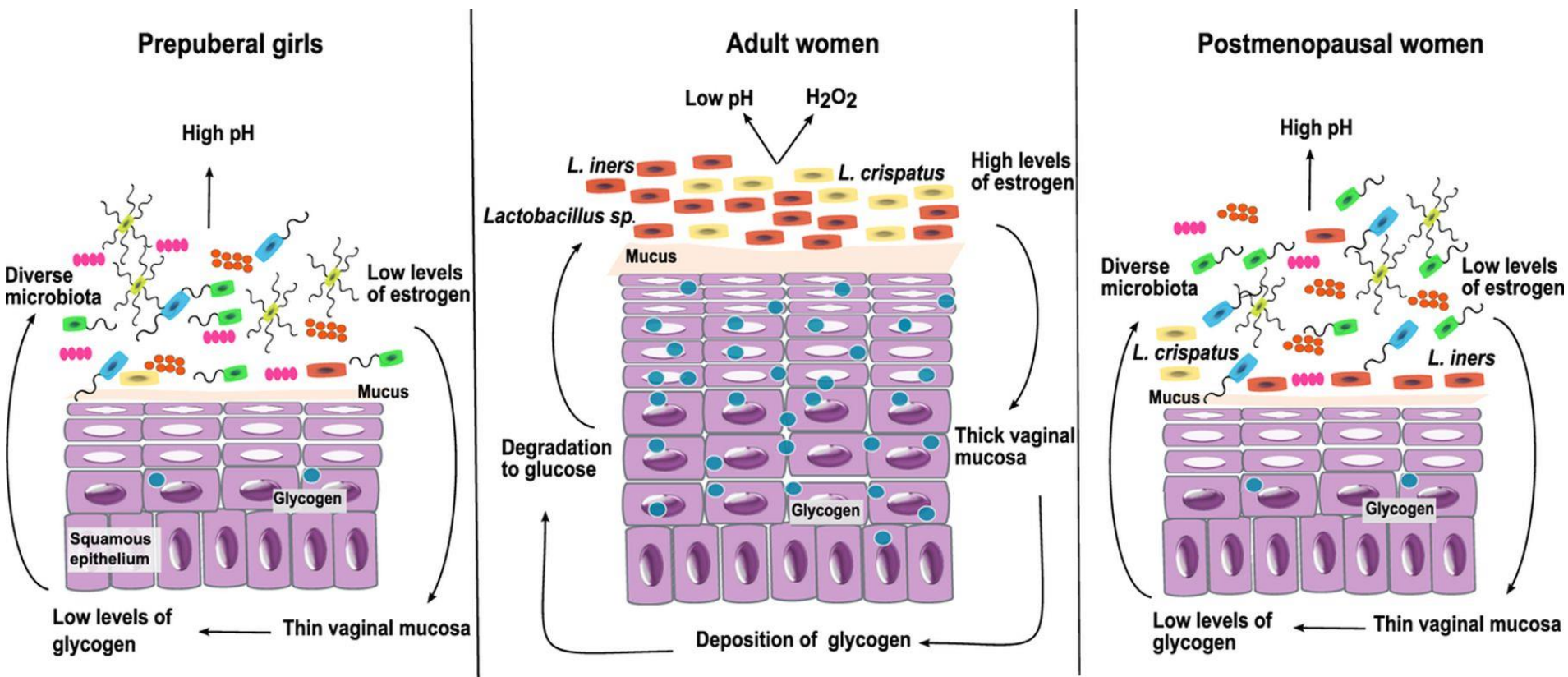
isoflavone	R ₁	R ₂
Biochanin A	OH	OCH ₃
Genistein	OH	OH
Formononetin	H	OCH ₃
Daidzein	H	OH

Međudodnos mikrobiote crijeva i estrogena

- Endogeni i egzogeni estrogeni i njima slični spojevi bivaju metabolizirani podu utjecajem crijevnih bakterija
- Nastali metaboliti djeluju pozitivno na metabolizam domaćina i smanjuju rizik obolijevanja od pojedinih bolesti
- Mikrobiota crijeva kao potencijalni biomerker pri vrednovanju i/ili u prevenciji bolesti izazvanih estrogenima



Promjene u vaginalnoj sluznici u različitim fazama u životu žene



- Microbiota zastupljena s *Lactobacillus sp.* - visoke razine estorogena
- Održavanje lagano kiselog pH sluznice - metabolizam glikogena - glukoze - laktata



