SEPTEMBER 8-10, 2013

7TH PROBIOTICS & PREBIOTICS *new foods*

ROME, UNIVERSITÀ URBANIANA

PROCEEDINGS AND ABSTRACTS

7TH PROBIOTICS & PREBIOTICS

new foods

SCIENTIFIC ORGANISERS

L. Capurso (Italy)

L. Morelli (Italy)

G. Delle Fave (Italy)

PEDIATRIC DAY

A. Guarino (Italy)

9.00-12.00 a.m. **JOINT MEETING: *PROBIOTIC STUDY GROUP OF SIGE (ITALIAN SOCIETY OF GASTROENTEROLOGY) AND MTCC (MEDITERRANEAN TASK FORCE FOR CANCER CONTROL)***

Gut microbiota and related diseases
President: M. Crespi (Italy)
Chairpeople: A. Gasbarrini (Italy), Z. Sharaiha (Jordan)

Diet, microbiota and colon cancer
N. Tozun (Turkey)

Probiotics and liver diseases: successes, problems and prospectives
A. N. Elzouki (Qatar)

Microbiota and Pancreatitis
G. Capurso (Italy)

Probiotics and IBD
F. Scaldaferri (Italy)

Probiotics and IBS
C. Severi (Italy)

Leaky gut, microbiota and cancer
A. Saggioro (Italy)

12.00 a.m.-1.00 p.m. **FREE COMMUNICATIONS**

FC 1.1 - PROBIOTIC *LACTOBACILLUS* STRAINS ACTIVITY AGAINST CANDIDA SPP.: IN VITRO ASSAYS
Maria Magdalena Coman* [(1)], Maria Cristina Verdenelli [(2)], Stefania Silvi [(3)], Cinzia Cecchini [(2)], Carla Orpianesi [(2)], Alberto Cresci [(3)]
[(1)] School of Advanced Studies, University of Camerino, Camerino, Italy
[(2)] Synbiotec S.r.l., Spin-off of UNICAM, Camerino, Italy
[(3)] School of Biosciences and Biotechnologies, University of Camerino, Camerino, Italy

FC 1.2 - PROTEOMIC RESPOND TO GROWTH UNDER LOW pH OF *LACTOBACILLUS CASEI* GCRL163 AND SHIROTA STRAINS
Ali Al-Naseri* [(1)], John P. Bowman [(1)], Margaret L. Britz [(1)]
[(1)] Tasmanian Institute of Agricultural Research, University of Tasmania, Hobart, Australia

FC 1.3 - SMALL INTESTINAL BACTERIAL OVERGROWTH IN PATIENTS WITH CHRONIC PANCREATITIS
Marianna Signoretti* [(1)], Serena Stigliano [(1)], Roberto Valente [(1)], Matteo Piciucchi [(1)], Gianfranco Delle Fave [(1)], Gabriele Capurso [(1)]
[(1)] Digestive and Liver Disease Unit, S. Andrea Hospital, University Sapienza, Rome, Italy

FC 1.4 - THE EFFECT OF 3 DIFFERENT PROBIOTICS ON THE PROPORTION OF DAYS OF COLD/FLU IN ACADEMICALLY-STRESSED UNIVERSITY STUDENTS: A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED STUDY

Amanda L. Ford* [1], Bobbi Langkamp-Henken [1], Cassie C. Rowe [1], Mary C. Christman [2], Carmelo Nieves, Jr. [1], Wendy J. Dahl [1]

[1] *Food Science and Human Nutrition Department, University of Florida, Gainesville, United States*

[2] *MCC Statistical Consulting and Departments of Biology and Statistics, University of Florida, Gainesville, United States*

FC 1.5 - ANTIOXIDATIVE ACTIVITY OF SYNBIOTIC FERMENTED DAIRY PRODUCTS CONTAINING PROBIOTIC *LACTOBACILLUS HELVETICUS* MTCC 5463

Vijendra Mishra* [1], Chandni Shah [2]

[1] *NIFTEM, NIFTEM, Kundli, Sonepat, India*

[2] *Anand Agricultural University, AAU, Anand, India*

FC 1.6 - *ENTEROCOCCUS FAECALIS* UGRA10: IN VITRO IMMUNOMODULATORY EFFECTS

Francesca Algieri* [1], Alba Rodriguez Nogales [1], Teresa Vezza [1], Natividad Garrido Mesa [1], Maria Elena Rodriguez Cabeza [1], Monica Comalada [2], Maria Pilar Utrilla [1], Manuel Martin-Bueno [3], Eva Valdivia [3], Mercedes Maqueda [1], Antonio Zarzuelo [1], Julio Galvez [1]

[1] *Department of Pharmacology, University of Granada, Granada, Spain*

[2] *Institute for Research in Biomedicine, Barcelona, Spain*

[3] *Department of Microbiology, University of Granada, Granada, Spain*

FC 1.7 - THE PROBIOTIC *LACTOBACILLUS CORYNIFORMIS* CECT5711 REDUCES ENDOTOXEMIA AND PREVENTS VASCULOPATHY IN OBESE MICE

Marta Toral* [1], Manuel Gómez-Guzmán [1], María Pilar Utrilla [2], Rosario Jiménez [1], Natividad Garrido-Mesa [2], María Elena Rodríguez-Cabezas [2], Mónica Olivares [3], Juan Duarte [1], Julio Gálvez [2]

[1] *Department of Pharmacology, School of Pharmacy, University of Granada, Granada, Spain*

[2] *CIBER-EHD, Department of Pharmacology, Center for Biomedical Research, University of Granada, Granada, Spain*

[3] *Research Department of Biosearch, Granada, Spain*

FC 1.8 - EFFECT OF *LACTOBACILLUS SALIVARIUS* STRAIN LPLM-01 IN A MURINE MODEL OF SALMONELLA TYPHIMURIUM INFECTION

Erica Castro*[1-4], Juan P. Mellado [1], Pamela Contreras [1], Maria J. Aguayo [1], Karen Pardo [1], Elizabeth Monsalves [1], Fernando Cárcamo [1], Rodrigo Vera [1], Jaime Cofré [1], Hernán Montecinos [2], Margarita González [3]

[1] *Laboratory of Lactic Bacteria, University of Concepción, Concepción, Chile*

[2] *Department of Biological Sciences, University of Concepción, Concepción, Chile*

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[4] *Department of Medicine, St. Sebastian University, Concepción, Chile*

2.00-3.00 p.m.

OPENING CEREMONY

Chair: G. Delle Fave (Italy)

L. Capurso (Italy)
L. Morelli (Italy)
R. Marabelli - Ministero della Salute (Italy)
B. Burlingame - FAO
D. Conte - SIGE (Italy)
A. Guarino - ESPGHAN

3.00-6.00 p.m.

MICROBIOME, PROBIOTICS, NUTRACEUTICALS AND SKIN

Chairpeople: D. Conte (Italy), D. Del Rio (Italy)

- Skin microbiome
  L. Morelli (Italy)

  Gut skin axis
  R. Paus (Germany)

- Skin microbiome and skin disease: the example of rosacea
  M. Picardo (Italy)

- Probiotics and allergy
  L. Drago (Italy)

- Nutraceutical and acne
  E. Camera (Italy)

- Nutraceuticals and skin protection
  F. Giampieri (Italy)

  Nutraceuticals and photoageing
  L. Korkina (Italy)

  Closing remarks
  D. Del Rio (Italy)

WELCOME COCKTAIL

Chairpeople: G. Delle Fave (Italy), G. Ippolito (Italy)

8.30-9.00 a.m. **OPENING LECTURE**
Microbes inside: funcion of mucosa - interacting bacteria
W. M. de Vos (Finland)

9.00-10.30 a.m. **MICROBIOTA AND HEALTH**

From basic to applied research: lessons from the human microbiome projects
F. Guarner (Spain)

From basic to applied research: prospectives from gut microbiota studies for pharma or food applications
J. Doré (France)

Microbes and immune regulation
G. Rook (United Kingdom)

10.30 a.m.-12.30 p.m. **NEW FOODS**
Chairpeople: N. Caporaso (Italy), V. Fogliano (The Netherlands)

Food properties affecting satiety
P. Riso (Italy)

How food aroma modulate satiation
P. Luning (The Netherlands)

Whole grain and weight management
F. Thielecke (Switzerland)

Food and modulation of gut peptides
P. Vitaglione (Italy)

Coffee and liver
F. Morisco (Italy)

Excess in food intake and genetic susceptibility to cancer
S. Romeo (Sweden)

New foods for CRC prevention
L. Ricciardiello (Italy)

Incorporating stevia into your product reformulation through
D. Machiels (Belgium)
- *Understanding the science of sugar and sweeteners*
- *Analysing consumers' preferences*
- *Finding the right balance between sugar/caloric reduction and product acceptability*

12.30-1.30 p.m. **FREE COMMUNICATIONS**

FC 1.9 - METABOLOMICS APPROACH FOR UNDERSTANDING THE VIABILITY AND ACTIVITY OF PROBIOTICS IN SET-YOGHURT: DETERMINATION OF VOLATILE AND NON-VOLATILE POLAR METABOLITE PROFILE

Sarn Settachaimongkon* [(1)], Hein Van Valenberg [(1)], Eddy Smid [(2)], Robert Nout [(2)], Toon Van Hooijdonk [(1)], Marcel Zwietering [(2)]

[(1)] Dairy Science & Technology group, Wageningen University, Wageningen, Netherlands
[(2)] Laboratory of Food Microbiology, Wageningen University, Wageningen, Netherlands

FC 1.10 - IMPACT OF A COMPLEX FOOD MICROBIOTA ON ENERGY METABOLISM IN THE MODEL ORGANISM CAENORHABDITIS ELEGANS

Chiara Devirgiliis* [(2)], Elena Zanni [(1)], Chiara Laudenzi [(1)], Claudio Palleschi [(1)], Giuditta Perozzi [(2)], Daniela Uccelletti [(1)]

[(1)] Department Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Rome, Italy
[(2)] CRA-NUT, Centro di Ricerca Alimenti e Nutrizione, Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Rome, Italy

FC 1.11 - *MELANZANA ROSSA DI ROTONDA*: PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY TOWARDS ITS VALIDATION FOR NUTRACEUTICALS MARKET

Marcello Nicoletti* [(1)], Chiara Toniolo [(1)], Gabriela Mazzanti [(1)], Antonella Di Sotto [(1)], Lorena Abete [(1)]

[(1)] Sapienza University of Rome, Rome, Italy

FC 1.12 - STRUCTURE-FUNCTION RELATIONSHIP OF ANTIOXIDANT PEPTIDES ISOLATED FROM WHEAT SPROUTS. FUNCTIONAL BINDING WITH PHOSPHOLIPIDS

Gian Luigi Gianfranceschi* [(1)], Isabella Calzuola [(1)], Valeria Marsili [(2)]

[(1)] NPP Nutraceutical & Phytochemical Products, 3A Parco Tecnologico Agroalimentare dell'Umbria, Pantalla di Todi (PG), Italy
[(2)] Dipartimento di Biologia Cellulare e Ambientale, Università degli Studi di Perugia, Perugia, Italy

FC 1.13 - FERMENTED MILK DAIRY PRODUCT CONTAINING *BIFIDOBACTERIUM LACTIS* CNCM I-2494 ENHANCES GUT IMMUNE BARRIER FUNCTION VIA A REG-TH17 IMMUNE PATHWAY

Simona Agostini* [(1)], Monique Goubern [(2)], Mathilde Leveque [(1)], Vassilia Theodorou [(1)], Raphael Moriez [(2)], Sophie Legrain-Raspaud [(2)], Helene Eutamene [(1)]

[(1)] UMR 1331 Toxalim, INRA/INP/UPS, Neurogastroenterology & Nutrition Group, Toulouse, France
[(2)] Danone Research, Center Daniel Carasso, Palaiseau, France

FC 1.14 - POLYDEXTROSE, A DIETARY FIBER, INCREASES POSTPRANDIAL GLP-1 IN OBESE SUBJECTS

Arthur Ouwehand* [(1)], Alvin Ibarra [(1)], Kirsti Tiihonen [(1)], Kaisa Olli [(1)], Essi Sarkkinen [(2)], Niina Tapola [(2)], Esa Alhoniemi [(3)], Sofia Forssten [(1)]

[(1)] DuPont Nutrition and Health, Danisco Sweeteners Oy, Kantvik, Finland
[(2)] Foodfiles Ltd., NA, Helsinki, Finland
[(3)] Pharmatest Ltd., NA, Helsinki, Finland

FC 1.15 - BERBERINE, INTERACTING WITH THE P2X7 PURINERGIC RECEPTOR, AMELIORATES EXPERIMENTAL LIVER INJURY

Elisa Vivoli* [1], Stefano Milani [2], Angela Provenzano [1], Andrea Cappon [1], Alessio Masi [3], Roberto Narducci [3], Guido Mannaioni [3], Gloriano Moneti [4], Erica Novo [5], Maurizio Parola [5], de Oliveira Claudia PMS [6], Fabio Marra [1]

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[3] NEUROFARBA, Università di Firenze, Firenze, Italy
[4] CISM, Università di Firenze, Firenze, Italy
[5] Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Torino, Italy
[6] Dipartimento di Gastroenterologia, University of Sao Paolo, Sao Paolo, Brazil

1.30-2.30 p.m. **LUNCH**

2.30-3.30 p.m. **NEWS IN PEDIATRIC CHRONIC INFLAMMATION**
Chairwoman: A. Staiano (Italy)

Interactions between intestinal microbiota and epithelium in pediatric inflammatory bowel disease
L. Stronati (Italy)

Intestinal microbioma signature of pediatric patients with chronic gut disorders
S. Cucchiara (Italy)

Probiotics and asthma
M. Miraglia del Giudice (Italy)

3.30-5.00 p.m. **MICROBIOTA AND IBD**
Chairpeople: R. Caprilli (Italy), F. Pallone (Italy)

Microflora and immunity
I. Monteleone (Italy)

Faecalibacterium prausnitzii
J. M. Wells (The Netherlands)

Is there a role for Probiotics in IBD?
M. Rescigno (Italy)

Probiotics for IBD
T. Karakan (Turkey)

Peroxisome proliferator-activated receptor gamma in the colon: inflammation and innate antimicrobial immunity
P. Desreumaux (France)

LECTURE
Faecal microbiota transplantation
A. Gasbarrini (Italy)

5.00-5.30 p.m. **FREE COMMUNICATIONS**

FC 1.16 - ADHESION AND MUCOSAL EFFECT OF *LACTOBACILLUS RHAMNOSUS GG* IN NORMAL AND INFLAMED COLONIC MUCOSA EVALUATED BY AN EX-VIVO ORGAN CULTURE TECHNIQUE
Cristiano Pagnini* [(1)], Michela Martorelli [(1)], Gianenrico Rizzatti [(1)], Vito D. Corleto [(1)], Stefano Festa [(1)], Francesca Menasci [(1)], Stefano Angeletti [(1)], Emilio Di Giulio [(1)], Gianfranco Delle Fave [(1)]
[(1)] Sapienza University of Rome, Faculty of Medicine and Psychology, S. Andrea Hospital, Rome, Italy

FC 1.17 - THE COMMENSAL BACTERIUM *FAECALIBACTERIUM PRAUSNITZII* A2-165 IS PROTECTIVE IN MURINE CHRONIC COLITIS MODELS

Rebeca Martin Rosique* [1], Florian Chain [1], Sylvie Miquel [1], Jun Lu [2], Jean Jacques Gratadoux [1], Harry Sokol [1], Elena F Verdue [2], Premysl Bercik [2], Luis G Bermudez Humaran [1], Philippe Langella [1]

[1] *INRA Jouy en Josas, Institut MICALIS, Jouy en Josas, France*

[2] *Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Canada*

FC 1.18 - ROLE OF HUMAN ENTEROGLIAL CELLS IN MEDIATING PATHOGENIC AND PROBIOTIC BACTERIA EFFECTS ON INTESTINAL EPITHELIAL CELLS

Fabio Turco* [1], Ilaria Palumbo [1], Marco Della Coletta [1], Cira Buonfantino [1], Giovanni Sarnelli [1], Rosario Cuomo [1]

[1] *Department of Clinical Medicine and Surgery, University of Napoli Federico II, Napoli, Italy*

FC 1.19 - *FAECALIBACTERIUM PRAUSNITZII* EXHIBITS ANTINOCICEPTIVE EFFECT IN A NON-INFLAMMATORY VISCERAL PAIN MODEL

Sylvie Miquel* [1], Rebeca Martin-Rosique [1], Agathe Gelot [2], Alain Eschalier [2], Denis Ardid [2], Muriel Thomas [1], Philippe Langella [1], Frédéric Antonio Carvalho [2]

[1] *Commensal and Probiotics-Host Interactions Laboratory, INRA UMR1319 Micalis, Jouy en Josas, France*

[2] *Pharmacologie Fondamentale et Clinique de la Douleur, UMR1107 NEURO-DOL Inserm/UdA, Clermont-Ferrand, France*

5.30-6.30 p.m.

NO CONVENTIONAL PROBIOTIC

Chairwoman: A. Castellazzi (Italy)

Bacterial spores as probiotics: mode of action
S. Cutting (United Kingdom)

Phagebiotics: bacteriophages as new class of probiotics for managing gut microflora
A. Sulakvelidze (USA)

Evaluation of *Bacillus Subtilis R0179* in healthy young adults
W. J. Dahl (USA)

PEDIATRIC DAY

9.00-10.30 a.m. **MEASURING THE EFFICACY OF NUTRITIONAL INTERVENTIONS: THE COMMENT INITIATIVE**
Chairpeople: A. Guarino (Italy), J. A. Vanderhoof (USA)

The initiative
H. Szajewska (Poland)

Outcome measures in functional disorders
M. M. Tabbers (The Netherlands)

- Respiratory infections
A. Lo Vecchio (Italy)

Gastroenteritis
H. Szajewska (Poland)

10.30-11.00 a.m. **LECTURE**
- Programming of the intestinal immune response through modulation of intestinal microflora
W. A. Walker (USA)

11.00 a.m.-1.00 p.m. **ROLE OF PROBIOTICS IN SPECIFIC CONDITIONS**
Chairpeople: S. Cucchiara (Italy), H. Szajewska (Poland)

- Probiotics for pediatric IBD
A. Staiano (Italy)

- The rational use of probiotics in abdominal-pain related functional gastrointestinal disorders in childhood
Z. Weizman (Israel)

- Changes in microflora composition and inflammation in cystic fibrosis
E. Bruzzese (Italy)

- Indications to probiotic therapy in children receiving antibiotics
Y. Vandenplas (Belgium)

The new ESPGHAN guidelines for European children with gastroenteritis
A. Guarino (Italy)

1.00-2.30 p.m. **LUNCH**

2.30-4.00 p.m. **FUNCTIONAL EFFECTS OF NUTRITION AT DIFFERENT AGES**
Chairpeople: M. De Curtis (Italy), Z. Weizman (Israel)

- Effects of mother's microflora on immunological programming on the child and risk of atopy: an epigenetic paradigm
E. Isolauri (Finland)

Nutritional interventions in neonates
P. Manzoni (Italy)

Tolerance acquisition in early infancy: role of intestinal microflora
R. Berni Canani (Italy)

The gut-liver axis and intestinal microflora
P. Vajro (Italy)

Functional effects of early nutritional interventions on later performance
J. A. Vanderhoof (USA)

4.00-5.00 p.m. **DEBATE: INDICATIONS TO PROBIOTICIS FOR PREVENTION OF NECROTIZING ENTEROCOLITIS**
Chairpeople: P. Manzoni (Italy), R. Berni Canani (Italy)

F. Indrio (Italy), M. A. Rojas (USA), G. Terrin (Italy)

5.00-6.00 p.m. **FREE COMMUNICATIONS**
President: A. Diamanti (Italy)
Chairpeople: R. Berni Canani (Italy), Z. Weizman (Israel)

FC-PD 1.1 - A DOUBLE-BLIND PLACEBO-CONTROLLED RANDOMIZED TRIAL ON PROBIOTICS IN SMALL BOWEL BACTERIAL OVERGROWTH IN CHILDREN TREATED WITH OMEPRAZOLE
Yvan Vandenplas* [(1)], Badriul Hegar [(2)], Esther I Hutapea [(2)]
[(1)] *Department of Pediatrics, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium*
[(2)] *Department of Child Health University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta*

FC-PD 1.2 - FRUCTOOLIGOSACCHARIDES PURIFICATION
Clarisse Nobre* [(1)]
[(1)] *Thermodynamique Department, Faculté Polytechnique de Mons, University of Mons, Mons, Belgium*

FC-PD 1.3 - THE EFFECT OF *LACTOBACILLUS CASEI* AND *LACTOBACILLUS PARACASEI* STRAINS ON CYTOKINE RESPONSE IN CHILDREN WITH ATOPIC DERMATITIS
Bozena Cukrowska* [(1)], Ilona Rosiak [(1)], Aldona Ceregra [(2)], Ilona Motyl [(3)]
[(1)] *Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland*
[(2)] *Department of Allergology, The Children's Memorial Health Institute, Warsaw, Poland*
[(3)] *Institute of Fermentation Technology and Microbiology, Technical University, Lodz, Poland*

FC-PD 1.4 - EFFECTS OF PROBIOTICS ON INTESTINAL COLONIZATION AND INFLAMMATORY BOWEL DISEASE IN PRETERM INFANTS WITH BW<1500 G.
Morena De Angelis* [(1)], Maria Grazia Capretti [(1)]
[(1)] *NICU, University and General Hospital, Bologna, Italy*

FC-PD 1.5 - PROTECTIVE EFFECTS OF PROBIOTICS IN PRESCHOOL CHILDREN IN AN URBAN SLUM IN INDIA
Arthur Ouwehand* [(1)], Sofia Forssten [(1)], Rajkumar Hemalatha [(2)], Upadrasta Venkata Prasad [(2)], Gummuluri Krishna Swetha [(2)], Raja Sriswan Mamidi [(2)], Jag eevan Babu Geddam [(2)], Bhaskar Varrnasi [(2)], Alvin Ibarra [(1)], Kankipati Vijaya Radhakrishna [(2)]
[(1)] *Active Nutrition, DuPont, Kantvik, Finland*
[(2)] *Clinical Division, Immunology and Microbiology, National Institute of Nutrition, Hyderabad, India*

FC-PD 1.6 - ANTIBIOTIC ASSOCIATED DIARRHEA AND PROBIOTICS IN CHILDREN: AN ITALIAN SURVEY

Francesca Penagini* [1], Fabio Meneghin [1], Dario Dilillo [1], Giulia Ramponi [1], Valentina Fabiano [1], Chiara Mameli [1], Silvia Salvatore [2], Elisa Bergozzi [2], Michela Gaiazzi [2], Sabrina Cardile [3], Claudio Romani [3], Licia Pensabene [4], Valentina Mancini [5], Gian Vincenzo Zuccotti [1]

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[3] *Department of Pediatrics, Messina Hospital, Messina, Italia*
[4] *Department of Pediatrics, University of Catanzaro, Catanzaro, Italia*
[5] *Department of Pediatrics, Parma Hospital, Parma, Italia*

FC-PD 1.7 - PROBIOTICS USE IN CHILDHOOD: A SURVEY ON OPINION AND RECOMMENDATIONS BY PEDIATRICIANS

Roberto Romano* [1], Carmen Napolitano [1], Miriam Mariano [1], Laura Di Florio [1], Marcello Manchisi [1], Andrea Lo Vecchio [2], Alfredo Guarino [2], Antonietta Giannattasio [1]

[1] *Medicine and Health Sciences Department, University of Molise, Campobasso, Italia*
[2] *Department of Translational Medical Science, Section of Pediatrics, University Federico II, Naples, Italia*

8.30-9.15 a.m. **LECTURES**
Chairpeople: M. Anti (Italy), P. Nisticò (Italy)

- Immunomodulatory effects of probiotics
  E. Mengheri (Italy)
- Distinct immunomodulatory properties of *Lactobacillus paracasei* strains
  M. Rossi (Italy)

9.15-10.45 a.m. **MICROBIOTA AND AGEING**
Chairwoman: P. Brigidi (Italy)

Gut microbiota, host gene expression and ageing
P. Patrignani (Italy)

Metagenomics in ageing
C. Franceschi (Italy)

Diet and microbiota in elderly
P. W. O'Toole (Ireland)

- Metabolomics in elderly
  F. P. Martin (Switzerland)

10.45-11.45 a.m. **MICROBIOTA HOST METABOLIC INTERACTION**
Chairpeople: E. Mengheri (Italy), I. Rowland (United Kingdom)

Prebiotics vs fibers: a review of available effects
K. Tuohy (Italy)

- Microbiota and probiotics in obesity: a NMR-based metabolomic approach
  R. Calvani (Italy)

Microbiota-phytochemical interactions
I. Rowland (United Kingdom)

11.45 a.m.-1.00 p.m. **MICROBIOTA AND IBS (GUT-BRAIN AXIS)**
Chairpeople: G. Gasbarrini (Italy), M. Koch (Italy)

Gut barrier in health and diseases
A. Gasbarrini (Italy)

- Long term consequences of a gut barrier damage
  V. Stanghellini (Italy)

Mucosal permeability in IBS
G. Barbara (Italy)

Probiotics: a new tool to target gut barrier functions in IBS?
M. Neunlist (France)

1.00-2.00 p.m. **FREE COMMUNICATIONS**

FC 1.20 - *LACTOBACILLUS RHAMNOSUS GG*-DERIVED FACTORS PROTECT HUMAN COLONIC SMOOTH MUSCLE FROM PATHOGEN LIPOPOLYSACCHARIDE-INDUCED DAMAGE

Carola Severi* [1], Annunziata Scirocco [1], Gennaro Prota [2], Massimo Marignani [3], Gianni Pozzi [2]
[1] Dipartimento di Medicina Interna e Specialità Mediche, Università Sapienza di Roma, Roma, Italy
[2] LAMMB, Dip artimento di Biotecnologie Mediche, Università di Siena, Siena, Italy
[3] UOC Malattie dell'apparato digerente e fegato, Azienda Ospedaliera S. Andrea, Roma, Italy

FC 1.21 - INTESTINAL MUCUS ALTERATIONS INDUCED BY A CHRONIC STRESS ARE LINKED TO A SHIFT IN O-GLYCOSYLATION RATHER THAN TO MUCIN EXPRESSION CHANGES: PREVENTION BY A PROBIOTIC TREATMENT

Stéphanie Da Silva* [1], Catherine Robbe-Masselot [2], Alessandro Mancuso [2], Myriam Mercade-Loubière [3], Christel Cartier [4], Marion Gillet [4], Afifa Ait-Belgnaoui [5], Pascal Loubière [3], Etienne Dague [6], Vassilia Théodorou [4], Muriel Mercier-Bonin [3]
[1] LISBP, CNRS/INRA, INSA, Toulouse, France
[1] Unité de Glycobiologie Structurale et Fonctionnelle, CNRS, Université de Lille 1, Villeneuve d'Ascq, France
[3] LISBP, UMR CNRS/INRA, INSA de Toulouse, Toulouse, France
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[6] LAAS CNRS, ITAV-UMS3039, Université de Toulouse, Toulouse, France

FC 1.22 - EVALUATION OF THE *LACTOBACILLUS FERMENTUM* IN THE DCA EXPERIMENTAL MODEL OF IRRITABLE BOWEL SYNDROME

Alba Rodríguez Nogales* [1]
[1] Department of Pharmacology, University of Granada, Granada, Spain

FC 1.23 - PROBIOTIC-ENRICHED FOODS AND DIETARY SUPPLEMENTS CONTAINING SYNBIO® POSITIVELY AFFECT BOWEL HABITS OF HEALTHY ADULTS

Stefania Silvi* [1], Maria Cristina Verdenelli [2], Cinzia Cecchini [2], Maria Magdalena Coman [3], Maria Simonetta Bernabei [4], Jessica Rosati [4], Renato De Leone [4], Carla Orpianesi [2], Alberto Cresci [1]
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FC 1.24 - PROBIOTICS CAN IMPROVE GUT-RELATED ANXIETY AND HEALT-RELATED QUALITY OF LIFE IN IBS PATIENTS WITH A DIARRHEA COMPONENT: A MULTICENTER, DOUBLE BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

Jordi Espadaler* [1], Vicente Lorenzo-Zuñiga [2], Elba Llop [3], Luis Abreu [3], Jordi Serra [2]
[1] AB-Biotics SA, Cerdanyola del Valles, Spain
[2] Hospital Universitari Germans Trias i Pujol, Badalona, Spain
[3] Hospital Puerta de Hierro, Madrid, Spain

9.30-11.00 a.m. **BOTANICAL NUTRITIONAL FOOD: HOW AND WHY**
Chairpeople: A. Ghiselli (Italy), M. Serafini (Italy)

European legislation
V. Silano (Italy)

Italian legislation
B. Scarpa (Italy)

Tradition of use: the main question
M. Serafini (Italy)

Quality control: the scientific approach
C. Bicchi (Italy)

11.00-12.30 a.m. **VAGINAL MICROBIOTA AND PROBIOTICS IN GYNAECOLOGY**
Chair: R. Della Loggia (Italy)

Probiotics in recurrent urinary tract infections in women
R. Della Loggia (Italy)

Treatment of vaginal ecosystem disorders with lactobacilli and bovine lactoferrin
A. Cianci (Italy)

Vaginal environment and microbiota
S. Lello (Italy)

POSTER PRESENTATION

1 PROBIOTICS FOR INFANTILE COLIC: A SYSTEMATIC REVIEW OF THE CURRENT EVIDENCE

Jasim Anabrees [1], Flavia Indrio [2], Bosco Paes [3], Khalid AlFaleh [4]
[1] *Sulaiman Al Habib Medical Group, Arrayan Hospital, Riyadh, Saudi Arabia*
[2] *University of Bari, Bari, Italy*
[3] *McMaster University, Hamilton, Canada*
[4] *King Khalid University, Riyadh, Saudi Arabia*

2 COULD PROBIOTICS STIMULATE IMMUNE RESPONSE IN HUMAN IN VITRO MODEL?

Stephanie Beauchemin [1], Francois A. Leblond [1], Marie-Eve Boyte [2], Chad MacPherson [2], Pierre Burguiere [2], Thomas A. Tompkins [2], Vincent Pichette [1]
[1] *Centre de Recherche de l'Hopital Maisonneuve-Rosemont, Universite de Montreal, Montreal, Canada*
[2] *Lallemand Health Solutions, R&D, Montreal, Canada*

3 ANTI-LISTERIAL AND ANTI-BIOFILM ACTIVITIES OF POTENTIAL PROBIOTIC *LACTOBACILLUS* STRAINS ISOLATED FROM TUNISIAN TRADITIONAL FERMENTED FOOD

Rihab Ben Slama [1], Bochra Kouidhi [1], Tarek Zmantar [1], Sihem Bayar [1], Kamel Chaieb [1], Amina Bakhrouf [1], Abdelkarim Mahdhi [1]
[1] *Faculty of Pharmacy, Monastir, Monastir, Tunisia*

4 ANTIBACTERIAL ACTIVITY OF *LACTOBACILLUS PARACASEI* SUBSP. PARACASEI BMK2005 AGAINST ENTEROPATHOGENIC E. COLI AND ITS POTENTIAL FOR USE AS AN ANTIDIARRHEAL PROBIOTIC STRAIN

Kamel Bendjeddou [1]
[1] *University of Bejaia, Bejaia, Algeria*

5 ENCAPSULATION OF PROBIOTIC MICROORGANISMS IN AQUEOUS DISPERSIONS OF CELLULOSE DERIVATIVES

Milos Beran [1], Marian Urban [1], Frantisek Toman [1], Josef Drahorad [1], Lubomir Adamek [1]
[1] *Food Research Institute Prague, Radiova 7, Prague*

6 EFFECTIVENESS OF MULTIPROBIOTIC "SYMBITER® *ACIDOPHILUS*" TO PREVENT ANTIBIOTIC-INDUCED HEPATIC AND COLONIC DYSFUNCTION

Tetyana Beregova [2], Lyudmyla Zakordonets [1], Taisa Dovbynchuk [2], Andriy Putnikov [2], Tetyana Furzikova [2], Olena Perepelytsina [2], Liudmyla Ostapchenko [2], Sergiy Kramarev [1], Ganna Tolstanova [2]
[1] *Department of Pediatric Infectious Diseases, Bogomolets National Medical University, Kyiv, Ukraine*
[2] *Educational and Scientific Centre "Institute of Biology", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine*

7 EFFECT OF TEMPERATURE, HUMIDITY AND OTHER INGREDIENTS ON STABILITY OF PROBIOTIC FORMULAE

Elena Bessi [1]
[1] *HPI, Humana Pharma International, Casorate Primo (PV), Italy*

8 INFLUENCE OF A NEW SYNBIOTIC BEVERAGE ON THE HUMAN GUT MICROBIOTA IN SIMULATOR OF THE HUMAN INTESTINAL MICROBIAL ECOSYSTEM (SHIME®)

Fernanda Bianchi [1], Elizeu Rossi [1], Maria Angela Adorno [2], Isabel Sakamoto [2], Erica Kuba [1], Katia Sivieri [1]
[1] *Faculty of Pharmaceutical Sciences (UNESP), Júlio de Mesquita Filho University, Araraquara, Brazil*
[2] *São Carlos School of Engineering EESC/USP, São Paulo University, São Carlos, Brazil*

9 PROBIOTIC SUPLEMENTATION FOR PREVENTION OF FUNCTIONAL GASTROINTESTINAL DISORDERS IN THE FIRST MONTH OF LIFE WITH

Massimo Bisceglia [1], Pasquale Comberiati [1], Anastasia Cirisano [1], Serafina Barberio [1], Francesco Paravati [1], Vincenzo Poerio [1], Flavia Indrio [2]
[1] *U.O. Pediatria, S. Giovanni di Dio, Crotone, Italia*
[2] *Dipartimento di Pediatria, Università di Bari, Bari*

10 PHAGEBIOTICS IN PROPHYLAXIS AGAINST FOOD-BORNE INFECTIONS

Svetlana Bochkareva [1]
[1] *Bphage LLC, R&D company, Moscow, Russian Federation*

11 CHLORIDE SECRETION INDUCED BY ROTAVIRUS NSP4 IS OXIDATIVE STRESS-DEPENDENT AND IS INHIBITED BY *SACCHAROMYCES BOULARDII* IN HUMAN ENTEROCYTES

Vittoria Buccigrossi [1], Carla Russo [1], Serena Orlando [1], Alfredo Guarino [1]
[1] *Dept. Translational Medical Science, Section of Pediatrics, University of Naples "Federico II", Naples, Italy*

12 EFFECTS OF *BIFIDOBACTERIUM* SUPPLEMENTATION ON PLASMA LIPID PROFILE IN DYSLIPIDEMIC CHILDREN

Paola Cagliero [1], Francesca Abello [1], Ornella Guardamagna [1]
[1] *Department of Pediatrics, University of Turin, Turin, Italy*

13 EFFECT OF A PROBIOTIC FERMENTED SOY PRODUCT ON COLONIC INFLAMMATION IN DEXTRAN SODIUM SULFATE-INDUCED COLITIS IN RATS

Daniela Cardoso Umbelino Cavallini [1], Larissa Sbaglia Celiberto [1], Mariana Nougalli Roselino [1], Raquel Bedani [2], Nadiége Dourado Pauly-Silveira [1], Graciela Font de Valdez [3], Roseli Aparecida Pinto [1], Elizeu Antonio Rossi [1]
[1] *UNESP - Univ Estadual Paulista, School of Pharmaceutical Sciences, Araraquara, Brazil*
[2] *University of Sao Paulo, School of Pharmaceutical Sciences, Sao Paulo, Brazil*
[3] *Reference Center for Lactocacilli, Cerela, San Miguel de Tucuman, Argentina*

14 INTAKE OF PASSIFLORA EDULIS PEEL BY TNBS-INDUCED COLITIS RATS: APPROACHES IN PREBIOTIC AND ANTIOXIDANT EFFECTS

Cinthia B. B. Cazarin [1], Juliana K. Silva [1], Angela G. Batista [1], Anderson L. Ferreira [2], Karina Fukuda [3], Fabio Augusto [3], Ricardo L. Zollner [4], Mario R. Marostica Jr [1]
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[4] *Faculty of Medical Science, University of Campinas, Campinas/SP, Brazil*

15 EFFECT OF A PROBIOTIC SOY BEVERAGE AND SULFASSALAZINE ON FECAL MICROBIOTA OF ANIMALS WITH COLITIS INDUCED BY DEXTRAN-SODIUM SULFATE

Larissa Sbaglia Celiberto [1], Mariana Nougalli Roselino [1], Raquel Bedani [2], Elizeu Antonio Rossi [1], Roseli Aparecida Pinto [1], Nadiége Dourado Pauly-Silveira [1], Graciela Font de Valdez [3], Daniela Cardoso Umbelino Cavallini [1]
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[3] *Centro de Referência para Lactobacilos, CERELA, Tucumã, Argentina*

16 *LACTOBACILLUS PARACASEI* F19 MODULATES THE INFLAMMATORY RESPONSE IN AN EX VIVO/IN VITRO MODEL OF BIOPSIES SAMPLES FROM DIARRHEA-PREDOMINAT IBS PATIENTS

Alessandra Cianflone [1], Debora Compare [1], Marco Sanduzzi Zamparelli [1], Emilia Russo [1], Maria Timone [1], Alberto Martino [1], Gerardo Nardone [1]
[1] *University of Naples Federico II, Department of Clinical Medicine and Surgery, Naples, Italy*

17 THE PROBIOTIC STRAIN *B. ANIMALIS SUBSP. LACTIS BI07* DIFFERENTLY MODULATES THE INFLAMMATION DEPENDENT UNBALANCES OF THE MUCOSA-ASSOCIATED INTESTINAL MICROBIOTA OF BREAST-FED INFANTS AND ADULTS

Clarissa Consolandi [2], Manuela Centanni [1], Silvia Turroni [1], Simone Rampelli [1], Clelia Peano [2], Marco Severgnini [2], Elena Biagi [1], Gianluca De Bellis [2], Patrizia Brigidi [1], Marco Candela [1]
[1] *Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy*
[2] *Institute of Biomedical Technologies, Italian National Research Council, Segrate, Milan, Italy*

18 EFFECT OF TWO PROBIOTICS AND DATE PALM EXTRACT ON SKIN AND GUT GENE EXPRESSION OF GILTHEAD SEABREAM (SPARUS AURATA, L.)

Hector Cordero [1], Abdelkarim Mahdhi [2], Francisco Antonio Guardiola [1], Alberto Cuesta [1], Amina Bakhrouf [2], María Ángeles Esteban [1]
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[2] *Laboratory of Analysis, Treatment and Valorization of Pollutants of the Environment and Products (LATVPEP), University of Monastir, Monastir, Tunisia*

19 DEVELOPMENT OF INTESTINAL FLORA IN NEWBORNS BORN TO MOTHERS WHO RECEIVED INTRAPARTUM ANTIBIOTIC PROPHYLAXIS

Luigi Corvaglia [1], Giacomo Tonti [1], Silvia Martini [1], Morena De Angelis [1], Azzurra Orlandi [1], Concetta Marsico [1], Giacomo Faldella [1]

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20 CORRELATION BETWEEN CHRONIC TREATMENT WITH PROTON PUMP INHIBITORS (PPIs) AND BACTERIAL OVERGROWTH IN THE STOMACH: ANY POSSIBLE BENEFICIAL ROLE FOR PROBIOTICS?

Mario Del Piano [1], Mario Migliario [2], Filomena Sforza [3], Luca Mogna [4], Giovanni Mogna [5]

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21 CORRELATION BETWEEN SPECIFIC BACTERIAL GROUPS IN THE ORAL CAVITY AND THE SEVERITY OF HALITOSIS: ANY POSSIBLE BENEFICIAL ROLE FOR PROBIOTICS?