Review

Modulating Composition and Metabolic Activity of the Gut Microbiota in IBD Patients

Mario Matijašić ^{1,*}, Tomislav Meštrović ², Mihaela Perić ¹, Hana Čipčić Paljetak ¹,
Marina Panek ¹, Darija Vranešić Bender ³, Dina Ljubas Kelečić ³, Željko Krznarić ^{3,4,5}
and Donatella Verbanac ¹

¹ Center for Translational and Clinical Research, University of Zagreb School of Medicine, 10000 Zagreb, Croatia; mihaela.peric@mef.hr (M.Pe.); hana.paljetak@mef.hr (H.Č.P.); marina.panek@mef.hr (M.Pa.); donatella.verbanac@mef.hr (D.V.)

² Clinical Microbiology and Parasitology Unit, Polyclinic "Dr. Zora Profozić", Bosutska 19, 10000 Zagreb, Croatia; tomislav.mestrovic@gmail.com

³ Department of Internal Medicine, Division of Clinical Nutrition, Clinical Hospital Centre Zagreb, 10000 Zagreb, Croatia; dvranesi@kbc-zagreb.hr (D.V.B.); ljubas.dina@gmail.com (D.L.K.); zeljko.krznaric1@zg.t-com.hr (Ž.K.)

⁴ Department of Internal Medicine, Division of Gastroenterology and Hepatology, Clinical Hospital Centre Zagreb, 10000 Zagreb, Croatia

⁵ Department of Internal Medicine, University of Zagreb School of Medicine, 10000 Zagreb, Croatia

* Correspondence: mario.matijasic@mef.hr; Tel.: +385-01-4590-070

Academic Editors: Terrence Piva and Denis Girard

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Kliničke studije koje ukazuju na korist od uzimanja probiotika

Probiotik	Način korištenja	Učinak
<i>L. rhamnosus</i> , <i>B. animalis</i>	Oralni probiotik/1x, 256 trudnica, 1 tromj.	Smanjenje gastacijskog dijabetesa
<i>L. rhamnosus</i>	Oralni dodatak probiotika, 6-mjesečne bebe	Povećanje otpornosti na perturbacije mikrobiote i prerastanje patogena, sprječavanje alergijskih bolesti
<i>B. longum</i> , <i>B.</i> <i>animalis</i> , <i>L.</i> <i>acidophillus</i>	Oralni probiotik, trudnice/dojilje/dojenčad 1-2 mj. prije i 6 mj. nakon poroda	Smanjena učestalost ekcema kod novorođenčadi
<i>L. rhamnosus</i> , <i>L. reuteri</i>	5 dana/1x/vaginalete	Veća uspješnost izlječenja bakterijske vaginoze nego s metronidazolom

Intervencija probioticima- primjer hipertenzija

Effect of Probiotics on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials

Saman Khalesi, Jing Sun, Nicholas Buys and Rohan Jayasinghe

Hypertension, 2014

- Meta-analiza d 9 randomiziranih studija koje su pratile utjecaj intervencije probioticima na krvni tlak
- Značajno smanjenje sistoličkog krvnog tlaka uočeno je u pacijenata koji su unosili dnevno $\geq 10^9$ CFU probiotika
- Mogući mehanizmi djelovanja
 - Modulacija **lipidograma** (Gou, 2011)
 - Djelovanje na renin–angiotensin; inhibicija angiotenzin konvertirajućeg enzima preko **tripeptida VPP i/ili IPP** (Ong, 2008)
 - Apsorpcija bioloških tvari i **fitoestrogena iz s vazodilatacijskim svojstvima** (Lye, 2009)
 - Modulacija metabolizma **glukoze, smanjenje pretilosti i upale općenito** (Valentini, 2014)

The Gut Microbiota Regulates Bone Mass in Mice

Klara Sjögren,¹ Cecilia Engdahl,¹ Petra Henning,¹ Ulf H Lerner,^{1,2} Valentina Tremaroli,³ Marie K Lagerquist,¹ Fredrik Bäckhed,³ and Claes Ohlsson¹

¹Centre for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

²Department of Molecular Periodontology, Umeå University, Umeå, Sweden

³Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

Journal of Bone and Mineral Research, Vol. 27, No. 6, June 2012, pp 1357–1367

Review

CellPress

Effects of the gut microbiota on bone mass

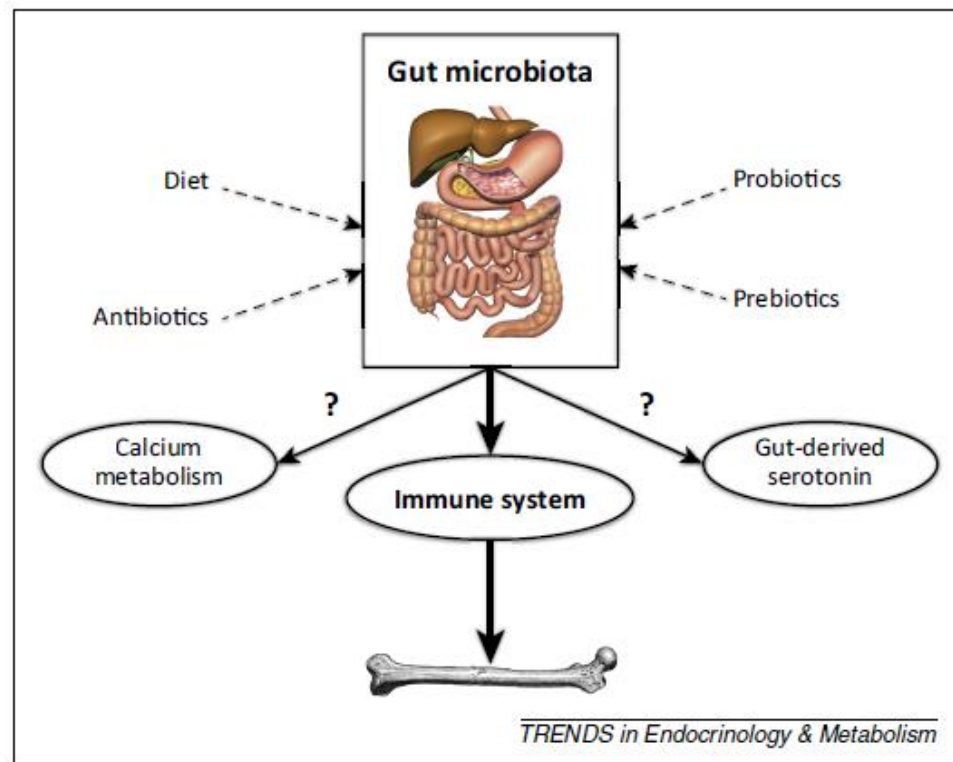
Claes Ohlsson and Klara Sjögren

Centre for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, 413 45 Gothenburg, Sweden

Trends Endocrinol Metab. 2015 Feb;26(2):69-74.

Mogući mehanizam djelovanja

- Mikrobiota crijeva (MC) jedan je od predloženih regulatora koštane mase
 - Nedavna istraživanja na germ-free miševima to potvrđuju
- Međuodnos je posredovan imunološkim sustavom odgovornim za regulaciju osteoklastogeneze
- Dodatno se istražuje metabolizam kalcija i serotonin sintetiziranog u crijevima
- Prehrana i okolišni čimbenici utječu na sastav MC.
- Intervencijska istraživanja **autora pokazala su da antibiotici, probiotici i prebiotic koji mijenjaju MC, reguliraju i metabolizam kosti**



- ✓ **Autori zagovaraju istraživanja MC – novi pristup u iznalaženju terapijskih opcija za osteoporozu**

Projektni tim – MINUTE for IBD

Assessment of Microbiota, Inflammatory Markers, Nutritional and Endocrinological Status in IBD Patients - HRZZ – 2014-2018

- **Medicinski fakultet SuZ**- Mihaela Perić, Mario Matijašić, Hana Čipčić Paljetak, Marina Panek, Vera Kufner, Donatella Verbanac
- **Imperial College London, UK** - Anja Barešić
- **KBC Zagreb** - Željko Krznarić, Silvija Čuković Čavka, Darija Vranešić Bender, Marko Brinar, Mirjana Kalauz, Dora Grgić, Nikša Turk, Ivana Kraljević, Dunja Rogić, Dina Ljubas Kelečić, Ana Kunović
- **KB Dubrava** - Marija Crnčević Urek, Marko Banić
- **Poliklinika „Dr. Zora Profozić”** – Tomislav Meštrović
- **Fidelta d.o.o.** - Karmen Brajša
- **Xellia Pharmaceuticals** - Gabrijela Ergović