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22 ACT AND NOT REACT: PROPHYLACTIC USE OF PROBIOTIC IN COLIC, REGURGITATION AND FUNCTIONAL CONSTIPATION, CLINICAL AND SOCIO-ECONOMIC IMPACT

Antonio Di Mauro [1], Giuseppe Riezzo [2], Elisa Civardi [3], Cristina Intini [4], Luigi Corvaglia [5], Elisa Ballardini [6], Massimo Bisceglia [7], Mauro Cinquetti [8], Emanuela Brazzoduro [9], Antonello Del Vecchio [10], Silvio Tafuri [11], Ruggiero Francavilla [1], Indrio Flavia [1]

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[7] Department of Pediatrics, Neonatology Division, San Giovanni di Dio Hospital, Crotone, Italy
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[10] Division of Neonatology, Di Venere Hospital, Bari, Italy
[11] Section of Hygiene, Aldo Moro University of Bari, Bari, Italy

23 INHIBITORY EFFECT OF POTENTIAL BACILLUS PROBIOTIC STRAINS AGAINST PATHOGENIC BACTERIA AND YEAST ISOLATED FROM ORAL CAVITY

Walid Fdhila [1], Sihem Bayar [1], Bochra Khouidi [1], Fethi Maâtouk [2], Feten Ben Amor [2], Hajer Hentati [2], Abdelkarim Mahdhi [1]

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[2] P.H.U Faculty of Dentistry of Monastir 5000, Monastir University, Monastir, Tunisia

24 INFLUENCE OF THE MICROBIAL COMMUNITY IN KEFIR GRAINS ON THE SURVIVAL AND GROWTH OF STAPHYLOCOCCUS AUREUS IN MILK

Sabina Fijan [1], Sonja Sostar Turk [1]

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25 EVALUATION OF PROBIOTICS AND DATE PALM EXTRACT IN INNATE DEFENCE PRESENT IN EPIDERMAL MUCUS OF GILTHEAD SEABREAM *(Sparus aurata L.)*

Francisco Antonio Guardiola [1], Hector Cordero [1], Abdelkarim Mahdhi [2], Alberto Cuesta [1], Jose Meseguer [1], Amina Bakhrouf [2], María Angeles Esteban [1]

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26 ORAL PROBIOTIC CAPSULES AS SUPPLEMENTATION OF THE STANDARD ANTIBIOTIC THERAPY TO PATIENTS WITH BACTERIAL VAGINOSIS OR AEROBIC VAGINITIS: RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

Piotr Heczko [1]

[1] *Chair of Microbiology, Jagiellonian University Medical College, Krakow, Poland*

27 PREBIOTIC AND PROBIOTIC SUPPLEMENTATION PREVENTS HUMAN RHINOVIRUS INFECTIONS IN PRETERM INFANTS: A RANDOMIZED, PLACEBO-CONTROLLED TRIAL

Erika Isolauri [1], Raakel Luoto [1], Olli Ruuskanen [1], Matti Waris [2], Marko Kalliomäki [1], Seppo Salminen [3]

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28 GOOD SELECTION OF *BIFIDOBACTERIUM LONGUM* STRAINS PREDICT THEIR EFFICACY IN PREVENTION OF COLITIS IN MICE EXPERIMENTAL MODEL

Hana Kozakova [1], Dagmar Srutkova [1], Tomas Hudcovic [1], Martin Schwarzer [1]

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29 *LACTOBACILLUS* STRAINS AS A POTENTIAL PROBIOTIC TREATMENT FOR GNOTOBIOTIC ARTEMIA CULTURE

Faouzi Lamari [1], Abdelkarim Mahdhi [1], Kais Fdhila [1], Sadok Khouaja [1], François-Joël Gatesoupe [2]

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30 PUBLIC HEALTH AND BUDGET IMPACT OF PROBIOTICS IN CONTROLLING UPPER RESPIRATORY TRACT INFECTIONS IN FRANCE

Irene Lenoir-Wijnkoop [1], Laetitia Gerlier [2], Jean-Louis Bresson [3], Claude Le Pen [4], Gilles Berdeaux [2]

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31 SELECTION OF A PROBIOTIC STRAIN POTENTIATING THE IMMUNOMODULATORY PROPERTIES OF AN ELDERBERRY EXTRACT (EE)

Christine Libon [1], Marie-Francoise Aries [1], Laila Haddioui [2], Christian Latge [1]

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[2] *Fonderephar, Paul Sabatier University, Toulouse, France*

32 PROBIOTICS FOR THE TREATMENT OF IRRITABLE BOWEL SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS OF EVIDENCE IN CHILDREN AND ADULTS

Andrea Lo Vecchio [1], Bruzzese Eugenia [1], Viscovo Sara [1], Chiatto Fabrizia [1], Fedele Cristina [1], Guarino Alfredo [1], Giannattasio Antonietta [2], Sarnelli Giovanni [3]

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33 INTERACTION OF *LACTOBACILLUS FERMENTUM* BGHI14 WITH RAT COLONIC MUCOSA – IMPLICATIONS FOR COLITIS INDUCTION

Jovanka Lukic [1], Ivana Strahinic [1], Marina Milenkovic [2], Natasa Golic [1], Milan Kojic [1], Ljubisa Topisirovic [1], Jelena Begovic [1]

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[2] Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

34 SURVIVAL AND RETENTION OF THE PROBIOTIC PROPERTIES OF *BACILLUS SP.* STRAINS UNDER MARINE STRESS STARVATION CONDITIONS AND THEIR POTENTIAL USE AS A PROBIOTIC IN ARTEMIA CULTURE

Abdelkarim Mahdhi [1]

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35 ON THE INTERACTION OF LISTERIA MONOCYTOGENES AND TUNISIAN PROBIOTIC *LACTOBACILLUS PLANTARUM* EXTRACTS

Abdelkarim Mahdhi [1], Rihab Ben Slama [1], Bochra Kouidhi [2], Tarek Zmantar [1], Kamel Chaieb [3], Sihem Bayar [1], Amina Bakhrouf [1]

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[3] College of Arts and Sciences, Yanbu, Kingdom of Saudi Arabia, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia

36 PRODUCTION OF NEW OAT GRAINS PROBIOTIC YOGHURT

Hoda Mahrous [1], Amal Abd El Razek [2], Wedad M. El-Kholy [3]

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[2] Food Science and Technology Dept., Fac. of Agric., El–Shatby, Alexandria University, Alex, Egypt

[3] Dairy Research Dept., Food Technology Research Institute, A.R.C., Alex, Egypt

37 GLYCAEMIC RESPONSE TO FOUR DIFFERENT SWEETENERS IN TIPE II DIABETES (SUCROSE IN THREE DIFFERENT PREPARATION VERSUS ACALORIC SWEETENER)

Patrizia Marrino [1], Dazzi Davide [2], Giulio Marchesini [3]

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38 PROTECTIVE EFFECTS OF *LACTOBACILLUS ACIDOPHILUS* CRL 1014 AND YACON *(SMALLANTHUS SONCHIFOLIUS)* ON DMH-INDUCED COLON CARCINOGENESIS IN WISTAR RATS

Camilla Martins Avi [1], Ana Paula Silva Almeida [1], Maria Angela Tallarico Adorno [2], Elizeu Antônio Rossi [1], Katia Sivieri [1]

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[2] Department of Hydraulic and Sanitation, University of São Paulo, São Carlos, Brazil

39 DOES EARLY OLIGOSACCHARIDES CONSUMPTION AFFECT PANCREAS MATURATION?

Catherine D. Michel [1], Pauline Theulier [1], Sophie E. Blat [2], Gwenola J. Le Drean [1], Patricia L. Parnet [1]

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40 CAPABILITY OF THE TWO MICROORGANISMS *BIFIDOBACTERIUM BREVE* B632 AND *BIFIDOBACTERIUM BREVE* BR03 TO COLONIZE THE INTESTINAL MICROBIOTA OF CHILDREN: THEIR PROSPECTIVE USE IN COLICKY INFANTS

Luca Mogna [1], Mario Del Piano [2], Giovanni Mogna [3]

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[2] Gastroenterology Unit, Maggiore della Carità Hospital, Novara, Italy

[3] Probiotical SpA, Novara, Italy

41 DECAFFEINATED COFFEE RESTORES GUT PERMEABILITY IN A RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

Filomena Morisco [1], Giovanna Mazzone [1], Vincenzo Lembo [1], Giuseppe D'Argenio [1], Paola Vitaglione [2], Maria Guarino [1], Silvia Camera [1], Eduardo Clery [1], Carmine Ferraiuoli [1], Vincenzo Fogliano [2], Nicola Caporaso [1]
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42 MECHANISM OF PROLONGEVITY INDUCED BY *LACTOBACILLUS GASSERI* SBT2055 IN C. ELEGANS

Hisako Nakagawa [1], Tomohiro Hosoya [2], Tomohiro Moriya [2], Fumihiko Sakai [2], Yosuke Nakayama [1], Tadaaki Miyazaki [1]
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[2] Milk Science Research Institute, Megmilk Snow Brand Co.,Ltd., Kawagoe, Saitama, Japan

43 PRELIMINARY RESULTS ON CLINICAL EFFECTS OF THE PROBIOTIC *L.SALIVARIUS* LS01 IN CHILDREN AFFECTED BY ATOPIC DERMATITIS

Antonio Augusto Niccoli [1], Lorenzo Drago [2], Niccoli A. [1], Artesi A.L., Candio F., Ceccarelli S. [1], Cozzali R. [1], Ferraro L.[1], Fiumana D., Mencacci M., Morlupo M., Pazzelli P. [1], Rossi L., Drago L. [2]
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[2] Department of Biomedical Science for Health, University of Milan, Milan, Italy

44 CAPABILITY OF EXOPOLYSACCHARIDE-PRODUCING *LACTOBACILLUS PARAPLANTARUM* BGCG11 TO COUNTERACT THE EFFECT OF ENTEROPATHOGENS ON THE INTESTINAL CELL LINE HT29-MTX

Milica J. Nikolic [1], Natasa E. Golic [1], Milan O. Kojic [1], Ivana D. Strahinic [1], Claudio Hidalgo Cantabrana [2], Patricia Ruas-Madiedo [2]
[1] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Instituto de Productos Lácteos de Asturias - Consejo Superior de Investigaciones Científicas (IPLA-CSIC), University of Oviedo, Villaviciosa, Spain

45 LIVE *LACTOBACILLUS GG* COUNTERACTS THE EFFECTS INDUCED BY GLIADIN ON THE CELLULAR POLYAMINE CONTENT AND INTESTINAL EPITHELIAL PARACELLULAR PERMEABILITY OF CACO-2 CELLS

Antonella Orlando [1], Michele Linsalata [1], Benedetta D'Attoma [1], Maria Notarnicola [2], Francesco Russo [1]
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[2] Laboratory of Nutritional Biochemistry, National Institute for Digestive Diseases I.R.C.C.S. "Saverio de Bellis", Castellana Grotte (Ba), Italy

46 EFFECTS OF MULTISTRAIN PROBIOTIC PREPARATION ON THE CYTOKINE PROFILE IN RAT LIVER AND PANCREAS UPON LONG-TERM GASTRIC HYPOCHLORHYDRIA

Lyudmila Ostapchenko [1], Kateryna Dvorshchenko [1], Oksana Bernyk [1], Sergii Vakal [1]
[1] ESC "Institute of Biology", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

47 EFFECTS OF EARLY PREBIOTIC AND PROBIOTIC INTERVENTION ON DEVELOPMENT OF GUT MICROBIOTA AND CRYING IN PRETERM INFANTS - A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Anna Pärtty [1], Raakel Luoto [1], Marko Kalliomäki [1], Seppo Salminen [2], Erika Isolauri [1]
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48 IMPACT OF THE PROBIOTIC *LACTOBACILLUS PLANTARUM* TENSIA (DSM 21380) COMPRISING YOGHURT ON BLOOD INDICES AND BLOOD PRESSURE OF HEALTHY VOLUNTEERS

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66 EFFECTIVENESS OF THE TWO MICROORGANISMS *L. FERMENTUM* LF15 AND *L. PLANTARUM* LP01, FORMULATED IN SLOW RELEASE VAGINAL TABLETS, IN WOMEN AFFECTED BY BACTERIAL VAGINOSIS (BV): A PILOT STUDY

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PROBIOTICS AND IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is a common disorder characterized by abdominal discomfort associated to altered bowel function (diarrhea- or constipation-predominant IBS). Its pathogenesis remains poorly understood since the heterogeneous nature of this syndrome. Pathophysiology of IBS is multifactorial, including roles for visceral hypersensitivity, gastrointestinal dysmotility, alteration of intestinal permeability, immune function and the intestinal microbiota. Recent research into the etiology of IBS has centered on the interaction between the gastrointestinal (GI) tract and the central and enteric nervous system considering a role of abnormal pain processing and behavioural pathways.

The microbiome may contribute to IBS symptoms by altering gut neuromotorsensory function, barrier function and/or the brain-gut axis. The current working hypothesis is that abnormal microbiota activate mucosal innate immune responses which increase epithelial permeability, activate nociceptive sensory pathways and dysregulate the enteric nervous system. It has to be noted that alterations in microbiota in IBS could be abnormal *per* se or secondary to alterations in the availability of substrate related to dietary abnormalities or changes in gut transit. There is increasing evidence of an activation of the intestinal immune system in IBS. These include evidence for immune activation, changes in cytokine levels, low-grade intestinal inflammation, and an altered stress response. Many of the studies that have demonstrated an up-regulation of the GI mucosal immune system have been in post-infectious IBS, in which there is a clear infective trigger.

Earlier studies using conventional culture-based techniques had suggested that IBS patients had fewer Lactobacillus spp., coliforms and Bifidobacteria spp., the genera frequently used in probiotic products. Subsequent studies, which subdivided IBS patients according to predominant bowel habit, showed that diarrhea-predominant IBS patients had lower numbers of Lactobacilli spp., while constipation-predominant IBS had increased amounts of Veillonella spp. Furthermore bacterial microbiota in IBS patients presents a greater temporal instability than controls.

This evidence has highlighted the potential for therapeutic manipulation of the GI microbiota in particular with probiotics. Indeed, probiotics possess a number of properties that may prove of benefit to patients with IBS. Some strains have considerable metabolic activity, including the fermentation of nondigested carbohydrates and their conversion into short-chain fatty acids, the deconjugation of bile salts and vitamin synthesis. A number of probiotics modify the inflammatory response to some enteropathogens. Some probiotics have been shown to produce chemicals (e.g. neurotransmitters, neuromodulators) that can modify gastrointestinal functions such as motility or sensation and some have been shown to enhance mucosal barrier function and modulate inflammation.

However if probiotics are to be used to treat IBS and alter the gut microbiota, it is important to explore the mechanisms behind their beneficial effects pointing to an etiopathogenic approach. Probiotics benefits are likely to be strain specific. Features of probiotics possibly relevant to treatment of IBS are: (i) modification of mucosal adherence to inhibit pathogenic bacteria adhesion; (ii) enhancement of barrier function of epithelium; (iii) secretion of bacteriocins; (iv) acidification of the colon by nutrient fermentation; (v) immunomodulatory actions: (vi) alteration in mucosal response to stress; (vii) inhibition of visceral hypersensitivity.

There have been a number of meta-analyses on probiotics in IBS recently, all of which agree that probiotics are beneficial to varying extents even if, like most therapies in IBS, probiotics are unlikely to be beneficial for all patients. Most of the meta-analyses of probiotics in IBS report a

beneficial impact on global symptoms, abdominal pain and flatulence, whereas the impact on bloating is equivocal. However, any benefit is likely to be strain specific.

In conclusion, the evidence from clinical trials and systematic reviews are largely supportive of the use of probiotics in IBS, but only for specific strains. However, head-to-head comparisons between different probiotic products would be useful and future trials need to be large scale, high quality and use valid end points. Furthermore, given the wide availability of products to the public, patients need careful guidance as to which product is likely to be of benefit. Finally further research is needed to predict which patient groups are most likely to respond to probiotics, perhaps through faecal microbial profiling, .

REFERENCES

Parkes GC, Sanderson JD, Whelan K. Treating irritable bowel syndrome with probiotics: the evidence. Proc Nutr Soc. 2010;69:187-94.

Simren M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut 2013; 62:159–176.

Spiller R. Review article: probiotics and prebiotics in irritable bowel syndrome. Aliment Pharmacol Ther. 2008;28: 385-96.

Whelan K. Probiotics and prebiotics in the management of irritable bowel syndrome: a review of recent clinical trials and systematic reviews. Curr Opin Clin Nutr Metab Care. 2011;14:581-7.

PROBIOTIC LACTOBACILLUS STRAINS ACTIVITY AGAINST CANDIDA SPP.: IN VITRO ASSAYS

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Objective: To analyze *Lactobacillus* strains for properties related to vaginal mucosa colonization and antagonism towards *Candida* species. The aim of the study was to confirm the efficacy of these strains and to use them for the design of a product for local application to the vaginal tract.
Methods: Several probiotic lactobacilli strains were screened for their antipathogenic potential against *Candida* spp. by four different methods of assay. In addition we investigated the *in vitro* adherence blockage of eight *Candida* pathogens by eight *Lactobacillus* strains to HeLa cells, considering three possible mechanisms: exclusion by adhered lactobacilli, displacement of adhered pathogens and competition for receptor sites (inhibition test). Vaginal ovules and douches were produced using lyophilized/microgranulated SYNBIO®, a 1:1 combination of *Lactobacillus rhamnosus* IMC501® and *Lactobacillus paracasei* IMC502® as preliminary evaluation of different matrix and cell viability during 6 months.
Results: All strains showed an antipathogenic activity against the *Candida* strains tested. Six probiotic strains demonstrate a high adhesion capacity to HeLa cells. The inhibition results highlight a significant ($P<0.05$) competition of *L. plantarum* 319, *L. rhamnosus* IMC501®, *L. paracasei* IMC502® and SYNBIO® against all the *Candida* strains. During the storage of vaginal ovules at 25°C for 6 months, Witepsol® was the matrix that showed the highest suitability to preserve viable microorganisms thus increasing the shelf-life of the product respect to PEG formulation.
Conclusions: The probiotic strains analyzed demonstrate an inhibitory action against *Candid*a spp. growth and a high adhesion capacity to HeLa cells. The results suggest that the probiotic strains used in the present study could prevent colonization of the urogenital tract by relevant pathogens such as *Candida* strains through barrier and interference mechanisms (mainly displacement and competition). An advantage for women is that they can self-administer the probiotics.

PROTEOMIC RESPOND TO GROWTH UNDER LOW pH OF LACTOBACILLUS CASEI GCRL163 AND SHIROTA STRAINS

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Objective: To investigate how cell physiological functions can predict the stability of acid stress Lactobacilli.
Lactobacillus casei is widely used in fermented dairy food products. On this basis, maximising viability throughout the physiological challenges of food processing and storage is desirable so that they may be delivered to the host gastrointestinal (GI) tract in a maximally viable state. One of these challenges is survival of low pH conditions. Acidic environments in food products and during GI tract passage impact the survival of probiotic bacteria.
Methods: In this study, the global proteome responses of *L. casei* strains from Cheddar cheese (GCLR163) and fermented milk (Shirota) grown under different pH conditions (pH 4.5 and pH 6.5) were studied using comprehensive proteomics via a combination of liquid chromatography and tandem mass spectrometry. The strains were cultured in MRS medium with pH strictly mintained within an anaerobic bioreactor system.
Results: In response to low pH (pH 4.5) conditions, the abundance of proteins participating in nucleotide biosynthesis and protein synthesis were significantly repressed in both strains. *L. casei* GCRL163 appeared to tolerate low pH better than the Shirota strain since Shirota exhibited higher levels of stress proteins and proteins associated with protein turnover (Clp-system proteins) and proton extrusion (via the F0F1-ATP synthase complex) as well as proteins associated with energy generation via pyruvate metabolism and glycolysis/gluconeogenesis at low pH.
Conclusions: The growth under low pH can provide useful evelauation the stability and survival of Lactobacilli and proteomic data provides clearer comprehension of acid stress adaptation responses in fermented foodderived probiotic lactobacilli.

SMALL INTESTINAL BACTERIAL OVERGROWTH IN PATIENTS WITH CHRONIC PANCREATITIS

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Objective: Small intestinal bacterial overgrowth (SIBO) is considered as a factor possibly worsening symptoms and nutritional status in patients with chronic pancreatitis (CP) and pancreatic exocrine insufficiency (PEI) not responding to treatment. However, few studies evaluated the rate of SIBO in CP patients employing different substrates, and non-standardized procedures, with a wide range of positivity (0-92%). Moreover, those studies often investigated CP patients with previous resective surgery (cause of SIBO *per se)*. The primary aim of the study is to assess the prevalence of SIBO in CP patients without history of resective surgery, as compared to a control group. The secondary aim is to analyze clinical and biochemical factors related with SIBO in CP patients.

Methods: CP patients with a certain diagnosis at imaging and functional procedures, and controls enrolled at the GI outpatients clinic with non-specific, non-chronic GI complaints.

SIBO evaluated by H2 glucose breath test (GBT) with a standard protocol according to Rome consensus conference. Positivity rate, basal, peak over basal (HOB) and H2 values at 120 minutes were evaluated. Patients with positive GBT were treated with high doses of Rifaximin.

For CP patients, relation between BT results, abdominal symptoms, clinical and biochemical variables were analyzed.

Results: 36 CP patients (55% male, mean age 54) and 43 controls (45 % male, mean age 45) enrolled. Of the 36 CP patients (alcoholic aetiology in 14) ,15 had PEI, 5 advanced CP (defined by M-ANNHEIM severity index) and none had previous resective surgery. GBT positivity rate was higher in CP than controls (6/36, 17% vs 3/45, 7%), albeit without a significant difference (p=0.3).

HOB mean values (4.95 ppm in CP vs. 3.1 ppm in controls; p=0.1) were not different in the two groups, however mean H2 basal excretion (8.09 ppm in CP vs.2.9 ppm in control; p=0.015), H2 peak (9.7 in CP vs. 4.8 in controls; p=0.009) and H2 at 120 minutes (4.1 in CP vs. 1.3 in controls; p=0.01) were significantly higher in CP than controls .

There were no differences in terms of rate of PEI, different severity of disease, abdominal symptoms, pancreatic enzymes or proton pump inhibitors therapy or biochemical values between CP patients with or without SIBO.

Conclusions: Our findings suggest that SIBO is not uncommon in CP, even in a population of uncomplicated patients. The lack of a significant difference with controls suggest that the study is currently underpowered. The higher values of H2 excretion (basal, peak and after 120 minutes) in CP compared to controls suggest an increased production of H2 in CP patients, for which the underlying mechanisms is unclear. SIBO in CP patients does not seem related with peculiar clinical features or with worse nutritional status.

THE EFFECT OF 3 DIFFERENT PROBIOTICS ON THE PROPORTION OF DAYS OF COLD/FLU IN ACADEMICALLY-STRESSED UNIVERSITY STUDENTS: A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED STUDY

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Objective: Academic stress is directly related to the percentage of days with cold/flu, and supplementation of galactooligosaccharides reduces the number of cold days. It was hypothesized that daily consumption of probiotics before, during and after academic exams would reduce the proportion of sick days.
Methods: Undergraduate students [n=581] were randomized to receive one of 3 probiotics (*Lactobacillus helveticus* R0052 [n=145], *Bifidobacterium bifidum* R0071 [n=142], *Bifidobacterium longum* ssp *infantis* R0033 [n=147]) or placebo [n=147] daily for 6 wk. Participants recorded cold/flu symptom intensity (SI; 0=not experiencing to 3=severe) daily for 9 cold/flu symptoms. An SI score >6 was counted as a cold day.
Results: During the study, 177 participants reported 884 cold days. The proportion of total days with cold was smaller with *B. bifidum* (140/6707, 2.1%) and *B. infantis* (186/6847, 2.7%) vs. placebo (281/6878, 4.1%) but not *L. heveticus* (277/6821, 4.1%). The proportion of participants within each treatment with ≥1 d of cold was significantly lower for participants receiving *B. bifidum* (n=34, 23.9%) versus placebo (n=55, 37.4%). Across all participants, the average number of cold episodes per participant was lower in the *B. bifidum* (0.4±0.1, $P<0.05$) and *B. infantis* groups (0.5±0.1, $P=0.07$) vs. placebo (0.6±0.1). On days where cold symptoms were reported, participants receiving *B. infantis* or *B. bifidum* were least likely to miss class ($P<0.05$) or do poorly on an assignment ($P<0.05$), respectively.
Conclusions: These data suggest that daily intake *B. bifidum* R0071 and *B. infantis* R0033 may reduce the proportion of sick days and affect academic performance during academic stress.

ANTIOXIDATIVE ACTIVITY OF SYNBIOTIC FERMENTED DAIRY PRODUCTS CONTAINING PROBIOTIC LACTOBACILLUS HELVETICUS MTCC 5463

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Objective: The present study has been undertaken to check the antioxidative activity of raw materials used for preparation of synbiotic dairy products, to test the antioxidative activity of selected synbiotic fermented dairy products and to study the variation in antioxidative activity of selected synbiotic fermented dairy products during storage at temperature 5 ± 1^0 C.

Methods: Two synbiotic products i.e. Synbiotic Lassi with Honey as Prebiotic & Synbiotic Whey Drink with Inulin & Orange Juice, were prepared as per protocol developed. The products were distributed in sterile plastic cups & glass bottles respectively and stored at refrigerated temperature (5 ± 1^0 C) for 28 days. During storage analysis of Physico-Chemical parameters (Titratable Acidity, pH), Microbial counts (LAB) and Antioxidative Activity (Hydroxyl Radical Scavenging Activity, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) cation (ABTS) assay, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay,) was done on 0, 7, 14, 21, 28th day of the storage. The antioxidative activity of Honey, Inulin, Tropicana Orange juice, Milk, Intracellular and Extracellular extract of *Streptococcus thermophilus* MTCC 5460 (MD2), *Lactobacillus helveticus* MTCC 5463 was also determined.

Results: In case of honey based synbiotic lassi, the initial acidity was 0.70% L.A. and on the day of 28th it was 0.83% L.A. Lactobacilli count of freshly prepared honey based synbiotic lassi was Log 8.64cfu/ml which declined to Log 8.21cfu/ml on 21st day while on 28th day saw slight increase to Log 8.36 cfu/ml. Hydroxyl radical scavenging activity of honey based synbiotic lassi was 107.76% on 0 day while 105.53%, 100.92%, 96.20% & 79.41% on 7, 14, 21 & 28th day. Activity of freshly prepared whey based drink was 100.32% which declined sharply to 79.21% on 7th day. The activity further increased to 102.59% on 14th day & 100.68% & 94.67% on 21 & 28th day of storage respectively. The DPPH activity of freshly prepared honey based synbiotic product was 28.43% which slightly decreased on 23.03% on 7th day & further increased to 35.93% on 28th day of storage respectively. The ABTS activity of freshly prepared honey based and whey based synbiotic products was 56.23% and 55.11% respectively. Thereafter further storage showed contrasting results in activity.

Conclusions: The study concluded that the synbiotic products posses antioxidative property which can be affected by storage duration of the product

ENTEROCOCCUS FAECALIS UGRA10: IN VITRO IMMUNOMODULATORY EFFECTS

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Objective: Probiotics have shown to exert beneficial effects due to their immunomodulatory properties. The aim of the present study was to evaluate the *in vitro* effects of probiotic *Enterococcus faecalis* UGRA10 (live or death) in two different cell types involved in the immune response: Caco-2 cells (intestine epithelial cells) and RAW 264.7 cells (macrophages). In addition, we investigated the effects of these probiotics on the signaling pathways associated to mitogen-activated protein (MAP) kinases in Caco-2 cells.
Methods: Cells were incubated for 3 hours with each probiotic (live or death) (10^8 CFU/ml), and stimulated with LPS (100 ng/ml) or IL-1β (1ng/ml) for 30 minutes (western blot) or 24 h (IL-8 or nitrite determination). Western blots were performed with protein extracts to analyze phosphorylated or total forms of p38 MAP kinase, p42/44 ERK or SAPK/JNK.
Results: *Enterococcus faecalis* UGRA10, live or death, inhibited the stimulated production of either IL-8 (Caco-2 cells) and nitric oxide (RAW 264.7). In the epithelial cells, this inhibitory effect was associated with a reduced phosphorylation of the three MAP kinases evaluated of p38 MAP kinase, p42/44 ERK or SAPK/JNK when it compared with stimulated cells without probiotic.
Conclusions: *Enterococcus faecalis* UGRA10 was able to downregulate the stimulated immune response by interfering with cell signaling associated with MAP kinase pathways. The viability of this probiotic seems not to be essential in its immunomodulatory effect.

THE PROBIOTIC LACTOBACILLUS CORYNIFORMIS CECT5711 REDUCES ENDOTOXEMIA AND PREVENTS VASCULOPATHY IN OBESE MICE

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Objective: Obesity is associated with intestine dysbiosis, characterized by a low grade inflammatory status, which affects vascular function. In this study we evaluate the effects of a probiotic with immunomodulatory properties, *Lactobacillus coryniformis* CECT5711, in obese mice fed a high (60%) fat diet (HFD).
Methods: C57BL/6J mice were divided into 4 groups: control, control-treated, obese, and obese-treated. Treated mice received orally *L. coryniformis* at a dose of 10^8 colony forming units per day, during 12 weeks. At the end of the treatment, plasma lipopolysaccharide (LPS) concentration, vascular reactivity, in situ detection of vascular reactive oxygen species contents and microbial analysis of the colonic contents were analyzed.
Results: The probiotic treatment did not affect the weight evolution, although it reduced basal glycemia and insulin resistance. *L. coryniformis* administration to HFD-induced obese mice induced marked changes in microbiota composition and reduced the metabolic endotoxemia since it decreased the LPS plasma level, associated with an improvement of the gut barrier disruption. Furthermore, it lowered TNF-alfa expression in liver, improving the inflammatory status and thus the glucose metabolism. Additionally, the probiotic reversed the endothelial dysfunction observed in obese mice when the endothelium- and nitric oxide-dependent vasodilatation induced by acetylcholine in aortic rings was studied. It also restored the increased vessel superoxide levels derived from NADPH oxidase observed in obese mice.
Conclusions: The study demonstrates an endothelial-protective effect of *L. coryniformis* CECT5711 in obese mice by increasing nitric oxide bioavailability, suggesting the therapeutic potential of this gut microbiota manipulation to prevent vasculopathy in obesity.

EFFECT OF *LACTOBACILLUS SALIVARIUS* STRAIN LPLM-O1 IN A MURINE MODEL OF *SALMONELLA TYPHIMURIUM* INFECTION

Microbial diversity and functionality in health and disease

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The prevention of bacterial infections with probiotics is an interesting study field. The objective of this study was to research the preventive effect of *Lactobacillus salivarius* LPLM-O1, isolated from breast milk, in a murine model of infection with *Salmonella typhimurium* ATCC 14028. Balb/c mice (n=76), 6-8 weeks old, were grouped on: G1 (base diet), G2 (10^9 CFU of LPLM-O1, once daily for 7 days), G3 (10^8 CFU of *S. typhimurium*), and G4 (daily oral dose of 10^9 CFU of LPLM-O1 for 7 days, and then 10^8 CFU of *S. typhimurium*). Survival rate, splenic index (SE), specific growth rate (SGR), differential leukocyte count and bacterial count were quantified. The survival rate in groups G1 and G2 was 100%. In G3 and G4, there was a 40% mortality rate at days 7 and 10, with 100% and 86% rates at day 16, respectively. SGR was for G1=1.6, G2=1.3, G3=0.1, and G4=0.6, with no diarrhoea or bleeding. Differential leukocyte count was indicative of acute bacterial infection. The highest *Salmonella* counts were on infected mice that had not been previously treated with the strain LPLM-O1. *S. typhimurium* count in faeces was significantly lower in G4 (10^5 CFU/gr) than in G3 (10^7 CFU/gr) ($p \leq 0.05$). Once *Salmonella* had colonized organs like the liver, spleen and intestines, LPLM-O1 administration did not have an impact on the count. Taking into account the high infection rate, preventive administration of LPLM-O1 slightly raised the SGR and the survival rate, while lowering the pathogenic microbial load in the intestines.

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SKIN MICROBIOME

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The *classical, text-book* definition of the microbial communities inhabiting the human skin could was like the following:" skin flora= bacterial populations of the skin, locally dense or scarce, according to the site, with Gram-positive organisms (e.g., staphylococci, micrococci, diphtheroids) usually predominating." Only two data were relevant in this definition: the skin is a complex ecological niches for microbes and Gram-positive are the most abundant organisms inhabiting this environment. In 2009 a review (1) suggested that:" There is surprisingly little literature that has systematically evaluated the influence of the resident cutaneous microflora in skin health". In the last four years, however, the scenario has been dramatically changed and several papers have been published on this matter, some of them in journals such as *Science* or *Nature*. In the following lines an update on what is going on in this area.

Three major questions are generally raised about skin-inhabiting microbes:

- What microbes are present on the skin surface?
- How do they contribute to health and disease states?
- How do dermatologic practices alter microbial diversity?

As a microbial ecologist I will try, in the following lines, to address the first question, focusing on the bacterial components of this ecosystem.

The first improvement in the comprehension of the microbial component of the skin ecosystem, which it is estimated to harbor an average of 1 billion bacterial cells per square centimeter (2) has been provided by culture independent methods, able to detect the presence on the human skin of microbes difficult to reproduce in laboratory conditions.

First approaches, based on 16SRNA analysis, (3,4) indicated a marked increase in diversity of the skin microbiota composition when compared to patterns derived from the culture methods.

Four phylotypes (Actinobacteria, Firmicutes Proteobacteria and Bacteroidetes) were found to be dominant and it was also detected a large presence of Gram negative proteobacteria such as *Methylophilus methylotrophus* (2). In addition temporal and site stability was also shown (4).

In order to survey all depths of the skin, some authors (5) sampled some volunteers using three methods: swab, scrape, and punch biopsy and they found that *Proteobacteria* dominated the skin microbiota at all depths of sampling. They also confirmed interpersonal variation and temporal stability. Data obtained by this pioneering use of molecular techniques then provided a change in the scenario of the skin microbiota, where phyla of Gram negative were more abundant than Gram positive and a temporal stability of specific site was shown.

However, a really new scenario was provided by first outcomes of the Human Microbiome Project (HMP), launched by NIH, reported in a row of papers, from 2009 and up to now (6,7).

Assessment of temporal and site specific stability of the skin microbiota was one of the first achievement of this project (reviewed in 7); genomic analysis revealed that *Staphylococcus* and *Corynebacterium* spp. are the most abundant organisms in moist areas, with the surprising relevant presence of *Corynebacteria*, which are extremely fastidious and slow-growing organisms in culture, and therefore difficult to detect without using molecular techniques.

Dry areas of skin show a more wide biodiversity, with a mixture of Actinobacteria, Proteobacteria, Firmicutes and Bacteriodetes, with a large presence of Gram negative, undetected with the classical plate counting methods.

Genomic data also revealed that, even if when compared with the intestinal microbiota, the skin bacteria had a greater variability over time (7), there are sites in which the microbial composition is

quite stable. Moreover, the microbial profile of the skin seems to be so related to each single individual to be useful also for forensic purposes (8).
In a way very similar to that showed for gut microbiota it was also possible to detect age-related shifts in the skin microbiota, linked to the sexual maturation (9).

A surprising outcome, recently published (10) shows evidence of a previously unknown physical interaction between commensal bacteria and dermal cells including deep dermal stroma and superficial adipose tissue, areas that were assumed to be devoid of a microbial community. To determine the relative abundance of microbial DNA below the superficial barrier of the skin stratum corneum, subject skin was subjected to surgical scrubbing and sterile swabbing prior to biopsy for DNA analysis. The study does not provide indications that live bacteria colonize or inhabit the dermis, but that bacterial components and/or metabolites are present in massive amounts in deep dermis.
Another new set of data (11) shows that viruses represent a significant part of the cutaneous flora, with an asymptomatic carriage of beta and gamma-human papillomaviruses on the healthy skin.
The interaction between skin commensal bacteria and the immune system are under intensive investigations (12,13) as well as changes of skin microbiota composition due to pathological conditions (14,15,16) .
The impressive amount of data recently accumulated by genomics researches about skin microbiota and microbiome is also summarized in recent reviews (17,18.19) which in turn could be the basis for future investigations in this challenging but promising specific section of the human microbiota and microbiome.

References.

1. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? Br J Dermatol. 2008 Mar;158(3):442-5
2. Kong HH, Segre JA. Skin microbiome: looking back to move forward. J Invest Dermatol. 2012 Mar;132(3 Pt 2):933-9
3. Dekio I, Hayashi H, Sakamoto M, Kitahara M, Nishikawa T, Suematsu M, Benno Y. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. J Med Microbiol. 2005 Dec;54(Pt12):1231-8.
4. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci U S A. 2007 Feb 20;104(8):2927-32.
5. Grice EA, Kong HH, Renaud G, Young AC; NISC Comparative Sequencing Program,Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA. A diversity profile of the human skin microbiota. Genome Res. 2008 Jul;18(7):1043-50.
6. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC; NISC Comparative Sequencing Program, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA. Topographical and temporal diversity of the human skin microbiome.Science. 2009 May 29;324(5931):1190-2.
7. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009 Dec 18;326(5960):1694-7.
8. Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R. Forensic identification using skin bacterial communities. Proc Natl Acad Sci U S A. 2010 Apr 6;107(14):6477-81
9. Oh J, Conlan S, Polley EC, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. Genome Med. 2012 Oct 10;4(10):77.
10. Nakatsuji T, Chiang HI, Jiang SB, Nagarajan H, Zengler K, Gallo RL. The microbiome extends to subepidermal compartments of normal skin. Nat Commun 2013;4:1431.
11. Foulongne V, Sauvage V, Hebert C, Dereure O, Cheval J, Gouilh MA, Pariente K, Segondy M, Burguière A, Manuguerra JC, Caro V, Eloit M. Human skin microbiota: high diversity of

DNA viruses identified on the human skin by high throughput sequencing. PLoS One. 2012;7(6):e38499
12. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, Deming C, Quinones M, Koo L, Conlan S, Spencer S, Hall JA, Dzutsev A, Kong H, Campbell DJ, Trinchieri G, Segre JA, Belkaid Y. Compartmentalized control of skin immunity by resident commensals. Science. 2012 Aug 31;337(6098):1115-9.
13. Kosiewicz MM, Zirnheld AL, Alard P. Tuning of skin immunity by skin commensal bacteria. Immunotherapy. 2013 Jan;5(1):23-5.
14. Dekio I, Sakamoto M, Hayashi H, Amagai M, Suematsu M, Benno Y. Characterization of skin microbiota in patients with atopic dermatitis and in normal subjects using 16S rRNA gene-based comprehensive analysis. J Med Microbiol. 2007 Dec;56(Pt 12):1675-83
15. Redel H, Gao Z, Li H, Alekseyenko AV, Zhou Y, Perez-Perez GI, Weinstock G, Sodergren E, Blaser MJ. Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. J Infect Dis. 2013 Apr;207(7):1105-14.
16. Fahlén A, Engstrand L, Baker BS, Powles A, Fry L. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. Arch Dermatol Res.2012 Jan;304(1):15-22
17. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011 Apr;9(4):244-53
18. Kong HH. Skin microbiome: genomics-based insights into the diversity and role of skin microbes. Trends Mol Med. 2011 Jun;17(6):320-8.
19. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M; NIH Intramural Sequencing Center Comparative Sequencing Program, Kong HH, Segre JA. Topographic diversity of fungal and bacterial communities in human skin. Nature. 2013 Jun 20;498(7454):367-70

SKIN MICROBIOMA AND SKIN DISEASE: THE EXAMPLE OF ROSACEA

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Introduction: The skin is the human body largest organ and represent the first barrier to environmental exposure acting as physical barrier and protecting from the assault of foreign organisms or toxic substances. Moreover, the skin is also colonized by a complex microbial populations that include bacteria, fungi and viruses most of which are harmless or in some cases even beneficial to their host. The complex microbial populations that colonize the human body form the human microbiome The composition of these microbial communities depends on skin characteristics such as sebaceous gland concentration, moisture content, temperature as well as host genetics and environmental factors.. A complex relationship exists between host and microrganisms and for this reason both the presence of pathogens and the imbalance and/or perturbation in the commensal ecosystem have been associated with skin disease (Chen YE and Tsao H 2013; Kong HH and Segre JA 2012). These complex microbial communities may contribute even in the development of non infectious pathologies such as atopic dermatitis, psoriasis, acne and rosacea. Rosacea in particular seems to represent an interesting model to evaluate the correlation between microbioma and skin disease.

Rosacea: Rosacea, one of the most common dermatoses affecting primarily adults of 30-60 years of age, is a chronic cutaneous disorder characterize by centrofacial persisting erythema, telangiectases, papules, pustules and phymas. Four different subtypes of the disease have been recognized, i.e. erythematotelangiectatic (ETR), papulopustular (PPR), phymatous and ocular, distinguished on the of specific clinical manifestations and morphological characteristics (Lazaridou E et al, 2010). The pathogenesis has not yet been clarified but several factors have been identified as triggering ones such as solar exposure, dietary agents and drugs. Abnormalities of the cutaneous vascular and lymphatic system, dermal matrix degeneration and in some cases abnormalities of the sebaceous gland have also been described as factors implicated in the pathophysiology of rosacea.

Rosacea and microorganisms: Microbes have long been addressed as having a role in rosacea but the association between specific microbial colonization and incidence of the disease showed contradictory results. No one among the microrganism considered such as *Helicobacter pylori, Demodex folliculorum, Staphylococcus epidermidis*, and *Chlamydia pneumonia* has been identified as having a real causative role in the disease. Probably the collection of microbes populating the skin, more than a single microrganism population has to be considered. Microbiome composition is crucial for the correct skin immune functions and together with the physical barrier functions account for the capability of the skin to detect potential dangerous events such as trauma and infection. Toll-like receptors (TLRs) represent one of the mechanism by which innate immune system trigger inflammation by the recognition of specific microbial products or products of host injury (Takeda K et al, 2003; Jiang D et al, 2005; Taylor KR et al, 2007). Recent findings have shown that rosacea patients present abnormal activation of innate immune pattern recognition receptors. The epidermis of subjects affected by rosacea express higher amount of TLR2 than healthy subjects indicating a possible explanation for the enhanced inflammatory responses to external stimuli. Moreover, enhanced expression of TLR2 can lead to abnormal production of cathelicidin antimicrobial peptides and increased expression and activity of serine protease kallikrein (KLK5) characteristic of the disease. This finding suggest a role for the microbiome and TLR2 in controlling epidermal inflammation considering that in normal condition the collection of

microbes populating the skin activate TLRs but do not promote inflammation (Yamasaki K et al, 2011).
Additionally, recent data indicate the potential pathogenetic role in the development of rosacea of small intestinal bacterial overgrowth (SIBO). Rosacea patients have a significantly higher SIBO prevalence than healthy subjects and its eradication leads to a significative regression of skin lesions. Moreover, rosacea patients SIBO negative do not obtain any improvement after antibiotic therapy (Parodi A et al, 2008).

Conclusion: The role of microorganisms in the development of rosacea as well as other skin diseases has not been clearly defined. The data available to date suggest that they may have a potential role, which seems to be rather synergistic with other factors, unless the real causative microorganism has not been identified yet.
Recent metagenomic studies have uncovered a surprising diversity within microbiome and have indicated a much larger role in immune modulation and epithelial health than previously expected.
Defining the complexity of human microbiome and understanding microbe-host interactions and the factors driving microbial colonization could probably increased our knowledge about pathogenesis of different skin diseases and could help in the development of novel diagnostic tools and therapeutic treatment.

References

- Chen YE, Tsao H. The skin microbiome: Current perspectives and future challenges. J Am Acad Dermatol. 2013 Jul;69(1):143-155.
- Kong HH, Segre JA. Skin microbiome: looking back to move forward. J Invest Dermatol. 2012 Mar;132(3 Pt 2):933-9.
- Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, Noble PW. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med. 2005 Nov;11(11):1173-9.
- Lazaridou E, Giannopoulou C, Fotiadou C, Vakirlis E, Trigoni A, Ioannides D. The potential role of microorganisms in the development of rosacea. J Dtsch Dermatol Ges. 2011 Jan;9(1):21-5.
- Parodi A, Paolino S, Greco A, Drago F, Mansi C, Rebora A, Parodi A, Savarino V. Small intestinal bacterial overgrowth in rosacea: clinical effectiveness of its eradication. Clin Gastroenterol Hepatol. 2008 Jul;6(7):759-64.
- Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003;21:335-76.
- Taylor KR, Yamasaki K, Radek KA, Di Nardo A, Goodarzi H, Golenbock D, Beutler B, Gallo RL. Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2. J Biol Chem. 2007 Jun 22;282(25):18265-75.
- Yamasaki K, Kanada K, Macleod DT, Borkowski AW, Morizane S, Nakatsuji T, Cogen AL, Gallo RL. TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. J Invest Dermatol. 2011 Mar;131(3):688-97.

PROBIOTICS AND ALLERGY

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In the last decades the prevalence of eczema and allergic diseases such as asthma, allergic rhinitis and atopic dermatitis (AD) raised significantly in industrialised countries. At least 20% of European is affected by allergic diseases, and all over the world allergies are the most common chronic pathologies affecting children. The increase in the prevalence of allergic diseases has been associated with improvement in food hygiene and a subsequent change in the gastrointestinal microflora (hygiene hypothesis). As a consequence, a relative lack of immune system stimulation by pathogens has contributed to the allergic diseases rise. Atopic dermatitis is characterized by chronic inflammation, intense pruritus, eczema and increasing in intestinal permeability. Recently, it has been demonstrated that the skin barrier dysfunction not only enhances allergen sensitization but also leads to systemic allergic responses such as an increased immunoglobulin (Ig) E levels and airway hyperreactivity. Yet, despite almost four decades of research since the first observation of environmental associations of atopy, there is no a single intervention with proven efficacy for primary prevention. Perhaps, the most promising intervention is probiotic bacteria usage. Probiotics, defined as living microrganisms which, when ingested in adequate amounts, may have a healthy effect on the host, have been shown to reduce intestinal permeability, limiting the absorption of noxious molecules from the gut lumen.
Previous studies highlighted the beneficial role of a specific lactobacilli strain in the treatment of moderate/severe atopic dermatitis. In particular, *Lactobacillus salivarius* LS01 DSM 22775 led to a reduction in microbial translocation and a significative decrease in staphylococcal load after 16 weeks of probiotic treatment. Furthermore, the treatment with *L. salivarius* LS01 was able to reduce the production of type Th2 cytokines, maintaining stable the production of type Th1 cytokines, which increase typically represents a cause of clinical worsening in patients affected by AD. Recently, it has been demonstrated that the combination of probiotics, in particular *L. salivarius* LS01 DSM 2275 and *Bifidobacterium breve* BR03 DSM 16604, was able to improve clinical and immunological parameters in adults suffering from moderate to severe AD. After 12 weeks of probiotic treatment, patients showed a significative increase in regulatory T cells (Treg) percentage and a reduction in plasmatic LPS concentration, a marker of altered gut barrier permeability. As a consequence, a significant reduction of microbial translocation was observed during and after probiotic treatment. These data indicate that microbial translocation is effectively involved in the AD pathogenesis and that the recovery of gut barrier functions could improve the clinical outcome of AD. Moreover, the probiotic formulation have been seen to upregulate Th1 functions and downregulate Th2 and Th17 activity, improving Th1/Th2 and Th17/Treg ratios. Th2 and Th17 were positively correlated with plasma LPS level, suggesting an interaction between microbial translocation and the activation of unfavourable Th subtypes in the pathogenesis of this disease. With regard to the intestinal flora composition, the decrease in staphylococcal load after probiotic treatment observed in previous studies was confirmed. Staphylococci were found to be correlated with disease severity that could be due to IgE hypersensitivity or the production of exotoxins with superantigen properties, playing a crucial role in AD development and maintenance. Finally, it has been observed that the 2 bacterial strains used in the probiotic mixture were able to colonize the intestine for long time, also after the suspension of treatment. In conclusion, all these results together demonstrate that the combination of probiotics has strong immunomodulatory activity as a consequence of their ability to improve gut barrier function. Probiotics intake represents an encouraging and healthy option in the treatment and alleviation of allergic diseases, in particular atopic dermatitis, leading to a stimulation of immune system and an improvement of intestinal functionality.

NUTRACEUTICALS AND ACNE

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Acne is a complex and multifactorial skin disease accounting for the most common dermatological disorder of the developed nations. Epidemiological studies have estimated that in the western world acne affects 79% to 95% of the adolescent population. In recent years, we have assisted to an increase of acne incidence also in the post-adolescential population, with 40% to 54% of individuals older than 25 years. In contrast, rates of acne have been documented to be extremely low in hunter-gatherer communities. The precise mechanisms concerned with the onset of acne symptoms remain elusive. Classically, four major factors are called into play in acne pathogenesis, such as sebum overproduction, bacterial colonization (Propionibacterium Acnes – P. Acnes), follicular hyperkeratinization, and increased output of pro-inflammatory signals. Androgens and other influences are likely to participate in the development of acne. The sebaceous gland activity initiated at puberty by androgens sets out the conditions for the colonization of P. Acnes. However, factors determining the selection of more virulent strains of P. Acnes in acne remain unclear. In the past, it was believed that comedones preceded the P. Acnes colonization, which consequently triggered inflammation. This oversimplified scheme has been questioned in recent years due to recognition of multiple factors impacting the pathogenic events in acne.
Due to the higher incidence of the disease in the industrialized countries, epidemiological and association research have focused on the role of western diet in the pathogenesis of acne, although familial and ethnic factors are also implicated in the acne prevalence. The diet styles are pivotal in determining the correct supply of calories, macro- and micro-nutrients, antioxidants, and essential fats. Additionally, diet has a strong impact on the gut microflora, which is in metabolic equilibrium with skin. Biochemical alterations associated with acne have been primarily searched in sebum, which is the main product of the sebaceous gland activity. Sebum is an elaborated lipid mixture synthesized in the sebaceous gland cell units, namely sebocytes, and delivered to the skin surface though a peculiar mechanism known as holochrine secretion. Sebum discharge represents a major step in the final stages of differentiation of sebocytes and results from the accumulation of cytoplasmic lipid droplets and subsequent cell disintegration and release of their content into the follicle. Human sebum consists of squalene, esters of glycerol (notably triglycerides and diglycerides), wax, and cholesterol esters, as well as free cholesterol and fatty acids. Triglycerides and fatty acids, taken together, account for the predominant proportion (57,5%), followed by wax esters (26%) and squalene (12%). The least abundant lipid in sebum is cholesterol, which with its esters, accounts for the 4,5% of total lipids. The most characteristic products of the sebaceous secretion are squalene and wax esters. They are unique to sebum and likely the major sebum components with skin protective functions. Squalene is a linear intermediate in the biosynthetic pathway leading to cholesterol. The reason why cholesterol is not synthesized in the sebaceous gland, in favor of squalene accumulation, is still unclear. Branched chain fatty acids and particular pattern of unsaturation are also characteristic features of sebum. The “sebaceous-type” reaction of desaturation is catalyzed by Δ6 desaturase enzyme (fatty acid desaturase-2, FADS-2), which preferentially converts palmitic acid (16:0) to sapienic acid (16:1n-10), accounting for ca. 25% of the total fatty acids. Sebum overflow and acne grade appear to be associated with an increased proportion of monounsaturated fatty acids suggesting a possible role for desaturase enzyme in the sebaceous lipogenesis and acne onset. Elongation of 16:1n-10 by 2-carbon unit and further unsaturation leads to the formation of sebaleic acid (18:2n-10,13), also found in human sebum. Decreased concentration of linoleic acid (18:2n-6,9) has been detected in skin surface lipids of acne patients. In particular, depletion of 18:2n-6,9 affects WE significantly making it reasonable to

assume that the essential FA linoleic acid is directly involved in the sebaceous lipid synthesis. Moreover, low level of linoleic acid also produces impairment of the epidermal barrier function, which might account for major permeability of comedonal wall to inflammatory substances. Other lipids have been proposed as involved in the development of comedone lesions. In particular, the attention has been pointed to the increase of other fatty acids and on by-products of squalene peroxidation. Upon oxidative challenge, squalene is readily oxidized giving rise to different squalene peroxidation by-products associated with harmfulness to skin cell cultures and *in vivo*, histologic changes and immune suppression. The primary peroxidation product in skin surface lipids is squalene monohydroperoxide, which causes up-regulation and release of inflammatory mediators, and keratinocytes proliferation. The skin surface and comedones lipids collected from acne patients are enriched in polar lipids mainly derived from squalene oxidation. The strategy that skin adopts to limit the potentially harmful effects of peroxidated squalene relies on the vitamin E supply at the skin surface. Vitamin E is found at the skin surface as a significant constituent of human sebum. In sites with elevated sebaceous glands density continuous secretion of vitamin E is detected in amounts associated with the levels of cosecreted squalene. Skin vitamin E delivery through the sebaceous gland secretion may be considered as a physiological anti-oxidant mechanism to prevent squalene from oxidation oxidation. More recent data have demonstrated that higher amounts of squalene peroxide and consequently decreased levels of vitamin E in acne patients vs healthy controls further supporting the role of squalene peroxidation and, in general, of lipid peroxidation in acne development. Low glycemia load diet has been demonstrated to be able to correct the increased sebum production and compositional changes proper of acne, indicating the need to point to diet habits as possible concurrent factors influencing sebaceous gland physiology.

Diet and nutraceuticals versus sebum quality and acne

The link between acne and diet has been the focus of medical and dermatological investigation throughout decades to our days. In spite of numerous studies aiming at the identification of mechanistic roles of dietary components in the acne risk, complexity of the matter and limitations in the scientific rigor applicability have left many hypotheses open.

Several lines of evidence place on the stage of the diet habits predisposing to acne the modern lifestyles that lean over sophisticated foods with poor nutriceutical value and high in insulinotropic effects. Additionally, it is well documented that a period of insulin resistance occurs during puberty, one coinciding with the development of acne. In recent years it has become evident that there may be a connection between low-fiber carbohydrates and the risk of acne. Diets low in processed food and sugars (with an overall low glycemic load) are associated with decreased acne risk. Skin inflammation is partly hinged on gastrointestinal mechanisms. Interestingly, the gut microbiota contributes to glucose tolerance, and oral administered probiotics can improve fasting insulin levels and glucose turnover rates even in presence of high fat diet. High fat, sugar – leads to increased intestinal permeability giving access to LPS endotoxins through the intestinal barrier, which in turn leads to low-grade inflammation, oxidative stress and insulin resistance. Such dietary factors play a role in the alteration of the sebaceous gland output. Sebum production and quality can be increased by the consumption of dietary fat or carbohydrate. In contrast, caloric restriction decreases dramatically sebum secretion rate. These findings are suggestive of involvement of dietary factors in the sebaceous lipid synthesis. The western diet typically provides a higher supply of n-6 over n-3 PUFA, with a ratio between 10:1 and 20:1, which is higher than the 2:1 ratio found in the non-westernized diet.

Epidemiological studies have shown that increasing the intake of polyunsaturated fatty acids (PUFA) through a diet rich in fish and seafood results in a lower rates of acne. Intake of n-3 PUFA have an impact on the inflammatory pathways resulting in a lower pro-inflammatory cytokines secretion and the leukotriene B_4 (LTB_4) synthesis, mechanisms demonstrated to be beneficial in acne. Fortification of the n-3 PUFA levels in association with to antioxidants and zinc sources have

been explored for their beneficial effects on acne symptoms. Increased levels of reactive oxygen species (ROS) produced by neutrophils are found in inflammatory acne lesions. Skin antioxidants are found at a lower level in acne patients. By contrast, biomarkers of lipid peroxidation and oxidative damage, due to increased ROS levels, are a common finding in acne. It is likely that antioxidants on one hand prevent oxidative consumption of antiinflammatory lipids (e.g. n-3 PUFA), on the other hand interfere with the production of proinflammatory lipid bioproducts. Circulating levels of vitamin E, a major lipofilic dietary antioxidant, has resulted significantly lower in acne compared to healthy controls in cross sectional studies. Similar evidence are unclear for food hydrophilic antioxidants, including as vitamin C. Nevertheless, vitamins E and C cooperate in determining the skin redox status. Moreover, vitamin C regenerates redox active vitamin E, which is consumed due to free radical chain reaction. Flavonoids of vegetable origin with well-characterized antioxidant properties have proven highly active in inhibiting lipogenesis and promoting excretion of sebum from mature sebocytes. Flavonoids also exert antibacterial properties against P. Acnes, activity shared with resveratrol, a phytoalexin found in the skins of red grapes. Among oligoelements, zinc is at the frontline of the antinflammatory, antioxidant and antimicrobial acivities demonstrated to be advantageous to contrast acne manifestations. Moreover, zinc exerts a significant sebum normalization effect. Given the level of evidence available, several food antioxidants together with microelements are likely to be beneficial in the management of acne symptoms, especially in the milder grades of the disorder, where diet habits and onset of low-levels of oxidative stress have a major relevance. In a group of acne patients we have evaluated the effects of an oral combination of vitamin C, grape polyphenols and zinc on the acne grade. Parameters of sebum outflow, biomarkes of oxidative modifications, and levels of inflammatory cytokines were evaluated in patients affected with mild acne following 8 weeks treatment. Results demonstrated the relevance of controlling the extent of sebum oxidation in though the oral administration of nutraceuticals with a prominent antioxidant activity.

NUTRACEUTICALS AND SKIN PROTECTION

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Skin is constantly exposed to a wide variety of toxic agents as environmental insults and genotoxic factors. Smoke, pollutants, UV radiation, stress and microorganisms can induce oxidative damage through generation of free radicals, that can interact with cellular biomolecules such as nucleic acids (leading to mutations, renewal failure and cell death), proteins (leading to collagen degradation, elastin alteration) and fatty acids (provoking especially membrane lipid oxidation): all these phenomena alter the redox status of the intracellular milieu and lead to many skin disorder including hyperplasia, erythema, aging and cancer. To mitigate such damage the skin possesses extremely efficient defence mechanisms, including antioxidant enzymes and nonenzymatic antioxidant molecules. However, because of constant environmental exposure to physical and chemical agents, an oxidant/antioxidant imbalance may have key effect on skin well-being**.** A strategy to protect against, or to prevent, the toxic effects of free radicals could be the systematic consumption or the topical use of natural compounds derived from the diet. Fruits and vegetables represent important sources of bioactive compounds, such as vitamins, mineral salts, phenols and other phytochemicals, which act directly against biological oxidative status and therefore can be considered nutraceuticals. A nutraceutical can be defined in fact as "any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease". Among food with healthy benefits, berries represent an innovative healthy choice due their interesting biological properties and nutritional value of these fruits. Among berries, strawberry (*Fragaria x ananassa*) is of the most commonly consumed and is, by far, the most studied berry from the agronomic, genomic and nutritional view point. Its remarkable nutritional composition has been correlated to the high content of vitamin C, folates and to the high levels and variety of phenolic constituents. The major class of these compounds is represented by flavonoids, mainly anthocyanins, that possess strong anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, and photo-protective properties and are able to modulate enzymatic pathways; thus, they may play a role in preventing skin diseases related to oxidative stress. Only recently, fruit breeding programs have begun to take into account the importance and the necessity to combine production efficiency traits with improved fruit quality, including the new concept of improved fruit sensorial, nutritional and nutraceutical quality. During the last 5 years more than 100 new selections have been created within the breeding program of the Polytechnic University of Marche to improve the nutritional quality of fruit and among these new selections the cultivar *Sveva* has shown to be one of the most promising in terms of nutraceutical compounds such as vitamin C, flavonoids, anthocyanins.

Recently, the possible photoprotective capacity of this cultivar was analyzed on human dermal fibrostasts (HuDe) against UVA radiation. This component of the solar UV spectrum is able to penetrate through the dermis to subcutaneous tissue and may affect both epidermal and dermal skin components. Incubation for 24 hours with different concentrations of the strawberry extracts showed a photoprotective activity in HuDe cells exposed to UV-A radiation, increasing cellular viability as well as diminishing DNA damage in a dose-responsive manner as compared to control cells.

This cultivar showed also a relevant protection on HuDe cells stressed with two different oxidant stressors: H_2O_2, a reactive oxygen specie administered in a single bolus, and AAPH, a radical that decomposes constantly and with known kinetic features over time. These two types of oxidizing

agent mimic two different conditions of oxidative stress: (i) the former, an acute condition in which cells are subjected to high doses of free radicals in a single time, and (ii) the latter, a chronic condition in which the action of radical species persists over time. The pre-treatment with *Sveva* showed a significant cytoprotective effect on cells stressed alternatively with the two oxidants, maintaining cell viability much higher than control cells. The increased cell viability was accompanied by a lower level of intracellular ROS, a lower extent of membrane lipid peroxidation and a lower extension of DNA damage. Moreover, strawberry compounds were involved in cell proliferation and recovery, showing significant restoring of vitality and cell function from induced oxidative stress. Finally, strawberry compounds were able to modulate both aerobic and anaerobic metabolism, enhancing glycolytic performance and mitochondrial respiratory function especially in stressed cells.

Overall, routine consumption/intake or topical treatment with nutraceuticals could be effective to enhance resistance to oxidative stress and prevent/improve skin disorders. In this regards, strawberry contains bioactive compounds that may confer protective activity in human skin, and among these elements polyphenols are the most promising group of compounds that can be exploited as ideal protective agents for a variety of skin disorders. Further studies are needed to verify if strawberry compounds are able to protect skin against other types of stress, such as lipopolysaccharide infections, and to understand which molecular mechanisms could be involved in their multiple healthy effects.

FROM BASIC TO APPLIED RESEARCH: LESSONS FROM THE HUMAN MICROBIOME PROJECTS

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Two large-scale initiatives of major funding agencies aimed at deciphering the structure and function of the human gut microbiota, namely the NIH's Human Microbiome project and the European MetaHIT project, finalized their research programme in 2012.

Until recent years, our knowledge on the human gut microbiota was largely limited to certain community members with potential pathogenicity by either translocation or production of toxins. Most of these potential pathogens were isolated in culture and recognized by traditional diagnostic techniques. However, culture-based techniques to identify bacteria have important limitations, and the large majority of bacteria in the human gut cannot be grown in culture media. Their potential role in health or disease has been ignored.

The advent of high-throughput technologies has changed our perspective dramatically. First, these technologies are culture independent and, remarkably, they allow the characterization of microbial communities as a whole, enabling a deeper and global view of all the community members and their relative abundance[1]. The novel approach for the analysis of microbial communities in environmental samples is called "metagenomics", and is defined as the study of all the genetic material recovered directly from environmental samples bypassing the need to isolate and culture individual community members[2]. The metagenome is the collective genetic content of the combined genomes of the constituents of an ecological community. The microbiome is defined as the collective genome of the microbial symbionts in a host animal[3].

The most common approach consist on the extraction of DNA from the biological sample, followed by the amplification and sequencing of 16S ribosomal RNA genes in the sample. The 16S rRNA gene is present in all bacteria and contains both conserved and variable regions. Thus, similarities and differences in the sequence of nucleotides of the 16S rRNA gene allow taxonomic identification ranging from the domain and phylum level to the species level. Currently, around 2.77 million aligned and annotated 16S rRNA sequences are available in DNA databases (http://rdp.cme.msu.edu/). Taxonomic identification is based on comparison of 16S rRNA sequences in the sample with reference sequences in the database. In this way, studies on the 16S rRNA gene provide information about microbial composition and diversity of species in a given sample.

A more powerful molecular approach is not limited to 16S rRNA sequencing but addresses all the genetic material in the sample. The decreasing cost and increasing speed of DNA sequencing, coupled with the advances in computational analyses of large datasets, have made it feasible to analyse complex mixtures of entire genomes with reasonable coverage. The resulting information describes the collective genetic content of the community from which functional and metabolic networks can be inferred. Thus, the full metagenomic approach has the advantage of not only providing the phylogenetical characterization of the microbial community but also telling about biological functions present in the community.

The human gut microbiota

Estimates suggest that the colon, the largest ecological niche for microbial communities in the human body, harbours over 10^{14} microbial cells, i.e. several hundred grams of microbes, most of them belonging to the domain Bacteria. Molecular studies based on 16S rRNA gene sequencing have highlighted that only 7 to 9 of the 55 known divisions or phyla of the domain Bacteria are detected in fecal or mucosal samples from the human gut[3-6]. Moreover, such studies also revealed that more than 90% of all the phylotypes belong to just two divisions: the Bacteroidetes and the Firmicutes. The other divisions that have been consistently found in samples from the human distal

gut are Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia. Of the 13 divisions of the domain Archea, only one or two species seem to be represented in the human distal gut microbiota. Thus, at the division level, the human intestinal ecosystem is less diverse than other ecosystems on earth, like soils and ocean waters which may contain 20 or more divisions. However, at a lower taxonomic level (species or strain), there is a considerable variation in the composition of the fecal microbiota among human individuals. Strain diversity between individuals is highly remarkable so that studies have found that a large proportion of the identified strain-level phylotypes are unique to each person. Each individual harbours his or her own distinctive pattern of bacterial composition.

In a cohort of 124 European adult subjects, a total of 3.3 million microbial genes were characterized by full metagenomic analysis of fecal samples[5]. This effort has provided the first gene catalogue of the human gut microbiome. Each individual carries an average of 600,000 non-redundant microbial genes in the gastrointestinal tract. This figure suggests that most of the 3.3 million genes in the catalogue are shared. It was found that around 300,000 microbial genes are common in the sense that they are present in at least 50% of individuals. Up to 98% of genes in the catalogue are bacterial, and the entire cohort harbours between 1,000 and 1,150 prevalent bacterial species, with at least 160 species per individual[5]. Interestingly, *Bacteroides*, *Faecalibacterium* and *Bifidobacterium* are the most abundant genera but their relative proportion is highly variable across individuals.

Network analysis of species abundance across different individuals suggested that the overall structure of the human gut microbiota in each individual conforms to discrete and distinct patterns defined by interactions within community members[6]. This hypothesis was investigated using a dataset of metagenomic sequences from American, European and Japanese individuals. Multidimensional cluster analysis and principal component analysis revealed that all individual samples formed three robust clusters, which have been designated as 'enterotypes'[6]. Each of the three enterotypes is identifiable by the variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). The basis for the enterotype clustering is unknown but appears independent of nationality, sex, age, or body mass index. Interestingly, it seems that the reported enterotype partitioning is related to long-term dietary patterns[7]. The *Bacteroides* enterotype was associated with diets enriched in protein and fat. In contrast, the *Prevotella* enterotype was linked to diets with predominance of carbohydrates and sugars.

The concept of dysbiosis

An imbalance of the normal gut microbiota composition is called dysbiosis. A number of disease states have been associated with changes in the composition of the gut microbiota. Some recent data on the metabolic syndrome suggest that changes in gut microbiome composition may play a role in the disorder. The mechanisms advocated are the provision of additional energy by the conversion of dietary fibre to short-chain fatty acids, effects on gut-hormone production, and increased intestinal permeability causing elevated systemic levels of lipopolysaccharides. The contact with these antigens seems to contribute to low-grade inflammation, a characteristic trait of obesity and the metabolic syndrome. Presumably obesity affects the diversity of the gut microbiota and probably, the way individuals harvest energy from nutrients.

One of the major hypotheses underlying the pathogenesis of inflammatory bowel disease (IBD) is the presence of abnormal communication between gut microbial communities and the mucosal immune system[8]. Luminal bacteria appear to provide the stimulus for immune-inflammatory responses leading to mucosal injury. There is also some evidence showing that the microbiota of patients with IBD differs from that of healthy subjects. Differences include low biodiversity of dominant bacteria, highly variability over time, and changes both in composition and spatial distribution (high concentrations of mucosal adherent bacteria). The microbiota of Crohn's disease patients is characterized by a decreased in *Faecalibacterium prausnitzii* as well as increased numbers of the Proteobacteria and Actinobacteria phyla. Some other associations of human

conditions with particular microbiota characteristics have been described such as irritable bowel syndrome, colorectal carcinoma, autism, childhood-onset asthma and cardiovascular disease, but consistency among studies is still poor.

Therapeutic approaches to dysbiosis

Even if associations of dysbiosis with disease do not necessarily predict cause-effect relationships, there is growing interest in developing strategies to improve the 'physiological' quality of the human gut microbial ecosystem for health benefits. As suggested by experts, the future of a healthy human gut microbiota may include the restoration of our ancestral microbial ecology. According Cho and Blaser[9] there are two possible types of restoration. The first involves restoring ancient organisms in healthy hosts that lack them, as prophylaxis against future risk of disease. The second type of restoration could be therapeutic, when the etiological extinctions or imbalances are clearly identified. This scientific boundary will require an understanding of the biology of re-introductions, as well as developing microbial breeding programs[9].

References

1. Handelsman J, Rondon MR, Brady SF et al. (1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem *Biol* **5**(10), R245-9.
2. Frank DN, Pace NR. (2008) Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* **24**, 4–10
3. Turnbaugh PJ, Ley RE, Hamady M et al. (2007) The human microbiome project. *Nature* **18** 449(7164), 804-10.
4. Eckburg PB, Bik EM, Bernstein CN et al. (2005) Diversity of the human intestinal microbial flora. *Science* **308**, 1635-1638.
5. Qin J, Li R, Raes J et al. (2010) MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **4** 464(7285), 59-65.
6. Arumugam M, Raes J, Pelletier E et al. (2011) MetaHIT Consortium. Enterotypes of the human gut microbiome. *Nature* **12** 473(7346), 174-80.
7. Wu GD, Chen J, Hoffmann C, et al (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science **334**(6052):105-8.
8. Manichanh C, Borruel N, Casellas F & Guarner F (2012). The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* 9, 599-608.
9. Cho I, Blaser MJ. (2012) The human microbiome: at the interface of health and disease. *Nat Rev Genet* **13(**4):260-70.

MICROBES AND IMMUNE REGULATION; THE "OLD FRIENDS" MECHANISM.

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Selected immunoregulatory probiotics may provide an essential tool for reversing the increases in prevalence of many chronic inflammatory disorders in high-income countries. The potential of this approach emerges when one considers the gut microbiota in the context of human evolution, and recent changes in lifestyle. The original "hygiene hypothesis" was a narrow concept dealing with childhood infections and allergic disorders [1]. In recent years this concept has evolved into a branch of Darwinian medicine with a new conceptual framework [2], explained briefly here.

Mammals co-evolved with a multitude of micro- and macro-organisms that had to be tolerated, either because they were necessary parts of mammalian physiology (such as the various microbiotas) or because they were usually present in food, water and air (harmless environmental saprophytes) or because infection was inevitable and life-long: helminths are a good example of this last category. Many of these would have been picked up by the neonate, and attempts by the immune system to eliminate them merely caused severe immunopathology, such as elephantiasis. We have termed these categories of organism "Old Friends" to emphasise the evolutionary context. Because these organisms needed to be tolerated, co-evolutionary processes have caused them to drive immunoregulatory mechanisms. For example *Bacteroides fragilis* [3] and various helminths [4] secrete molecules that expand regulatory T cell (Treg) populations. Other Old Friends cause dendritic cells to mature into regulatory DC that tend to drive immunoregulatory rather than aggressive responses. A background level of regulatory DC is essential to ensure adequate processing of the "forbidden" targets of the immune system, and to drive appropriate immunoregulation. Thus helminth infection causes patients with early relapsing multiple sclerosis to develop circulating Treg that recognize myelin basic protein and that secrete IL-10 and TGF-β in the presence of this autoantigen [5]. The helminth has acted as a *Treg adjuvant*. We need to develop Treg specific for harmless allergens, gut contents, and self, in order to avoid allergic disorders, inflammatory bowel diseases and autoimmune diseases.

Genetic studies indicate that in parts of the world where the burden of immunoregulatory Old Friends was particularly high, there tended to be selection of single nucleotide polymorphisms (SNP) that were pro-inflammatory, and so partially compensated for excessive immunoregulation [2, 6]. This established an equilibrium as long as the load of Old Friends remained constant, but led to *inflammatory overshoot* when exposure to Old Friends was significantly diminished. This occurred at the so-called second Epidemiological transition …roughly equivalent to industrialization and urbanization, beginning in the early 19th Century when it was first noticed that allergies were increasing amongst wealthy town folk but not amongst farmers [2]. Modern urban life leads to loss of contact with most of these Old friends, notably the helminths. In the absence of the immunoregulatory Old Friends the pro-inflammatory SNP become risk factors for chronic inflammatory diseases [2, 6].

This is not a narrow phenomenon relevant only to allergic disorders. In high-, but not low-income countries there is a generalized failure of immunoregulation, so that many people live with raised C-reactive protein (CRP) even in the absence of clinically obvious inflammation [7]. But such raised CRP is a risk factor for depression [8], cardiovascular disease and other chronic inflammatory diseases. There is a simultaneous increase in allergic disorders, inflammatory bowel diseases, and autoimmune disease in high-income countries [8]. In sharp contrast, when CRP levels are followed longitudinally in a low-income country, with repeated blood samples from the same

individuals over time, it is found that CRP levels are close to zero when inflammation is not required, but rise rapidly during episodes of infection when inflammation is needed [7]. One serious consequence of the epidemic of failing immunoregulation in high-income countries deserves further emphasis. Persistently raised levels of proinflammatory mediators and cytokines cause a form of depression [8-10]. Psychosocial stressors drive release of proinflammatory mediators by pathways that involve the gut and the microbiota [11]. In individuals with poor immunoregulation (often manifested as persistently raised CRP) a given level of psychosocial stressor will lead to an exaggerated quantity and duration of raised proinflammatory mediators, thus increasing the risk of depression [8]. This *reduced stress resilience* of urban citizens of high-income countries might become a major issue. WHO predicts that within a decade or two depression will become the major medical scourge of mankind.

At this point we can return to the original "hygiene hypothesis". This "sound bite" was created when it was observed that allergic disorders were less prevalent in children with older siblings, especially older brothers [1]. But older siblings were merely one of many factors that cause, or correlate with, increased exposure to a diversity of gut microbiota. Other factors include cleaning a baby's pacifier/dummy by sucking it [12], the presence of antibodies to orofecally transmitted organisms [13, 14], keeping a dog [15], living in a low-income country [16], the presence of broad microbial biodiversity in the child's bedroom [17], and exposure to farms and cowsheds [18, 19]. Other well-recognised factors have the reverse effect, and reduce microbiota biodiversity, including birth by caesarian section [20], antibiotics [21] and social isolation [22].

Where allergies are concerned, recent work has revealed that much of the rigorously studied protective effect of the farming environment [18] is mediated by microbial biodiversity [17, 19]. Analysis of the microbiome of the mattress, or of organisms picked up by electrostatic dust collectors in childrens' bedrooms, show that the likelihood of developing allergic problems is inversely related to the diversity of harmless environmental bacterial and fungal taxa [17]. Interestingly exposure to this biodiversity can occur prenatally via exposure of the pregnant mother rather than the child [23], or during the first 2-3 years of childhood.

In high-income urban environments we no longer encounter most of the Old Friends, but nor do we want them back. Therefore we are more dependent that ever on biodiversity of the microbiotas, particularly the gut microbiota, which is the most crucial for immunoregulation. Similarly we are dependent on microbial inputs from the natural environment, which we suggest, explain the health benefits of exposure to green spaces [8]. We remain ignorant of the extent of the link between these two sources of microbial biodiversity. Does the natural environment provide organisms that colonise the human gut, or do these organisms merely modulate the immune system and host microbiota relationship, and so secondarily alter the gut microbiota and its immunoregulatory properties [8]?

In conclusion, a crucial role of appropriate probiotics might be to repair the faulty immunoregulation characteristic of people living in high-income urban centres. Carefully selected immunoregulatory probiotics might restore the biodiversity of our microbiota, and restore an evolutionarily determined requirement for exposure to microbial biodiversity from the natural environment.

1. Strachan DP. Hay fever, hygiene, and household size. *Brit Med J* (1989) **299**:1259-60
2. Rook GA. Hygiene hypothesis and autoimmune diseases. *Clin Rev Allergy Immunol* (2012) **42**:5-15
3. Round JL *et al*. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* (2011) **332**:974-7

4. Wilson MS *et al*. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med* (2005) **202**:1199-212
5. Correale J *et al*. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol* (2007) **61**:97-108
6. Fumagalli M *et al*. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. *J Exp Med* (2009) **206**:1395
7. McDade TW *et al*. Analysis of variability of high sensitivity C-reactive protein in lowland ecuador reveals no evidence of chronic low-grade inflammation. *Am J Hum Biol* (2012)
8. Rook GAW *et al*. Microbial Old Friends, immunoregulation & stress resilience. *Evolution, Medicine and Pubic Health* (2013) **EMPH (2013)**:46-64 doi: 10.1093/emph/eot004 http://emph.oxfordjournals.org/content/2013/1/46.full.pdf+html
9. Gimeno D *et al*. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med* (2009) **39**:413-23
10. Raison CL *et al*. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *Arch Gen Psychiatry* (2012) **Sep 3**:1-11
11. Bailey MT *et al*. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* (2011) **25**:397-407
12. Hesselmar B *et al*. Pacifier Cleaning Practices and Risk of Allergy Development. *Pediatrics* (2013)
13. Matricardi P *et al*. Exposure to foodborne & orofecal microbes versus airborne viruses in relation to atopy and allergic asthma; epidemiological study. *Brit Med J* (2000) **320**:412-7
14. Korhonen L *et al*. Enterovirus infections in early childhood and the risk of atopic disease - a nested case-control study. *Clin Exp Allergy* (2013) **43**:625-32
15. Aichbhaumik N *et al*. Prenatal exposure to household pets influences fetal immunoglobulin E production. *Clin Exp Allergy* (2008) **38**:1787-94
16. De Filippo C *et al*. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* (2010) **107**:14691-6
17. Ege MJ *et al*. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* (2011) **364**:701-9
18. Riedler J *et al*. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* (2001) **358**:1129-33
19. Hanski I *et al*. Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci U S A* (2012)
20. Magnus MC *et al*. Delivery by Cesarean section and early childhood respiratory symptoms and disorders: the Norwegian mother and child cohort study. *Am J Epidemiol* (2011) **174**:1275-85
21. Metsala J *et al*. Mother's and offspring's use of antibi2.5otics and infant allergy to cow's milk. *Epidemiology* (2013) **24**:303-9
22. Claesson MJ *et al*. Gut microbiota composition correlates with diet and health in the elderly. *Nature* (2012) **488**:178-84
23. Schaub B *et al*. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J Allergy Clin Immunol* (2009) **123**:774-82.e5

How food aroma modulates satiation

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Introduction

In view of the obesity epidemic, it is important to understand which factors influence food intake behaviour. In our modern society, humans are able to choose from a wide range of foods that vary in their physical/chemical product properties, nutrient composition, energy density, palatability, and sensory characteristics. These sensory characteristics are thought to influence meal termination and food intake.[1, 2] For example, an increase in viscosity leads to a longer oral exposure time and a decrease in food intake.[3] Oral exposure comprises taste, mouthfeel and retronasal aroma. Retronasal aromas pass through the pharynx after swallowing food and reach the olfactory epithelium via the retronasal route. So far, only a few studies focussed on the unimodal effect of *retronasal* aroma on satiation,[4-6] which includes processes that end a meal. The studies showed that aroma exposure time, complexity and type influenced satiation, measured on visual analogue scales (VAS).[4-6] However, it is not clear whether the increase in rated satiation would also lead to a decrease in food intake. It was suggested that the increase in satiation by the increase in aroma exposure time was caused by sensory-specific satiation (SSS),[5] which is the decrease in pleasantness of an eaten food, relative to the decrease in pleasantness of uneaten foods.[7, 8]

Orthonasal food aromas, perceived via the nose, are known as appetizer.[9] Smelling freshly baked bread or pizza,[10] for example, increase appetite. While hunger and satiation depend on physiological signals only, appetite refers also to eating in the absence of hunger and is co-regulated by other internal and external parameters, e.g. food cues like sight or aromas.[11] Most studies investigated the appetising effects of exposure to a combination of food cues, often including aroma, taste and sight, and found an increase in general appetite, food intake and appetite for the cued food.[12-15] In our project, the specific increase in appetite for the cued food is defined as sensory-specific appetite (SSA) and is the opposite of SSS. Other studies found a decrease in food intake[16] and development of SSS[17] after aroma exposure. Exposure time, aroma type and the way of smelling may affect the appetising or satiating effect.

In this project we investigated under which circumstances aromas are appetising and when they are satiating. We investigated the effects of retronasal aroma exposure time and concentration (PROFILE study), retronasal aroma type (TYPE study), orthonasal aroma exposure time (PASSIVE and ACTIVE studies) and orthonasal aroma types (PASSIVE study) on sensory-specific satiation/appetite, general satiation/appetite and *ad libitum* food intake.

Keywords: aroma properties, appetite, satiation, sensory-specific satiation, sensory-specific appetite, food intake, orthonasal, retronasal

Retronasal aroma exposure studies

We investigated the effects of retronasal aroma exposure time and concentration (PROFILE)[18] and retronasal aroma type (TYPE)[19] on *ad libitum* food intake and general satiation (Figure 1). In two within-subject cross-over studies, non-restrained healthy female participants (age: 18-45y; BMI: 18.5-25kg/m^2) were asked to consume tomato soup during lunch time, until they felt comfortably full. Every 30 seconds, the

participants consumed 10 grams of a bland soup base, while a tomato soup aroma was delivered separately using an olfactometer (retronasal). This gave the impression of tomato soup.

In PROFILE (n=38), the retronasal aroma per sip of soup varied in exposure time (3s and 18s) and concentration (5 x), resulting in four different test conditions. We hypothesized that exposure to a higher concentration and/or longer exposure time would increase satiation. The results demonstrated a 9% significant decrease in *ad libitum* soup intake when exposed to a higher concentration and a longer exposure time of the aroma as compared to the other three conditions (all $p<0.05$). However, hunger and satiation ratings (VAS) did not reveal any differences between conditions. A SSS test showed the development of SSS.

In TYPE (n=45), the quality of the tomato soup aroma varied: one tomato soup aroma rich in creamy notes and one without creamy notes. We hypothesised that the creamy notes were associated with energy dense soup and therefore would increase satiation. The results showed no differences in soup intake between conditions. However, the hunger and satiation ratings (VAS) showed that subjects felt less hungry ($p < 0.10$) and more satiated ($p < 0.05$) when creamy notes were added, but only between 6 and 13 min after start of the soup intake. The difference in hunger and satiation disappeared after 13 min of soup intake.

Orthonasal aroma exposure studies

We hypothesised that food aromas are appetising after a short exposure (of circa 1-3 minutes), but become satiating over time (of circa 10-20 minutes). Furthermore, the way of smelling may affect any appetising/satiating effects. Two studies investigated the appetising/satiating effects of several aroma types over time (Figure 1). The aromas were smelled passively (PASSIVE; n=21)[20] or actively (ACTIVE; n=61).

In two within-subject cross-over studies (PASSIVE and ACTIVE), women (age: 18-45y; BMI: 18.5-25kg/m^2) were exposed for respectively 20 and 10 minutes to different aromas during separate sessions. The investigated aromas in PASSIVE were meat, tomato soup, banana, chocolate, bread, pine tree, green/grassy and no-aroma and in ACTIVE banana and no-aroma. All aromas in PASSIVE were distributed in a test room at supra-threshold levels. In ACTIVE, participants intensely smelled water (control) or real banana mashed into a cup covered with a tissue. Both studies measured general appetite and sensory-specific appetite on 100mm VAS over time. Additionally, food choice was measured using food photographs in PASSIVE and *ad libitum* intake of banana milkshake in ACTIVE after the aroma exposure.

Results from both studies showed an increase in sensory-specific appetite after one minute exposure to food aromas, meaning that the appetite for the smelled food increased in comparison to the appetite for other foods. Unexpectedly, the effects did not change over time. In addition, savoury aromas increased the appetite for other savoury foods (+5mm), but decreased the appetite for sweet foods (-5mm). The opposite was found for sweet aromas. The food choice task gave similar results, influencing food choice with 10-15%. The effects of food aroma exposure on general appetite were less clear than the increase in sensory-specific appetite (+12mm): PASSIVE smelling increased general appetite (+4mm) and ACTIVE smelling decreased general appetite (-2mm). The small effects are significant and consistent over time. ACTIVE smelling had no effect on subsequent *ad libitum* food intake.

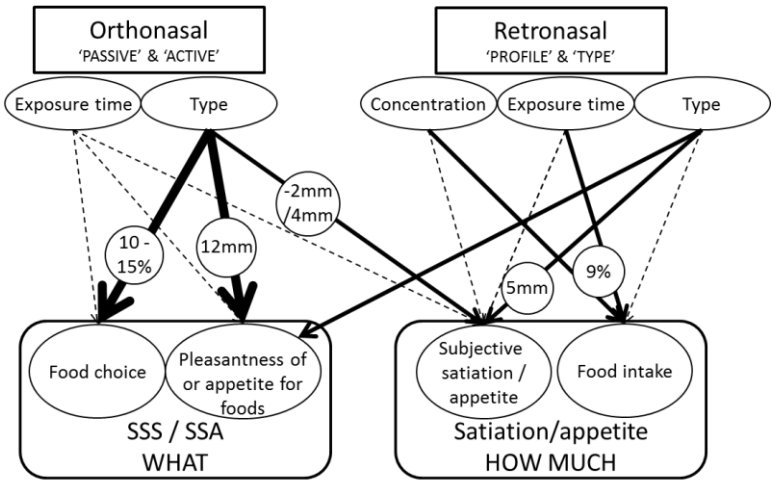

Figure 1: representation of all significant relations (straight lines) and insignificant relations (dashed lines) that were investigated in the present project.

Discussion and conclusion

The main objective of this project was to investigate under which circumstances aromas are appetising and when they are satiating. Exposure to the orthonasal food aromas showed larger effects on the development of SSA than on general appetite and food intake (Figure 1). Exposure to retronasal aromas had small or no effects on satiation and food intake, while SSS transferred to other foods with tomato aroma, demonstrating olfactory SSS. Other studies found similar effects for olfactory SSS,[17] validating the strong relationship between retronasal aroma type and SSS.

An increase in retronasal aroma increased satiation, whereas exposure to orthonasal food aromas increased appetite. However, in the retronasal studies the effect of aroma was measured during eating, whereas in the orthonasal studies no food was ingested during the aroma exposure. Some studies indicate that the neural response to orthonasal aromas in our brain is related to anticipation, whereas retronasal aromas are related to consumption and reward.[21, 22] Possibly, route of aroma exposure affects appetite and satiation.

Unexpectedly, *orthonasal* food aromas did not develop SSS over time. The existence of SSS was explained as the need for variety of foods in order to obtain a balanced diet.[23] When foods are merely smelled, no nutrients are ingested. Therefore, the consumption of the cued food remains desirable.[20] In addition, SSA generalised over the sweet and savoury category: e.g., sweet aromas increased the appetite for sweet foods, but decreased the appetite for savoury food. This category-specific response during smelling is similar to SSS during eating. SSS transfers to uneaten foods with similar properties.[8, 23]

Many factors influence satiation. Likely, major factors such as palatability and texture overrule the small effects of *retronasal* aroma on satiation as found in this project.

In conclusion, orthonasal food aromas had small effect on appetite and large effect on SSA, irrespective of exposure time. Retronasal aromas showed small effects on satiation, but play a role in the development of SSS. We suggest that aromas influence what you eat and less how much you eat (Figure 1).

References

1. Blundell JE, Rogers PJ, Hill AJ. Evaluating the satiating power of foods: implications for acceptance and consumption. In: etal. JS (ed) *Food acceptance and nutrition*. Academic Press: London, 1987, pp 205–19.
2. Hetherington MM. Sensory-specific satiety and its importance in meal termination. *Neuroscience & Biobehavioral Reviews* 1996; **20**(1)**:** 113-117.
3. Zijlstra N, Mars M, de Wijk RA, Westerterp-Plantenga MS, de Graaf C. The effect of viscosity on ad libitum food intake. *International Journal of Obesity* 2008; **32**(4)**:** 676-683.
4. Ruijschop R, Boelrijk AEM, Burgering MJM, de Graaf C, Westerterp-Plantenga MS. Acute Effects of Complexity in Aroma Composition on Satiation and Food Intake. *Chemical Senses* 2010; **35**(2)**:** 91-100.
5. Ruijschop RMAJ, Boelrijk AEM, de Ru JA, de Graaf C, Westerterp-Plantenga MS. Effects of retro-nasal aroma release on satiation. *British Journal of Nutrition* 2008; **99**(5)**:** 1140-1148.
6. Ruijschop R, Boelrijk AEM, de Graaf C, Westerterp-Plantenga MS. Retronasal Aroma Release and Satiation: a Review. *Journal of Agricultural and Food Chemistry* 2009; **57**(21)**:** 9888-9894.
7. Rolls BJ, Rolls ET, Rowe EA, Sweeney K. Sensory specific satiety in man. *Physiology & Behavior* 1981; **27**(1)**:** 137-142.
8. Rolls BJ, Rowe EA, Rolls ET, Kingston B, Megson A, Gunary R. Variety in a meal enhances food-intake in man. *Physiology & Behavior* 1981; **26**(2)**:** 215-221.
9. Rogers PJ, Hill AJ. Breakdown of dietary restraint following mere exposure to food stimuli: Interrelationships between restraint, hunger, salivation, and food intake. *Addictive Behaviors* 1989; **14**(4)**:** 387-397.
10. Ferriday D, Brunstrom JM. 'I just can't help myself': effects of food-cue exposure in overweight and lean individuals. *International Journal of Obesity* 2011; **35**(1)**:** 142-149.
11. Harrold JA, Dovey TM, Blundell JE, Halford JCG. CNS regulation of appetite. *Neuropharmacology* 2012; **63**(1)**:** 3-17.
12. Woods SC. The eating paradox - How we tolerate food. *Psychological Review* 1991; **98**(4)**:** 488-505.
13. Fedoroff I, Polivy J, Peter Herman C. The specificity of restrained versus unrestrained eaters' responses to food cues: general desire to eat, or craving for the cued food? *Appetite* 2003; **41**(1)**:** 7-13.
14. Ferriday D, Brunstrom JM. How does food-cue exposure lead to larger meal sizes? *British Journal of Nutrition* 2008; **100**(6)**:** 1325-1332.
15. Cornell CE, Rodin J, Weingarten H. Stimulus-induced eating when satiated. *Physiology & Behavior* 1989; **45**(4)**:** 695-704.
16. Jansen A, Theunissen N, Slechten K, Nederkoorn C, Boon B, Mulkens S *et al.* Overweight children overeat after exposure to food cues. *Eating Behaviors* 2003; **4**(2)**:** 197-209.
17. Rolls ET, Rolls JH. Olfactory sensory-specific satiety in humans. *Physiology & Behavior* 1997; **61**(3)**:** 461-473.
18. Ramaekers MG, Luning PA, Ruijschop RMAJ, Lakemond CMM, Bult JHF, Gort G *et al.* Aroma exposure time and aroma concentration in relation to food intake. *Submitted for publication* 2013.
19. Ramaekers MG, Luning PA, Ruijschop RMAJ, Lakemond CMM, Van Boekel MAJS. Temporary effect of cream aroma on satiation. *To be submitted for publication* 2013.
20. Ramaekers MG, Boesveldt S, Lakemond CMM, Van Boekel MAJS, Luning PA. Odors: appetizing or satiating? Development of appetite during odour exposure over time. *submitted for publication* 2013.
21. Negoias S, Visschers R, Boelrijk A, Hummel T. New ways to understand aroma perception. *Food Chemistry* 2008; **108**(4)**:** 1247-1254.
22. Small DM, Gerber JC, Mak YE, Hummel T. Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron* 2005; **47**(4)**:** 593-605.
23. Rolls BJ. Sensory-specific satiety. *Nutrition Reviews* 1986; **44**(3)**:** 93-101.