HVALA NA PAŽNJI



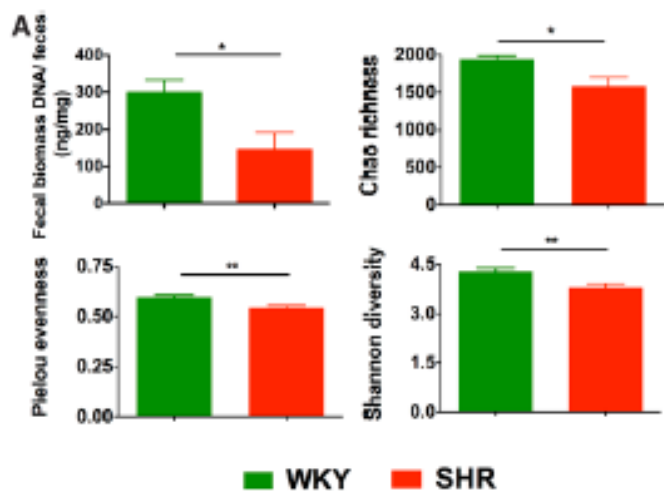
NASA is in
by adding
encoura

Microbiota and Hypertension

DOI: 10.1161/HYPERTENSIONAHA.115.05315

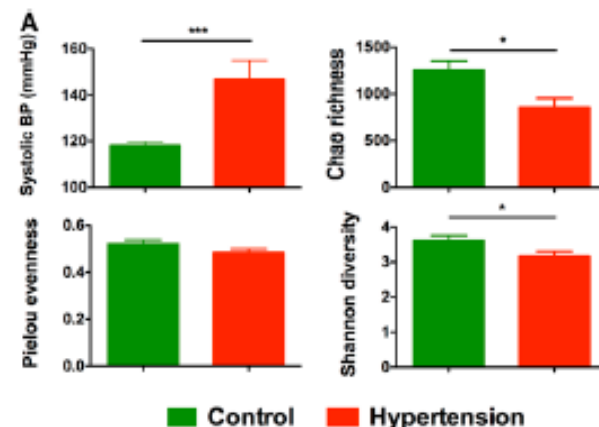
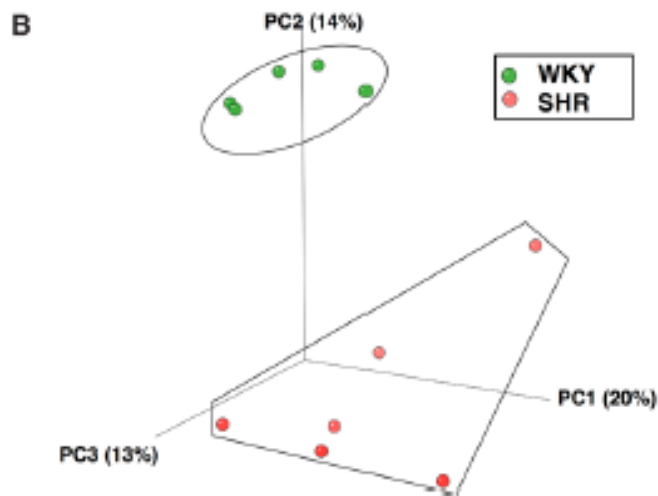
Gut Dysbiosis Is Linked to Hypertension

Tao Yang,* Monica M. Santisteban,* Vermali Rodriguez,* Eric Li, Niousha Ahmari, Jessica Marulanda Carvajal, Mojgan Zadeh, Minghao Gong, Yanfei Qi, Jasenka Zubcevic, Bikash Sahay, Carl J. Pepine, Mohan K. Raizada, Mansour Mohamadzadeh