WHAT IS KNOWN ABOUT THE ROLE OF WHOLE GRAINS IN WEIGHT MANAGEMENT?

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Background: Whole-grains have received increased attention for their potential health benefit, including their role in weight regulation. Epidemiological evidence consistently demonstrates that higher whole grain intake, when compared with lower whole grain intake, is associated with lower BMI, body weight, and abdominal adiposity, and smaller waist circumference and weight gain overtime. Evidence from randomized controlled intervention studies is less consistent.
Objective: Assess the scientific evidence, using a descriptive systematic approach, related to the effects of whole grain on weight management.
Methods: A search strategy was developed using the National Library of Medicine Medical Subject Headings (MeSH) key word nomenclature developed for Medline. This strategy was then used to search in Medline and Scopus, dating from 1980 to July 2013. Subsequently two researchers assessed independently the resulting abstracts, using hierarchically targeted selection criteria.
Results: The search process yields are shown in Figure 1. Articles were included if requested by one of the two reviewers, resulting in 66 articles.

Figure 1. Search strategy for assessing and identifying relevant whole grain weight management-related studies.

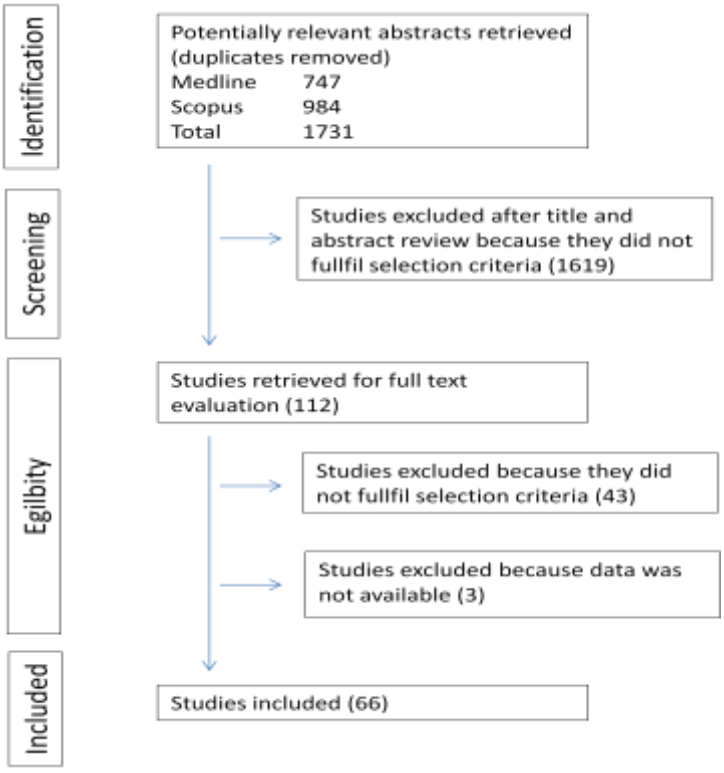

Table 1. Types of studies found.

Type of studies	Number
Epidemiological studies	27
Prospective	9
Cross sectional	18
Intervention studies	32
Randomized controlled trial$_a$	1
Randomized controlled trial$_b$	29

Not specified	2
Meta analyses	2
Reviews	5
Total	**66**

Of the 27 epidemiology studies, 7 prospective and 16 cross-sectional studies showed an inverse association between whole grain consumption and weight management outcomes. In general, these epidemiological studies showed that higher intake of whole grains (a daily intake of ~3 servings) is associated with lower BMI (kg/m^2) in adults; smaller waist circumferences and waist to hip ratio. Harland and Garton (2008), in a systematic review of the evidence, showed a modest reduction in BMI (0.63 kg/m^2) and waist circumference (2.7 cm) with the consumption of 3 servings/d of whole grains. Koh-Banerjee *et al*. (2004) estimated that for every 40 g increase in daily whole grain intake, the 8 year weight gain was lower by 1.1 kg. Recently, in a systematic review of longitudinal studies, Ye *et al*. (2012) reported an inverse association between whole grain and weight gain over time. Ye *et al*. found that compared with never/rare consumers of whole grains, those consuming 48-80 g whole grain/d (3-5 servings/d) had consistently less weight gain during 8-13 y (1.27 vs. 1.64 kg; p=0.001). A few studies have shown that higher intakes of whole grain foods were associated in a dose-dependent manner with lower abdominal fat, and subcutaneous and visceral adipose tissue volume. McKeown *et al*. (2009), reported whole grain intake in the top quintile was 3 compared with 0.5 servings/d in the lowest quintile. In contrast, no significant relationship was observed with refined grain intake.

These prospective studies suggest that weight gain and increases in abdominal adiposity over time are lower in people who consume more whole grains. However, they do not demonstrate causality. It should be noted that the majority of these studies were done in US cohorts and among adults. It is possible that the observed beneficial associations between higher whole grain intake and body measures are a reflection of healthier lifestyles and dietary patterns, with diets high in whole grains being more nutrient-dense and less energy-dense.

Of the 32 intervention studies, 19 were classified as short-term, acute response, satiety studies, 12 were classified as long-term, weight loss studies and 1 was a combination study that included satiety and weight loss. Fourteen out of the 19 satiety studies showed an effect of whole grain intake on subjective satiety measures (hunger, fullness and overall satiety) over the course of a few hours, a day or several days. However, very few of these studies demonstrated any impact of whole grains on energy intake, either at the next meal or over the course of the day. Hence, further research is needed to better understand the satiety effects of whole grains and their impact on weight management. Nine out of 12 weight loss studies showed an effect of whole grain consumption, in the context of hypocaloric diet, on body weight and/or body composition. A whole grain and restricted calorie diet does not appear to lead to greater weight loss compared with a diet of refined grain and caloric restriction, although it does improve overall diet quality and has added beneficial effects on metabolic risk factors. Some evidence suggests that incorporating whole grains into the diet may play a role in altering body composition or metabolism, irrespective of loss of body weight. However, the magnitude of the effect is small and the long-term relevance to overall (metabolic) health is unclear.

Several limitations in existing studies include: the lack of a consistent definition of whole grain foods; the lack of a method to validate the actual amount of whole grain consumed; the reliability of self-reported dietary intake assessment methods¸ epidemiological literature is predominantly from the US, and is mainly from Caucasian, adult cohorts; intervention studies exhibited considerable study duration heterogeneity, and a broad range of types and amounts of whole grain foods were included; substantial variability was also observed for study comparison groups and sample sizes.

Summary: A moderate body of evidence from epidemiological evidence suggests that higher whole grain intake is associated with improvements in body weight measures. Specifically, the evidence consistently demonstrates a higher intake of whole grains is associated with lower body weight, BMI, waist circumference, abdominal adiposity, and weight gain..

The evidence from intervention studies is, in comparison, limited and less consistent. Current evidence fails to clearly demonstrate that whole grain intake can contribute to weight loss independent of hypocaloric diets. The lack of consistency in intervention studies may partly be explained by heterogeneity in study duration, types and amounts of whole grain foods included, population, sample sizes.

Hence future epidemiological and intervention studies are needed to address the limitations observed in the current body of evidence, importantly using a consistent definition of whole grain foods, and the amount of whole grains consumed. Furthermore, studies need to be conducted on diets that potentially include single grain based. Additionally, to build a comprehensive picture of the effects of whole grains on weight management, studies will also need to take into consideration the impact of the food format and matrix.

FOOD INGREDIENTS TO CONTROL BRAIN FROM THE GUT: A SMART APPROACH TO MODULATE ENERGY INTAKE

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According to WHO (2012), over the past three decades, the prevalence of obesity and overweight has increased substantially. It's estimated that a total of 1.7 billion people are overweight while 310 millions are obese with an obesity growing rate in the poor countries three times bigger than 20 years ago. The greatest increases are now in China, Middle East, Southeast Asia and in the Pacific islands (Demerath, 2012). The trend is particularly alarming in children and adolescents as 170 million children (aged < 18 years) are estimated to be overweight (WHO, 2012).
Obesity and overweight pose a major risk for serious diet-related chronic diseases, including type-2 diabetes, cardiovascular disease, hypertension and stroke, and certain types of cancer. Moreover individuals with a body mass index (BMI) greater than or equal to 30 kg/m^2 accrued medical costs approximately 30% higher than those with BMIs lower than 25 kg/m^2 (Withrow and Alter, 2011).
The worldwide and rapid prevalence increase of obesity and its health consequences have turned it into one of the most serious health and societal challenges of the last years.
Obesity has multi-factorial causes that are not entirely clear. Although the susceptibility to obesity has been linked to genetic factors, recent evidence suggests that greater attention must be paid to gene-environment interaction, i.e environment-induced epigenetic changes (Demerath, 2012). Sedentary lifestyles and the wide availability of calorie-rich foods, inducing to overeat, are environmental factors finally inducing to overweight and obesity.
Limiting overeating by regulating food intake processes can be a nutritional strategy to counteract this social problem.
Food intake is driven by the homeostatic and the reward system. The first system controls energy balance by increasing the motivation to eat following endogenous signals of nutrient depletion; the second system drives eating in absence of metabolic needs. The two systems are strictly related by fine mechanisms constituting the gut-brain axis that modulates eating behavior and food intake in the short term as well as weight management in the long-term.
Post-prandial satiety is regulated by a sensory system that communicates between the gut and appetite-regulating centers in the brain, with the hypothalamus being responsible for nutrient and energy sensing and corresponding adjustments in food intake. In fact, in the gut there is a suite of endocrine cells, which synthesize and release various hormones in response to nutrient and energy intake, and it has been demonstrated that these hormones influence appetite in humans and rodents when administered at physiological levels (Janssen and Depoortere, 2013). Moreover some studies have demonstrated the possibility of some foods/ingredients to differentially modulate the GI response of these hormones, modifying accordingly hunger and satiety moods and/or subsequent energy intakes.
There is strong evidence for the role of endogenous cholecystokinin (CCK), glucagon-like-peptide (GLP-1) and peptide YY (PYY) in the regulation of the satiety enhancing effects of macronutrients. Food consumption (mainly protein and fat) stimulates the release of CCK while GLP-1 is released from the gut into the bloodstream in response to intestinal carbohydrate and fat, and PYY in response to intestinal fat and other nutrients (De Graaf et al., 2004; Halford and Harrold, 2008).
Thus GI hormones can be a suitable target for novel foods/ingredients aiming to regulate food intake through satiety modulation (see Table 1).

Table 1: Physiological characteristics of gastro-intestinal peptides modulating food intakes.

Name	Site of production	Effect on appetite	Mechanism	Additional effects
Ghrelin	Stomach	↑ hunger	• Ghrelin R (brain)	Long term effect on energy balance (inversely correlated with body fat)
CCK	Duodenum Jejunum	↑ satiation	• Vagus nerve	• Delays gastric emptying • Stimulates pancreatic enzyme secretion • Stimulates gallbladder contraction • Neurotransmitters
GLP-1	Intestine Brain	↑ satiety	• GLP-1R (brain)	• Incretin (insulin production) • Slows gastric emptying and modulates gastric acid secretion (ileal brake)
Oxynto modulin (OXM)	Intestine Brain	↑ satiety	• GLP-1R (brain) • ↓ ghrelin	• Slows gastric emptying • ↑ *weight loss* • ↑ *energy expenditure*
PYY	Ileum Colon Rectum	↑ satiety	• Y2 R (brain)	• Slows gastric emptying • Slows itestinal transport • Reduces gastric secretions
PP	Pancreas	↑ satiety	• Y2 R (brain) • Vagus nerve	----

Abbreviations: R, receptor; CCK, cholecystokinin; GLP-1. Glucagon-Like-Peptide11; PYY, peptide YY; PP, pancreatic polypeptide; ↑, increase; ↓, decrease.

Among dietary constituents that can modulate appetite and food intake, dietary fibre had documented effects on increasing satiety, thus playing a potential role in the control of energy balance. The evidence about the satiating efficacy of different types of dietary fibre, are not conclusive: results varied with the type of dietary fibre and with administration protocols (i.e. dietary fibre-rich foods vs pure supplements). As regards the types of dietary fiber, the amount of fibre consumed and its viscosity were likely to exert the highest contribution to stimulate satiety (Slavin and Green, 2007). However few studies focused on the role of different dietary fibres on GI hormone response in post-prandial phase.

In a study carried out by our research group it was demonstrated that consumption of 100 g of bread enriched with 3g of barley beta-glucans, reduced hunger and increased fullness and satiety and it reduced energy intakes at subsequent lunch. These findings were in accordance with a reduced ghrelin (orexygenic hormone) and increased PYY (anorexygenic hormone) response compared to a control bread (Vitaglione et al., 2009). When 3 g beta-glucans were consumed in a fruit-flavoured beverage it increased fullness and satiety and reduced energy intakes by increasing the response of PP compared to a control beverage (Barone Lumaga et al., 2012).

These studies showed that behind appetite moods and energy intakes elicited by the same dietary fiber (ingredient), different hormonal responses and physiological mechanisms can contemporary work in the post-prandial phase due to the influence of the whole food/meal including the potentially bioactive ingredient.

On the other hand, taking into account the gut-brain axis and the strict interplay between tonic (generated by the body's constant metabolic need for energy) and episodic (short-term inputs generated by meal intake) signals the hormonal response and the satiating efficacy of a novel food/ingredient may be different depending from nutritional status of subjects. In fact overweight and obese subjects experience reduced taste perception and blunted postprandial hormonal response to food compared to normal weight subjects; thus to get food satisfaction and pleasure overweight/obese people eat more frequently and in amount bigger than the necessary to cope their metabolic need. Hedonic hunger, that is the drive to eat in absence of metabolic need just for

pleasure (gustatory rewarding property of food), associated to a sedentary lifestyle and to a disinhibited eating behavior, in an environment rich of highly palatable foods, may be the major cause of calorie overload in overweight/obese subjects. Some works have been recently published aiming to understand the physiological mechanisms underlying this eating behavior. Data from a preliminary study conducted by Monteleone and co-workers (2012) showed that the consumption of food for pleasure was characterized by increased peripheral levels of both ghrelin and the endocannabinoid 2-arachidonoyl-glycerol (2-AG); these two signals were also positively correlated. Indeed the levels of the other endocannabinoid anandamide (AEA) and of anandamide-related mediators oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), instead, progressively decreased after the ingestion of both highly pleasurable and isoenergetic nonpleasurable food.

These findings (even if preliminary) are of great importance as they suggest the involvement of endocannabinoids, lipid mediators produced in the intestine, in modulating eating behavior in absence of metabolic hunger. Biomarkers of food palatability (liking) that can influence food intakes together with biomarkers of satiety will give the possibility to have new targets for development of new foods/ingredients that can contemporary boost metabolic satiety guaranteeing hedonic aspect of eating.

In conclusion, GI peptides such as ghrelin, PYY, CCK, GLP-1, PP and OXM, can be considered as biomarkers of satiation/satiety and fine targets for developing new foods/ingredients to control energy intakes. A new perspective and challenge for physiologists and food scientists may be in individuating biomarkers of food reward to be used as targets for new foods/ingredients that may increase satiety preserving eating pleasure.

References

- Barone Lumaga, R., Azzali, D., Scalfi, L., Fogliano, V., Vitaglione, P. (2012). Sugar and dietary fibre composition influence, by different hormonal response, the satiating capacity of a fruit-based and a β-glucan-enriched beverage. Food Funct. 2012, 3(1):67-75.
- De Graaf, C., Blom, W. A. M., Smeets, P. A. M., Stafleu, A., & Hendriks, H. F. J. (2004). Biomarkers of satiation and satiety. American Journal of Clinical Nutrition, 79, 946–961.
- Demerath, E.W. (2012). The genetics of obesity in transition. Coll Antropol, 36(4), 1161-8.
- Halford, J.C., Harrold, J.A. (2008). Neuropharmacology of human appetite expression. Dev Disabil Res Rev, 14: 158 – 164
- Janssen, S., Depoortere, I. (2013). Nutrient sensing in the gut: new roads to therapeutics? Trends in Endocrinology and Metabolism, 24(2):92-100
- Monteleone, P., Piscitelli, F., Scognamiglio, P., Monteleone, A.M., Canestrelli, B., Di Marzo. V., Maj, M. (2012). Hedonic eating is associated with increased peripheral levels of ghrelin and the endocannabinoid 2-arachidonoyl-glycerol in healthy humans: a pilot study. J Clin Endocrinol Metab, 97(6), E917-24.
- Slavin, J., & Green, H. (2007). Dietary fibre and satiety. Nutrition Bulletin, 32, 32–42.
- Vitaglione, P., Barone Lumaga, R., Stanzione, A., Scalfi, L., Fogliano, V. (2009). β-Glucan-enriched bread reduces energy intake and modifies plasma ghrelin and peptide YY concentrations in the short term. Appetite, 53, 338–344.
- Withrow, D., Alter, D.A. (2011). The economic burden of obesity worldwide: A systematic review of the direct costs of obesity. Obesity Reviews, 12, 131-141.

METABOLOMICS APPROACH FOR UNDERSTANDING THE VIABILITY AND ACTIVITY OF PROBIOTICS IN SET-YOGHURT: DETERMINATION OF VOLATILE AND NON-VOLATILE POLAR METABOLITE PROFILE

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Objective: This study aimed to apply a metabolomics approach for investigating the activity of two dairy-applied probiotics, *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB12, and a probiotic candidate, *Lactobacillus plantarum* WCFS1, in co-culture with traditional yoghurt strains (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) during set-yoghurt fermentation and storage.
Methods: Bacterial population dynamics, pH and titratable acidity were monitored during set-yoghurt fermentation (42 °C for 4 h) and throughout refrigerated storage for four weeks. The production of yoghurt aroma volatiles and dynamic changes in non-volatile polar metabolites were investigated. Headspace SPME-GC/MS and ^{1}H-NMR spectroscopy were used as non-targeted analytical tools to monitor the changes in milk compositions associated with microbial metabolism and their interaction.
Results: Yoghurt bacteria showed active growth during fermentation and remained quite stable throughout storage while probiotic bacteria expressed difficulty to develop in milk with strain-dependent viability patterns. The GC/MS and ^{1}H-NMR-derived data allowed observing the changes of volatile and non-volatile polar metabolite profiles in yoghurt during fermentation and storage. A number of 35 volatiles and 40 non-volatile polar metabolites were presumptively identified. Furthermore, multivariate statistical analysis allowed discriminating yoghurts with different types of probiotics incorporated according to their volatile and non-volatile polar metabolite profiles.
Conclusions: This study demonstrates an application of complementary metabolomics-based techniques for global characterization of biochemical changes associated with probiotic strains and their activity during set-yoghurt fermentation and storage. The advantages are (1) minimal pre-treatment required and (2) simultaneous measurement of overall metabolites present in yoghurt matrix.

IMPACT OF A COMPLEX FOOD MICROBIOTA ON ENERGY METABOLISM IN THE MODEL ORGANISM CAENORHABDITIS ELEGANS

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Objective: The nematode *C.elegans* is widely used as a model system for research on aging, development and host-pathogen interaction. Little is currently known about the mechanisms underlying the beneficial effects exerted by many foodborne microbes. We took advantage of *C.elegans* to evaluate the effects of the microbiota derived from a traditional Italian dairy product on longevity, larval development, fertility, lipid accumulation and gene expression.
Methods: Lactic acid bacteria (LAB) derived from Mozzarella di Bufala Campana cheese were grown in MRS medium and used to feed worms. Animals were analyzed in terms of life span, larval development, brood size, fat storage. Expression profile of genes involved in lipid metabolism was carried out by RT-qPCR. The composition of foodborne microbial consortium was evaluated before and after the colonization of worms' gut by 16S rDNA analysis and strain typing.
Results: LAB supplementation decreased lifespan in the nematodes and impaired larval development compared to the animals fed with conventional *E.coli* nutrient source. Moreover, massive accumulation of lipid droplets was revealed by Nile Red staining. Alteration of the expression of genes involved in lipid metabolism was also observed. Characterization of food microbiota revealed the presence of *Lactobacillus delbrueckii*, *L. fermentum* and *Leuconostoc lactis* as the main species, displaying different effects as well as worms' gut colonization capacity.
Conclusions: We demonstrated the involvement of genes related to lipid homeostasis as a host response to supplementation with foodborne LAB. Since several pathways are evolutionary conserved in *C. elegans*, our results highlight the nematode as a valuable model system to shed light on the mechanisms underlying the effects of a complex microbial consortium on host energy metabolism.

MELANZANA ROSSA DI ROTONDA: PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITY TOWARDS ITS VALIDATION FOR NUTRACEUTICALS MARKET

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Variability is the main character of life and is highly expressed in plants. Among the human mission, we must consider the capacity of expanding natural variability to the possible limits. This peculiar attitude is evident in the agriculture cultivars. Selection and development of these cultivars is the result of talent and application of generations of farmers, which have domesticated wild types, generating, with patient work, completely different productive plants. This enormous work and indispensable treasure is now in danger. Concentration of production and market control on food acts against the survival of many cultivars, despite their potential economic value. This potentiality could reach new interest and possibility in the recent nutraceuticals and botanicals market. However, these products need urgently scientific validation and technology.
We report the case of an ecotype of *Solanum aethiopicum* L., cultivated in Basilicata region, where it is known as "melanzana rossa di Rotonda". It received the DOP character, being essentially present in this part of Italy. It belongs to the aubergine category, but differs for many characters, like the shape and colour resembling those of tomato. The taste is practically unique and it is enhanced by the cooking in different ways.
We report the results of the composition analysis of this species, as whole and of different parts, mainly based on HPTLC devices and showing a high content in polyphenols. This account is in accordance with the pharmacological tests, showing a strong antioxidant activity. Data were compared with the aubergines cultivars actually utilized in the Italian market, i.e. *Solanum melongena* var. bianca di Senise and *S. melongena* var. purpose, utilizing certified ALSIA products. We were able to show the different composition of melanzana rossa, in particular the content in chlorogenic acid, using the HPTLC analysis and comparing the fingerprints. The same method was also experimented for quantitative determinations. A remarkable antioxidant activity was detected, in different amounts for the different parts of the fruits.
These results encourage further study in order to use "melanzana rossa di Rotonda" for developing nutraceuticals or functional foods.

STRUCTURE-FUNCTION RELATIONSHIP OF ANTIOXIDANT PEPTIDES ISOLATED FROM WHEAT SPROUTS. FUNCTIONAL BINDING WITH PHOSPHOLIPIDS.

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Wheat sprouts extract was studied to characterize peptide and phospholipid fractions. The aim of this research was prompted by evidences we reported that the peptide molecules present in the extract are responsible of specific biological activities including the control of cell proliferation in cancer cells *in vitro*. Recently we observed that the strong antioxidant activity exerted by wheat sprout extract is for 70% due to antioxidant peptides. Consequently the structure of the peptides extracted from wheat sprouts and fractionated by HPLC was analyzed by mass spectrometry and mass spectrometry of second order (MS/MS). The mass/mass spectrometry results were analysed by a recently reported (J.Pep.Sci. 2005, 11: 225-234) automatic combinatorial method that carries out the computation of all amino acid sequences compatible with a given molecular ion. The possible sequences are automatically obtained by considering all the sequences compatible with the molecular weights of the peptide compound (MH^+) and of its potential breakdown products present in the MS/MS spectrum. The designed sequences were scanned to recognize potential antioxidant sequences. The evidence of peptides with antioxidant activity is quite recent, but the number of papers demonstrating a strong antioxidant activity by peptides is quickly increasing. In this last decade more than 800 papers have been reported in the international literature. From the analysis of reported data is difficult to recognize a peptide sequence specific towards an antioxidant function. It is probable that the specific antioxidant activity is linked both to sequence and structure of the peptide. We can see that the antioxidant peptides some time do not contain antioxidant amino acids (such as cystein, methionin, taurine); in this case the presence of aromatic amino acids appears necessary.

Some we obtained results are reported :

MH^+ : 541.0 ; sequence : AcPhe-Cys-Ala-Gly-CysNH_2

MH^+ : 663.5 ; sequence : AcAsn-Val-Ala-Leu-Cys-CysNH_2

MH^+ : 785.5 ; sequence : NH_2Tyr-Met-Thr-Val-Val-Ala-CysNH_2

It is noteworthy that the C-terminal of many sequences is necessarily constituted by amidated cysteine. Moreover the results show another peculiarity : the presence of domains with two residues of cysteine, some time together with aromatic aminoacids. A question associated to biologically active peptides is their poor bioavailability. It has been reported that the complex with phospholipids can significantly increase this bioavailability. In this context the phospholipids molecular species extracted from wheat sprouts were characterized. The peptide sequences were scanned also to recognize binding sites for phospholipids following the vector machine prediction method. The results show several sequences suitable for the binding with phospholipids, according to previously reported results (J.Pep.Sci 2011, 17 : 741-760). Peptide sequences containing both potential antioxidant domains and interaction sites for phospholipids are selected. Some of the above reported sequences are synthesized. The synthetic peptides shows a strong antioxidant activity "in vitro" evaluated as radical scavenging of the superoxide ion and capacity to inhibit the production of thiobarbituric acid reactive substances (TBARS) (J. Clin. Pathol. 2001, 54: 356-361).

FERMENTED MILK DAIRY PRODUCT CONTAINING BIFIDOBACTERIUM LACTIS CNCM I-2494 ENHANCES GUT IMMUNE BARRIER FUNCTION VIA A REG-TH17 IMMUNE PATHWAY

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Objective: Irritable Bowel Syndrome (IBS) is characterized by visceral hypersensitivity, increased intestinal permeability and gut immune cells activation. A fermented milk (FM) product containing *Bifidobacterium lactis* CNCM-I2494 suppresses visceral hypersensitivity by normalizing intestinal epithelial barrier dysfunction induced by acute stress in rats. Recent outcomes showed that balance between Tregs and Th17 cells is critical for maintaining gut barrier integrity. Our aim was to investigate the impact of FM product on gut immune barrier in basal and acute stress conditions.
Methods: Wistar rats were orally given (15 days) FM product or saline. At day 15, paracellular and transcellular permeability were measured in Ussing chamber on ileal Peyer's patches, ileum and colonic segments in basal and after 2h of acute stress. IL10, IL12, TGF-β and IL-17A levels were measured in ileal Peyer's patches and $CD4^+CD25^+Foxp3^+$ cells were quantified by flow cytometry both in ileal Peyer's patches and in mesenteric lymph nodes (MLN).
Results: FM product counteracted the increase of para- and trans-cellular permeability induced by acute stress at all the gut segments tested. In Peyer's patches, the ratio IL10/IL12 reduced by stress was restored to control values after FM consumption. In basal condition, FM product increased IL10, IL-17A and TGF-β levels and significantly decreased Treg cells ($CD4^+CD25^+Foxp3^+$) population in Peyer's patches without affecting their population in MLN.
Conclusions: A chronic consumption of a marketed FM product containing *Bifidobacterium lactis* CNCM-I2494 seems to up regulate gut local reg-TH17 immune pathway contributing in turn on restoring epithelial gut permeability integrity altered by acute stress.

POLYDEXTROSE, A DIETARY FIBER, INCREASES POSTPRANDIAL GLP-1 IN OBESE SUBJECTS

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Objective: Dietary fibers are known to be associated with enhanced satiety. However, the mechanism of the different dietary fibers contributing to the satiety-related gastrointestinal (GI) peptide release, especially in obese population, is still poorly understood. In this study, we evaluated the effects of polydextrose (PDX), a soluble fiber, on postprandial satiety-related GI peptides in overweight, non-diabetic males and females.
Methods: 18 volunteers (means: 42 y; BMI 33.6 kg/m^2) consumed twice daily a standard hamburger meal with and without a supplementation of PDX (Litesse® 15g) in a double-blinded, crossover and randomized design. For GI peptide (Glucagon-like peptide-1, GLP-1; Peptide tyrosine tyrosine, PYY; cholecystokinin, CCK; and ghrelin) measurements, venous blood samples were drawn before the consumption of the test meal, and at 30, 60, 120, 240 and 360 min post meal. The GI peptide response curves were modeled using mixed-effects linear models.
Results: PDX supplementation gave higher values for the GLP-1 response than the placebo treatment ($p<0.01$). PDX had also a decreasing trend on ghrelin ($p=0.0575$) and PYY ($p=0.0788$) responses. There were no differences in postprandial CCK between the placebo and PDX treatments.
Conclusions: The intestine plays a key role in postprandial metabolism and satiety through various peptides secreted in response to food. In this study, PDX induced enhanced GLP-1 response after a high-fat meal in obese subjects. GLP-1 secretion is known to decrease gastric emptying and contribute to the decreased food intake. Thus, as a dietary fiber, PDX may offer additional means to improve postprandial metabolism in obese subjects.

BERBERINE, INTERACTING WITH THE P2X7 PURINERGIC RECEPTOR, AMELIORATES EXPERIMENTAL LIVER INJURY

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Objective: The alkaloid berberine (BRB) is commonly used as a supplement in the US, and possesses a wide range of pharmacological effects, including protection against liver injury. In this study, we analyzed the effects of BRB in different murine models of liver injury, and investigated the underlying mechanism of action.
Methods: BRB was tested in steatohepatitis induced by a methionine and choline deficient (MCD) diet and in acute acetaminophen (APAP) intoxication. The mechanism of action of BRB was further investigated in LPS-stimulated murine macrophages (RAW 264.7) in vitro. Activation of the P2X7 receptor was assessed by measurement of calcium transients in response to benzyl-ATP.
Results: BRB markedly suppressed ALT elevation and necroinflammation in MCD-fed mice. In addition, intrahepatic gene expression of inflammatory and profibrogenic markers were significantly downregulated in MCD-fed, BRB-treated mice.
Feeding a MCD diet produced activation of the inflammasome pathway, and these effects were limited by BRB. In the model of APAP-induced hepatotoxicity, BRB also reduced ALT elevation, upregulation of NALP3 inflammasome components, and mortality. Inflammasome activation in LPS-stimulated RAW 264.7 was decreased by BRB. BRB did not interfere with the activation of NF-κB pathway. However, in RAW 264.7 cells, BRB limited the elevation of intracellular calcium caused by a selective ligand of P2X7, a purinergic receptor involved in inflammasome assembly.
Conclusions: Administration of BRB ameliorates necro-inflammation and cytokine expression in experimental steatohepatitis. We demonstrate for the first time that BRB modulates the inflammasome pathway, interfering with activation of the purinergic receptor, P2X7.

PROBIOTICS AND ASTHMA

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A possible, significant beneficial effect of probiotics has been suggested in subjects with bronchial asthma. Various scientific studies using a range of probiotics have been performed, but the results have not been unequivocal. In a mouse model of bronchial asthma, it has been demonstrated that the oral administration of probiotics, before and during sensitisation with allergens and airway challenge, results in a suppression of all characteristics of the asthmatic phenotype, including the production of specific IgE, inflammation of the airways and development of airway hyperresponsiveness (AHR). Furthermore, this effect is associated with the suppression of the allergen-induced Th2 immune response, along with induction of TGF-β in the intestines.

Experimental studies have demonstrated the ability of *L. casei* to increase the activity of the NK cells in the lungs and to increase the production of IFNγ and TNF10 by nasal lymphocytes. This probiotic has been the subject of clinical trials conducted by Giovannini et al., which involved the administration of fermented milk containing *Lactobacillus casei* to 187 children aged between two and five years in order to study the effect of this probiotic on the number of episodes of asthma and allergic rhinitis. After twelve months of study, the investigators did not find any statistically significant differences between the treatment and control groups of asthmatic children. However, the number of episodes of rhinitis was lower in the group taking the probiotic. This led to the conclusion that treatment with *Lactobacillus casei* may influence the number of episodes of allergic rhinitis but not asthma.

Wheeler et al. (98) conducted a clinical study to evaluate the possible beneficial effects of treatment with yoghurt containing *L. acidophilus* in 15 adult subjects (mean age 33 years) with moderate asthma. In this case, also, there were no obvious significant clinical effects provided by this treatment on symptoms, respiratory function tests or immunological parameters (eosinophils, total IgE or production of IL-2, IL-4 and IFNγ by lymphocytes).

On the other hand, it has recently been demonstrated, in a mouse model, that the oral administration of *Lactobacillus reuteri* is able to alleviate the asthmatic response induced by allergens, reducing eosinophilia and the production of proinflammatory cytokines in bronchoalveolar lavage (BAL) fluid and improving bronchial hyperreactivity measured using a metacholine test. The mechanism through which *L. reuteri* is able to achieve these results seems to be tied to its ability to increase the expression of CD4+ and CD25+ T-cells, which are able to reduce inflammation and bronchial hyperreactivity, reducing the levels of TNF and IL5 in the BAL fluid in animal models.

Another study conducted in school-aged children (age 6-12 years) with asthma and allergic rhinitis using *L. gasseri* for two months found an improvement in lung function and asthmatic symptoms measured on the basis of scores compared to placebo. In addition, in subjects treated with *L. gasseri*, there was a significant reduction in TNF-α, IFN-γ, IL-12 and IL-13.

In our recent study, we found that *L. reuteri* is effective in reducing asthmatic inflammation in allergic children. These subjects, aged between six and 14 years, were suffering from persistent mild/moderate asthma, defined according to the Global Initiative for Asthma (GINA) Guidelines, and were exhibiting an allergy to house dust mites (*Dermatofagoides farinae* and *Dermatofagoides pteronissinus* ++/+++). Through measurement of nitric oxide in exhaled air and FEV1 using spirometry, we were able to identify a reduction in bronchial inflammation and an improvement in respiratory performance in the treatment group.

Unfortunately, the scientific literature in the field of application of probiotics in the treatment of individuals with allergic asthma is still somewhat scanty, and, therefore, we do not currently have

sufficient data on experiments in mouse models of asthma that have shown the effectiveness of probiotic treatment to justify the use of probiotics in clinical practice in individuals with asthma.

Bibliography

Forsythe P, Inman MD, Bienenstock J. Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. *Am J Respir Crit Care Med* 2007 Mar 15; 175 (6): 561-9.

Giovannini M, et al. A randomized prospective double blind controlled trial on effects of long-term consumption of fermented milk containing *Lactobacillus casei* in pre-school children with allergic asthma and/or rhinitis. *Pediatr Res* 2007, 62 (2): 215-20.

Rose MA, Stieglitz F, Köksal A, Schubert R, Schulze J and Zielen S. Efficacy of probiotic *Lactobacillus GG* on allergic sensitization and asthma in infants at risk. *Clinical & Experimental Allergy* 2010; 40, 1398-1405.

Yue-Sheng Chen, Ren-Long Jan, Yen-Lin Lin, Hsin-Hung Chen and Jiu-Yao Wang. Randomized Placebo-Controlled Trial of Lactobacillus on Asthmatic Children With Allergic Rhinitis. *Pediatric Pulmonology* 2010; 45: 1111-1120.

Miraglia Del Giudice M, Maiello N, Decimo F, Fusco N, D'Agostino B, Sullo N, Capasso M, Salpietro V, Gitto E, Ciprandi G, Marseglia GL, Perrone L. Airways allergic inflammation and *L. reuterii* treatment in asthmatic children. *J Biol Regul Homeost Agents* 2012 Jan-Mar; 26 (1 Suppl): S35-40.

PEROXISOME-PROLIFERATOR-ACTIVATED RECEPTOR GAMMA IN THE COLON: INFLAMMATION AND INNATE ANTIMICROBIAL IMMUNITY

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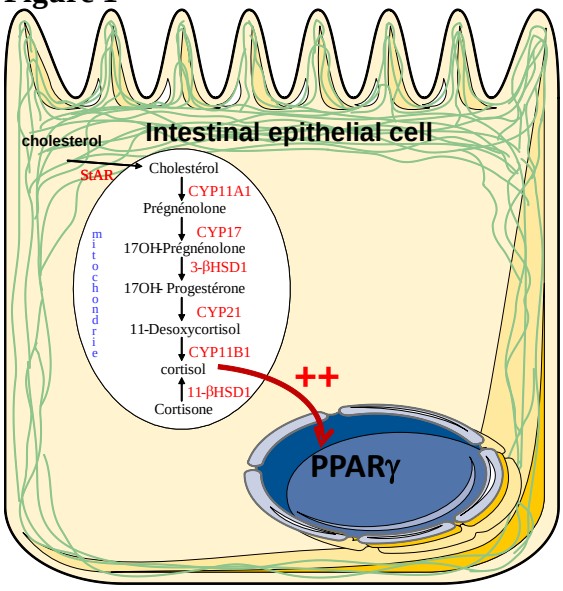
Introduction: Peroxisome proliferator–activated receptor gamma (PPARγ) is a nuclear receptor, originally described in adipose tissue, that controls the expression of a large number of regulatory genes in lipid metabolism and insulin sensitization. Well known by endocrinologists, thiazolidinediones (TZDs) are classical PPARγ synthetic agonists which were currently used as insulin-sensitizing agents in the treatment of type 2 diabetes. While the clinical benefits of TZDs in treating metabolic disorders have been clearly demonstrated, new studies performed in animal models of colitis and in patients with ulcerative colitis have also revealed the key roles of PPARγ activation in the regulation of inflammation and immune response, notably in the colon through epithelial cells.

Mechanisms of action and regulation: During inflammation, PPAR acts directly to negatively regulate gene expression of proinflammatory genes in a ligand-dependent manner by antagonizing the activities of other transcription factors such as members of the NF-κB and AP-1 families. A major mechanism that underlies the ability of PPARs to interfere with the activities of these transcription factors has been termed transrepression. PPARγ acts by inhibiting signal-dependent transcription factors that mediate inflammatory programs of gene activation. Several mechanisms underlying negative regulation of gene repression by PPARs have been described in recent studies using siRNA, microarray analysis, and specific knockout mice. Also consistent with the role of PPARγ for intestinal homeostasis, there is a cross-talk between intestinal microbial flora and PPARγ which is involved in intestinal anti-microbial barrier function through induction of β-defensin expression.

The regulation of PPARγ in colon epithelial cells remains poorly known. Intestinal flora, probiotics or bacterial products, such as LPS or butyrate are able to increase PPARγ expression in epithelial colonic cells. Based on in vitro and in vivo experiments, recent data also suggest that glucocorticoids and particulalrly endogenous glucocorticoids produced by colonic are potent inducers of PPARγ expression in the gut (Figure 1).

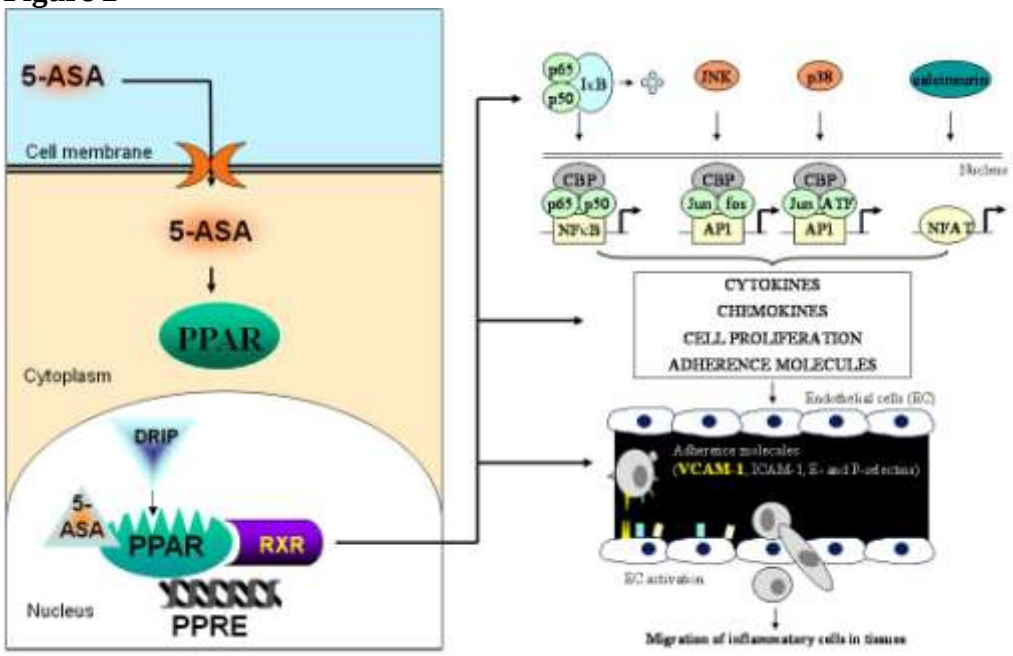
Figure 1



Intestinal epithelial cells represent a significant source of extra-adrenal glucocorticoids having the ability to induce locally PPARγ expression

Therapeutic development: Due to safety issues concerning particularly the greater risk of myocardial infarction, use of TZDs has been severely limited for the treatment of type 2 diabetes and/or inflammatory diseases, justifying the development of a new family of PPARγ agonists with major transrepressive effects and without toxicity. By the demonstration that the anti-inflammatory effects of 5-aminosalicylic acid (5-ASA) in patients with ulcerative colitis were mediated by PPARγ activation (Figure 2), several molecules having 5-ASA similarities have been developed and screened leading to the selection of a aminophenyl-alpha-methoxy-propionic acids named GED-0507-34-Levo (GED). This compound activating PPARγ has 100-to 150-fold higher anti-inflammatory activity than 5-ASA. This new PPAR modulator is giving promising results both in vitro and in vivo, without toxicity and is currently evaluated in a phase 2 clinical trial.

Figure 2



ADHESION AND MUCOSAL EFFECT OF LACTOBACILLUS RHAMNOSUS GG IN NORMAL AND INFLAMED COLONIC MUCOSA EVALUATED BY AN EX-VIVO ORGAN CULTURE TECHNIQUE.

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Background: Probiotic bacteria have shown to promote beneficial effect in several pathologic conditions, but their mechanisms of action are still to unravel. In particular, probiotic bacteria have been recently proposed in ulcerative colitis (UC) patients. To exert their beneficial effect, adhesion of the bacteria to colonic mucosa and cytokines modulation appear to be important factors, but specific studies are scanty. Lactobacillus rhamnosus GG (LGG) represents one of the probiotic species more widely investigated and commercially used. We developed a simple ex-vivo organ culture technique and we evaluated adhesion of LGG to colonic mucosa and the effect on mucosal TNF expression.

Aim: To investigate the adhesion and the effect on cytokines expression of LGG in proximal and distal colonic mucosa of ulcerative colitis (UC) patients and normal controls, by means of a simple ex-vivo organ culture technique.

Methods: endoscopic biopsies from proximal and distal colon were collected from patients with UC (n=10) and normal controls (n=12). To evaluate LGG adhesion, bioptic specimens were washed and incubated for 2 h at 37° with and without LGG (6 x 10^6 CFU/200µL each reaction), and then total DNA was extracted and mucosal adherent LGG was quantified by real-time PCR with specific primers. To evaluate the mucosal effect of LGG, bioptic specimens were incubated for 24h at 37° with and without LGG conditioned media, and then total RNA was extracted and TNF expression quantified by RT-PCR. Adherent LGG and cytokines levels were compared in colon of UC patients and normal control.

Results: In the normal colon, LGG showed a better adhesion in distal compared with proximal speciments (23.5±6.9 vs. 9.1±2.5, relative increment 2.6, $p<0.05$). In UC patients, LGG adhesion was not impaired by active inflammation (3.2±1.3 vs. 3.5±0.9 in inflamed vs. not inflamed mucosa, p=ns). In normal colon, preliminary investigation showed that LGG conditioned media suppressed the TNF expression in rectum-sigma specimens.