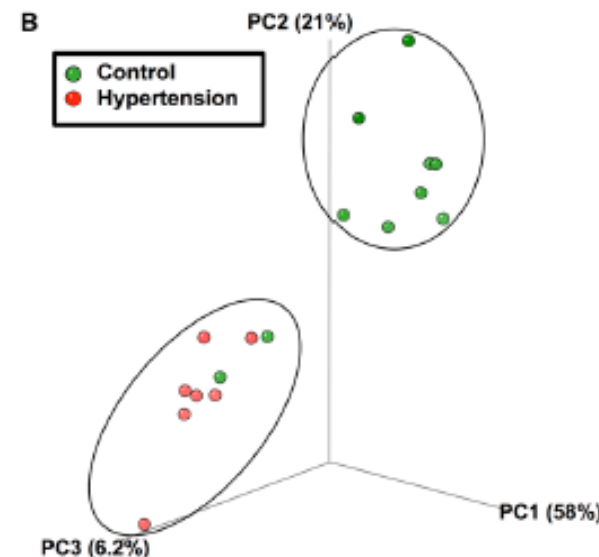


životinjski modeli

TRANSLACIJSKA ISTRAŽIVANJA

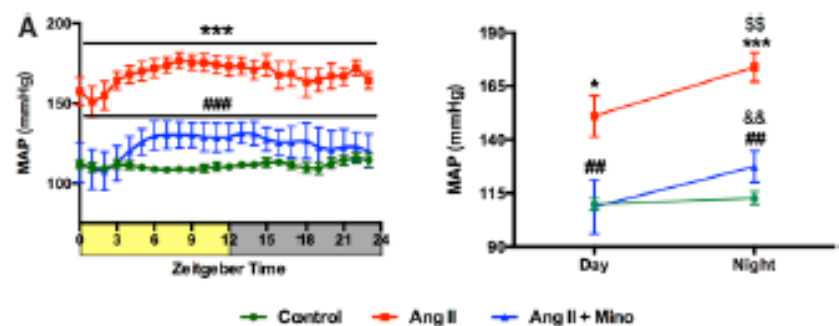


pacijenti

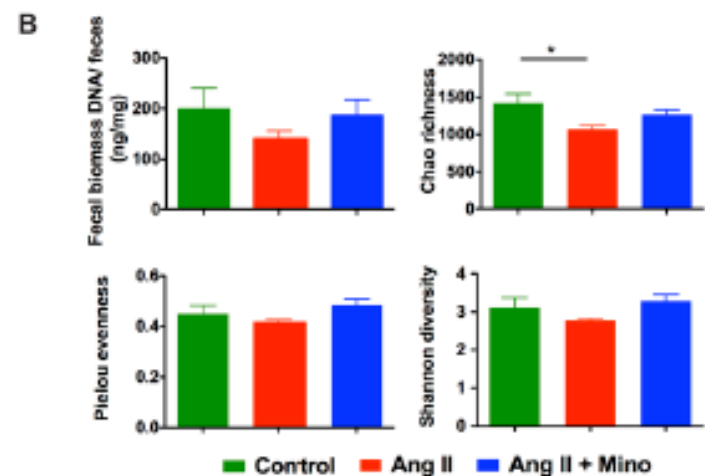


Mikrobiota crijeva u Wistar Kyoto (WKY) štakora i u spontano hipertenzivnih životinja (SHRs).

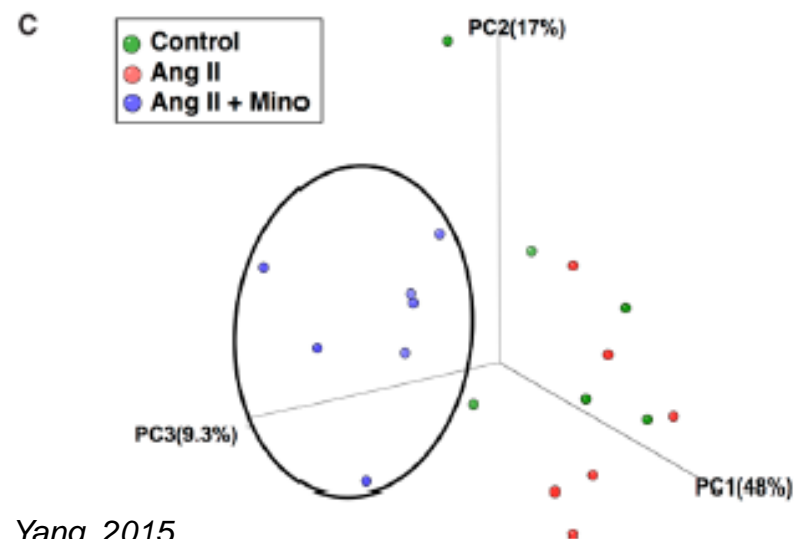
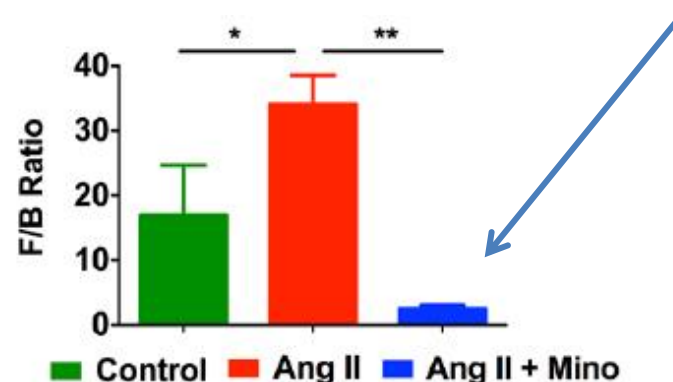
Smanjenje raznolikosti mikrobiote u pacijenata s hipertenzijom (n=7) u odnosu na kontrolnu skupinu (n=10)