Conclusion: In an ex-vivo organ culture model, LGG showed better adhesion to distal compared with proximal colonic specimens, and displayed comparable adherence both in inflamed and not inflamed mucosa. Ex vivo organ culture may represent a useful and simple method to evaluate probiotic bacteria characteristics and to lead to a more focused utilization in clinical setting.

THE COMMENSAL BACTERIUM FAECALIBACTERIUM PRAUSNITZII A2-165 IS PROTECTIVE IN MURINE CHRONIC COLITIS MODELS

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Objective: A decrease in the abundance and biodiversity of the commensal bacterium *Faecalibacterium prausnitzii* has been consistently observed in the gut microbiota of Crohn's disease patients[1]. In previous studies, both *F. prausnitzii* and its supernatant (SN) have shown protective effects in acute TriNitro-Benzene Sulfonic acid (TNBS)-colitis model[2]. Here, we tested for the first time the effects of both *F. prausnitzii* and its SN in murine chronic colitis models.
Methods: Colitis was induced in male C57BL/6 mice by intrarectal administration of DiNitroBenzene-Sulfonic acid (DNBS). After a 4-days recovery period from acute colitis, mice were gavaged with either *F. prausnitzii* A2-165 or its SN for 7 days. In additional mice, a 10 days-recovery period was followed by the probiotic therapy for 10 days. Three days before sacrifice, colitis was reactivated by a lower dose of DNBS. The severity of colitis was assessed by weight loss, macroscopic and microscopic scores, myeloperoxidase (MPO) activity and serum and colon cytokines levels.
Results: Intragastric-administration of either *F. prausnitzii* or its SN led to a significant decrease in colitis severity. In addition, down-regulation of tissue inflammatory markers (MPO and pro-inflammatory cytokines) was correlated with the reduction in colitis severity.
Conclusions: We have shown for the first time protective effects of both *F. prausnitzii* and its SN during recovery from acute colitis and colitis reactivation. These results reinforce the potential of *F. prausnitzii* as an anti-inflammatory bacterium with therapeutic potential in Inflammatory Bowel Diseases.

[1]Miquel *et al*. *Current opinion Microbiol.* 2013
[2]Sokol *et al*. *PNAS USA.* 2008

ROLE OF HUMAN ENTEROGLIAL CELLS IN MEDIATING PATHOGENIC AND PROBIOTIC BACTERIA EFFECTS ON INTESTINAL EPITHELIAL CELLS

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Objective: Human enteroglial cells (EGC) recognize healthy and harmful bacteria. The effects of mediators released by EGC on the surrounding cells have not been explored yet. We aimed to evaluate the effects of soluble factors released by EGC, upon probiotic and pathogenic bacteria stimulation, on intestinal epithelial cells (IEC) viability and differentiation.
Methods: EGC were exposed to probiotic (Lactobacillus Paracasei F19, 10^8 bacteria/mL) and pathogenic (Enteroinvasive E. Coli, 10^8 bacteria/mL) bacteria. After 24h, conditioned media (CM) from these cultures was used to challange CaCo2 cells, in presence or absence of an anti-RAGE antibody. CaCo2 viability and differentiation, after 24 h, were studied by MTT vitality test and lactase/sucrase activity test, respectively. CaCo2 cells with medium alone: control; data expression: mean±SD; n = 3.
Results: CM from unstimulated EGC or stimulated with both probiotics and pathogens did not affect CaCo2 viability. CM from unstimulated and from probiotic-stimulated EGC, significantly increased lactase (+1.15±0.17 and +1.29±0.19 vs control; $p<0.05$) and sucrase activity (+5.25±1.12 and +4.89±0.95 vs control; $p<0.05$). CM from pathogen-stimulated EGC significantly decreased lactase and sucrase activity (2.85±0.23 and 3.15±0.79 fold decrease vs control; $p<0.05$). When cells were pre-treated with anti-RAGE, the increase in lactase and sucrase activity induced by probiotics was abolished.
Conclusions: EGC medium, conditioned with pathogens and probiotics, differently modulate IEC differentiation without exerting cytotoxic effects, with a mechanism involving RAGE. Our results provide further evidence on the role of EGC in mediating the host-bacteria interaction and highlight the major contribution of EGC in controlling epithelial barrier function.

FAECALIBACTERIUM PRAUSNITZII EXHIBITS ANTINOCICEPTIVE EFFECT IN A NON-INFLAMMATORY VISCERAL PAIN MODEL

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Objective: Visceral pain is a diffuse and stabbing sensation which may be associated with functional gastrointestinal disorders such as Irritable Bowel Syndrome (IBS) or Inflammatory Bowel Disease (IBD). This complaint is a crucial feature because of its significant impact on patients' quality of life and lack of efficient therapies. Moreover, IBS and IBD are characterized by a dysbiosis suggesting a long term impact of the intestinal microbiota on inflammation, but also on colonic hypersensitivity (CHS). Interestingly, diminished abundance of *Faecalibacterium prausnitzii*, an anti-inflammatory commensal bacterium, has been reported. The aim of this study was to determine if *F. prausnitzii* could have an impact on CHS, independently to its anti-inflammatory properties.
Methods: We assessed the effect of *F. prausnitzii* in Neonatal Maternal Separation (NMS) CHS mouse model. Adult mice were orally treated, nine days, with *F. prausnitzii* A2-165 strain (10^9 CFU/day). Visceral pain was measured by electromyographic abdominal contractions induced by colorectal distension. The absence of inflammation in this model was confirmed and intestinal permeability was evaluated.
Results: NMS treatment induced an increased visceromotor response (VMR) in the absence of alteration in gut wall macroscopic integrity or colonic mucosa inflammation status. However, a slight increase of colonic permeability has been measured. *F. prausnitzii* treatment significantly decreased VMR in NMS model, without effect in control mice.
Conclusions: These results suggested a protective role of *F. prausnitzii* on CHS in a non-inflammatory model. This study could optimize the potential future use of this bacterium as a probiotic to treat abdominal pains of patients.

BACTERIAL SPORES AS PROBIOTICS: MODE OF ACTION

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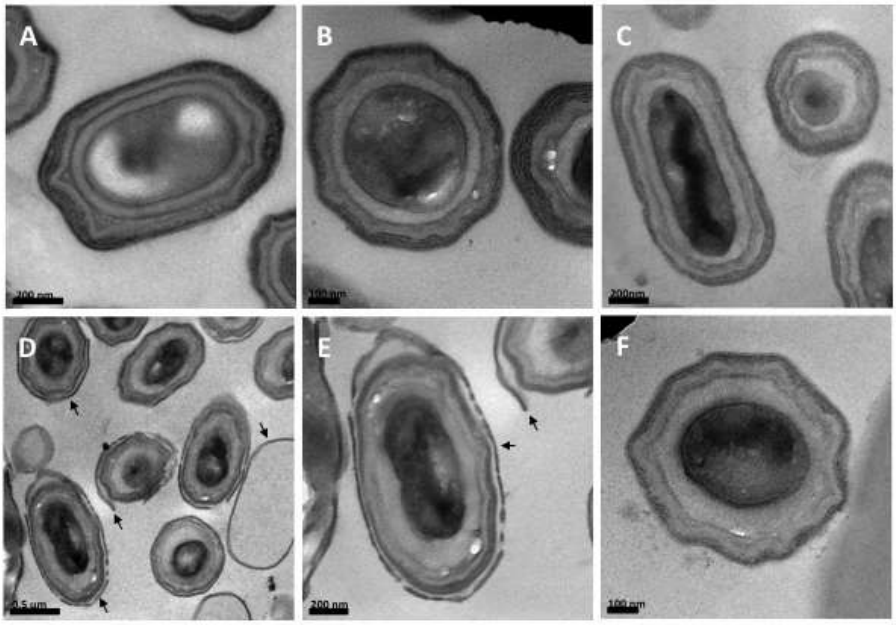
Members of the spore-forming genus *Bacillus* have long been used as probiotic supplements for human and animal use. Species such as *Bacillus clausii*, *Bacillus coagulans*, *Bacillus subtilis* and *Bacillus licheniformis* are found in a number of products and some, such as *B. clausii* that are produced as GMP products with proven efficacy in the prevention of gastrointestinal illness.



The compelling aspect of spore forming bacteria is the spore which is produced at the end of the life cycle of this organism and enables a product to be produced that is both heat and desiccation resistant. This enables a product to be produced that can be stored indefinitely at ambient temperature and survive passage through the gastro-intestinal tract. This in turn simplifies storage and distribution of these products and enables spores to be used in ways that are not possible with the more common products such as the lactobacilli etc. For example, spores can be stored in aqueous solution, incorporated in foods included baked products and used in animal feed products where the formulation process requires extensive incubation at high temperatures.

Despite these attributes our understanding of spores and how they exert a probiotic effect is less apparent. What we do know and which will be covered in this presentation is as follows:

Innate Immunity: Spores are able to interact with Toll-like Receptors and induce an innate immune response leading to the production of IFN-γ and other cytokines. In animal studies as few as two nasal doses of *B. subtilis* spores can provide 100% protection to influenza (H5N2). Interestingly, this protection can be achieved using inactivated or killed spores.

Clostridium difficile: *B. subtilis* spores have been used to evaluate protection against *C. difficile* infection in animal models. Our data shows that pre-dosing and concurrent dosing of mice infected with *C. difficile* provides varied levels of protection and illuminates the potential of using spores for treatment of this important nosocomial infection.

PHAGEBIOTICS: BACTERIOPHAGES AS NEW CLASS OF PROBIOTICS FOR MANAGING GUT MICROFLORA

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Probiotics offer the promise of health benefits by altering the commensal gut microbiota, and they continue to gain in popularity. However, one approach for a "probiotic intervention" that has not received much prior attention is to use lytic bacteriophages (or phages) to target and remove pathogenic bacteria from the gastrointestinal (GI) tract before they can cause disease. Lytic phages are bacterial viruses that attach to and kill their bacterial hosts by lysing them during their internal replication cycle. Bacteriophages are arguably the oldest (ca. 3.5 billion-years-old) and most ubiquitous organisms (10^{30}-10^{32} phage particles) on Earth, and they have remarkable bactericidal activity against their specific bacterial hosts; i.e., phages attack their targeted bacterial hosts but cannot infect human or other eukaryotic cells. The approach of using lytic phages as part of a probiotic diet is similar to that used for bacteria-based probiotics, which are administered during a period of time and act by favorably conditioning the gut microflora. The key difference between bacteria-based probiotics and lytic phage-based probiotics (designated "phage-biotics") is that the former introduce nonpathogenic bacteria into the GI tract (in order to interfere with the ability of pathogenic bacteria to colonize the GI tract and cross the intestinal mucosa); whereas, the latter use lytic phage to kill specific pathogenic bacteria in the GI tract. The approach of using orally administered lytic phages to improve / fine-tune the GI tract's microbiota (by specifically killing specific bacteria in the gut, without disturbing the normal microflora) is protected by two patents (#7,459,272 and 8,003,323) granted to Intralytix.

We began exploring the feasibility of lytic phage-based probiotics by using phages with potent lytic activity against *Shigella* spp. *Shigella* are some of the most common and deadly bacterial pathogens in the world, and they are responsible for approx. 165 million worldwide cases of shigellosis annually, ca. 163 million of which occur in developing countries and cause 1.1 million deaths (of which an estimated 650,000 are among children <5 years of age). The problem is of somewhat less magnitude in developed countries; however, even there its impact is very significant, with an estimated 1.5 million cases of shigellosis occurring annually in industrialized countries. Thus, there is an urgent and real need for new approaches that can help to reduce the incidence and severity of shigellosis.

We have identified a potent phage cocktail (tentatively designated ShigActive™) consisting of 5 lytic phages possessing excellent lytic activity against a collection of 62 genetically-diverse strains of *Shigella* spp. isolated from clinical cases of shigellosis in various countries (including Mali, Chile, Pakistan, Peru, and Japan). The cocktail kills 92% of all of the *Shigella* strains in our collection, and 100% of all of the *Shigella* strains *if* we exclude *S. boydii* (the latter is almost never associated with shigellosis, in contrast to pandemic *S. dysenteriae* type 1, *S. sonnei*, and classical *S. flexneri* serotypes, which are lysed by ShigActive™). Currently, a good animal model for shigellosis is not available. Thus, the efficacy of ShigActive™ was further evaluated in an (i) *in vitro* system (the HeLa cell invasion assay), and (ii) *in vivo* system (C57BL6 mice that are colonized by and shed *Shigella* without exhibiting the signs of shigellosis). ShigActive™ provided 100% protection in the HeLa cell model when used in a 1,000:1 phage:bacteria ratio (which would be expected during a typical human infection) (Dr. Eileen Barry, personal communication). During our *in vivo* studies, ShigActive™ was administered (*via* oral gavage) to groups of 9- to 16-weeks-old C57BL6 mice (i) 1 h before, (ii) 1 h

after, (iii) 3 h after, and (iv) 1 h before and 1 h after challenge with *S. sonnei* strain S43 Nal^R (ca. 1 x 10^8 CFU/mouse). The mice treated with ShigActive™ shed significantly less *Shigella* (P = <0.05) than did the control mice treated with sterile PBS or saline. Also, the mice treated with ShigActive™ and sacrificed 2 days postchallenge had significantly lower concentrations (P = <0.05) of viable shigellae in specimens of their small intestines than did the mice treated with sterile PBS/saline. The effect was dependent on the regimen of phage administration; the efficacy increased with more concentrated ShigActive™ preparations and repeat administration. In order to determine whether exposure to ShigeActive™ elicits any side effects in mice, we administered the cocktail during (i) short-term (7 days) and (ii) long-term (28 days) studies. The tested preparation (ca. 1 x 10^{10} PFU/mL, a dose 10 times higher than what is needed for efficacy) was given orally, by gavage (0.1 mL/mouse), twice a day for 7 days, followed by once a day every other day for 3 weeks. No significant difference was noted in the animals' weight gain, urine tests were normal, and leucocytes, nitrite, urobilinogen, blood, bilirubin, and glucose were not detected in any of the urine specimens. Also, significant differences were not observed in the pH, specific gravity, and protein and ketone concentrations in the urine of the control and phage-treated groups. After 7 days of treatment, hematological differences were not detected between the control and phage-treated mice. Finally, when the main bacterial composition of the GI microflora was analyzed using 16S rRNA pyrosequencing, there was no differences in the proportions of the sequences on the phylum level in control and ShigActive™-treated groups. The latter observations further supports the idea that phage-based probiotics will have a very gentle effect on the overall gut microflora, which may further enhance their protective effects.

The presentation will give the audience a current, novel perspective about the history of bacteriophage therapy research and the crucial regulatory and human safety issues concerning the use of bacteriophages in various foods and as phage-biotics. The presentation will then focus on using bacteriophages to manipulate GI microflora, with the emphasis on using them to prevent and/or treat major bacterial diseases of the GI tract; e.g., cholera and shigellosis. Phage-based probiotics are expected to have, because of their high specific activity, a very gentle effect on the overall gut microflora, which may further enhance their protective effects. They are also expected to be compatible – and, in fact, synergistic – with all classical bacteria-based probiotics. Thus, the phage-based probiotic approach may serve as a platform technology for developing a new class of probiotics for managing bacterial infections whose etiologic agents have an oral portal of entry and require GI tract colonization in order to cause disease. Moreover, phage-biotics may be valuable tools for advancing our understanding of the important physiological roles that some normal flora bacteria have in the mammalian GI tract. That knowledge may help to identify new strategies for improving human health in areas not involving the prevention and treatment of bacterial diseases; e.g., reducing the occurrence of obesity and some forms of cancer, where the GI tract's normal microbiota may have a role.

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DEFINITIONS AND OUTCOME MEASURES FOR TRIALS ASSESSING NUTRITIONAL INTERVENTIONS IN CHILDREN WITH RESPIRATORY INFECTIONS: A SYSTEMATIC REVIEW.

Andrea Lo Vecchio, Eugenia Bruzzese, Alfredo Guarino

Acute respiratory infections (ARI), including both upper respiratory tract infections (URTI) and lower respiratory infections (LRTI), are the most common illnesses during infancy, worldwide (1). The high rates of respiratory infections are associated with high social and family costs (2). ARI are a major cause of missed work days by parents and are responsible for a massive use of drugs and investigations.

Risk factors for ARI, in addition to host-related condition, include environmental conditions, such as seasonality (i.e. winter time with higher rates of influenza, RSV and other viruses), and selected settings such as day care centers, schools and hospitals. Children attending daycare centers are at 2-3 times greater risk for developing ARI than children at home (3). Host-related conditions such as immune-compromising conditions, underlying chronic diseases and atopy are related with an increased incidence and severity of ARI (4).

Scattered data suggest that selected nutritional interventions may reduce the risk to develop ARI. Probiotics, prebiotics, vitamins and micronutrients such as zinc, have been used to reduce the incidence of gastrointestinal and respiratory infections in pediatric populations. Results are sometimes conflicting and often difficult to generalize. This may depend on factors such as population features (e.g. age, location, health status, risk factors, other therapies) and type of intervention (e.g. type of supplementation, formulation, dosage, duration of consumption), but may be even more strongly related to the difference in definitions and outcome measures that have been used.

The recently formed Consensus group on Outcome Measures Made in paediatric Enteral Nutrition clinical Trials (COMMENT) appointed specific working groups to identify and define criteria for assessing key outcomes in pediatric nutrition trials (5). Specific aims of the Working Group on respiratory infections were to define, through a systematic review of the available data, the most reliable definition of clinical respiratory outcomes and at defining standard end-points to evaluate the efficacy of nutritional interventions in prevention and treatment of respiratory tract infections in infancy and early childhood.

We critically reviewed clinical trials studying the impact of nutritional interventions on upper (URTI) and lower (LRTI) respiratory tract infections. We focused on definitions, key outcomes, settings and confounding factors.

The results showed a major heterogeneity with the use of a wide array of different definitions and clinical end-points and biomarkers. After abstract screening, 47 trials were included, 44 on prevention and 3 on treatment. Nutritional interventions ranged from enriched formulas to micronutrient given for broad ranges of time (**Fig 1**). Most data described specific nutrient administration for prevention of influenza-like-syndrome and URTI in children up to 6 years. The definitions of respiratory infections were highly heterogeneous. In 10/47 trials, URTI or LRTI were diagnosed by pediatrician (eg. rhinitis, laryngitis, pharyngitis, otitis, influenza). In 30 trials, definitions were less clear and were based on symptoms reported by family members or field workers (runny nose, cough, sore throat). Seven trials did not provide a specific outcome definition. Incidence was the most common outcome measure in prevention trials. Duration and illness severity were the main outcomes considered in trials on treatment (**Fig 2**).

Such variability in defining disease episodes could be partially related to the important role played by family members and/or field workers in reporting conditions, particularly in large field trials on prevention. In this specific setting, a definition needs to be broad and easy to measure with the risk to lose some sensitivity, but mainly specificity.

Different definitions of URTI and LRTI were used, based on the presence and association of specific symptoms the duration of which , however, was rarely reported. Diagnosis of URTI was often based on a single symptom or on the presence of a cohort of different respiratory symptoms (runny nose, nasal congestion, laryngitis, pharyngitis, tracheitis). The association and the duration of the symptoms varied between the studies. In most cases the presence of symptoms was recorded by a family member of the sick child (usually the mother), but in other trials the definition was based on the diagnosis made by the physician and a specific (segmental) definition of URTI, rather than specific symptoms, was reported.
Diagnosis of AOM was also poorly reported and heterogeneous throughout the studies. Tympanic membrane bulging is a key sign to differentiate AOM from otitis media with effusion (6) and the assessment of membranes through a pneumatic otoscopy is a pre-requisite for the diagnosis. However, this requires a medical staff well trained in pneumatic otoscopy (7), and may represent a barrier to include otitis among the outcome measures for large scale studies.
In case of LRTI, the definition was somehow more consistent in the few articles that included it, also because the diagnosis was based on physician consultation and specific symptoms of lower respiratory tract involvement (such as the increase of respiratory rate, crepitations to chest auscultation, chest indrawing) or on radiographic findings.
Fever was frequently used as outcome measure, being easy to measure and monitor, providing also information on the duration and somehow on the severity of the disease. However, the reliability of body temperature measure may widely change according to instrument, environment and site of measurement and, in addition, the definitions of fever were scattered with cut-off values ranging from 37°C to 38.5°C.
Overall, most studies focused on prevention, the heterogeneity for primary outcomes was limited and the incidence of new URTI or LRTI episodes was the outcome measure most frequently used, although definitions were not always sufficiently detailed. In the majority of trials, important information such as time limits of intervention and observation was not included. Other potentially relevant details were often missing. Reference to the season, that is a key risk factor for developing respiratory infections and is usually included in the definition of community acquired respiratory infections (such as winter season to define influenza or other respiratory viruses) was rarely found. Moreover, the wide age ranges of the study populations and the different settings in which the studies were performed, might be major biases for the susceptibility to respiratory infections.

Considering the high heterogeneity in the current literature on respiratory infections, and the problems in comparing the results of studies using different definitions and end-points, there is a major need of agreeing on definitions and outcomes in future clinical trials assessing respiratory outcomes in childhood.
The working group agreed on general indications/outcomes points to be considered in future trials.
Based on the outcome measures to be studied, two different study models may be proposed to effectively investigate the role of nutritional interventions in reducing occurrence and severity of respiratory infections: “trials of efficacy” and “trials of impact”.
The first may directly assess the role of a selected intervention on respiratory diseases; in that case, the incidence or severity of selected infections (otitis, URTI, pneumonia) may be considered as major end-point. The “outcomes of impact” such as the number of performed chest-X-ray, working-days loss, medical visits and interventions or hospitalization, may indirectly assess the effect of nutrition on respiratory illnesses, providing a reliable estimate of the burden of respiratory diseases on health-care. Outcomes of impact may be easily assessed in a large sample size, bypassing the need to use difficult or complex diagnostic criteria, or validated scores as needed for the “efficacy outcome” studied.

Figure 1.

Figure 2.

PRIMARY OUTCOMES*	**NUMBER OF TRIALS**
Prevention studies	**46**
Incidence of respiratory infections (URTI, LRTI, AOM)	34
Prevalence of respiratory symptoms (morbidity)	14
Duration of symptoms	6
Duration of hospitalization	1
Treatment studies	**4**
Duration of respiratory episodes in days	2

NUTRITIONAL INTERVENTIONS	**DURATION OF INTERVENTION (RANGE IN MONTHS)**	**NUMBER OF TRIALS**
Supplemented and modified infant formulas:		
With Probiotics	3-12 months	7
With Prebiotics	1-12 months	2
With both Prebiotics and Probiotics	3-12 months	3
Lipids (DHA)	12 months	1
Fermented probiotic dairy drink	3 months	1
Fermented milk product with probiotics	Few days- 12 months	3
Micronutrients supplementation (eg. Ca, Zn,Fe)	Few days -12 months	11
Vitamin+micronutrient	Few days-12 months	6
Vitamin supplementation	15 days-24 months	12
Lipids (eg. PUFA, cod liver oil, sesame oil)	3 days – 6 months	2
Other nutrients	4-6 months	2
Duration of hospitalization in days	2	
Severity of respiratory symptom (cough)	1	

REFERENCES

1. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet. 2010;375:1969-1987
2. Ortega-Molinari NA, Fairbrother G et al. Indirect, out-of-pocket and medical costs from influenza-related illness in young children. Vaccine. 2012 Jun 13;30(28):4175-81. doi: 10.1016/j.vaccine.2012.04.057. Epub 2012 Apr 27
3. Lu N, Samuels ME, Shi L et al. Child day care risks of common infectious diseases revisited. Child Care Health Dev. 2004;30:361-8.
4. Vaccines against influenza WHO position paper – November 2012 No. 47, 2012, 87, 461–476 http://www.who.int/wer
5. Koletzko B, Szajewska H, Ashwell M, Shamir R, Aggett P, Baerlocher K, Noakes P, Braegger C, Calder P, Campoy Folgoso C, Colomb V, Decsi T, Domellöf M, Dupont C, Fewtrell M, van Goudoever JB, Michaelsen KF, Mihatsch W, Guarino A, Koletzko S, Rigo J, Turck D, Taminiau J; Early Nutrition Academy and European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition – Consensus Group on Outcome Measures Made in Paediatric Enteral Nutrition Clinical Trials. Documentation of functional and clinical effects of infant nutrition: setting the scene for COMMENT. Ann Nutr Metab. 2012;60:222-32.
6. Shaikh N, Hoberman A, Kaleida P et al. Otoscopic signs of otitis media. Ped Infect Dis J 2011;30:822-26.
7. Pichichero ME, Poole MD. Comparison of performance by otolaryngologists, pediatricians and general practitioners on an otoendoscopic diagnostic video examination. Int J Ped Otorhinolaringol 2005;69:261-6.

PROGRAMMING OF THE INTESTINAL IMMUNE RESPONSE THROUGH MODULATION OF INTESTINAL MICROBIOTA

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In this presentation, I will consider how the newborn, full term, vaginally-delivered infant initially colonizes its gastrointestinal tract. With full colonization, a symbiotic relationship develops between colonizing bacteria and the underlying epithelial and lymphoid tissues. This relationship results in both non-specific and immunologic (innate and adaptive immune responses) defenses which collectively comprise the intestinal mucosal barrier to pathogens and harmful antigens. An important component of mature intestinal immune homeostasis is the development of oral tolerance to benign commensal bacteria and noxious antigens. This phenomenon can be achieved with complete colonization of the gut during the newborn period. With complete colonization and development of the mucosal barrier, immune homoestasis exists and there is no expression of disease. In contrast, circumstances exist in which inadequate colonization occurs (premature delivery, delivery by Cesarean section and excessive use of perinatal antibiotics). Under these circumstances, an inadequate colonization occurs leading to dysbosis and increased expression of immune-mediated and allergic disease states. This circumstance, dysbiosis of the gut, has become the basis for the "new" Hygiene Hypothesis. Fortunately, clinical evidence suggests that the use of pre- and probiotics can act as "surrogate" colonizers and help prevent the expression of these diseases. Each of these concepts will be discussed in detail in this presentation. If we examined a crosssection of small intestine in the human fetus *in utero* appears as an immature epithelial surface with prolonged cell turnover and a paucity of lymphoid elements. In contrast, an identical section of small intestine in the newborn infant in the extrauterine environment appears as an active, rapidly turning over structure, expressing the subtypes of epithelial cells and displaying a plethora of lymphoid elements **(Figure 1)**. The principal difference in these two situations is that the intrauterine environment is germ free whereas the extrauterine environment consists of abundant microbiota which colonize the gastrointestinal tract. This observation emphasizes the importance of initial intestinal colonization in the development of gastrointestinal protective functions.

Once a normal colonization has been achieved with diverse individual bacterial species, these microorganisms establish a symbiotic relationship with the underlying intestinal epithelial and lymphoid tissues. Conserved molecular patterns, either expressed on the surface of symbiotic bacteria or secreted into the gut, can interact with pattern recognition receptors (PRR) expressed on/or inside epithelial and lymphoid cells to initiate signal transduction and a transcription of a host of molecules which mediate host defense or metabolic activities within the intestine. The best known family of PRRs is the toll-like receptor (TLRs) family consisting of nine identified receptors which interact with components of gram positive and negative bacteria to mediate both innate and adaptive immune responses as well as other mucosal barrier cellular functions.

Maturation of mucosal immune function leading to immune homeostasis is not complete until the process of oral tolerance occurs. Oral tolerance is a systemic reduction in cellular and humoral

immunity to commensal bacteria and harmless antigens through perioral exposure to the intestine. **Figure 2** depicts our current understanding of oral tolerance. Antigens or non-pathogenic bacteria interacting with submucosal dendritic cells via TLRs in the presence of colonizing bacteria are stimulated to preferentially produce T-regulatory cells and a specialized microenvironment that facilitates the development of T-reg cells. These cells release TGF-β, an oral tolerogenic cytokine, which reduces the Th1, Th2 and Th17 response to antigen/bacteria. It has previously been shown that oral tolerance cannot be achieved in germfree animals and these animals must be conventionalized to full colonization during the neonatal period for tolerance to be effective, In our laboratory, we have shown that oral tolerance requires an intact TLR-4 to be effective and tolerance can be broken with extensive use of broad spectrum antibiotics. These observations suggest that normal initial intestinal colonization is needed to establish oral tolerance and tolerance once achieved can be broken by excessive use of antibiotics.

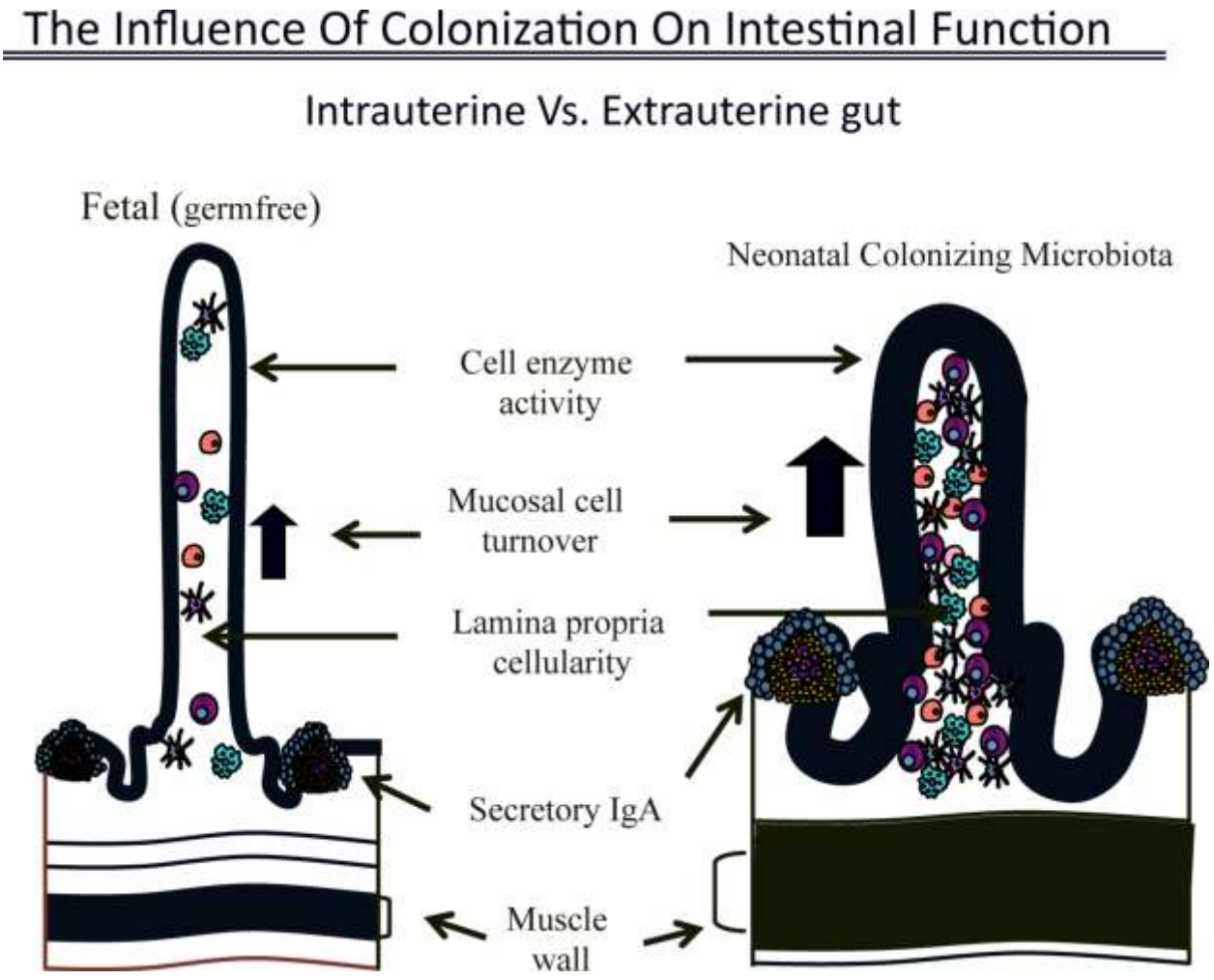
Fortunately there are possibilities of dealing with dysbiosis leading to clinical disease. Several clinical studies have been published which suggest that prebiotics and probiotics or a combination, e.g., symbiotics, may convert a dysbiosis to a symbiosis by balancing potential pathogens with health promoting bacteria. Two circumstances illustrate this approach to rectifying a dysbiosis of intestinal microbiota. A seminal study from Finland has shown that when *Lactobacillus acidophilus* (LGG) is given to pregnant women with a family history of allergy during the latter stages of pregnancy and during lactation, thus results in new infants having fifty percent less atopic dermatitis than control infants. Furthermore, this protective effect is still apparent at seven years after birth. However, when these studies were expanded to include multiple test sites using a single protocol, the results were not as clear-cut. However, a probiotic effect during pregnancy and lactation was helpful if the allergy-prone babies were born by Cesarean section. Another example of probiotics stabilizing a dysbiosis occurs with their use in premature infants to prevent necrotizing enterocolitis (NEC). Several studies have been done and when analyzed by meta-analysis seemed to both prevent and lessen the severity of NEC. A study done in Taiwan initially used a combination of *Lactobacillus acidophilus* and *Bifidobacteria infantis* in one nursery to significantly reduce the incidence and severity of the disease. This was followed by an expanded study in five nurseries with similar results. Since the FDA in the United States will not allow live organisms to be given to immune-compromised premature infants, we have tested, in human fetal intestinal models, the effect of secreted products from these two bacteria and then secretions from each grown separately. We have reported that secreted products of *Bifidobacteria infantis* has greater anti-inflammatory properties than *Lactobacillus acidophilus* and the anti-inflammatory function seems to be mediated through the stimulation of immature enterocyte genes in the innate inflammatory immune response. Further studies are planned to test the secreted factor mixed with expressed breast milk from mothers delivering premature infants to determine, if this combination of pre-(breast milk) and probiotic secretions may be protective.

In this presentation, it was emphasized that initial colonization was in part dependent on the infant diet, particularly breast feeding. Furthermore, it was shown that a symbiotic bacterial-host relationship determine immune homeostasis. An important component of immune homeostasis is the development of oral tolerance which can only occur with complete colonization of the intestine. Under conditions of abnormal colonization (dysbiosis), an increase in immune-mediated disease occurs. Fortunately, pre- and probiotics given to the infant can convert a dysbiosis to a symbiosis and potentially reduce the incidence of disease.

SUGGESTED REFERENCES

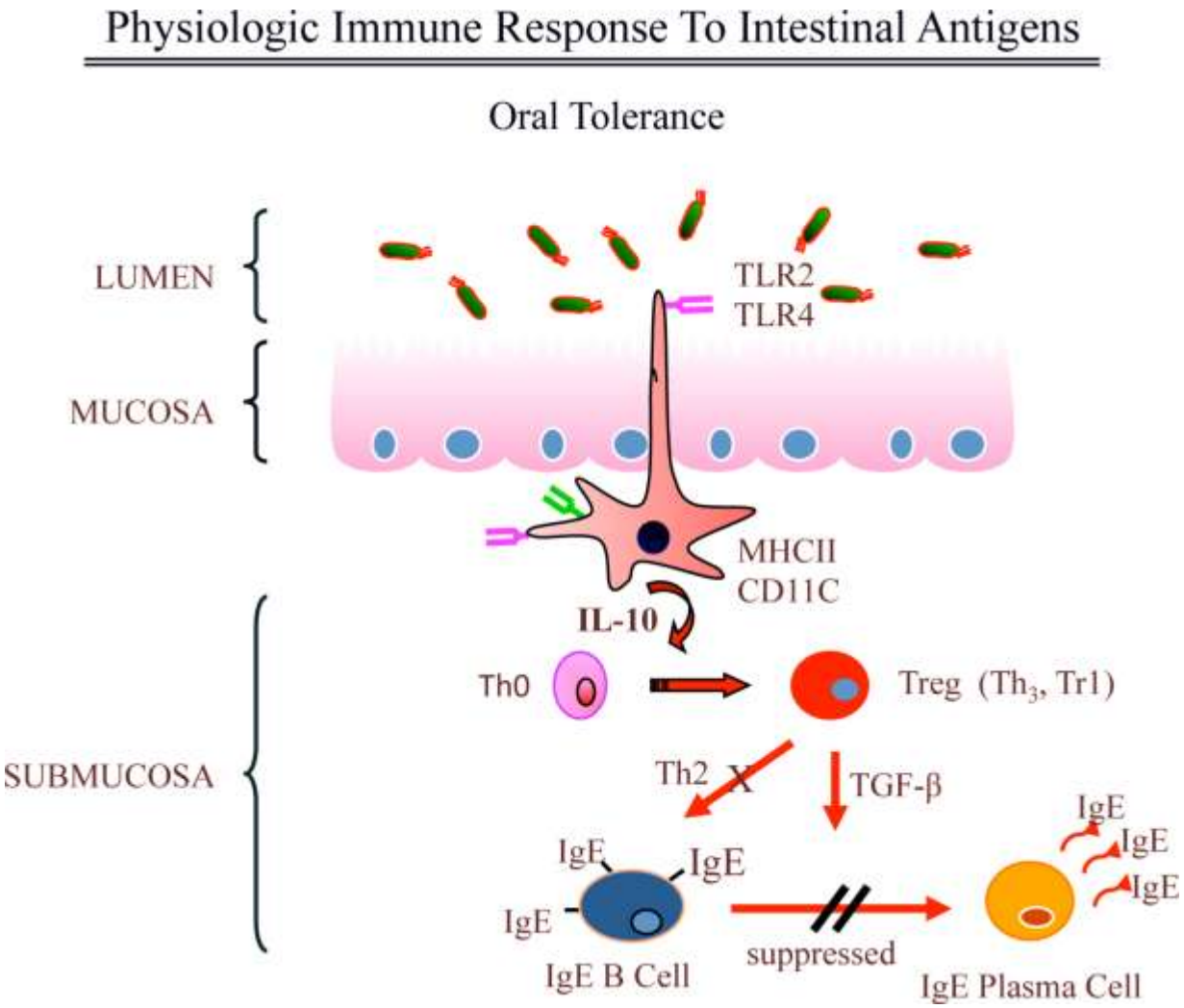
1. Walker WA. Bacterial colonization, probiotics and the development of intestinal host defense. Functional Food Reviews vol. 1(1), Spring 2009; 13-19.
2. Weng M, Walker WA. The role of gut microbiota in programming the immune phenotype. J Developmental Origin of Health and Disease 2013; 4:203-214; doi:10:1017/S20404174412000712.
3. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat Rev Immunol 2008 Jun;8(6):411-20. doi: 10.1038/nri2316.
4. Gareau MG Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. Nature Rev: Gastroenterol & Hepatol 2010; 7:503-514.

FIGURE 1



Legend: A schematic representation of a cross section of small intestine of human fetus *in utero* vs. the newborn human infant. Fetal intestine appears thin and exhibits a slow epithelial proliferation rate with a paucity of gut-associate- lymphoid-tissue (GALT), whereas infant intestine manifests a robust, diverse epithelium with a fast turnover rate and abundant GALT elements

FIGURE 2



Legend: Schematic representation of oral tolerance induction by gut microbiota. In the intestinal lumen, gut microbiota activate dendritic cells via the TLR2/TLR4 signaling pathways. Activated dendritic cells cause maturation of TH0 to subsets (TH3, Tr1) of Treg cells via release of IL-10 to stimulate TGF-β release and thereby suppress IgE production.

NEW PROBIOTICS FOR PEDIATRIC IBD

Annamaria Staiano

The conventional treatments for pediatric IBD, Ulcerative Colitis (UC) and Crohn's disease (CD) have focused on targeting inflammation and suppressing the enhanced immune response with steroids, thiopurines, and anti-tumor necrosis factor antibodies. Though these agents have reasonable efficacy, they may produce the significant side effects associated with chronic immune suppression. With recent evidence implicating a disruption in the balance of the gastrointestinal microbiome and intestinal immunity as a potential trigger for inflammatory bowel disease (IBD), there has been growing interest in using probiotics as an adjunct to standard anti-inflammatory and immune suppressing therapy. While the precise reasons for therapeutic benefit are incompletely understood, the proposed mechanisms include improving host mucosal barrier function leading to diminished immunologic reactivity, displacing deleterious microbes from the mucosal surface, and modulating the mucosal immune system. Probiotics can competitively exclude pathogenic bacteria by occupying the limited physical space or render their microenvironment inauspicious for pathogens by secreting antimicrobial substances. Lactobacillus and Bifidobacteria exert direct antibacterial effects on pathogens by producing bacteriocins and acid toxic to gram positive and gram negative bacteria. Probiotics can interact with epithelial cells, reducing secretion of water and enhancing production of mucus or anti-microbial peptides. For example, VSL#3 results in normalization of impaired colonic barrier function and restoration of intestinal epithelial integrity in IL-10 deficient mice and enhancement of epithelial resistance in T-84 cells. Studies have shown that the presence of probiotics modifies natural killer activity, modulates nuclear factor kappa-B (NFkB) pathway, and induces T cell apoptosis. Several probiotic bacteria, including Bifidobacterium breve, Streptococcus thermophilus, Bifidobacterium bifidum and Ruminococcus gnavus have been shown to secrete metabolites that reduce LPS-induced TNF-a secretion. Many probiotics strains have been used in clinical trials, including and the induction and maintenance of remission in UC, CD and pouchitis.

Ulcerative Colitis

Probiotics have been used in patients with UC, both to induce and maintain remission, with encouraging results in open label and randomized controlled trials. Miele et al. explored induction of remission in children with UC, adding VSL#3 to standard treatment with steroids and 5-aminosalicylic acid for the induction and maintenance of remission in 29 newly diagnosed patients. There was a significantly improved remission rate (92.8 %) compared to placebo (36.4 %). A Cochrane review examined probiotics' role in inducing remission in ulcerative colitis. The review concluded that adding probiotics to conventional treatment did not improve the overall remission rates in mild to moderate UC, but it was possible to obtain a slight benefit in decreasing disease activity. Some small pediatric series also support probiotic use for maintenance of remission in UC. Huynh et al. performed an open label trial of VSL#3 in addition to standard IBD care in eighteen consecutive pediatric patients and reported a remission rate of 61 %. Recently, a Cochrane Database Review by Naidoo and colleagues evaluated four studies with a total of 587 patients that compared probiotics to any other therapy in the maintenance of remission for UC. They found that all four studies had significant issues with execution including incomplete outcome data and unclear methods of blinding or randomization.

Crohn's disease

Limited studies are available on probiotic use for inducing remission and maintaining remission in patients with CD. For induction of remission, there is one randomized placebo controlled trial and a few open labeled studies. Schultz et al. conducted a placebo controlled trial employing Lactobacillus GG in 11 patients. Only 5/11 patients completed the study, and time to relapse was no different between the placebo and Lactobacillus GG groups. More literature exists regarding probiotics for maintenance of remission in Crohn's disease. In the largest maintenance trial to date,

Bousvaros et al. reported no significant difference in probiotic efficacy in those receiving L. rhamnosus GG compared with placebo. Unfortunately, the current evidence shows the efficacy of probiotics in CD to be much lower than in pouchitis and ulcerative colitis. This lack of treatment effect in patients with CD may reflect to the transmural nature of this disease, the poor design of the studies, or other as yet unidentified factors.

Pouchitis

Probiotics have been well studied for the prevention of pouchitis as well as for the induction and maintenance of remission. The product that has been most extensively evaluated for these indications is VSL#3 – a proprietary blend of eight bacteria. Almost, all the European randomized controlled studies of VSL#3 have shown clinical benefit, primarily for maintenance.

Conclusions

Although there are insufficient data to recommend probiotics in ulcerative colitis or Crohn's disease, good evidence supports the use of specific probiotics for maintenance of remission in pouchitis. Although there are limited regulatory standards for the agents, probiotics are relatively safe with minimal reported side effects or contraindications. More rigorous studies need to be published supporting efficacy and safety of these agents before they become a mainstay of IBD medical treatment.