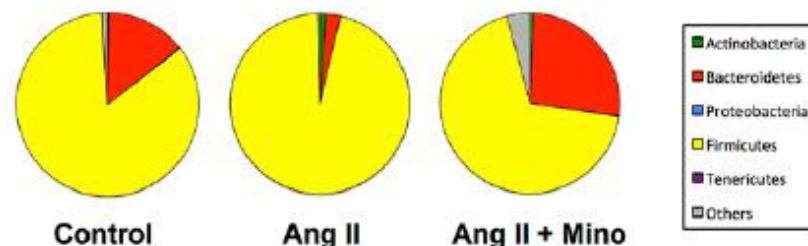
- Minociklin smanjuje angiotenzinom II izazvanu hipertenziju
- Sastav mikrobiote fecesa razlikovao se nakon tretmana s antibiotikom



A



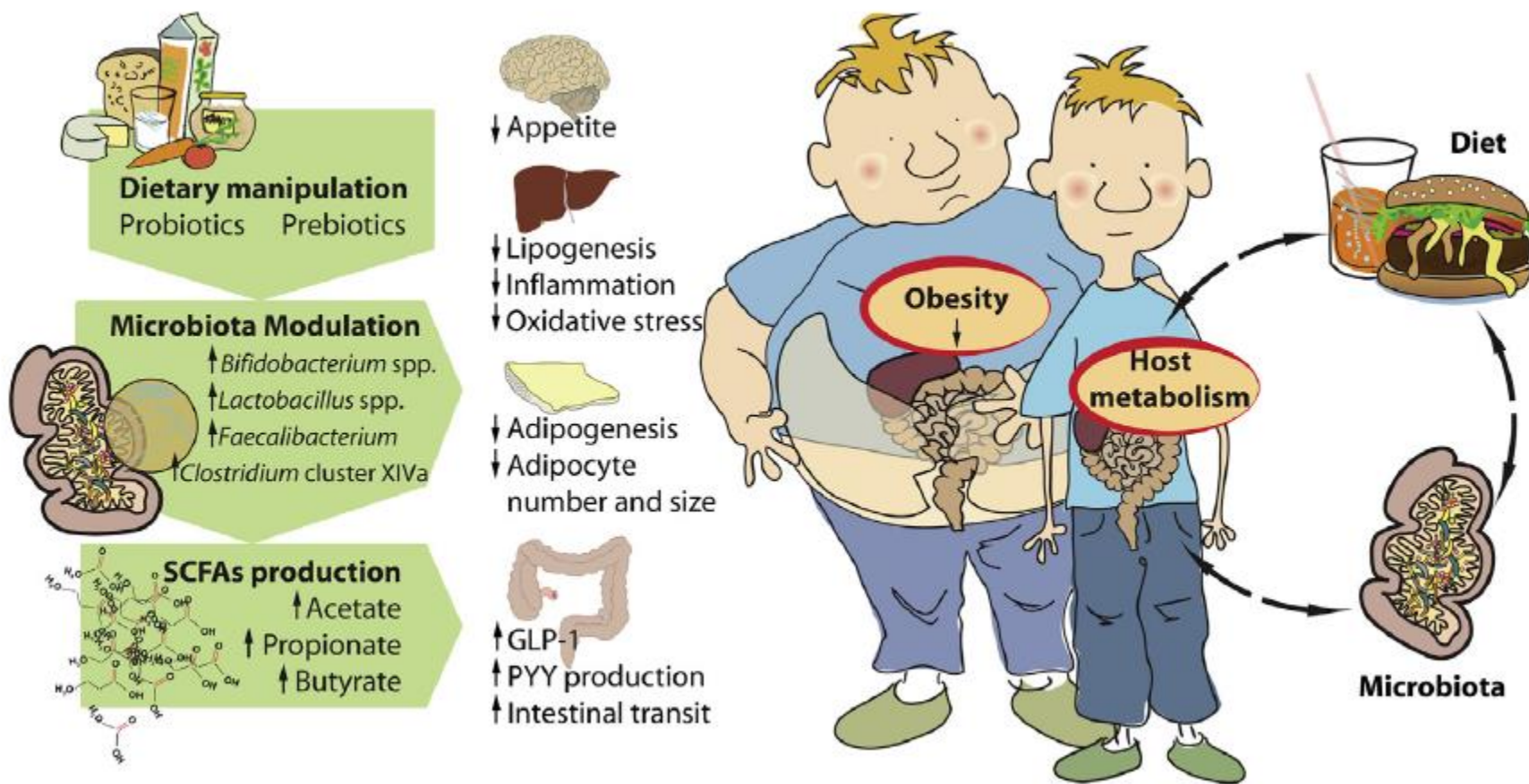
B



Phylum	P value			Mean value (%)		
	Con vs. AngII	Con vs. AngII + Mino	AngII vs. AngII + Mino	Control	AngII	AngII + Mino
Actinobacteria	0.1121	0.5609	0.4803	0.2167	1.096	0.6293
Bacteroidetes	0.4398	0.1425	0.0055**	13.03	2.581	27.20
Firmicutes	0.5251	0.0968	0.0045**	85.98	96.06	69.93
Proteobacteria	0.3006	0.9999	0.5631	0.1496	0.0508	0.1259

Prehrana općenito modulira metabolizam

Međudjelovanje prehrane i mikrobiote utječe na metabolizam domaćina





Gut bacteria blast off

Veteran astronaut twins Scott and Mark Kelly have 'gone' where few have before – the International Space Station toilet.

In 2015, Scott returned to the ISS where he will live until March 2016. Part of his mission is to send frozen poo back to Earth for analysis. Mark will also send poo, from Arizona, USA.

Gut bacteria make up most of the solids in poo. With the twins' contributions, scientists will be able to compare how gut bacteria behave in space and on Earth in genetically identical hosts – a historical first.


Why study space poo?

To send astronauts on longer NASA needs to know what bacteria in space.

Hernan says: 'It's a challenge for astronaut sampling tube containing liquid spilling, take the sample with one gloved hand. We tested and kit at the Johnson Space on simulated microgravity flight "Vomit Comet".'

Hernan Lorenzi's team developed sampling kit that Scott Kelly the International Space Station

Source: J Craig Venter Institute



Microbiome Fecal Reference

1. Fecal collection from the International Space Station (ISS) will be done in the Microbiome Fecal Reference Kit.
2. Empty 2 liters of glove bag for fecal storage at -80°C.
3. Double glove each time.
4. Differentiate **Do not collect anything else!**
5. Intentionally use one of gloves if dirty and use glove bag for disposal.
6. Seal the Fecal Sample and store in a bag of biohazardous waste (see NASA's biohazardous waste disposal manual).

Microbiome Fecal Reference

Try to use the Fecal Sample Reference Kit to collect fecal samples from the Fecal Sample Reference Kit.

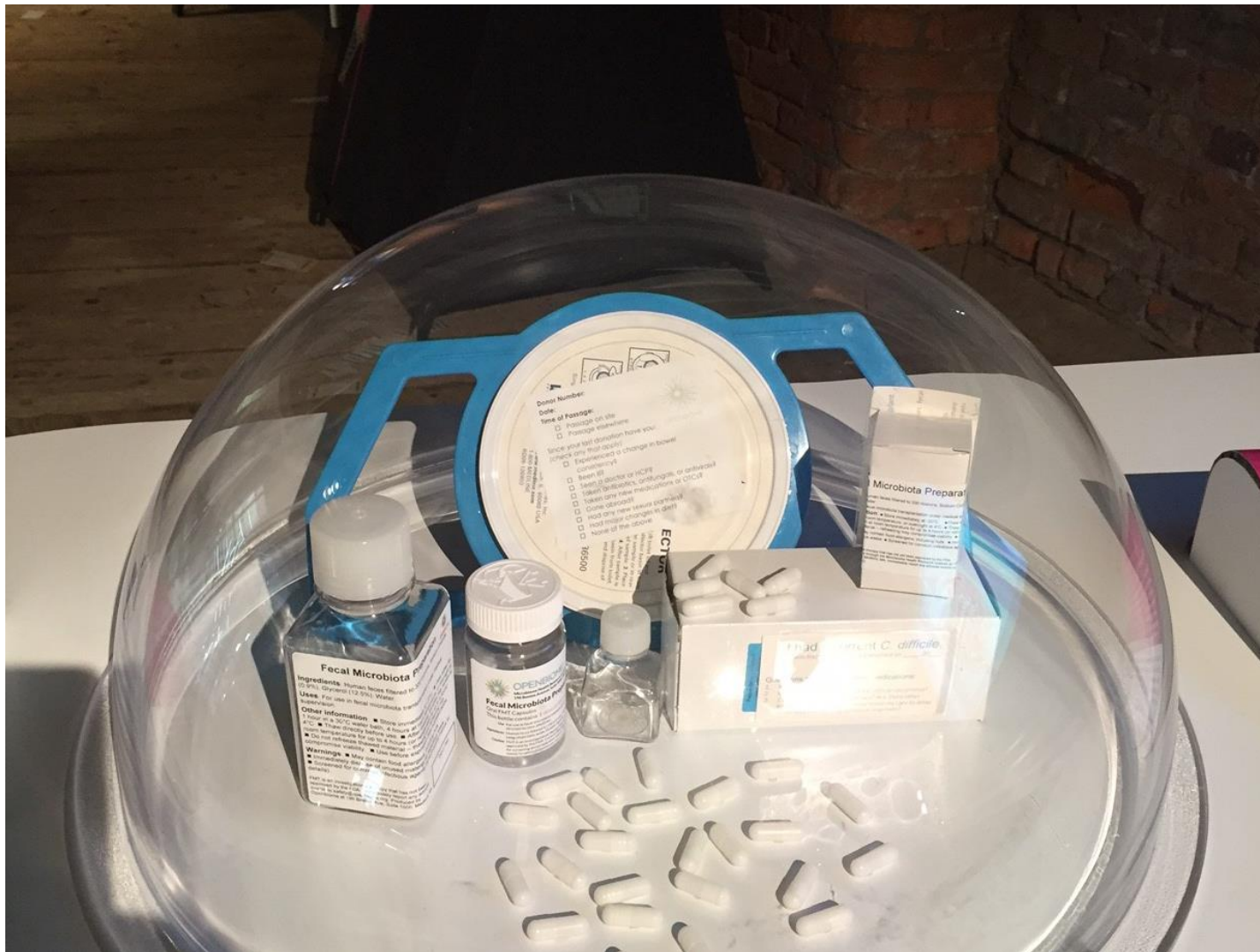
Label the Fecal Sample with information of the ISS.

Report the Fecal Sample to the NASA's Microbiome Fecal Reference Kit, along the number of crew members and the ISS's location.

For easy reference, NASA's Payload Operations Center has provided this handy poos collection guide for Mark on the space station.

To collect poos samples in the International Space Station toilet, Mark Kelly will require training, precision and the ability to multitask with one gloved hand.

Source: NASA's Payload Operations Center



Intervencije u sastav mikrobiote – Fekalna transplantacija

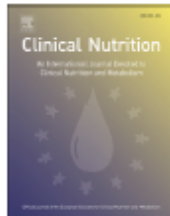


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Randomized control trials

Impact of personalized diet and probiotic supplementation on inflammation, nutritional parameters and intestinal microbiota – The “RISTOMED project” Randomized controlled trial in healthy older people

Luzia Valentini ^{a,*}, Alessandro Pinto ^b, Isabelle Bourdel-Marchasson ^{c,d,e}, Rita Ostan ^f, Patrizia Brigidi ^g, Silvia Turrone ^g, Silvana Hrelia ^h, Patrizia Hrelia ^g, Stefan Bereswill ⁿ, André Fischer ⁿ, Emanuela Leoncini ^h, Marco Malaguti ^h, Christèle Blanc-Bisson ^l, Jessica Durrieu ^l, Liana Spazzafumo ⁱ, Fabio Buccolini ^j, Florence Pryen ^k, Lorenzo Maria Donini ^b, Claudio Franceschi ^f, Herbert Lochs ^{b,m}

SUPPLEMENT

Proceedings of the IUNS 20th Congress of Nutrition

**Mediterranean Diet and Cardiovascular Health:
Teachings of the PREDIMED Study¹⁻³**

Emilio Ros,^{4,6*} Miguel A. Martínez-González,^{6,7} Ramon Estruch,^{5,6} Jordi Salas-Salvadó,^{6,9} Montserrat Fitó,^{6,10} José A. Martínez,^{6,8} and Dolores Corella^{6,11}