THE RATIONAL USE OF PROBIOTICS IN ABDOMINAL-PAIN RELATED FUNCTIONAL GASTROINTESTINAL DISORDERS IN CHILDHOOD.

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Functional gastrointestinal disorders are one of the most frequent complaints in children. Although benign in nature, they are commonly associated with an excessive anxiety, unnecessary lab tests and a significant economic burden.
According to the Rome III criteria these disorders may be classified into: functional dyspepsia (FD), irritable bowel syndrome (IBS), functional abdominal pain (FAP) and abdominal migraine.
Several studies in adults have demonstrated some clinical benefit of particular probiotic agents, mostly in IBS.
Pediatric literature data are scarce and controversial, as they present a wide variability of study design and type of microorganisms.
The present lecture will include the following topics:

1. Presentation of our own recent study.
2. Systematic literature review.
3. Potential mechanisms.
4. Useful guidelines and future directions.

The attached table presents a summary of all pediatric studies.
In conclusion, probiotic microorganisms may serve, to some extent, as a therapeutic aid in selected abdominal-pain related functional gastrointestinal disorders in childhood, mainly FAP and IBS.
These preliminary promising results should be validated in future well-designed large-scale trials.

Table. Probiotics in abdominal-pain related functional gastrointestinal disorders in childhood--Randomized controlled studies.

Study	Disorder	Agent	Supplementation (wks)	n	Age (yrs)	Pain relief	Other effects
Bausserman 2005	IBS	*L-GG*	6	50	6-20	No	Distention
Gawronska 2007	IBS FAP FD	*L-GG*	4	104	6-16	IBS - Yes FAP - No FD - No	
Francavilla 2010	IBS FAP	*L-GG*	8	136	5-14	IBS – Yes FAP - No	Improved permeability
Romano 2010	FAP	*L.reuteri*	4	52	6-16	Yes	
Guandalini 2010	IBS	VSL#3 (8 strains)	6x2 crossover	59	5-18	Yes	Bloating Stooling
Weizman 2013	FAP	*L.reuteri*	4	93	6-15	Yes	Bloating

CHANGES IN MICROFLORA COMPOSITION AND INFLAMMATION IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a complex, multi-system disease. It is becoming clear that colonization of CF airways is due to the presence of a dynamic polymicrobial community that typically includes a number of pathogenic species, and that bacterial diversity at this site is strongly influenced by host, environmental and clinical factors. The gastrointestinal tract is an important target of Cystic Fibrosis. About 60% of patients with severe CFTR born with exocrine pancreatic insufficiency, which progresses with age to include 85%–90% of patients. Children with CF are at risk of an abnormal intestinal microflora, as a consequence of impaired CFTR function, a heavy antibiotic load, pancreatic enzyme supplementation and acid suppression treatment. Loss of bicarbonate-rich pancreatic fluids in patients with pancreatic insufficiency alters the intestinal intraluminal milieu. The presence of intestinal inflammation in patients with CF has received little recognition until recently. In the last ten years data have been published demonstrating that intestine of CF children is a site of inflammatory processes. Several biomarkers, including calprotectin, S100A12, IL-8, IgM, IgG, neutrophil elastase, tumor necrosis factor-α, and eosinophil cationic protein have been used to detect intestinal inflammation in patients with CF . In particular, increased concentrations of fecal calprotectin (CLP) and rectal nitric oxide (rNO) were found in a series of CF children consecutively enrolled suggesting that intestinal inflammation is a frequent feature of CF . This was confirmed by endoscopy in a population of CF children . One of the hallmarks of CF lung disease is the colonization of the usually nearly sterile airways with bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, and a range of other microorganisms. Little is known about the intestinal flora composition in CF children. It is becoming clear that intestinal microflora is a complex functional unit that lives with the host in a symbiotic relationship. The gastrointestinal tract harbors more than 1000 bacterial species, whose composition is a result of several factors including age, diet, antibiotics and other drugs. There is evidence that a healthy intestinal microflora protects from allergy and other immune disorders by driving the development of immune response toward protection against intestinal and extraintestinal infections, including respiratory infections.

Because of the complexity of the gut microbiota and the fact that the majority of these bacteria have never been cultured, until five years ago the only information available, on the role of microbiota in CF were related to the presence of an increased rate of small intestinal bacterial overgrowth detected in patients with CF compared to healthy controls. Using breath testing, SIBO has been found in about one-half of CF patients and that the orocecal transit is frequently slower in CF. Recently, the use of molecular techniques has begun to be applied to stool samples from patients with CF and allow to demonstrate that patients with Cystic Fibrosis show a different pattern of intestinal microbial composition compared to healthy controls. Duytschaever et al. showed a decreased species richness and less temporal stability of the CF fecal microbiota as compared with healthy controls. In addition early life development of the gut and airway microbiota in CF was investigated and nutritional factors and gut colonization patterns seems to be determinants of the microbial development of respiratory tract microbiota in infants with CF. We studied the composition of intestinal microbiota in children with CF and observed that in basal conditions, CF children showed a significant reduction in Bacteroides , E.rectale and F. prausnitzii compared to healthy children. Compared to untreated CF, a further significant reduction in all bacterial species was observed in antibiotic treated CF patients. Conversely the administration of LGG to CF children induced a significant increase in Bacteroides counts compared to untreated CF children, although the Bacteroides loads were still lower than that detected in healthy controls

In conclusion intestinal microflora of CF children is different compared to healthy controls, with a consistent reduction of *Bacteroides, E.rectale* and *F.prausnitzii* species. Antibiotic treatment further

reduces the amount of healthy bacterial species. Probiotic supplementation seems to be able to partially restore the microbiota. These data suggest the efficacy of probiotic therapy in CF and suggest that this is linked with restoration of intestinal microflora and support the hypothesis that an earlier intervention maybe able to induce more relevant beneficial effects.

References

1. Lee JM, Leach ST, Katz T, Day AS, Jaffe A, Ooi CY. Update of faecal markers of inflammation in children with cystic fibrosis. Mediators Inflamm. 2012;2012:948367. doi: 10.1155/2012/948367. Epub 2012 Aug 21.
2. Raia V, Maiuri L, De Ritis G, De Vizia B, Vacca L, Conte R, et al. Evidence of chronic inflammation in morphologically normal small intestine of cystic fibrosis patients. Pediatric research 2000; 47:344–50.
3. 17. Bruzzese E, Raia V, Gaudiello G, Polito G, Buccigrossi V, Formicola V, et al. Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. Alimentary pharmacology & therapeutics2004 ;20:813–9.
4. Madan JC, Koestler DC, Stanton BA, Davidson L, Moulton LA, Housman ML, et al. Serial Analysis of the Gut and Respiratory Microbiome in Cystic Fibrosis in Infancy : Interaction between Intestinal and Respiratory. mBio 2012;3(4):1–10.
5. Werlin SL, Benuri-Silbiger I, Kerem E, et al. Evidence of Intestinal Inflammation in Patients With Cystic Fibrosis. J Pediatr Gastroenterol Nutr. 2010;51:304-308.
6. Scanlan PD, Buckling A, Kong W, Wild Y, Lynch SV, Harrison F. Gut dysbiosis in cystic fibrosis. J Cyst Fibros. 2012 Sep;11(5):454-5.
7. Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P. Cross-sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. Appl Environ Microbiol. 2011;77:8015-24.
8. Duytschaever G, Huys G, Bekaert M, Boulanger L, De Boeck K, Vandamme P Dysbiosis of bifidobacteria and Clostridium cluster XIVa in the cystic fibrosis fecal microbiota. J Cyst Fibros. 2012 Nov 11. pii: S1569-1993(12)00183-X. doi: 10.1016/j.jcf.2012.10.003.
9. Bruzzese E, Viscovo S, Scotto R, Chiatto F, Basile F, Nicastro E, et al. Structural abnormalities of intestinal microecology in cystic fibrosis and effects of probiotics and antibiotic administration. Journal of Pediatric Gastroenterology and Nutrition 2013;56; Suppl.2.

INDICATIONS TO PROBIOTIC THERAPY IN CHILDREN RECEIVING ANTIBIOTICS

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Probiotics are used since a long time in the prevention and treatment of diarrhea in infants and children. According to a recent meta-analysis, probiotics reduce the risk of antibiotic associated diarrhea (AAD) in children. Reduction of the risk of AAD was associated with the use of *L. caseii GG*, *S. boulardii* or *B. lactis* and *Str. thermophilus*. The number needed to treat is seven: 7 patients need to be given probiotics to have one patient less with AAD. Only *S. boulardii* was reported to be effective in the treatment of AAD caused by Clostridium Difficile (C. dif.). *S. boulardii* may be effective for secondary prevention in specific patient populations with particular concurrent antibiotic treatment. However, there is no evidence to support the use of any probiotic to prevent the recurrence of C. Dif. infection or to treat existing C. Dif. diarrhea.
The American Academy of Pediatrics concluded that RCTs showed a beneficial effect for probiotics in the prevention of AAD in children. This was also the result of an analysis of AAD independent of age. According to a Cochrane review published in 2011, including 16 pediatric studies, Bacillus spp., Bifidobacterium spp., Lactobacilli spp, Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp. or Streptococcus spp. alone or in combination have all been evaluated in the prevention of AAD. Nine studies used a single strain, four combined two and one combined three probiotic strains, one study was done with a probiotic food supplement containing ten strains, and one study had two probiotic arms that used three and two strains respectively. Overall, the incidence of AAD in the probiotic group was 9% compared to 18% in the control group. However, this benefit was not statistically significant in an extreme plausible (60% of children lost to follow-up in probiotic group and 20% lost to follow-up in the control group had diarrhea) intention to treat (ITT) sensitivity analysis. If the data are analyzed that way, the incidence of AAD in the probiotic group was 16% compared to 18% in the control group. However, ITT subgroup analysis was marginally significant for high dose probiotics: AAD in the probiotic group was 17% compared to 22% in the control group. None of the 11 trials that reported on adverse events documented any serious adverse events. *L. GG* was reported equally effective as *S. boulardii*. Age may as well be important. According to one meta-analysis, L. does reduce AAD in adults, but not in children. However, a recent meta-analysis did not find an age-related difference.
In most studies, the probiotic is started together with antibiotic treatment. Despite heterogeneity in probiotic strain, dose, and duration, as well as in study quality, the overall evidence suggests a protective effect of probiotics in preventing AAD. A GRADE analysis indicated that the overall quality of the evidence for the primary endpoint (incidence of diarrhea) was low due to issues with risk of bias (due to high loss to follow-up) and imprecision (sparse data). Another shortcoming is that the severity of the AAD is not considered: most of the time AAD is very mild and does not need any intervention.
Clinical trials should preferably be performed with the commercialized product.
Only these probiotics can be recommended in medical indications if scientific proof of efficacy of the same product with the same dose in the same indication is available. Strain and product specificity is considered to be of major importance, although high quality evidence for this statement is missing.
Probiotics have been shown to prevent the development of antibiotic associated diarrhea.
Probiotics are in general very safe. Regarding probiotics, cases of septicemia and fungemia have been reported in high-risk situations.

References

Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, Pieścik M, Puntis J, Shamir R, Szajewska H, Turck D, van Goudoever J; ESPGHAN Committee on Nutrition. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr. 2011;52:238-50.

D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-aanlysis. BMJ 2002;324:1361?

Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol. 2011;45 Suppl:S149-53.

Johnston BC, Goldenberg JZ, Vandvik PO, Sun X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev. 2011;11:CD004827

Kale-Pradhan PB, Jassal HK, Wilhelm SM. Role of Lactobacillus in the prevention of antibiotic-associated diarrhea: a meta-analysis. Pharmacotherapy 2010;30:119-26.

McFarland LV. Meta-analysis of probiotics for prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol 2006;101:812-22

Reid G. Microbiology: Categorize probiotics to speed research Nature 2012;485:446

Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. Plos One 2012;7:e34938

Szajewska H, Ruszczynski M, Radzikowski A. Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. J Pediatr 2006;149:367-72

Thomas DW, Greer FR. Clinical report: probiotics and prebiotics in pediatrics. Pediatrics 2010;126:1217-31

Tung JM, Dolovich LR, Lee CH. Prevention of Clostridium difficile infection with Saccharomyces boulardii: a systematic reviw. Can J Gastroenterol 2009;23:817-21

Turck D, Bernet JP, Marx J, Kempf H, Giard P, Walbaum O, Lacombe A, Rembert F, Toursel F, Bernasconi P, Gottrand F, McFarland LV, Bloch K. Incidence and risk factors of oral antibiotic associated diarrhea in an outpatient pediatric population. J Pediatr Gastroenterol Nutr 2003;37:22-6

GUT MICROBIOTA AND RISK OF DISEASE: AN EPIGENETIC PARADIGM EFFECTS OF MOTHER´S MICROBIOTA ON IMMUNOLOGICAL PROGRAMMING OF CHILD HEALTH

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Perhaps the greatest challenge of our time is reversing the increase of non-communicable diseases, of which allergic disease was the first to emerge. Indeed, modern civilization is faced with a progressive increase in immune-mediated health problems such as allergic, autoimmune and inflammatory diseases, as well as obesity. These share common environmental influences and immunological changes. While allergic diseases comprise the most common chronic disease in childhood, obesity is the most prevalent nutritional disorder among children throughout the world.

Although the human genotype affects individual susceptibility to disease, this is considered to have only a small direct influence on the immunological phenotype. Epigenetic mechanisms, i.e. influences in the development of an organism other than DNA sequences, might permanently affect the activity of human genes in the development of health, especially during the early critical stages of maturation. Consequently, interventions during these sensitive periods of development can have long-lasting effects in terms of reducing the risk of disease. This is the modern version of the programming theory: As adults, our health is determined by events *in utero* and in early childhood, especially with regard to the nutritional environment, which influences the maturation, structure and function of our immune system, metabolic health and the microbiological programming (Figure).

Recent demonstration that a growing number of clinical conditions, ranging from inflammatory bowel disease to allergic diseases and even obesity, are linked to aberrant gut microbiota composition, has led to active research interest in host-microbe crosstalk, characterizing and manipulating the gut microbiota at an early age. Accordingly, an extended version of the hygiene hypothesis has been introduced to emphasize the intimate interrelationship between diet, the immune system and microbiome and origins of human disease: The modern infant, particularly if caesarean section-delivered and devoid of the recommened exclusive breast-feeding, may lack sufficient stimulation of the mucosal immune system to generate a tolerogenic immune milieu and be prone to develop chronic inflammatory conditions, which may take the form of allergic or autoimmune disease, or predispose the child to obesity.

The intestinal microbiota provides maturational signals for the gut-associated lymphoid tissue. Gut microbiota composition can discriminate between allergic/ obese children and healthy children, and the distinction may precede clinical manifestations of disease. The mother provides the first inoculum of bacteria, which has been shown to influence the risk of disease in the child; *Bifidobacterium* species being the major determinants in this context. The infant's probability of being colonized by bifidobacteria is low if the mother has higher BMI and weight-gain during pregnancy and delivers by Caesarean section, while probability of *Bifidobacterium* colonization is higher in breast-fed infants (Figure). The recent scientific advances here, however, challenge the traditional thinking suggesting that the human fetus remains sterile and that the first pioneer bacteria directly originate from the vaginal and fecal microbiota of the mother. According to this theory, further sources of bacteria include the mammary glands through breast-feeding, the mother's skin and oral microbes, and the initial environment of the neonate. Accumulating evidence now suggests that traces of microbes are detectable in the placenta, amniotic fluid and

fetal membranes. Furthermore, microbial contact *in utero* has been shown to induce changes in the fetal intestinal innate immune gene expression. Microbial DNA has also been detected in the meconium of healthy term neonates, suggesting a prenatal origin. Taking these preliminary observations together (Figure), contact with the complex bacterial communities of the extra-uterine world may be initiated already *in utero*, which in turn are determined by the mothers´ intestinal microbiota, health and body composition during pregnancy.

After birth, the sources of environmental exposure directly shaping the risk of disease consist mainly of the step-wise development of the gut microbiota and breast-feeding. Human milk is rich in bioactive compounds conferring not only passive protection but also actively stimulating the development of the infant's immune system. Breast milk also contains health-promoting microbes, their optimal growth factors and components regulating host-microbe interaction. Thus the gut microbiota tends to interact with other elements in nutritional, immunological and microbiological programming of child health (Figure). The nutritional value of food is influenced by the gut microbiome, co-evolving with the immune system at an early age, which is again highly sensitive to diet. Furthermore, the gut microbiota regulates intestinal barrier function and immune responsiveness and can be affected by specific nutrients. The intimate interrelationship between diet, the immune system and microbiome and origins of human disease may be transferred from mother to infant.

The composition of the gut microbiota, and thus also the modification of the gut microbiota by specific probiotics or prebiotics early in life may have an impact on the risk of disease in the child. Above the impact on gut microecology, probiotic effects have been attributed to restoration to normal of increased intestinal permeability, improvement of the intestine's immunological barrier functions, alleviation of the intestinal inflammatory response, and reduced generation of proinflammatory cytokines characteristic of local and systemic allergic inflammation. Recent evidence from experimental and clinical studies indicate that the gut microbiota is also involved in the control of body weight and energy metabolism, affecting the two main causes of obesity: energy acquisition and storage, and contributing to insulin resistance and the inflammatory state characterizing obesity. Consequently, currently research is focusing both on characterizing specific probiotic strains and on how the food matrix and the dietary content interacts with the most efficient probiotic strains.

Figure legend

Figure 1. Epigenetic regulation and the developmental origins of disease programming

References

Isolauri E, Kalliomäki M, Laitinen K, Salminen S. Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease. Curr Pharm Des 2008;14:1368-75.
Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 2012; 96: 544-51.
Rautava S, Luoto R, Salminen S, Isolauri E. Perinatal microbial contact and the origins of human disease. Nature Reviews Gastroenterololy& Hepatololgy 2012; 9: 565-76.

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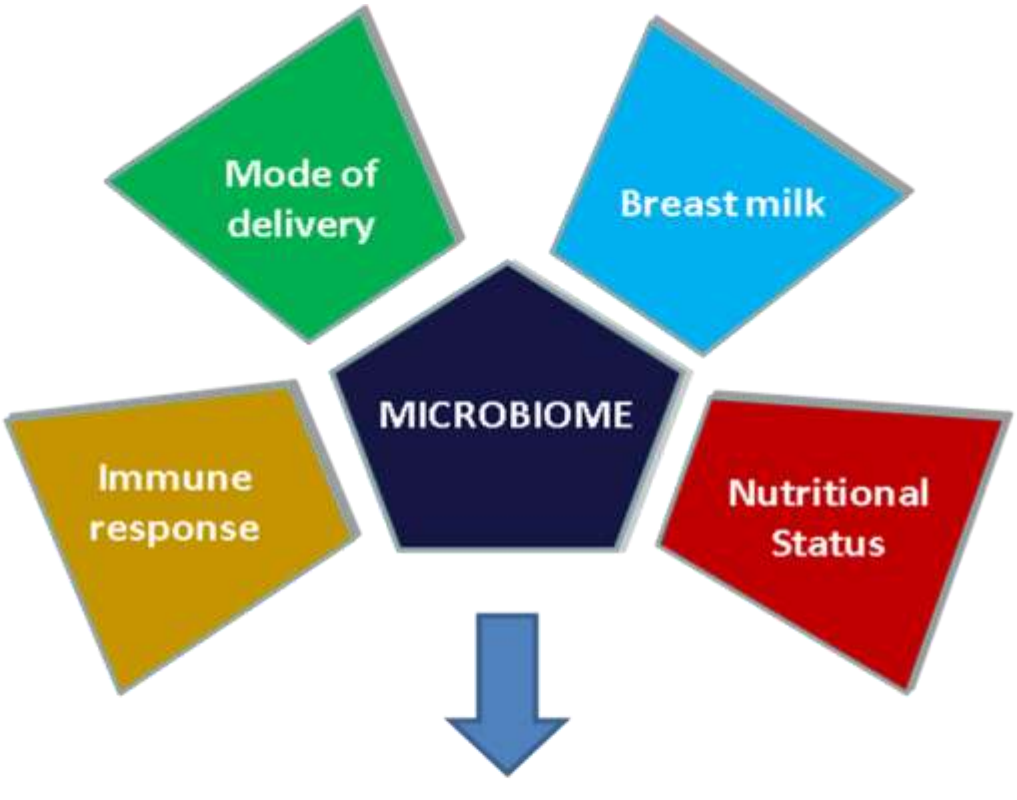

THE GUT-LIVER AXIS AND INTESTINAL MICROFLORA

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ABSTRACT

Intestinal bacteria influence gut permeability, systemic inflammation levels, and host metabolism. The contribution of gut microbiota to obesity, obesity related type 2 diabetes, cardiovascular diseases, and liver steatosis is becoming increasingly clear, although the causality remains to be proven in humans.

1. THE GUT-MICROBIOTA

Thanks to modern molecular microbiology techniques it has been possible to accurately profile the intestinal microbiota (IM). The main divisions that dominate the IM are **Bacteroidetes** and **Firmicutes**, representing the 93 % of all sequences recognized in fecal samples of healthy subjects. IM role in obesity was focused in animal models by Gordon's group, by using microbiota transplant from obese mice to germ-free mice. They showed that strains specific composition of gut microbiota (decrease of Bacteroidetes and consensual increase of Firmicutes) was one of the "transmissible" causes of obesity. Several other direct or indirect evidences have shown the role of IM also in obesity related liver diseases (NAFLD/NASH), and other components of the metabolic syndrome **(1)**

2. GUT- MICROBIOTA, GUT-LIVER AXIS, AND NAFLD

Intestinal Microbiota can be influenced by several factors and a more or less specific strain modulation may prevent or cause important complications concerning liver and cardiovascular system. Hepatic involvement in gut liver axis has recently been reviewed in depth by Vajro et al (**2**).

Firstly, the modifications occurring in IM appear able to promote hepatic lipogenesis, owing to an augmented uptake of free fatty acids (FFA) by the liver (3). Secondly, a growing body of evidence has confirmed that short chain fatty acids (SCFAs) derived from bacterial activity, induce secretion of peptide YY (PYY) by entero-endocrine cells, slowing down the intestinal motility and even promoting the development of Small Bacterial Overgrowth (SIBO). SIBO is characterized by the bacterial colonization of (normally sterile) small intestine, that increases its permeability ("leaky gut") and prejudices the integrity of TJ (tight junctions) of intestinal mucosa. This leads to subsequent increased trafficking of fat and waste materials (i.e. parasites, fungi, bacterial products as LPS, or undigested proteins) from the intestinal lumen to blood circulation, through submucosa absorption**.** Wigg's group showed an increased intestinal permeability, with trafficking of bacterial byproducts and high levels of TNF-α in patients affected by NASH *vs*. controls (**4**) .

The first important step of NAFLD to NASH progression with hepatic inflammation and fibrogenesis. appears related to an interplay between intestinal permeability and LPS, whose absorption is promoted by high fat diet. The alteration of mucosal permeability induces an endotoxemic metabolic state and insulin-resistance (2). In other words, the increased level of orexigenic adipokine leptin increases level of blood glucose and insulin levels, and intestinal expression of the lipoprotein lipase-related fasting-induced adipose factor (FIAF), that ultimately favors adipose tissue expansion, and FFA oxidation processes mediated by peroxisome proliferator-activated receptor (PPAR)**.**

IM has an important role also in the process of **choline** catabolism, an important precursor molecule useful in hepatic synthesis of VLDL for lipid excretion. The intestinal dysbiosis of obese patients may accelerate the catabolic process, bringing about hepatic damage through depletion of choline

itself and accretion of the hepatotoxicity of choline byproducts (dimethylamine and trimethylamine).
The interplay between IM and **bile acids** shows one more important function of IM and its implications in NAFLD/NASH pathogenesis through Farnesoid X receptor (FXR), an important nuclear receptor family responsible for lipids homeostasis, glucose homeostasis and hepatic inflammation, and fibrogenesis control.

3. MANIPULATION OF GUT-LIVER-AXIS THROUGH PROBIOTICS

Probiotics mainly act by changing the microflora of the host in a way he/she may take advantage in near and even far organ systems. Regarding NAFLD, they appear able to contrast the increased production of metabolic byproducts (e.g. SCFAs) by dysbiotic IM. SCFAs participate to obesity by increasing lipidogenesis and as immune modulators which influence gut immune responses. Below we will focus specifically studies of probiotics in experimental and human NAFLD.

a. Animal studies

Over the last years a number of animal model studies have recognized a direct role of IM on NAFLD/NASH, independently by body weight changes **(5).** This implied interesting prospective for the use of probiotics to treat these conditions. VSL#3 and Lactobacilli are the most used probiotic strains in the studies conducted in genetically obese or high-fat diet obese mouse **(2, 6)** .

b. Human studies

Probiotics studies in human NAFLD are scarce, but have shown interesting beneficial preliminary results. The 2007 Cochrane meta-analysis carried out to elucidate beneficial and harmful effects of probiotics in NAFLD/NASH could not give clear indications because of lack of randomized controlled trials (RCT) **(7).** The only two identified pilot non-randomised studies (both by Loguercio's group) however were either puzzled by co-treatment with other NAFLD therapeutic agents (i.e. anti-oxidants) or did not illustrate the results obtained with VSL#3 treatment.
Since the Cochrane publication (**7**) and subsequent ESPGHAN meta-analysis **(8),** 2 RCTs appeared.
1. A double blind RCT evaluating the efficacy of a 3 month course treatment with *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in adult patients with NAFLD, found a significant reduction of serum aminotransferases, while anthropometric parameters and cardiovascular risk factors remained unchanged in both treated and control groups **(9)**.
2. The first pediatric RCT carried out by group got similar results **(10)**. Obese children with NAFLD, unable to comply with lifestyle interventions, were given 12 billion CFU/day of *Lactobacillus GG* for 8 weeks. A significant decrease in alanine aminotransferase (with normalization in 80% of cases) was detected, with no changes in BMI zscore and visceral fat. TNFα and US bright liver parameters, as well, remained quite unmodified. A significant reduction of antipeptidoglycan-polysaccharide serum antibodies (a SIBO marker) probably mirrored improved intestinal dysbiosis and/or gut barrier.
A symbiotic treatment (probiotic *B. longum* + prebiotic fructo-oligosaccharides) vs. controls prescribed lifestyle changes, showed in the treated group a significant reduction in AST, LDL- chol, C-reactive protein, TNFα, serum endotoxin, insulin resistance, steatosis and NASH activity index **(11)**.

Despite these evidences, IM composition in human NAFLD patients was characterized only recently (**12),** showing an inverse and diet-/BMI-independent association with presence of NASH and % Bacteroidetes in stools. Studies looking at changes after probiotic treatment are needed.

REFERENCES

1. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006 ; 444:1027-31.
2. Vajro P, Paolella G, Fasano A. Microbiota and gut-liver axis: a mini-review on their influences on obesity and obesity related liver disease. J Pediatr Gastroenterol Nutr. 2013 56:461-8.

3. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis.Curr Opin Clin Nutr Metab Care. 2007 Nov; 10:729-34.
4. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut. 2001 48:206-11.
5. Li DY, Yang M, Edwards S, Ye SQ. Nonalcoholic Fatty Liver Disease: For Better or Worse, Blame the Gut Microbiota? JPEN J Parenter Enteral Nutr. 2013 (published online ahead of print).
6. Esposito E, Iacono A, Bianco G, Vajro P. et al. Probiotics reduce the inflammatory response induced by a highfatdiet in the liver of young rats. J Nutr. 2009; 139:905-11.
7. Lirussi F, Mastropasqua E, Orando S, et al. Probiotics for non-alcoholic fatty liver disease and/or steatohepatitis. Cochrane Database Syst Rev. 2007;CD005165.
8. Socha P, Horvath A, Vajro P, et al. Pharmacological interventions for nonalcoholic fatty liver disease in adults and in children: a systematic review.J Pediatr Gastroenterol Nutr. 2009; 48:587-96.
9. Aller R, De Luis DA, Izaola O, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011;15:1090-95.
10. Vajro P, Mandato C, Licenziati MR, et al. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr. 2011; 52:740-43.
11. Malaguarnera M, Vacante M, et al . Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012; 57:545-53.
12. Mouzaki M, Comelli EM, Arendt BM et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013 Feb 11 (published online ahead of print).

FUNCTIONAL EFFECTS OF EARLY NUTRITIONAL INTERVENTION ON LATER PERFORMANCE

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Many nutritional interventions early in infancy are capable of effecting later performance. Numerous processes may be affected, including immune function, central nervous system function, healthy growth, and others. Appropriate nutrition is critical early in life, when there is a window of opportunity to support normal development and function of the immune system. Probiotics, especially Lactobacillus GG, have been shown to play a role in such modulation.

DHA, or docosahexanoic acid , is another very good example of one such nutrient and will be used in this presentation as an example of how nutrition early in life can influence future outcomes. Here, using DHA as an example, we will present data that shows how early intake may influences–and affects cognition and psychomotor development, visual development, and function of the immune system.

DHA is a long chain polyunsaturated fatty acid of the containing 6 double-bonds (DHA, 22:6n-3), which makes the molecule very flexible. It belongs to the omega-3 (or n-3) family, the same as eicosapentaenoic acid (EPA, 20:5n-3) and the omega-6 (or n-6) arachidonic acid (ARA, 20:4n-6). These LCPUFA are synthesized endogenously from the precursors alpha linolenic acid (ALA, 18:3n-3) and linoleic acid (LA,18:2n-6) through a series of elongation and desaturation steps common to the omega-3 and omega-6 pathways. Alpha linolenic acid is transformed to EPA (eicosapentaenoic acid) from which 5 series prostaglandins (anti-inflammatory) are synthesized. EPA is then converted to DHA and from this compound, resolvins are synthesized which are known to inhibit the inflammatory process after it has been initiated. DHA itself is incorporated into membranes especially in the central nervous system, and in very large quantities in the retina. Consequently, its role in vision and cognition is important. DHA constitutes 30 to 40% of total brain lipids. Much of it concentrates around neural synaptic membranes making it important in facilitating synaptic function and assisting in neurotransmission in signal transduction. As one might expect, brain growth is very rapid especially during the last trimester of pregnancy and the first two years of life, and it is at this time that large quantities of DHA are deposited into membranes in the central nervous system. DHA is therefore the main polyunsaturated fatty acid found in the central nervous system, primarily in the gray matter and the retina. Adequate amounts of it are required during critical periods of development and growth. Most individuals can synthesize it but some require dietary intake to meet all the needs at this time. Smaller quantities are essential for a synthesis and maintenance of function of the brain and retina even when the brain is not actively growing. It is not only important in cellular structure and function but also may offer some protection against cell death. Research on LCPUFA early in life has focused on their roles in neurodevelopment, because DHA and ARA accumulate, especially during late pregnancy through about 2 years postnatally, in uniquely high concentrations in brain gray matter. DHA, EPA, and ARA serve as important cell membrane components as well as

precursors for an extensive network of biologic mediators with many effects in the body, including numerous roles in immune function and inflammation.

Infants fed DHA through human milk have enhanced brain function compared to infants fed standard infant formulas without DHA. They have higher scores for cognitive development, fewer behavioral and emotional problems, earlier motor skills, and more rapid visual maturation. Improvement in mental and verbal skills and a decrease in absenteeism can be seen throughout childhood. Birch *et al* performed a randomized controlled trial of early dietary supplementation of long chain polyunsaturated fatty acids, primarily including DHA , and its effects on mental development in term infants. Here a standard infant formula a formula supplemented with .35% DHA and a formula supplemented with .36% DHA and .72% arachadonic acid (ARA) were given within the first 5 days of life and continued for four months. Bailey mental development index was measured at 18 months and was significantly higher in the groups given DHA compared to the control group. Jensen at all demonstrated that maternal DHA supplementation improved Bailey PDI scores at 2 1/2 years of age when supplementation began at delivery and continued until 4 months of age. Agostoni showed in a study of 98 healthy term infants fed DHA for 4 months had significantly higher developmental quotient scores correlated closely with DHA erythrocyte phospholipids at 4 months of age. Colombo at all performed a dose response study comparing formulas with 0% DHA, .32% DHA, .64% DHA, and .96% DHA and demonstrated that there was little value and going above the .32% level. Drover *et al* demonstrated in 18-month-old term infants an enhancement of cognitive development, at all levels of DHA supplementation.

DHA also has significant influence on the development of vision. The studies of Birch *et al* clearly demonstrated in a randomized controlled trial that DHA supplementation with .36% DHA and .72% ARA resulted in significant improvement in visual acuity at 17, 26, and 52 weeks using visual evoked potential as an endpoint. The benefit persistent until 18 months, despite DHA being given only during the first 4 months. Makrides *et al* also demonstrated that visual evoked potential acuity was significantly enhanced relative to red blood cell DHA content in DHA supplemented infants. Data from the Birch dose response study likewise showed enhancement with DHA supplementation but no benefit related to doses higher than 0.32%.

Long chain polyunsaturated fatty acids are important structural components of immune cells, where they are concentrated heavily in cell membranes, effecting membrane fluidity, cells signaling, and gene expression. They are also metabolically important in eicosanoid and cytokine production affecting the immune cell response. Immune responses are coordinated through the secretion of a variety of compounds including prostaglandins, leukotrienes, thromboxanes, and other cytokines. Compounds synthesized from DHA and EPA are predominantly anti-inflammatory. A number of studies have now shown that administration of DHA and EPA can significantly influence symptoms of wheezing and potentially asthma. Hoppu reported lower levels of omega-3 fatty acids in breast milk in mothers of children with atopic disease. Salam has shown that children whose mothers ate oily fish high in omega-3 fatty acids during pregnancy were less likely to develop asthma. Hodge *et al* also showed a reduction of wheezing and asthma related to oily fish consumption.

Recently, Pastor *et al* demonstrated a significant reduction in incidence of bronchitis and bronchiolitis in infants at 5, 7 and 9 months of age who were fed DHA-containing formulas during the first year of life. In the cohort of patients originally described by Birch, infants not supplemented with DHA had a significantly higher incidence of allergic manifestations in the first 3 years of life compared to those who were supplemented early in life with DHA. The

impact of DHA and ARA supplementation on allergic manifestations during the first year of life was also measured by Lapillonne *et al*. Here again, there was a reduction in wheezing during the first year of life with DHA supplementation. D'Vas *et al* examined the effects of early postnatal fish oil supplementation on infant's cellular immune function and found that infants with high levels of DHA had lower TH2 responses to allergens.

Here, we have presented several examples of how one nutrient, DHA, appears to be quite important in early programming. Expert body reviews of these data have affirmed the benefits of supplementing infant feeding with DHA in formula fed infants early in life. These data suggests that DHA appears to have an early role an important role in programming of cognitive brain function, visual function, and immune system function as well. The immune modulatory effects appear to be predominantly anti-inflammatory. We must remember that that this is only an example of how one nutrient can affect early programming. It is quite likely we will find other nutrients which are similarly important.

References:

Martinez M. *J Pediatr.* 1992;120:S129-S138.;
Innis SM. *J Nutr.* 2007;137:855-859
Young C et al. *Synapse.* 2000;37:90-94
Carlson SE. *Semin Neonatal.* 2001;6:437-449
Jones CR et al. *Neurochem Res.* 1997;22:663-670
Salem N et al. *Lipids.* 2001;9:945-959
Anderson JW et al. *Am J Clin Nutr.* 1999;70:525-535
Vancassel S et al. *OCL* . 2007;14:164-170.
Birch EE et al. *Dev Med Child Neurol.* 2000;42:174-181
Jensen CL et al. *Am J Clin Nutr.* 2005;82:125-132
Agostoni C et al. *Lancet.* 1995;
Makrides M et al. *Lancet.* 1995; 345:1463-1468
Morale SE et al. *Early Hum Develop.* 2005;81:197-203
Calder PC. *Lipids*. 2003;38:343-352
Stulnig TM. *Int Arch Allergy Immunol.* 2003;132:310-321
Nagakura T et al. *Eur Respir J.* 2000;16:861-865.
Hoppu U et al. *J Pediatr Gastroenterol Nutr*. 2005;41:335-338
Salam MT et al. *J Asthma.* 2005;42:513-51.
Hodge L et al. *MJA.* 1996;164:137-140
Pastor N et al. *Clin Pediatr.* 2006;45:850-855
J.R. Drover et al. / Early Human Development 87 (2011) 223–230
J. Colombo, 0031-3998/11/7004-0406 PEDIATRIC RESEARCH Vol. 70, No. 4, 2011
D'Vaz N, et al. Clin Exp Allergy. 2012;42:1206–16

A DOUBLE-BLIND PLACEBO-CONTROLLED RANDOMZED TRIAL ON PROBIOTICS IN SMALL BOWEL BACTERIAL OVERGROWTH IN CHILDREN TREATED WITH OMEPRAZOLE

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Background: A decreased gastric acid secretion is known to favor small bowel bacterial overgrowth (SBBO). However, the impact of proton pump inhibitors on the development of SBBO in children have not been thoroughly studied. Moreover, the role of probiotics in the prevention of SBBO has not been evaluated in children.
Objective: To evaluate the incidence of SBBO in children treated with omeprazole and test if probiotics influence the incidence.
Methods: A double-blind, placebo-controlled trial was performed in 70 children treated orally during 4 weeks with 20 mg omeprazole per day. *Lactobacillus rhamnosus* R0011 (1.9×10^9 cfu) and *Lactobacillus acidophillus* R0052 (0.1×10^9 cfu) was given daily simultaneously to 36 subjects (probiotic group), while 34 subjects received placebo (placebo group). The diagnosis of SBBO was based on the development of suggestive symptoms in combination with a positive glucose breath test.
Results: After one month of PPI treatment, 30% (21/70) had a positive breathtest suggesting SBBO; of these 62% were symptomatic. Five children developed SBBO-like symptoms but had a negative breath test; 44 (63%) were symptom free and had a negative breathtest. There was no difference in the incidence of positive breath tests in the probiotic versus the placebo group (33% vs 26.5%; p: 0.13).
Conclusions: Since symptoms suggesting SBBO develop in 26% of PPI-treated children, and since a glucose breathtest is abnormal in 72% of these, this side-effect should be considered more frequently. The probiotic tested did not decrease the risk to develop SBBO.

FRUCTOOLIGOSACCHARIDES PURIFICATION

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Objective: As prebiotic sugars fructo-oligosaccharides (FOS) have gained an important place in the functional food market. Nevertheless, isolated FOS are only available for analytical purposes and even pure FOS commercialized mixtures are still very expensive. At large scale, FOS have been produced from sucrose by microbial enzymes resulting in mixtures containing significant amounts of salts and other non-prebiotic sugars, representing 40-60% (w/w) of total sugars, that must be removed. Industrially, sugars have been purified by simulated moving bed (SMB) chromatography. Since the success of chromatographic processes is highly dependent on the adsorbent used, in this study, the potential of commercial ion-exchange resins to separate FOS from smaller sugars was investigated.
Methods: A mixture of FOS was produced by fermentation of sucrose through Aureobasidium sp. Sugars contained in the fermentative broth were injected in a single-column working with Milli-Q water as eluent. From the chromatograms obtained, the influence of the resin structure and its cationic form in the separation performance were evaluated. The influence of the temperature and column length in the separation was also investigated.
Results: A demineralisation process was successfully developed. Resins in potassium form obtained the higher retention factor values for sugars when compared to the other ionic forms. However, when compared to calcium and sodium ones, shown to be the less efficient separating sugar mixtures. The resin with best separation performance was the Diaion UBK535Ca. A recovery yield of 92% (w/w) of FOS with 90% (w/w) of purity was obtained from batch experiments conducted at 25°C. The temperature shown did not influence the separation performance significantly. By increasing the column length, the purity of FOS increased to 92% (w/w), however the recovery yield decreased to 88% (w/w).
Finally, the separation of FOS from a fermentative broth has been optimized in a SMB pilot plant working with the selected resin.
Conclusions: Between the resins most referenced in the literature as having potential for oligosaccharides, the commercial Diaion UBK535Ca resin was selected for the separation of FOS from a fermentative broth.

THE EFFECT OF LACTOBACILLUS CASEI AND LACTOBACILLUS PARACASEI STRAINS ON CYTOKINE RESPONSE IN CHILDREN WITH ATOPIC DERMATITIS

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Objective: Recently, we have selected three *Lactobacillus* strains identified as *Lactobacillus casei* (LOCK 0900, LOCK 0908) and *Lactobacillus paracasei* (LOCK 0919), which given to children with atopic dermatitis (AD) significantly improved clinical symptoms compared with control group. The aim of this study was to determined the effect of probiotic application on cytokine production in children with AD.

Methods: The study included 60 children with cow's milk protein allergy presenting clinical symptoms of atopic dermatitis (AD) in mean age 10 months. The mixture of probiotics were applied for 3 months in daily dose $1x10^9$ in a randomized double-blind placebo controlled way. All children received hydrolyzed milk formula. Analyses were done before probiotic application, at the end of probiotic in-take (after 3 months), and then 5 months later. The severity of clinical symptoms was evaluated by international Scoring Atopic Dermatitis Index (SCORAD).

Results: Probiotics induced significant decreased in SCORAD after the end of bacteria in-take, but the improvement of clinical symptoms as compared with placebo group was found only in children with IgE-dependent AD (93% versus 54%, $p=0.033$). In probiotic group increased amounts of Th1 cytokines (IL-12, IFN-γ) and regulatory IL-10 were found in cell cultures, but only at the end of probiotic in-take ($p<0.05$). Pro-allergic IL-5 systematically decreased in probiotic group as compared with placebo group achieving statistical differences after 5 months.

Conclusions: The results show that the mixture of three *Lactobacillus casei* and *Lactobacillus paracasei* strains affects the immune system of children, particularly with IgE-dependent allergy, by induction of Th1 and regulatory cytokine production and by suppression of pro-allergic Th-2 response.

EFFECTS OF PROBIOTICS ON INTESTINAL COLONIZATION AND INFIAMMATORY BOWEL DISEASE IN PRETERM INFANTS WITH BW<1500 G.

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Objective: To assess the effectiveness of oral administration of *Lactobacillus reuteri* to reduce gut colonization with pathogenic bacteria in very low birth weight (BW) infants (BW<1500 g).
Methods: A prospective, randomized, blind study was performed in the NICU of Sant'Orsola-Malpighi Hospital, Bologna. VLBW infants were started on *Lactobacillus reuteri* (5 drops/day = 108 CFU) or placebo (5 drops/day) within the 7th day from birth. Culture for aerobic and anaerobic Gram-negative bacteria, and measurement of fecal calprotectin, were perfomed once a week, for four weeks, on rectal swab.
Results: Seventy-seven newborns (33 male) were evaluated: 37 received *L. reuteri* and 40 placebo. Treated and control infants did not differ in terms of gestational age (mean ±SD; 28±2.27 vs 29±2.18 weeks, respectively), birth weight (1003±294.44 vs 1076.45±260 g, respectively), and rate of cesarean section (35/37 vs 36/40).
Gut colonization by pathogenic species was different in the two groups: treated infants were colonized later than controls by *E.coli*, *Stenotrophomonas spp.* and *Pseudomonas spp.* (p=0.015). Average values of fecal calprotectin were higher in controls (177μg/g vs 140 μg/g, respectively). Fecal calprotectin values > 280 μg/g were detected in 8/150 (5.3%) samples from treated infants and in 21/148 (14.2%) samples from controls (p=0.031).
Conclusions: Probiotic supplementation influences gut colonization by pathogenic bacteria; further studies are needed in order to clarify its potential role in preventing intestinal inflammation in VLBW infants.

PROTECTIVE EFFECTS OF PROBIOTICS IN PRESCHOOL CHILDREN IN AN URBAN SLUM IN INDIA

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Objective: Supplementation of probiotics in healthy children for prevention of diarrhea has been shown to have a positive benefit. However, the studies have mostly been restricted to day care centers in developed countries. Thus, our objective was to determine the protective effects of probiotics on diarrhea and fever in preschool children in a community setting of a developing country.
Methods: The study was a double blind randomized controlled trial carried out in 379 healthy preschool children in an urban slum in India. Three randomly allocated groups of children received one of two probiotics (*Lactobacillus paracasei* Lpc-37 or *Bifidobacterium lactis* HN019) or the placebo for a period of nine months from August to April and the incidence of diarrhea and fever were assessed.
Results: During the wet season both probiotic strains, Lpc-37 and HN019, significantly reduced diarrhea in the month of September and fever in the month of August respectively in the children compared to the placebo group. Although there was no significant difference in incidence of diarrhea and fever among the groups during the whole period of supplementation, the children of the HN019 group had lower odds of diarrhea (0.81, 95%CI 0.37 – 1.73) compared to placebo group.
Conclusions: The overall non-significant effect on duration and episode of diarrhea and fever during the period of supplementation may be due to the low over all incidence of disease during the dry period. During the period of increased disease risk, i.e. the wet period, the tested probiotics may reduce the risk for diarrhea and fever.

ANTIBIOTIC ASSOCIATED DIARRHEA AND PROBIOTICS IN CHILDREN: AN ITALIAN SURVEY

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Aim: assess the incidence of AAD and the effect of concomitant probiotic administration in children.
Methods: All children discharged from Emergency Department with an antibiotic prescription were enrolled. Patients with diarrhea at admission and parents not understanding Italian were excluded. A phone recall with a standardized interview was made after 1 month. Age, site of infection, antibiotic treatment, probiotic supplementation, occurrence, duration and severity of diarrhea were recorded. AAD definition: presence of diarrhea (≥ 3 liquid stools/day) within 2 weeks of discharge without signs of new infection.
Results: 616 children (mean age±SD 56±45 months) completed the follow-up. AAD was reported in 62/616 (10%) children [33/176 (18.8%) < 24 months]. Duration of AAD was 1-15 days with 3-15 stools/day. Amoxicillin-clavulanate was the most frequently prescribed antibiotic and AAD occurred in 11% of cases. Probiotics were used in 381 (61%) children. AAD was reported in 39/381 (10%) children with probiotic compared to 22/235 (9%) without probiotic. The strains most commonly used were: Lactobacillus GG (N=176), Lactobacillus reuteri (N=98), Bacillus clausii (N=53) and Saccharomyces boulardii (N=18). In 36/381 a different strain or a mix product were used. Incidence of AAD was slightly (5% in both S. boulardii and mixed product vs. 10% of both L.GG and L.reuteri vs. 15% of B.clausii group) but not significantly different among the probiotic strains used.
Conclusion: AAD occurred in 1:10 subjects and in 1:5 children younger than 2 years. In our population probiotics were commonly used but did not provide a significant protective effect on AAD.

PROBIOTICS USE IN CHILDHOOD: A SURVEY ON OPINION AND RECOMMENDATIONS BY PEDIATRICIANS

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Objective: To investigate pediatricians' attitudes and prescribing practices for probiotics in gastrointestinal and non-gastrointestinal illnesses.
Methods: A questionnaire including questions on perceptions regarding the use of probiotics, recommendations for the use, and probiotics strains commonly prescribed was administered to primary care pediatricians (PCPs) and hospital pediatricians (HPs).
Results: 45 PCPs and 20 HPs returned the questionnaire. *Lactobacillus reuterii* and *Lactobacillus GG* were the most commonly used strains. The main gastrointestinal indication for probiotics use as therapeutic agents was acute gastroenteritis (90%), followed by antibiotic-associated diarrhea (78%) and constipation (60%). HPs used probiotics mainly for the treatment of acute gastroenteritis (95% vs 62% of PCPs; p=0.006), antibiotic-associated diarrhea (90% vs 40% of PCPs; p=0.0002) and functional intestinal disease (50% vs 22% of PCPs; p=0.02). The main therapeutic indication for non-gastrointestinal conditions in PCPs group was urinary tract infections (38% versus 10% of HPs; p=0.02), while in HPs group was atopic disease (45% vs 24% of PCPs; p=0.09). As for the use of probiotics as preventive therapy, the most common indications in gastrointestinal disorders were antibiotic-associated diarrhea (PCPs 73% vs HPs 35%; p=0.003), functional intestinal disorders (PCPs 65% vs HPs 40%; p=0.05) and constipation (48%). In non-gastrointestinal disorders, probiotics were mainly prescribed for urinary tract infection and atopic disease. The main perceived barrier to probiotic use was the lack of strong scientific evidence.
Conclusions: Pediatricians recognize a role for probiotics in a variety of illnesses. Programs to implement guidelines are strongly needed to guide the use probiotics in gastrointestinal and non-gastrointestinal conditions.

IMMUNOMODULATORY EFFECTS OF PROBIOTICS

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A complex dialogue exists between intestinal microbiota and the host immune system that allows maintenance of immune homeostasis, provides immune tolerance to food proteins and commensal bacteria, and at the same time protects against pathogenic infections. Perturbation of this dialogue may lead to development of food allergy, autoimmunity and inflammatory intestinal diseases. Evidence is increasing that it is possible to influence human health through modulation of the microbiota by administration of probiotics, which are live microorganisms that confer health benefits on the host when administered in appropriate amounts. A growing body of work now supports the efficacy of various probiotic strains in ameliorating chronic intestinal inflammation, diarrhea, constipation, irritable bowel syndrome, atopic dermatitis, allergy, vaginitis and liver disease.

The first barrier against the entry of pathogens is constituted by intestinal epithelial cells and mucus layer, representing the outer curtain of defense. Probiotics provide help by maintaining barrier function and integrity and secreting defensins/bacteriocins. For microbes eluding the barrier, the active immune response comprising both innate and adaptive responses, represents an inner defense. The innate immune response includes neutrophils, dendritic cells, macrophages and the toll-like receptor (TLR) family that recognizes conserved structures of microrganisms activating cytokine/chemokine signaling pathways. Among the TLRs, TLR4 specifically recognizes Gram-negative bacteria, while TLR2 is the receptors for Gram-positive bacteria including probiotics. There is some evidence that probiotic bacteria can inhibit the TLR signaling pathways. We found that *L. amylovorus* was able to counteract the various steps of inflammatory signaling induced by enterotoxigenic *E. coli*,(ETEC), on one hand by inhibiting the TLR4 mediated activation of NF-kB signaling, and on the other hand by up-regulating the cascade inhibitor Tollip or inhibiting the ETEC induced decrease of the inhibitor IRAK-M.

Apart the defense against pathogenic bacteria, the intestinal immune system should coexist with innocuous resident microbiota, and this is achieved through the tolerance induction. There is increasing evidence of the importance of probiotics in establishement of tolerogenic immune response, although their efficacy may depend on the strain, since it is widely recognized that each probiotic strain influences the host immune system by a variety of ways and specific activities. We found a novel activity of *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* Bb12 in the modulation of tolerance. These strains induced intestinal but not systemic antigen-specific hyporesponsiveness in ovalbumin-immunized rats through different mechanisms. The hyporesponsiveness was associated with an expansion of regulatory T cells and increased IL-10 and TGFβ after LGG but not *B. animalis* treatment, and increased apoptosis after *B. animalis* but not LGG treatment. These results suggest a potential probiotic therapeutic strategy to prevent undesirable reactions to immunogenic antigens in the gut.

Dysregulated intestinal immune response and imbalanced microbiota composition have been recognized to play an important role in the development of intestinal inflammatory pathologies. Recently, investigators related the imbalances in gut microbiota with obesity and its associated inflammation which promotes insulin resistance and type-2 diabetes. The increase in macrophage infiltration into adipose tissue is a main factor in the development of inflammatory state in obesity. A role of probiotics in reducing obesity and the associated pathologies is now emerging and our results support this role by indicating a reduction of adipocytes and macrophage infiltration in adipose tissue.

DISTINCT IMMUNOMODULATORY PROPERTIES OF *LACTOBACILLUS PARACASEI* STRAINS

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The intestinal microbiota interacts with the local immune system promoting the mechanisms of intestinal homeostasis in health. In this regard, many studies have provided evidence that also probiotics can modulate the gut immune system (1). Actually, probiotic bacteria influence both the development and regulation of intestinal immune and non-immune defenses (2). This symbiosis has risks and benefits for the host organism because bacteria continuously challenge the fine balance of intestinal immune homeostasis with their load of microbial-associated molecular patterns (MAMPs). The risk of an exaggerated inflammatory response and chronic inflammation is however limited by the polarized expression of pattern recognition receptors on the basolateral membrane (or intracellularly) in intestinal epithelial cells (IECs) and dendritic cells (DCs) that can intercalate between IECs for direct bacterial uptake (3).

In studying host responses there is a tendency to focus on the cell types that comprise the biological barriers to microbes. The main advantage of *in vitro* models based on single cell types lies in their ability to provide key information on a particular cell type's reaction to a microbe. In particular, studies have focused on probiotic interaction with enterocytes and DCs. In addition to its barrier function, the immunomodulatory role of intestinal epithelium is attracting considerable attention. By using enterocytic cells a previous work showed that *Bifidobacterium infantis* and *Lactobacillus salivarius* did not induce proinflammatory response in human IECs as compared to *Salmonella typhimurium*, suggesting that IECs display immunological unresponsiveness when exposed to lactic acid bacteria (LAB) (4). A more active role of IECs was suggested by Haller et al. (5). Adopting a co-culture model of Caco-2 cells (a human IEC line) and peripheral blood mononuclear cells, they observed discriminative activations of Caco-2 cells between *Escherichia coli* and LAB strains (*Lactobacillus johnsonii* or *Lactobacillus gasseri*). Interestingly, Rimoldi et al. (6) reported that the release of pro-inflammatory mediators by IECs, in a different human co-culture system, occurred in response to bacteria and it was dependent on bacterial invasiveness and on the presence of flagella.

DCs are potent antigen-presenting cells which can effectively induce primary immune responses against microbial infection and other stimuli. Activation of DCs can be induced by infectious agents and inflammatory products. In addition, single probiotics of the *Lactobacillus* group can regulate DC surface marker expression and cytokine production (7). By using human DCs as a cellular model it was provided evidence that the examined bacterial species displayed distinct immunomodulatory effects (8). Furthermore, different strains of the same species differentially polarized the phenotype of the immune response (9, 10). In line with these findings, current research is focused on peculiar activities of different *Lactobacillus paracasei* strains, by considering the importance of this species in promoting immune functions and decreasing infections. Specifically, *in vitro* studies have mainly analyzed the effects on DCs. *L. paracasei* strain B21060 showed a strong anti-inflammatory activity on DCs when co-incubated with *S. typhimurium* (11).

Similarly, *L. paracasei* CNCM I-4034, decreased production of pro-inflammatory cytokines in human intestinal DCs challenged with *Salmonella typhi* (12). Studying strains of the *L. paracasei* species we previously found that they induced the highest levels of maturation among the tested probiotic species in DCs isolated from a mouse model of food antigen sensitivity (13). Furthermore, we observed a different ability among five genetically characterized *L. paracasei* strains to modulate the activity of mouse DCs (14). The strains were genetically differentiated by using the F-AFLP technique which led to the identification of several molecular markers unique to each strain. The functional studies indicated that all the strains stimulated the phenotypic maturation of DCs, but they acted differently on DCs in relation to the other tested properties. In particular, the analysis of the cytokine profile revealed a different secretion of interleukin (IL)-2, IL-12 and IL-10. The last cytokine has an anti-inflammatory effect and it is critical for the maintenance of tolerance to commensal intestinal bacteria. On the contrary, IL-12 has functions mutually antagonistic to IL-10 and induction of a high ratio IL-10/IL-12 is considered predictive of efficient *in vivo* anti-inflammatory properties. In fact, the ability of LAB strains to induce a high ratio of IL-10/IL-12 production in human PBMC correlated with their capacity to provide significant protection from TNBS-induced colitis (15). Specifically two strains, IMPC 4.1 and ATCC 334, stimulated the highest levels of IL-2 and IL-10 in our study. In this regard, IMPC 4.1 can be considered particularly efficient as an anti-inflammatory/regulatory strain. Other two strains, IMPC 2.1 and LMG P-17806, were characterized by an intermediate ability to induce cytokine secretion. While a very low ability to induce IL-10 and IL-12 secretions in DCs differentiated another tested strain, LMG 23554 (= strain YS8866441), from the others. This feature could be related to the potential pathogenic behavior of LMG 23554, isolated from a blood culture of a patient with infective endocarditis, which was also able to exacerbate colitis in TNBS-treated mice and to translocate to extra-intestinal organs (16). We speculated that a low ability to stimulate the immune system could allow strain LMG 23554 to cross the intestinal mucosal barrier and/or persist in the extraintestinal organs or circulation.

Whether specific metabolites can be responsible of highlighted immunomodulatory effects remains to be addressed. Indeed it is known that probiotic effects arise not only from whole microorganisms and wall components, but also from metabolites such as proteins, peptides and extracellular polysaccharides produced in conditioned cell culture media (17, 18). Notably, Von Schillde et al. (19) recently addressed this specific point by showing that a *L. paracasei* strain from the VSL#3 mixture secreted a protease selectively degrading proinflammatory chemokines.

In conclusions, differential immunomodulatory properties shown by various strains of *L. paracasei* were reported in our, as well as, in other studies. Therefore, a more functional use of probiotics should be based on its pro- or anti-inflammatory properties, that are closely related to their ability to induce a specific cytokine profile in DCs and IECs. The analysis of this profile could also be instrumental in predicting a potential pathogenic behaviour of a novel probiotic strain, possibly resulting from its low ability to induce cytokine secretion.

References

1. Borchers AT et al. Probiotics and immunity. J Gastroenterol 2009;44:26-46.
2. Tlaskalova-Hogenova H et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett 2004; 93,97-108.
3. Rescigno M et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361-367.
4. O'Hara AM et al. Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. Immunology 2006;118:202-215.
5. Haller D et al. IL-10 producing CD14low monocytes inhibit lymphocyte-dependent activation of intestinal epithelial cells by commensal bacteria. Microbiol Immunol 2002; 46:195-205.
6. Rimoldi, M et al. Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. Blood 2005;106:2818-2826.
7. Christensen HR et al. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol 2002;168:171-178.
8. Hart AL et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 2004;53:1602-1609.
9. Medina M et al. Differential immunomodulatory properties of *Bifidobacterium longum* strains: relevance to probiotic selection and clinical applications. Clin Exp Immunol 2007;150:531-538.
10. Menard O et al. Gnotobiotic mouse immune response induced by Bifidobacterium sp. strains isolated from infants. Appl Environ Microbiol 2008;74:660-666.
11. Mileti E et al. Comparison of the immunomodulatory properties of three probiotic strains of lactobacilli using complex culture systems: prediction for *in vivo* efficacy. PLoS One 2009;4(9):e7056.
12. Bermudez-Brito M et al. Human intestinal dendritic cells decrease cytokine release against Salmonella infection in the presence of Lactobacillus paracasei upon TLR activation. PLoS One 2012; 7(8): e43197.
13. D'Arienzo R et al. Modulation of the immune response by probiotic strains in a mouse model of gluten sensitivity. Cytokine 2009;48:254-259.
14. D'Arienzo R et al. Distinct immunomodulatory properties of *Lactobacillus paracasei* strains. J Appl Microbiol 2011;111:1482-1491.
15. Grangette C et al. Enhanced anti-inflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids. Proc Natl Acad Sci USA 2005;102:10321–10326.
16. Daniel C et al. Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. Appl Environ Microbiol 2006;72:5799-5805.
17. Otte J-M et al. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. Am J Physiol Gastrointest Liver Physiol 2004;286:G613–G626.
18. Ganguli K et al. Probiotics prevent necrotizing enterocolitis by modulating enterocyte genes that regulate innate immune-mediated inflammation. Am J Physiol Gastrointest Liver Physiol 2013;304:G132-G141.

19. von Schillde M-A et al. Lactocepin secreted by Lactobacillus exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. Cell Host & Microbe 2012;11:387–396.

METABONOMICS IN ELDERLY: A TOOL TO COMPREHENSIVELY MODEL HOST AND GUT MICROBIAL METABOLIC TRAJECTORIES FOR HEALTHY AGEING

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The aging phenotype in humans is very heterogeneous and can be described as a complex mosaic resulting from the interaction of a variety of environmental and genetic-epigenetic factors. Decades of research on aging have revealed hundreds of genes and many biological processes associated with the aging process, but each of these candidate signatures typically represent only a few molecular readouts, therefore lacking a general molecular footprint of aging. In parallel, the improvements in nutrition, sanitary conditions and medical care have led to a general increase in human longevity, with subsequent effects of the host-gut microbial metabolic interactions in health and disease (1). There is nowadays increasing evidence that intestinal microbiota is an influential actor in health ageing and maybe a key determinant in longevity (2). Gut function is generally well preserved with healthy ageing. However, age-related structural and functional impairment of the metabolic adaptive response and repair mechanisms of the mucosa makes gastrointestinal tract (GIT) more susceptible to the risk of malnutrition, systemic infection, enteropathogens but also adverse events related to medication (3). In particular, thorough investigation of gastrointestinal disturbances in elderly patients couples with intensive nutritional support can make a very real impact on their outcome (4).

Therefore, it is today critical to understand the molecular foundations of the impact of the gut microbial activity on human health and nutritional status (5). The current omics revolution offers an unprecedented opportunity to explore how our gut symbionts contribute to our physiology and human health. Future systems biology approaches combining state-of-the-art microbial and metabolic readouts modeling, including metagenomics and Metabonomics, will help deciphering the molecular foundations of these transgenomics interactions and understanding how gut microbiota specificities could be exploited to develop new therapeutic and nutritional strategies.

In particular, demographics have made ageing and age-related chronic disease an enormous and growing biomedical and societal challenge (6). The immune system undergoes profound and multifaceted changes with ageing. In particular, the homeostatic balance between the pro-inflammatory and the anti-inflammatory arms of the immune system is skewed resulting in a state of persistent low-grade systemic inflammation. In this the origins and drivers of the “inflammaging” process are still poorly understood (7), but intestinal dysbiosis is a feature of old age. The age related chronic inflammation is believed to be pathogenic especially with regards to its contribution to frailty and degenerative disorders.

Metabonomics has emerged over the last two decades as a novel way able to provide insights into the role of mammalian gut microbial metabolic interactions in individuals, with specific readouts in health and disease. It is likely that gut microbiome required for proper functioning of the gut ecosystem in the elderly is different than in young subjects, therefore knowledge and systems models will help assessing early deviations from healthy ageing trajectories. In particular, both system wide (i.e., whole organism) and organ-specific metabolism may have components driven by gut microbial activities (8), which suggests that the dynamics of the gut microbiome could help to maintain or re-establish host metabolic homeostasis in disease and early onsets of metabolic deregulations. Gut microbial activities can be extremely specific, as for development and maintenance of the mucosal innate and adaptive immune system, but also very complex, such as in

the etiology and development of several chronic inflammatory disorders and gastrointestinal cancers.

Metabonomics is considered today a well-established system approach to characterize the metabolic phenotype, which results from a coordinated physiological response to various intrinsic and extrinsic parameters including environment, drugs, dietary patterns, lifestyle, genetics, and microbiome (9). Despite recent applications, a comprehensive metabolic phenotype of longevity in humans has not yet been reported. In particular, profiling data on extreme longevity is missing, where biological markers conducive of exceptional longevity could provide insights into mechanisms that protect the host from common diseases and/or slow the biological processes of aging.

By combining NMR/MS based metabonomics and targeted lipidomics approaches (Figure 1) we report for the metabolic phenotype of longevity and aging in a well characterized human cohort compromising centenarians, elderly and young individuals (10). Specifically, untargeted metabonomics profiling of urine revealed that the longevity process is marked by changes in gut microbial metabolism, which may relate to specific gut functionality. Lipidomics reveals unique changes in eicosanoids synthesis in centenarians, whom despite having high levels of pro-inflammatory markers; also exhibit a well-defined anti-inflammatory network. Our metabonomics and lipidomics results underpin profound differences in the longevity phenotype, where dynamics of the interaction between intestinal microbiota and the host, and activation of specific lipid mediator mechanisms with a balanced network among accumulation of inflammatory response and an efficient anti-inflammatory cascade are much more pronounced in centenarians.

Future research efforts are needed to determine how nutritional and medical management, linked to system biology approaches will help not only guide personalized management programs, but aid in the understanding on specific requirements for non-responders. It can be forecasted that integrative system biology approaches, together with the multifactorial origins of the age related disorders, would help delineate different behavioral and response phenotypes, with tailored nutritional and exercise programs aims at healthy ageing

References

1. Tiihonen, K., Ouwehand, A. C. & Rautonen, N. Human intestinal microbiota and healthy ageing. Ageing Res Rev 2010;9:107-116.

2. Biagi, E., Candela, M., Turroni, S., Garagnani, P., Franceschi, C. & Brigidi, P. Ageing and gut microbes: perspectives for health maintenance and longevity. Pharmacol Res 2013;69:11-20.

3. Rayner, C. K. & Horowitz, M. Physiology of the ageing gut. Curr Opin Clin Nutr Metab Care 2013;16:33-38.

4. Lovat, L. B. Age related changes in gut physiology and nutritional status. Gut 1996;38:306-309.

5. Martin, F. P., Collino, S., Rezzi, S. & Kochhar, S. Metabolomic applications to decipher gut microbial metabolic influence in health and disease. Front Physiol 2012;3:113.

6. Rehman, T. Role of the gut microbiota in age-related chronic inflammation. Endocr Metab Immune Disord Drug Targets 2012;12:361-367.

7. Franceschi, C., Capri, M., Monti, D. *et al.* Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev 2007;128:92-105.

8. Martin, F. P., Sprenger, N., Yap, I. K. *et al.* Panorganismal Gut Microbiome-Host Metabolic Crosstalk. J Proteome Res 2009;8:2090-2105.

9. Nicholson, J. K., Holmes, E. & Wilson, I. D. Gut microorganisms, mammalian metabolism and personalized health care. Nat Rev Microbiol 2005;3:431-438.

10. Collino, S., Montoliu, I., Martin, F. P. *et al.* Metabolic signatures of extreme longevity in northern italian centenarians reveal a complex remodeling of lipids, amino acids, and gut microbiota metabolism. PLoS ONE 2013;8:e56564.

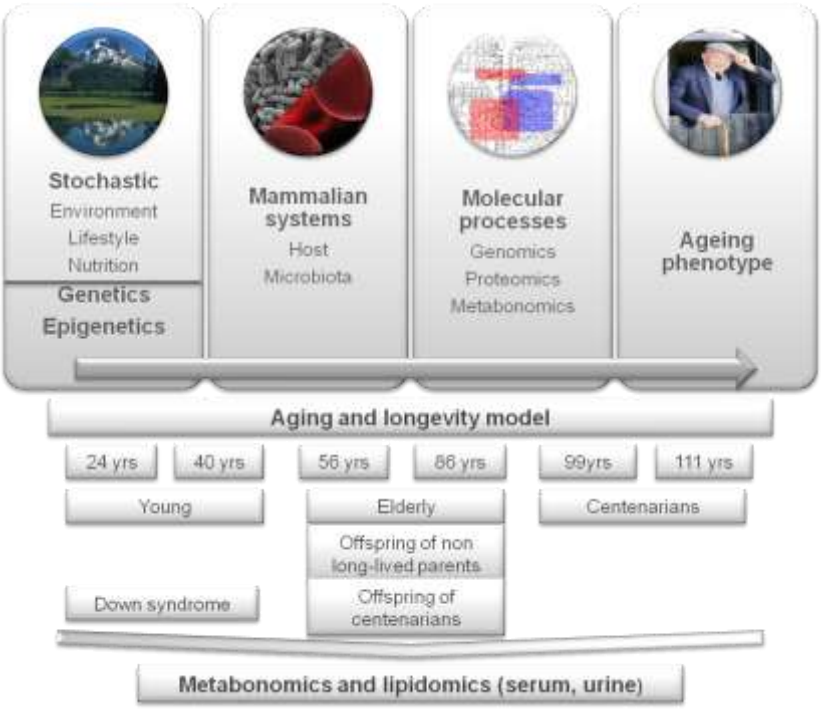

Fig. 1. The aging mosaic and composition of studies human aging cohort. Aging is a complex interaction of genetics, biochemistry, and physiology factors affecting all organs in the body. Using LC-MS/MS and ^{1}H-NMR metabonomics approach in a powerful human aging model we depicted the metabolic blueprints of healthy aging.

"MICROBIOTA AND PROBIOTICS IN OBESITY: A NMR-BASED METABOLOMIC APPROACH"

In a recent editorial appeared in the New England Journal of Medicine, Joseph Loscalzo brilliantly highlighted that *"The Galtonian distinction between the influence of genetics and environment on phenotype is now widely recognized as an overly simplistic dichotomy. Genes and environmental factors interact in myriad ways to modulate and modify the biology of all living organisms, challenging the notion that these two principal determinants of phenotype can ever truly act independently of each other. Environmental exposures and experiences can have a direct influence on the expression of genes through epigenetic processes or on the function of gene products through post-translational modification. Likewise, genetic factors influence the consequences of environmental exposures or stresses on the organism"* (Loscalzo 2013).

Obesity is the prototype of a condition deriving from alterations in the multifactorial context depicted so elegantly by Loscalzo. The condition arises from the interplay among a wide range of variables, including genetic predisposition, nutritional habits and lack of physical activity, set within a social, cultural and environmental landscape (Bleich 2008). Obesity-susceptibility loci have been identified and confirmed across diverse populations (Lu 2013). Accumulating evidence suggests that pre- and postnatal nutritional environment crucially "programs" early growth and the long-term risk of developing obesity, mainly through epigenetic mechanisms (Duque-Guimarães 2013). Animal and human data also demonstrate that phylogenic changes occur in the gut microbiota composition in obese versus lean individuals, and suggest that the count of specific bacteria is inversely related to fat mass development, diabetes, and/or obesity-associated low levels of inflammation (Delzenne 2011).

In this scenario, a growing interest has converged on the interrelationship among the triad - host (genome), diet, and gut microbiota - as central players in obesity. All three elements are inextricably linked, integrating and mechanistically conflating genetic and environmental determinants of phenotype.

The recognition of human beings as "superorganism" entertaining close symbiotic relationships with microbial ecosystems imposes the adoption of comprehensive analytical approaches to capture the dynamic interaction(s) among diet, microbiota and human host (Calvani 2013).

The combination of different "omic" sciences [e.g., (meta)genomics, microbiomics, trascriptomics, proteomics, and metabolomics] is receiving considerable interest in obesity research. Sophisticated high-throughput analytic platforms have recently been developed to characterize the phenotype of obese individuals from their genetic background to the nearly complete repertoire of molecules representing crucial metabolic processes of the human-microbe hybrid (Kurland 2013).

In particular, NMR-based metabolic profiling of biological fluids has emerged as a valuable tool to investigate the metabolic derangements associated with obesity development and progression both in animals and humans (Calvani 2010, Boulangé 2013, An 2013, Xie 2012). The characterization of the metabolic phenotype in biological fluids may allow monitoring the metabolic effects elicited by specific weight-loss strategies – e.g., nutritional approaches, including diet and/or supplementation with probiotics, prebiotics or synbiotics, and bariatric surgery – in obese subjects (Laferrère 2011, Dewulf 2012, Friedrich 2012).

Current challenges and future opportunities of metabolomics in the field of obesity research will be discussed.

Loscalzo J. Gut microbiota, the genome, and diet in atherogenesis. N Engl J Med. 2013 Apr 25;368(17):1647-9. doi: 10.1056/NEJMe1302154.

Bleich, S.; Cutler, D.; Murray, C.; Adams, A. Why is the developed world obese? Annu. Rev. Public Health 2008, 29, 273 − 95.

Lu Y, Loos RJ. Obesity genomics: assessing the transferability of susceptibility loci across diverse

populations. Genome Med. 2013 Jun 28;5(6):55. [Epub ahead of print]
Duque-Guimarães DE, Ozanne SE. Nutritional programming of insulin resistance: causes and consequences.Trends Endocrinol Metab. 2013 Jun 18. doi:pii: S1043-2760(13)00088-X. 10.1016/j.tem.2013.05.006. [Epub ahead of print]
Delzenne NM, Cani PD. Interaction between obesity and the gut microbiota: relevance in nutrition. Annu Rev Nutr. 2011 Aug 21;31:15-31. doi: 10.1146/annurev-nutr-072610-145146.
R. Calvani, A. Miccheli, F. Landi, M. Bossola, M. Cesari, C. Leeuwenburgh, C. C. Sieber, R. Bernabei, E. Marzetti, 2013. Current nutritional recommendations and novel dietary strategies to manage sarcopenia, J. Frailty Aging 2,38-53.
Kurland IJ, Accili D, Burant C, Fischer SM, Kahn BB, Newgard CB, Ramagiri S, Ronnett GV, Ryals JA, Sanders M, Shambaugh J, Shockcor J, Gross SS. Application of combined omics platforms to accelerate biomedical discovery in diabesity. Ann N Y Acad Sci. 2013 May;1287(1):1-16. doi: 10.1111/nyas.12116. Epub 2013 May 9.
Calvani, R.; Miccheli, A.; Capuani, G.; Tomassini, M. A.; Puccetti, C.; Delfini, M.; Iaconelli, A.; Nanni, G.; Mingrone, G. Gut microbiome-derived metabolites characterize a peculiar obese urinary metabotype. Int. J. Obes. 2010, 34 (6), 1095 − 1098.
Boulangé CL, Claus SP, Chou CJ, Collino S, Montoliu I, Kochhar S, Holmes E, Rezzi S, Nicholson JK, Dumas ME, Martin FP. Early Metabolic Adaptation in C57BL/6 Mice Resistant to High Fat Diet Induced Weight Gain Involves an Activation of Mitochondrial Oxidative Pathways. J Proteome Res. 2013 Mar 8. [Epub ahead of print]
An Y, Xu W, Li H, Lei H, Zhang L, Hao F, Duan Y, Yan X, Zhao Y, Wu J, Wang Y, Tang H. High-Fat Diet Induces Dynamic Metabolic Alterations in Multiple Biological Matrices of Rats. J Proteome Res. 2013 Jun 27. [Epub ahead of print]
Xie B, Waters MJ, Schirra HJ. Investigating potential mechanisms of obesity by metabolomics. J Biomed Biotechnol. 2012;2012:805683. doi: 10.1155/2012/805683. Epub 2012 May 16. Review.
Laferrère B, Reilly D, Arias S, Swerdlow N, Gorroochurn P, Bawa B, Bose M, Teixeira J, Stevens RD, Wenner BR, Bain JR, Muehlbauer MJ, Haqq A, Lien L, Shah SH, Svetkey LP, Newgard CB.
Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. Sci Transl Med. 2011 Apr 27;3(80):80re2. doi: 10.1126/scitranslmed.3002043.
Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut. 2012 Nov 7. [Epub ahead of print]
Friedrich N, Budde K, Wolf T, Jungnickel A, Grotevendt A, Dressler M, Völzke H, Blüher M, Nauck M, Lohmann T, Wallaschofksi H. Short-term changes of the urine metabolome after bariatric surgery. OMICS. 2012 Nov;16(11):612-20. doi: 10.1089/omi.2012.0066. Epub 2012 Oct 24.

LONG TERM CONSEQUENCES OF A GUT BARRIER DAMAGE

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In humans, the gastrointestinal tract represents the largest body surface in contact with the environment. The mucosal barrier is positioned at the body-environment interface. It plays a crucial role in selecting luminal factors allowed to enter in the body (e.g., fluids, electrolytes, and nutrients), but at the same time it prevents the access of potentially dangerous factors (e.g., pathogens and their toxins). The mucosal barrier is composed by the intestinal microbial flora, now referred to as microbiota, the mucus layer, the epithelial cells and the intercellular tight junctions (TJ) positioned between them. (1) TJ are the key molecules involved in the control of paracellular permeability. They comprise a complex protein system organized in the transmembrane proteins occludin and claudins interacting with zonula occludens (ZO) proteins which bind to the actin cytoskeleton. Contraction of actin leads to the opening of TJ allowing increased permeability to electrolytes and small molecules. (2)

Several diseases can directly or indirectly affect the function and or the integrity of the intestinal epithelial barrier. Some examples include gastrointestinal infections, inflammatory bowel diseases, celiac disease, liver failure, diabetes and metabolic syndrome. Evidence suggests that following recovery from acute phases of these intestinal disorders symptoms may persist and in several instances they can either fulfil the diagnosis for the irritable bowel syndrome (IBS) or resemble IBS. Examples include the occurrence of IBS in IBD in remission or the development of IBS symptoms following an acute bout of infectious gastroenteritis. The persistence of increased intestinal permeability and low grade intestinal inflammation along with changes in intestinal microbiota have been identified in these patients believed to be central in symptom pathogenesis. Here we will briefly report this evidence.

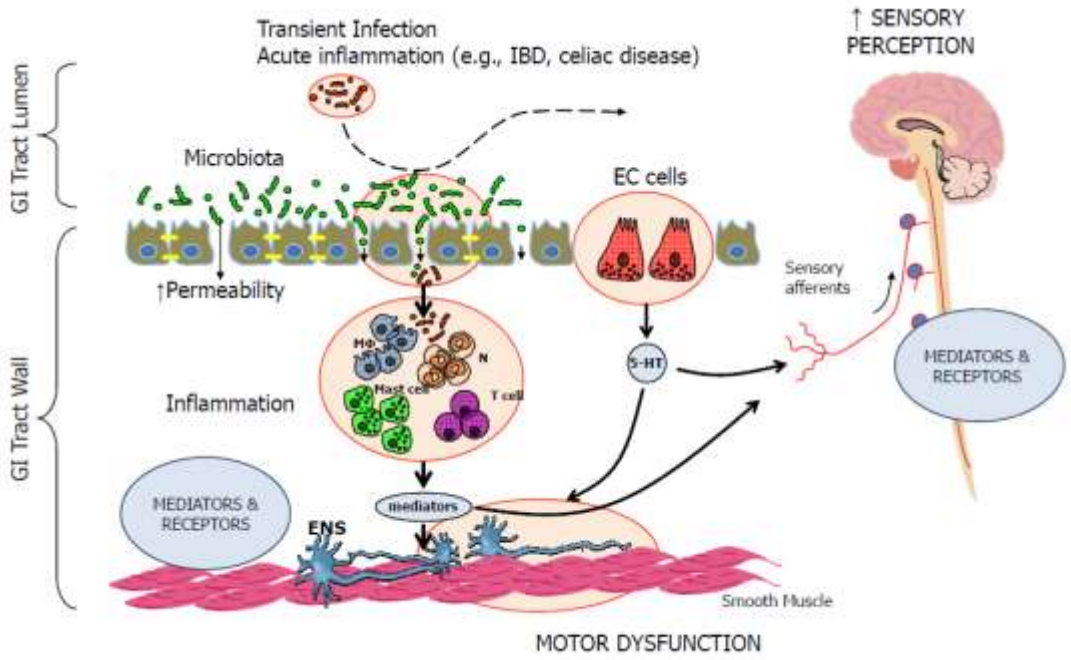
Post-infectious IBS. Following a bout of infectious gastroenteritis, a small but significant proportion of subjects go on to develop post-infectious IBS, dyspepsia or both (for review, see 3). A recent systematic review and meta-analysis identified that the pooled incidence for IBS development after infectious gastroenteritis was about 10% (95% CI: 9.4-85.6) (4). Recent studies focused on a waterborne outbreak of gastroenteritis involving around half of the population of the small town of Walkerton in Canada. Residents of this community developed gastroenteritis as a consequence of accidental contamination of the municipal water supply with *Escherichia coli* O157:H7 and *Campylobacter jejuni.* Two years later, 36% of those infected developed IBS. Similarly to previous smaller sample size reports risk factors for the development of IBS symptoms included: female gender and the severity of the initial illness, (ie, weight loss, bloody diarrhea and long duration of the infectious episode) (5, 6). Previous work also demonstrated the importance of psychological co-morbidity for the development of IBS postinfection (7). The mechanisms underlying the persistence of symptoms remain poorly defined, but may include genetic factors, particularly related polymorphisms for genes involved in pro-inflammatory cytokine production

[interleukin(IL)-6] and host-bacteria interactions (one of the bacterial recognition receptor, toll-like receptor 9) as well as molecules involved in the control of mucosal permeability (tight junctions) (8). Intestinal tissue analysis identified the persistence of low-grade immune activation and enteroendocrine abnormalities. Spiller et al. showed increased numbers of intraepithelial lymphocytes, lamina propria T cells, calprotectin-positive macrophages and enteroendocrine cells who failed to decline in patients who develop postinfectious-IBS (9). Others have reported an increased number of mast cells in the terminal ileum (10), while the number of these cells resulted normal in the rectum (10, 11).

IBS-like symptoms in patients with IBD, microscopic colitis and celiac disease. IBS-like symptoms develop in about 50% of patients with microscopic colitis (12) and in 33-57% of patients with ulcerative colitis or Crohn's disease in remission (13, 14). These figures are substantially higher than the expected prevalence of IBS in the general population and support the assumption that human low-grade mucosal inflammation could play a role on IBS symptom development. Interestingly, IBS-like symptoms in patients with IBD were significantly associated to the presence of psychological impairment and poor quality of life (14, 15). IBD is thought to result from inappropriate, ongoing activation of the mucosal immune system driven by the intestinal microbiota. This aberrant response is facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system, likely influenced by genetic factors (16). A recent study assessed the occurrence of IBS-like symptoms in patients with IBD in remission and the role of low grade intestinal inflammation and alterations in the integrity of mucosal permeability. IBS-like symptoms were present in 35.4 and 38% of CD and UC patients, respectively. Paracellular permeability was significantly increased in both quiescent IBD with IBS-like symptoms and IBS compared with quiescent IBD without IBS-like symptoms ($p<0.01$, respectively) or controls ($p<0.01$, respectively). Significantly lower expression of ZO-1 and α-catenin was detected in IBS and quiescent IBD with IBS-like symptoms. IELs and TNF-α were significantly increased in quiescent IBD with IBS-like symptoms, but not in IBS. These results suggest that in quiescent IBD, IBS-like symptoms related to persistent subclinical inflammation associated with increased colonic paracellular permeability (17).

Similarly to IBD and microscopic colitis a high rate of IBS-like symptoms has been reported in patients with celiac disease on a gluten free diet and after apparent resolution of mucosal damage and immune activation (18, 19). Similarly, patients with non celiac gluten sensitivity showed increased small bowel and large bowel permeability compared with controls (20) Whether changes in mucosal permeability are involved in the symptom persistence in these patients remains to be determined.

FIGURE 1. Mechanisms involved in long term consequences of gut barrier damage



REFERENCES

1. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009;9:799-809.
2. Nusrat A, Turner JR, Madara JL. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G851-7.
3. Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology 2009;136:1979-1988.
4. Thabane M, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. Aliment Pharmacol Ther 2007;26:535-44.
5. Marshall JK, Thabane M, Garg AX, Clark W, Meddings J, Collins SM; WEL Investigators. Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. Aliment Pharmacol Ther 2004;20:1317-22.
6. Marshall JK, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM; Walkerton Health Study Investigators. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. Gastroenterology 2006;131:445-50.
7. Dunlop SP, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology 2003;125:1651-9.
8. Villani AC, Lemire M, Thabane M, Belisle A, Geneau G, Garg AX, Clark WF, Moayyedi P, Collins SM, Franchimont D, Marshall JK. Genetic risk factors for post-infectious irritable bowel syndrome following a waterborne outbreak of gastroenteritis. Gastroenterology 2010;138:1502-13.
9. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804-11.
10. Wang LH, Fang XC, Pan GZ. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. Gut 2004;53:1096-101.

11. Dunlop SP, Jenkins D, Spiller RC. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. Am J Gastroenterol 2003;98:1578-1583.
12. Isgar B, Harman M, Kaye MD, Whorwell PJ. Symptoms of irritable bowel syndrome in ulcerative colitis in remission. Gut 1983;24:190-192.
13. Limsui D, Pardi DS, Camilleri M, Loftus EV Jr, Kammer PP, Tremaine WJ, Sandborn WJ. Symptomatic overlap between irritable bowel syndrome and microscopic colitis. Inflamm Bowel Dis 2007;13:175-181.
14. Simrén M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol 2002;97:389-396.
15. Farrokhyar F, Marshall JK, Easterbrook B, Irvine EJ. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. Inflamm Bowel Dis 2006;12:38-46.
16. Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 2006;101:1288-1294.
17. Vivinus-Nébot M, Frin-Mathy G, Bzioueche H, Dainese R, Bernard G, Anty R, Filippi J, Saint-Paul MC, Tulic MK, Verhasselt V, Hébuterne X, Piche T. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. Gut. 2013 Jul 22. [Epub ahead of print]
18. O'Leary C, Wieneke P, Buckley S, O'Regan P, Cronin CC, Quigley EM, Shanahan F. Celiac disease and irritable bowel-type symptoms. Am J Gastroenterol 2002;97:1463-7.
19. Midhagen G, Hallert C. High rate of gastrointestinal symptoms in celiac patients living on a gluten-free diet: controlled study. Am J Gastroenterol 2003;98:2023-6.
20. Vazquez-Roque MI, Camilleri M, Smyrk T, Murray JA, Marietta E, O'Neill J, Carlson P, Lamsam J, Janzow D, Eckert D, Burton D, Zinsmeister AR. Gastroenterology. 2013 May;144(5):903-911.e3. doi: 10.1053/j.gastro.2013.01.049. Epub 2013 Jan. A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function.

LACTOBACILLUS RHAMNOSUS GG-DERIVED FACTORS PROTECT HUMAN COLONIC SMOOTH MUSCLE FROM PATHOGEN LIPOPOLYSACCHARIDE-INDUCED DAMAGE

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Objective: Probiotics-derived factors released from living microorganisms culture have been shown to exert beneficial properties on pathogen-induced oxidative inflammation suggesting that probiotics supernatants could be a rich source of new-antipathogenic compounds. Pathogen Gram negative-derived lipopolysaccharide (LPS) produced an inflammatory NFkB-mediated cytopathic oxidative imbalance in human colonic smooth muscle cells (SMC) that persists after LPS-washout and contributes to SMC morpho-functional alterations. Aim of this study was to evaluate if supernatants harvested from LGG cultures protect human SMC from LPS-induced myogenic damage.
Methods: *Lactobacillus rhamnosus GG* (ATCC 53103 strain) was grown in MRS medium at 37°C in sealed bottles. Samples were collected from bacterial cultures in middle and late exponential phases as well as in early and late stationary phase. Bacterial cells were eliminated from the samples by a centrifugation step followed by filtration of the supernatant through a 0.20 µm filter. Samples were stored at -20°C before use. Highly pure human SMC culture was then exposed for 24h to highly purified LPS (1µg/ml) obtained from a pathogen strain of *Escherichia coli* (O111:B4) in the absence and presence of supernatants harvested during the different phases of growth of LGG cultures. Supernatants effects were evaluated on LPS-induced SMC morpho-functional alterations. Data are expressed as mean±SE, $p<0.05$ considered significant.
Results: LPS induced persistent significant ($p<0.05$) 14.6%±0.59 cell shortening and 47.2%±7.8 decrease in acetylcholine-induced contraction of human colonic SMC. These LPS effects were not reverted by the sole LGG culture medium or by supernatants harvested during the middle exponential phase of LGG cultures. In turn LPS-induced morpho-functional SMC alterations were inhibited by supernatants harvested during the late exponential or stationary growth phases. Supernatants from the late exponential phase inhibited LPS-induced cell shortening by 27.8%±1.0 and that from the late stationary phase induced a further inhibition that reached 41.2%±2.14. LPS effects on Ach-induced contraction were inhibited by 33.6%±2.6 in the presence of supernatants from the late exponential phase with no further effects observed with supernatants harvested during the late stationary phase.
Conclusions: LGG secreted products are able to directly protect human colonic smooth muscle from LPS- induced myogenic damage providing novel insights about the possibility that LGG-derived products could reduce the risk of progression to a post-infective motor disorder.

INTESTINAL MUCUS ALTERATIONS INDUCED BY A CHRONIC STRESS ARE LINKED TO A SHIFT IN O-GLYCOSYLATION RATHER THAN TO MUCIN EXPRESSION CHANGES: PREVENTION BY A PROBIOTIC TREATMENT

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Objective: In contrast to well-known disruption of intestinal epithelial barrier in irritable bowel syndrome (IBS), changes in structure and physical properties of the mucus layer remain unknown. Despite efficacy of probiotics on IBS symptoms, their influence on the mucus barrier integrity has not been yet investigated.
Methods: Thus, we aimed at evaluating in rats whether (i) a chronic stress (Water Avoidance Stress, WAS) modifies the number of intestinal goblet cells, Muc2 expression, the biochemical structure of mucin O-glycans, as well as the mucus layer morphology, and (ii) a probiotic treatment with *Lactobacillus farciminis* prevents these alterations.
Results: WAS did not modify neither the number of goblet cells nor Muc2 expression in the colon and ileum. In contrast, WAS strongly affected O-glycosylation of ileal and colonic mucins, as reflected by the appearance of more elongated polylactosaminic O-glycan structures. Mucin sialylation and sulfation remained unchanged. Scrapped mucus of stressed rats was flattened and less cohesive *vs.* controls, as shown by Atomic Force Microscopy. Such structural and morphological modifications influence physico-chemical interactions within the mucin fiber network, negatively impacting the mucus barrier integrity. The probiotic treatment prevented the WAS-induced changes in O-glycan structure and partially restored the mucus gel morphology.
Conclusions: In conclusion, we have shown for the first time that a chronic stress in rats induces changes in O-glycan structure and mucus layer morphology without affecting mucin expression. The probiotic treatment with *L. farciminis*, by preventing these changes, contributes to the enhancement of the mucus barrier function.

EVALUATION OF THE LACTOBACILLUS FERMENTUM IN THE DCA EXPERIMENTAL MODEL OF IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is a high prevalent functional gut disorder. Since disappointing results are frequently obtained with the available pharmacological treatments, the use of alternative medicines is becoming attractive options for many patients. The aim of the study was to evaluate the effects of *Lactobacillus fermentum* in an experimental model of irritable bowel syndrome (IBS) in rats induced by intracolonic administration of deoxycholic acid (DCA). Male Sprague Dawley rats (240–320 g) were administered DCA once daily on 3 consecutive days, and then divided into the different treated experimental group (n=10), which received orally the probiotic at 10^9 CFU per day; a non IBS and an untreated control IBS group. One and two weeks after, abdominal withdrawal reflex to colorectal distension (CRD) was semiquantitatively scored, and those rats receiving DCA showed higher values in comparison with non-IBS rats. After one or two weeks of treatment, the treated group showed reduced CRD score values than IBS control. Also, we evaluated the referred pain with Von frey filaments; the IBS control showed higher values in comparison with non-IBS and treated group. After two weeks of treatment, all rats were sacrificed and the expression of different markers evaluated in the colonic tissue by qPCR. The results revealed that *Lactobacillus fermentum* ameliorated the increased expression of COX-2, as well as that related with the toll like receptors TLR3 and TLR4. In addition, *Lactobacillus fermentum* was able to significantly counteract the reduced expression of the mucins, MUC-2 and MUC-3. In conclusion, *Lactobacillus fermentum* was able to reduce the visceral hypersensitivity as well as to improve the altered immune response clearly involved in IBS.

PROBIOTIC-ENRICHED FOODS AND DIETARY SUPPLEMENTS CONTAINING SYNBIO® POSITIVELY AFFECT BOWEL HABITS OF HEALTHY ADULTS

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Objective: The present study was a randomised, double-blind, placebo-controlled, parallel group study assessing the effect of daily consumption of the combination of two probiotic bacterial strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, named SYNBIO®, by probiotic-enriched foods (dairy and not-dairy foods) or by dietary supplement (capsules) on the bowel habits of healthy adults.
Methods: The study was performed with a 4-week run-in period followed by a 12-week intervention period. Primary and secondary outcomes assessed by a questionnaire gave the overall assessment of bowel well-being, a self-administration of Psychological General Well-Being Index (PGWBI) estimated the health-related quality of life and gastrointestinal tolerance was determined with Gastrointestinal Symptom Rating Scale (GSRS). Support Vector Machine models for the classification problem have been used to validate the total outcomes of the bowel well-being.
Results: SYNBIO® consumption positively affect bowel habits of volunteers consuming either probiotic-enriched foods or probiotic capsules. The intestinal regularity, the stool volume and the PGWBI global score were significantly improved in the supplemented groups respect to controls. Support Vector Machine models validated the accuracy of these features. The persistence of strains in the gastrointestinal tract of people were confirmed by an high percentage of recovery of probiotic bacteria from the faeces of subjects for each supplemented group.
Conclusions: The results indicate that the probiotic combination SYNBIO® is effective in colonising the gut by either functional foods or capsules. The persistence in the intestinal tract favoured the intestinal regularity and contributed to maintain and improve intestinal well-being of consumers.

PROBIOTICS CAN IMPROVE GUT-RELATED ANXIETY AND HEALT-RELATED QUALITY OF LIFE IN IBS PATIENTS WITH A DIARRHEA COMPONENT: A MULTICENTER, DOUBLE BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

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Objective: To evaluate the dose and time-dependent effect of a commercial probiotic formula (IBS.31, AB-Biotics, Spain) in the gut-specific anxiety (GSA) and health-related quality of life (HRQOL) of IBS patients, using validated questionnaires (VSI and IBSQOL, respectively)
Methods: Multicenter, randomized, double blind, placebo-controlled clinical trial. 84 consecutive IBS-patients with a diarrhea component (Rome-III criteria, 63% female; age range 20-70 yrs.) were randomly allocated to receive one capsule daily containing: a) $1x10^{10}$ cfus/capsule (n=28), b) $2x10^{9}$ cfus/capsule (n=27), or c) placebo (n=29), for 6 weeks. At baseline, 3 and 6 weeks patients filled the IBSQOL and VSI questionnaires.
Results: No diferences were observed at baseline between groups. At 3 weeks, a statistically significant improvement in HRQOL vs. placebo was achieved in the high dose group, but not in the low dose group, and no statistically significant changes were observed in GSA. At six weeks, statistically significant improvements vs. placebo were observed for the high and low probiotic doses, both for GSA and HRQOL (post-hoc $P < 0.05$ for all cases), without differences between probiotic doses. Moreover, changes in HRQOL and GSA were higly correlated ($r=0.69$; $P < 0.001$). No adverse events were reported, and there were no differences in the number of patients discontinuing from the study between groups.
Conclusions: A higher probiotic dose might achieve an earlier improvement, but after 6 weeks both doses achieved the same effect. Besides, improvement in HRQOL and GSA were highly correlated, consistently with a key involvement of the brain-gut axis.

+TRADITION OF USE: THE MAIN QUESTION

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Botanical preparation have traditionally been used in European Union (EU) for maintaining or optimizing health as well as for the preventing and treatment of disease. Their medicinal use has evolved more recently, and the traditional use, based on bibliographical or expert evidence, if is available for a period of at least 30 years, allow a simplifies registration as traditional herbal medicinal product.
The use as food need to be proven under the new health claims directives: randomised controlled trials, while evidence from traditional use is not considered.
The manufacturers of botanical food supplements must observe critical and essential requirements in order to verify and assure the safety of their products.
In the recent years, several publications have examined the safety assessment of botanicals, explaining the data required to perform such risk assessment. But in all these reports, the traditional use of the plants or plant preparations are often ignored.
Communication on the properties of plants is an important factor. For non medicinal use the communication is focused on nutritional and/or health benefits. For botanical foods supplements it is therefore essential that such communication is possible under the form of a claim.
The 1924/2006 EU directive cover the use of plants as food supplements, in which the article 13.4 display the health claims for botanicals. These claims must be pre-approved by EFSA (European Food Safety Authority) which utilize a assessment methodology that not consider tradition of use as valid proof.
This presentation wants to provide a framework for data collection in support of traditional use for botanicals, explores the various systems proposed and aims to identify the critical and essential requirements for manufactures, and describes how traditional use can be combined with the applicable legislation on botanical food supplements in EU to ensure meaningful information to consumers

References

R .Anton et al. 2012.2012 Traditional Knowledge for the assessment of health effects for botanicals- A framework for data collection. EFFL 2, 74-80

R. Anton et al. 2012. The role of Traditional Knowledge in the safety assessment of Botanical Food Supplements- Requirements for manufactures. EFFL, 5, 241-250

QUALITY CONTROL OF VEGETABLE MATRICES: THE CONTRIBUTION OF NEW APPROACHES AND TECHNOLOGIES

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The objective of this lecture is to provide a short overview about the evolution of quality control of matrix of vegetable origin involved with food supplement on the basis of the most recent achievements in this research field, in particular in view of the approaches developed for the so-called omics sciences and metabolomics
The leading concept is that quality must be controlled with quick, simple, cheap and automated (if possible) methods. A survey of the approaches at present available and of the possibilities offered by the most recent technologies in sample preparation and analysis and statistical methods will therefore be presented. Some examples ranging from simple problems of plant identification to detect possible adulteration to the evaluation of bioequivalence of complex plant extracts will be discussed. In particular, the example will concern discrimination of plant material belonging to different species of the same genus (*Cimicifuga racemosa*) by TLC, detection of adulteration on essential oils of different origin and economical value (*Mentha x piperita*) by quantitative GC-MS, determination of phytoequivalence of a plant extract (*Ginko biloba*) by a NMR fingerprinting approach, definition of the representative composition of a Natural Complex Sample (NCS) (absolute of *Populus nigra* buds) by derivatization-GC-FID and GC-MS, HPLC-UV, HPLC-MS.
The contribution of advanced approaches and technologies deriving from basic research is mandatory to satisfy the ever increasing request of controls to guarantee consumers about safety, genuineness, origin and against possible adulteration of ingredients of vegetable origin for food supplements.

PROBIOTICS IN RECURRENT URINARY TRACT INFECTIONS IN WOMEN

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Typically, an urinary tract infection (UTI) in the female patient results from colonization of the vagina and urethra with fecal flora and subsequent ascent into the bladder. Recurrent UTI are one of the most common health problems for women: it has been evaluated that the lifetime risk of contracting a UTI in females is over 50 % and that about 25% of women diagnosed with a primary UTI will suffer a recurrence [1,2]. There is no single definition of recurrent UTI to date but we can accept that given by Hooton[3] who considers recurrent UTIs as symptomatic infections that follow adequate treatment and proven resolution of a previous infection. Most recurrences have been shown to occur within 2–3 months of initial infection and the majority are thought to be caused by reinfection rather than relapse[3]. *Escherichia coli* is the leading uropathogen isolated (80%) in acute and recurrent UTIs in women, followed by *Staphylococcus saprophyticus* (10%–15%). Other potential but less common uropathogens include *Enterococcus, Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas,* and *Serratia*. Community acquired multidrug-resistant *Staphylococcus aureus* remains quite rare[4].

Clinically, predominant risk factors for recurrent UTIs vary in different age groups. It is a common misconception that voiding patterns, personal hygiene, and prolonged exposure to moisture promote the development of UTIs. In addition, body mass index, delayed voiding, pericoital voiding patterns, urinary frequency, wiping patterns, douching, use of tight clothing were not found to be risk factors associated with recurrent UTIs. On the contrary, the top three reported behavioral risk factors for recurrent UTIs in young women show sexual basis, as recent intercourse, new partners, and the use of spermicide[2]. UTIs remain common throughout a woman's lifetime but nonbehavioral risk factors, such as urinary incontinence, history of UTI before menopause, pelvic organ prolapse and incomplete bladder emptying play a larger role in recurrent UTIs in postmenopausal women[5]. Anyway, one of the most relevant risk factor of recurrent UTIs remains an abnormal vaginal microbial flora since the microbial species that inhabit the vaginal tract play an important role in the maintenance of health and prevention of infections.

Unfortunately, well-established interdisciplinary guidelines for the management of recurrent UTIs are lacking: treatment of this disease remains complex in clinical practice and the use of antibiotics does not appear as the optimal solution. Indeed, there may be many reasons to avoid antibiotics for prevention of UTIs: multiple drug allergies and sensitivities, patient acceptance, compliance, and comorbidities. But the most relevant is the risk of development of antimicrobial resistance[4]. As a consequence, other measures such as cranberry products, local estrogen replacement and, with high interest, probiotics have been taken in consideration.

Around 50 microbial species inhabit the vagina - compared to the 800 species inhabiting the gut - but the species present in the vaginal mucosa vary between pre- and post-menopausal woman. Generally, the dominating species of a healthy premenopausal woman belong to the *Lactobacillus* genus, the most common of which are *L. iners*, *L. crispatus*, *L. gresseri* and *L. jensenii*. Many factors such as estrogen changes, vaginal pH and glycogen content can affect the colonization of lactobacilli in the vaginal mucosa. The interest in the potential role of the vaginal flora began in the 1970s with the results of Bruce and coworkers[6] who found that women suffering from recurrent UTIs showed low lactobacilli counts. The protective effect of these microbial species depends on various factors: i) the ability to maintain a low vaginal pH (≤ 4.5); ii) the production of hydrogen peroxide and other antibacterial materials such as bacteriocins; iii) the production of surfactants that inhibit pathogen adherence; iv) the ability to prime host defenses such as macrophages, cytokines and others[7]. Recently, the immunomodulating role of probiotics has been emphasized by the study of the Toll-like receptors (TLRs) which functions are to activate the immune system to check the

presence of microorganism. Indeed, TLRs are activated by some probiotics and leads to the production of various cytokines, chemokines and effector molecules, that may culminate in clearance of uropathogen from the urinary tract[8].

All these facts form the rational basis for the proposed use of probiotics in order to normalize the vaginal flora and, by this way, to strengthen the barrier against the invasion by bacteria of intestinal origin. Actually, *not all probiotics may be probiotic*[9] and it should be emphasized that for a successful prevention of UTIs it is necessary that the used lactobacilli species and strains possess the aforementioned proprieties, since not all the strains has the same ability to colonize the host, to inhibit pathogen binding and growth, and to create a balanced flora that resists spermicidal killing. Most of commercially available probiotics contain strains that have been selected for their intestinal activity and not necessarily they possess good activity in the vaginal environment. Following Reid & Bruce[10], a number of commercial strains were found to lack at least one of the important properties required of a urogenital probiotic, whereas good clinical potential for the urogenital tract is shown by *L. crispatus* CTV-05, *L. rhamnosus* GR-1, and *L. fermentum* RC-14.

The ability of lactobacilli to colonize the vaginal mucosa depends on the route of delivery and extent of adhesion to vaginal epithelial cells. Vaginally inserted capsules are an effective way of introducing or boosting the local Lactobacillus content. With vaginal formulations, local colonization by these strains was noted after 3 days and continued to be evident at 12 days[11], whereas evidence of fecal and vaginal colonization by some strains was observed after 14 days of oral administration[12]. However, while these studies determined the presence of the strains, most did not quantify the extent of colonization, which may be an important component of the antibacterial effects of lactobacilli[13].

Very few clinical trials have tested probiotics for prevention of recurrent UTI, and these studies are small in size and a recent meta-analysis was able to collect just 6 acceptable published studies[14]. The first one was performed by Reid and coworkers[15] in 1992, who treated 41 adult women with acute lower urinary tract infections with vaginal suppositories containing *L. rhamnosus GR-1* and *L. fermentum* B-54, after eradication by trimethoprim/sulfamethoxazole; the treatment resulted in a recurrence rate of 21% in probiotic treated patients, while in patients given sterilized skim-milk suppositories the recurrence rate was 47%. Some years later a similar study was conducted by Baerheim and coworkers using *L. casei* in vaginal suppositories but no significant difference was found between treated and controls. More recently, Stapleton and coworkers[16] treated 96 young women with vaginal suppositories containing *L. crispatus* CTV-05 or placebo suppositories by self-administration once daily, for 5 days. Women who received the treatment and achieved a high-level *L. crispatus* vaginal colonization pattern throughout the course of the study had a significant reduction in the risk of recurrent UTI, whereas when this high-level colonization pattern occurred in women who received placebo, it was not protective.

A treatment by oral administration has been proposed by Beerepoot and colleagues[17], who report a comparative effectiveness study of oral capsules containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 twice daily with trimethoprim-sulfamethoxazole, 480 mg, once daily for UTI prevention in postmenopausal women. The study included 252 postmenopausal women who had experienced at least 3 self-reported, symptomatic UTIs in the year prior to enrollment and evaluated the rates of recurrent UTI and antimicrobial resistance. During 12 months of prophylaxis, the mean number of clinical recurrences in the trimethoprim-sulfamethoxazole group was 2.9 in comparison with 3.3 in the lactobacilli group. No significant differences in the adverse effects were seen between the 2 groups. Thus, following the authors, lactobacilli therapy was clearly second-best to trimethoprim-sulfamethoxazole for the primary outcome of UTI prevention. Nevertheless, the study confirms the efficacy of the tested strains and within the context of preserving the health of the microbiome, antibiotic-sparing approaches to UTI prevention become very appealing.

The use of lactobacilli offers an alternative for use in the prevention of recurrent UTI but further research is needed in this area, using strains of probiotics known to colonize vaginal epithelial cells and inhibit uropathogens, in order to define optimal dosing, duration and administration way.

1. Griebling TL. *Urologic diseases in America project: trends in resource use for urinary tract infections in women*. Urology **173**, 1281–1287 (2005).
2. Scholes D, Hooton TM, Roberts PL, Stapleton AE, Gupta K, Stamm WE. *Risk factors for recurrent UTI in young women*. J Infect Dis **182**, 1177–1182 (2000).
3. Hooton TM. *Recurrent urinary tract infection in women*. Int J Antimicrob Agents **17**, 259–268 (2001).
4. Nosseir SB, Lind LR, M.D., Winkler HA. *Recurrent Uncomplicated Urinary Tract Infections in Women: A Review*. J Women Health **21**, 347-354 (2012).
5. Raz R, Gennesin Y, Wasser J. *Recurrent urinary tract infections in postmenopausal women*. Clin Infect Dis **30**, 152–156 (2000).
6. Bruce AW, Chadwick P, Hassan A, van Cott GF. *Recurrent urethritis in women*. Can Med Assoc J **108**, 973-976 (1973).
7. Waigankar SS, Patel V. *Role of probiotics in urogenital healthcare*. J Midlife Health **2**, 5-10 (2011).
8. Amdekar S, Singh V, Singh DD. *Probiotic Therapy: Immunomodulating Approach Toward Urinary Tract Infection*. Curr Microbiol **63**, 484–490 (2011)
9. Reid G, Dols J, Miller W. *Targeting the vaginal microbiotsa with probiotics as a mean to counteract infections*. Curr Opin Clin Nutr Metabol Care 12, 583-587 (2009)
10. Reid G, Bruce AW. *Selection of* Lactobacillus *strains for urogenital probiotic applications*. J Infect Dis **183** suppl. S77-80 (2001).
11. Gardiner GE, Heinemann C, Bruce AW, Beuerman D, Reid G. *Persistence of* Lactobacillus fermentum *RC-14 and* Lactobacillus rhamnosus *GR-1 but not* L. rhamnosus *GG in the human vagina as demonstrated by randomly amplified polymorphic DNA*. Clin Diagn Lab Immunol **9**, 92-96 (2002).
12. McLean NW, Rosenstein IJ. *Characterisation and selection of a* Lactobacillus *species to re-colonise the vagina of women with recurrent bacterial vaginosis*. J Med Microbiol 49. 543-52 (2000).
13. Barrons R, Tassone D. *Use of Lactobacillus Probiotics for Bacterial Genitourinary Infections in Women: A Review*. Clinical Therapeutics 3, 453-468 (2008)
14. Grin PM, Kowalewska PM, Alhazzan W, Fox-Robichaud AE. *Lactobacillus for preventing recurrent urinary tract infections in women: meta-analysis*. Can J Urol 20, 6607-6614 (2013).
15. Reid G, Bruce AW, Taylor M. *Influence of three-day antimicrobial therapy and lactobacillus vaginal suppositories on recurrence of urinary tract infections*. Clin Ther **14**, 11-16 (1992).
16. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, Yarova-Yarovaya Y, Fiedler T, Cox M, Stamm WE. *Randomized, Placebo-Controlled Phase 2 Trial of a Lactobacillus crispatus Probiotic Given Intravaginally for Prevention of Recurrent Urinary Tract Infection*. Clin Infect Dis 52, 1212–1217 (2011).
17. Beerepoot MAJ, ter Riet G, Nys S, et al. *Lactobacilli vs antibiotics to prevent urinary tract infections: a randomized, double-blind, noninferiority trial in postmenopausal women*. Arch Intern Med **172**, 704-712 (2012).

PROBIOTICS FOR INFANTILE COLIC: A SYSTEMATIC REVIEW OF THE CURRENT EVIDENCE

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Objective: The objectives of this systematic review are to evaluate the efficacy of probiotic supplementation in the reduction of crying time and successful treatment of colic

Methods: Literature searches were conducted of MEDLINE, EMBASE and the Cochrane Central Register of Controlled Trials. Only randomised controlled trials enrolling term, healthy infants with colic were included. A meta-analysis of included trials was performed utilizing the Cochrane Collaboration methodology.

Results: Three trials that enrolled 220 infants met inclusion criteria, of which 209 infants were available for analysis. The studies were assessed as good quality. Lactobacillus reuteri was the only subspecies utilized in the therapeutic intervention. Two of the trials were industry funded. Probiotic supplementation significantly and progressively shortened crying times to 7 days reaching a plateau at three weeks post initiation of therapy [mean difference -56.03 minutes; 95% CI (-59.92, -52.15)]. Similarly, successful treatment of infantile colic was significantly increased with a relative risk (RR) of 0.06; 95% CI (0.01, 0.25) and a number needed to treat of 2.

Conclusions: Our review supports the beneficial effects of probiotic supplementation in infantile colic in predominantly breast fed infants. Larger independent studies in different populations and longer follow up are still required prior to the implementation of a practice change recommendation.

COULD PROBIOTICS STIMULATE IMMUNE RESPONSE IN HUMAN IN VITRO MODEL?

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Objective: We evaluated the capacity of probiotic strains to stimulate colon and immune system cells in an *in vitro* human model searching for an efficient strategy to compare probiotic strains immunomodulatory potential.
Methods: In the co-culture model, T84 human cell line is used to represent the intestinal barrier and freshly isolated human Peripheral-Blood-Mononuclear-Cells (PBMC) are used as immune cells. These two cell types are separated by a semipermeable membrane. Probiotics are co-cultured (10^7 CFU of *Lactobacillus* or *Bifidobacterium*) with T84 cells for 6 and 48 hours and cells and media were screened during 48 hours. Cytokine secretion is evaluated by immunoassays, shift in PBMC's proportion by FACS and gene modulation by PCR.
Results: Amongst the twelve inflammatory cytokines evaluated, few were not stimulated by the the tested probiotics (IL-2, IL-4, IL-5, IL-12 and IFNgamma). Others were induced but did not allow strain discrimination (e.g. IL-1beta peaking at 200-300pg/mL after 12 hours for both strains). Finally, TNF secretion is more stimulated (peaks at 100ng/mL) and longer sustained (more than 15ng/ml after 48 hours) when in contact with *Lactobacillus* in comparison to *Bifidobacterium* (35ng/mL and less than 2,5ng/ml after 24 hours). Amongst PBMC, only T_{reg} sub-population was found significantly modulated by both tested probiotic strains (up to 3-fold increase; $p<0.05$).
Conclusions: Our model proved that a measurable immune response could be elicited by probiotic strains. This model could discriminate probiotic strains by induced inflammatory response. This model could be used as a tool to predict the potential of a probiotic strain to induce an immune response.

ANTI-LISTERIAL AND ANTI-BIOFILM ACTIVITIES OF POTENTIAL PROBIOTIC LACTOBACILLUS STRAINS ISOLATED FROM TUNISIAN TRADITIONAL FERMENTED FOOD

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Objective: A wide range of lactic acid bacteria (LAB) produce bacteriocins, which were essentially active against the food-borne pathogen. This study aims to find a possible bioprotective LAB culture to prevent *Listeria* growth in cheese ripening.
Methods: *Lactobacilli s*pecies were isolated from traditional Tunisian fermented food and then characterized for its ability to inhibit *Listeria monocytogenes* growth. Antagonistic effect of *Lactobacillus plantarum* on four *L. monocytogenes* strains was tested in soft artisanal cheese as well as the anti-biofilm activity of *Lactobacillus* extracts.
Results: Our results demonstrate that the selected lactic acid bacteria extract exhibited a good antibacterial effect against *L. monocytogenes* ATCC 19115 with low MICs values: 8.33 ± 2.1, 20 ± 1.2 and 23.33 ±1, respectively. *L. plantarum* also exhibited a stronger inhibitory effect against *L. monocytogenes* when grown in cheese. Moreover, a potential anti-biofilm effect of the three LAB extracts with BIC_{50} values ranging from 5% to 15% for *L. monocytogenes* ATCC 19115 was demonstrated. Although LAB extracts were able to eradicate significantly a preformed *L. monocytogenes* biofilm ($P < 0.05$). Growth inhibition of preformed biofilm was more difficult to achieve.
Conclusions: LAB could be used as a bioprotective culture in cheese ripening to prevent *Listeria* growth.

ANTIBACTERIAL ACTIVITY OF LACTOBACILLUS PARACASEI SUBSP. PARACASEI BMK2005 AGAINST ENTEROPATHOGENIC E. COLI AND ITS POTENTIAL FOR USE AS AN ANTIDIARRHEAL PROBIOTIC STRAIN

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Objective:

- Production of an infantile fermented milk with a lactic acid bacteria presenting an antibacterial activity against enteropathogenic *E. coli*
- Determination of some probiotic aptitude of this lactic acid bacteria

Methods: The antagonism of *Lactobacillus paracasei* subsp. *paracasei* BMK2005 (identified by 16S rDNA) against enteropathogenic *E. coli* (EPEC) was tested, *in vitro*, by using an infantile milk made out by addition of whey to reconstituted skimmed milk (50/50, v/v). The antagonist effect has been determined by wells diffusion agar and mixed culture methods. In addition, some probiotic aptitudes of the bacterial strain, as the effect of acidic pH, gastro-intestinal proteolytic enzymes and biliary salts on the growth of the lactobacilli strain, was determined *in vitro* by using of MRS broth. In another hind, an *in vivo* study carried out on holoxenic rabbits, made diarrheic with EPEC, was realized to confirm the results obtained in the *in vitro* study.

Results: The tests showed that the lactobacilli strain presents a good antibacterial activity against EPEC. In the wells diffusion agar method, *Lb*. *paracasei* subsp. *paracasei* BMK2005 has shown an important antibacterial activity against EPEC. Therefore, the mixed culture, using the prepared infantile milk, has presented that the lactobacilli strain caused a highly significant decrease in EPEC accounts at the end of 8 hours of incubation. The *in vivo* study carried out on holoxenic rabbits, has clearly demonstrated a highly significant reduction of the faecal accounts of EPEC in the treated rabbits compared to those untreated (negative control) where the number of EPEC did not decrease. In addition, Lactobacilli strain has presented a good resistance to acidic pH, biliary salts and gastro-enetestinal proteolytic enzymes.

Conclusions: The *in vitro* and *in vivo* tests was demonstrated that *Lactobacillus paracasei* subsp. *paracasei* BMK2005 was endowed with a good antibacterial activity against EPEC in an infantile probiotic formula. In addition, this strain has presented good probiotic aptitudes (resistance to acidic pH, biliary salts and gastro-enetestinal proteolytic enzymes).

ENCAPSULATION OF PROBIOTIC MICROORGANISMS IN AQUEOUS DISPERSIONS OF CELLULOSE DERIVATIVES

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Objective: The objective of this work was development of a new technology of enteric probiotic encapsulation and verification of the ability of the polymeric coating to protect the probiotic microorganisms against the deleterious effects in gastro-intestinal tract and to prolong their shelf-life and durability in different food matrices.
Methods: Two different methods of encapsulation of *Lactobacillus acidophilus* and *Bifidobacterium lactis* bacterial strains with commercial enteric FMC's Aquacoat® ECD (ethylcellulose) and Aquacoat® CPD (cellulose acetate phthalate) pseudo-latex preparations were tested and compared - conventional spray drying and nebulisation by pressured carbon dioxide. Prebiotic Frutafit® HP inulin was added to the pseudo-latex preparations as a bacterially-degradable polysaccharide to facilitate release of the probiotic microorganisms from the coating in the colon.
Results: Spray drying provided larger particle aggregates and decreased bacterial vitality by one or two orders in comparison with the nebulisation method. The survivability of the encapsulated probiotics *in vitro* in bile salt solution and simulated gastric juice were improved significantly in comparison with control bacterial sample by several orders.
Conclusions: The composite of inulin and enteric cellulosic derivatives combines the enzymatic susceptibility of inulin and the protective properties of ethyl cellulose. **Kinetics of the probiotic bacteria release by the time-dependent erosion of the coating and by enzymatic activities of the colon microflora can be adjusted in a wide range by changing Aquacoat® ECD, Aquacoat® CPD and inulin ratios.**

This work was supported by the research grant QJ1210093 of the National Agency for Agriculture Research, Czech Republic.

EFFECTIVENESS OF MULTIPROBIOTIC "SYMBITER® ACIDOPHILUS" TO PREVENT ANTIBIOTIC-INDUCED HEPATIC AND COLONIC DYSFUNCTION

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Objective: Colonic dysfunction, e.g. diarrhea, and hepatic failure are common side-effects of long term antibiotic therapy in children. In present study we tested the effectiveness of multiprobiotic "Symbiter® acidophilus" (CFU/ml: lactobacillus and lactococcus (10^9), bifidobacterium (10^8), propionobacterium ($3x10^7$), acetic acid bacteria (10^5)) to prevent beta-lactam antibiotic-induced hepatic and colonic dysfunction.

Methods: 1) Wistar rats (180-230 g) were treated with ceftriaxone (300 mg/kg, i.m.) or ceftriaxone+Symbiter (0.16 ml/kg, per os) for 14 days. On the 1st day after ceftriaxone withdrawal Cl^- transport by isolated colonic loop perfusion technique *in vivo*; parietal microflora and bacterial translocation by bacteriological culture method; the serum levels of ALT, AST, GGTP, triglycerides were measured. 2) Children (0-13 y.o.) with acute infectious diseases were treated with either beta-lactam antibiotics (penicillin, ceftriaxone, cefazolin, cefataxime) alone (n=36) or in combination with Symbiter (n=34) (10^{12} CFU/dose/day). Frequency and duration of bloating, diarrhea, vomiting, and stomach pain were recorded.

EFFECT OF TEMPERATURE, HUMIDITY AND OTHER INGREDIENTS ON STABILITY OF PROBIOTIC FORMULAE

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Objective: Evaluate the effect of ingredients and packing on viability of probiotic mixtures to use on pediatric area.
Methods: microbial analysis (Methods from EU pharmacopeia or ISS Guideline) of different mixtures containing probiotics (powder formulations or oil suspension) were compare to investigate the effect of temperature, humidity or other mixed ingredients on microbial stability
Results and conclusions: Our data shows a strong influence of temperature and humidity on microbial surviving; humidity of the environment is a very important parameter to consider during the formulation of a new probiotic product.

INFLUENCE OF A NEW SYNBIOTIC BEVERAGE ON THE HUMAN GUT MICROBIOTA IN SIMULATOR OF THE HUMAN INTESTINAL MICROBIAL ECOSYSTEM (SHIME®)

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Objective: Evaluate the effect of a new potentially synbiotic beverage fermented *with Lactobacillus casei* LC-01 based on aqueous extracts of soy and quinoa with added fructooligosaccharides in the gut microbiota by a Simulator of the Human Intestinal Microbial Ecosystem (SHIME®).

Methods: Beverage with *Lactobacillus casei* LC-01 and fructooligosaccharides (B1), beverage with *Lactobacillus casei* (B2), beverage with fructooligosaccharides (B3) and beverage without *Lactobacillus casei* LC-01 and fructooligosaccharides (B4) were investigated by applying a dynamic model of the human gastrointestinal tract (SHIME®). Plate counts, ammonium concentration analysis, short chain fatty acids (SCFA) and PCR-DGGE from the ascending, transverse and descending colon were weekly performed.

Results: The synbiotic beverage (B1) showed the best microbiological results in the ascending colon stimulating the growth of *Lactobacillus* spp and *Bifidobacteria* spp and reducing *Clostriduim* spp, *Bacteroides*, *Enterobacteria* and *Enterococcus* spp. There was no statistical difference between the B2, B3 and B4 beverages in ammonium ion concentration, however all beverages differed from the B1, which provided significant reduction of ammonium concentration in the three regions of the colon. There wasn't statistical difference between the treatments for SCFA. Plate count and Denaturing Gradient Gel Electrophoresis (DGGE) showed the survival of *L. casei* in the colon. DGGE patterns confirmed that various treatments affected the microbial community within the simulator of the human intestinal microbial ecosystem

Conclusions: This study showed the beneficial influence of the synbiotic beverage on microbial metabolism and lactobacilli community composition for improving human health.

PROBIOTIC SUPLEMENTATION FOR PREVENTION OF FUNCTIONAL GASTROINTESTINAL DISORDERS IN THE FIRST MONTH OF LIFE WITH

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Background: Colic, regurgitation and constipation are common feeding problems in infants. The onset of these disturbances in the neonatal period not only required a over load work for pediatrician but also could act as a n early traumatic experience that might influence the onset of gastrointestinal tract disturbances late in life. In animal models the role of microbiota has been demonstrated to be crucial for the adaptation to environmental stresses but is not been studied in infant.

Aims: The aim of this prospective study was to evaluate the effects of probiotic supplementation reduce the onset of these "minor" gastrointestinal disorders.

Study design and patients: A double blind placebo controlled multicentre study was performed in our Hospital. Both formula fed-infants and breast fed-infants a term were enrolled in the study at the 3rd day of life from January to December 2012. The infant were randomly assigned in a double-blind manner to receive either *L. reuteri* at dose of $1x10^8$ CFU a day (5 drops of an oil suspension) or placebo for 30 days. . Parents were given a structured diary to record daily episodes of colic, regurgitation and number of stools.

Results: Demographic characteristic of the infants were similar. Of the 330 infants enrolled, 241 completed the study. The newborns receiving probiotics had a significant decrease in mean of minute of crying time per day (48,07 ± 10,24 vs 112,24 ± 44,67 $p<0.01$) and a larger number of stools per day compared to the placebo group. (5.12± 1.2 days vs 2.4±0.8 $p<0.01$) . The number of regurgitation per day did not show any statistical significance between the two group of infant at one month of follow up. No infant showed any adverse effect related to the trial.

Conclusions: These findings show and confirm our previous studies that supplementation with *L. reuteri* DSM 17938 reduce the onset of colic and constipation in the first month of life. This could represent a new therapeuthical strategy for preventing these potential harmful condition.

PHAGEBIOTICS IN PROPHYLAXIS AGAINST FOOD-BORNE INFECTIONS

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Objective: The risk of infection caused by the food contaminated with food-borne pathogens is very high. Peroral form of phagebiotics will allow to reduce the risks of outbreaks and sporadic cases of these infections.

Methods: Using the clinical material a collection of strains of enterobacteria and other medically significant microorganisms was created. Bacteriophages, active against these pathogens, were detected and isolated, focusing close attention to their lytic activity, productivity, ability to retain their characteristics in storage as well as safety for lab animals and humans.

Results: The phage cocktail (PhC) against S. aureus, E. coli, S. enterica and L. monocytogenes with titre 10^6 pfu/ml was created. None of the phages carry any known undesirable genes. The PhC was purified from endotoxins. We didn't detect any intoxication symptoms during estimation of acute and chronic toxicity in outbred mice and guinea pigs. Specific efficiency of PhC was studied during experimental salmonellosis infection of 20 outbred white mice. When the PhC was used 70% of them survived. Complex safety and efficiency assessments of PhC were carried out within the rehabilitation program of 46 men with IBS. Thirty patients who took the PhC stopped complaining sooner, dyspepsia and meteorism seased, their stool normalized, tong plaque and bowel sounds disappeared; the number of patients with dysbacteriosis stages 2 and 3 decreased by 33%, whereas 37% of the patients showed complete normalization of microbiocenosis.

Conclusions: The introduction to the world market of the novel class of probiotics: phagebiotics, will reduce the risk of sporadic cases and outbreaks of food-borne infections.

CHLORIDE SECRETION INDUCED BY ROTAVIRUS NSP4 IS OXIDATIVE STRESS-DEPENDENT AND IS INHIBITED BY SACCHAROMYCES BOULARDII IN HUMAN ENTEROCYTES

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Objective: Rotavirus (RV) infection leads to watery diarrhea through multiple mechanisms, the main being the chloride secretion which may depend on NSP4 enterotoxic activity in human enterocyte but its mechanisms are largely unknown. Redox unbalance is a common event in cells infected by viruses, but the role of oxidative stress in RV infection is still unknown. The aim of this study was to investigate the link between the oxidative stress and chloride secretion induced by RV. In addition, because *Saccharomyces boulardii* (Sb) has been effectively used in RV diarrhea, we tested its effects in RV-infected cells.

Methods: Intestinal ion transport was evaluated in Caco-2 cells infected with RV or exposed to its enterotoxin NSP4 in Ussing chambers. Reactive oxygen species (ROS) and reduced (GSH)/ oxidized (GSSG) glutathione ratio were assessed using dichlorofluorescein and a colorimetric assay, respectively. *S.boulardii* culture supernatant (SbS) was added to Caco-2 cells before and after RV infection.

Results: Chloride secretion was associated with ROS increase in Caco-2 cells infected with RV SA11 strain. Caco-2 cells treated with NSP4 produced a dose-dependent chloride secretion and this was associated with an increase in reactive oxygen species (ROS). RV increased ROS levels and reduced reduced (GSH)/oxidized (GSSG) glutathione ratio inducing oxidative stress. N-acetylcysteine (NAC), a potent antioxidant, strongly inhibited ROS increase and GSH unbalance induced by the virus. The inhibition of NSP4-induced chloride secretion by NAC preincubation suggests that there is a link between oxidative stress and RV-induced diarrhea. SbS prevented RV-induced oxidative stress and chloride secretion in Caco-2 cells. And these results were confirmed in human intestinal biopsies.

Conclusions: These results demonstrate that chloride secretion induced by NSP4 is oxidative stress-dependent and inhibited by *S.boulardii* acting on oxidative stress through its soluble metabolites

EFFECTS OF BIFIDOBACTERIUM SUPPLEMENTATION ON PLASMA LIPID PROFILE IN DYSLIPIDEMIC CHILDREN.

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Objective: Preclinical scientific evidences support the use of probiotics in the treatment of hypercholesterolemia, but clinical findings are often contrasting. This pilot prospective study was undertaken to evaluate the effects of an association of *Bifidobacterium lactis* MB 2409 (DSM 23733), *B. bifidum* MB 109 (DSM 23731) and *B. longum* BL 04 (DSM 23233) on lipid profile in children affected by primary dyslipidemia.
Methods: After a 4 week diet run-in period, 38 dyslipidemic children, aged 10.8 ± 2.1 yrs, were randomized to receive the 3 bifidobacteria or a placebo for three months. Subjects showing a decrease of lipid profile continued with probiotic supplementation for additional three months. A dietary evaluation was performed by a dietician and the lipid profile (TC, HDL-C, TG) was assessed at baseline and after each treatment period by automatic analyzer.
Results: Baseline lipid profile examination showed: TC 223.4±23.3 mg/dl, HDL-C 56.4±11.8 mg/dl, TG 93.1(37-184), LDL-C 148.4±20.9 mg/dl. After three months of treatment 16 children showed: TC 198.5±20.3 mg/dl, HDL-C 60.5±14.8mg/dl, TG 75.3(44-128), LDL-C 122.9±17.2 mg/dl. Bifidobacteria reduced TC by 12.3% (p=0.05) and LDL-C by 10.7% (p=0.05) compared to placebo. Data analysis concerning lipid profile are in progress. A strict dietary control demonstrated that no significant change occurred through the study.
Conclusions: Treatment with the three *Bifidobacterium* strains was well-tolerated and it should be hypothesized that hypercholesterolemic children could benefit from this approach, when preliminary results will be confirmed also by additional larger controlled studies. The variability observed among responders should be related to the colonization time.

EFFECT OF A PROBIOTIC FERMENTED SOY PRODUCT ON COLONIC INFLAMMATION IN DEXTRAN SODIUM SULFATE-INDUCED COLITIS IN RATS

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Objective: The purpose of this study was to investigate the effect of a probiotic soy product, fermented with *Enterococcus faecium* CRL183 and *Lactobacillus helveticus* 416 with addition of *Bifidobacterium longum* ATTC15707, in a dextran sodium sulfate-induced ulcerative colitis model in rats.
Methods: Forty rats were randomly assigned to 4 treatments for 30 days: C– control; CL- colitis (DSS 4% in the drinking water for 7 days); CLF– colitis plus probiotic fermented soy product (2mL; 10^8 CFU/mL); CLP– colitis plus unfermented soy product (2mL without probiotic addition). All groups received a basal diet and the treatment starting one week before colitis induction. The colonic damage was monitored both macroscopically and histologically at the end of the experiment.
Results: Probiotic product reduced the symptoms of colitis during the induction period and after that the probiotic treated rats exhibited the lowest colonic damage score (0.8±0.63) compared to CL group (3.44±0.73; $p<0.01$). Colon of CLP animals presented edema areas without ulceration, with a mean score of 1.2±0.87 ($p<0.01$ vs CL). Histological analyses revealed that colon of CLF and CLP animals exhibited an infiltrate of inflammatory cells, but showed no crypt hyperplasia, alterations in epithelium or areas of ulceration.
Conclusions: Regular intake of the probiotic fermented soy product, and to a lesser extent, unfermented soy product lowers the risk for development of ulcerative colitis in rats, since integrity of the colon was improved. Our finding suggests that protective effect observed include the anti-inflammatory properties of the probiotic strain and the soy bioactive components.

INTAKE OF PASSIFLORA EDULIS PEEL BY TNBS-INDUCED COLITIS RATS: APPROACHES IN PREBIOTIC AND ANTIOXIDANT EFFECTS

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Objective: The *Passiflora edulis* peel (PEP) is a source of fibers and compounds with antioxidant action. The aim of this study was to evaluate the prebiotic potential and antioxidant activity of this food industry byproduct in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model.
Methods: Thirty-six Wistar rats were divided into two groups: AIN and Peel (AIN+PEP). Each group was divided: Non-Colitis and Colitis. It was replaced 50% of fibers from the original diet (AIN) for PEP fiber. The animals were fed for 10 weeks. Colitis was induced by intracolonic administration of TNBS, and after 7 days, the damage score and biochemical parameters were evaluated. ANOVA and Tukey tests were used to compare statistics differences ($P<0.05$).
Results: The intake of PEP diet was able to improve in 10% the beneficial bacterial flora in Non-Colitis and Colitis groups when compared to AIN groups. However, it was observed no differences in the fecal pH or in short chain fatty acids contents among the healthy and colitis groups. The consumption of PEP did not decrease the damages caused by TNBS; which was evidencied by the increase in colitis macroscopic score (1.24x), lipid peroxidation (2.63x), and decrease in the colon superoxide dismutase activity (1.64x) in Peel Colitis group. On the other hand, it was observed an increased serum antioxidant activity (FRAP) (1.21x Non-Colitis and 1.31x Colitis) among the animals fed with PEP.
Conclusions: The PEP intake showed prebiotic effects and an improvement in the serum antioxidant status of the colitis-induced animals, in comparison to the AIN-group.

EFFECT OF A PROBIOTIC SOY BEVERAGE AND SULFASSALAZINE ON FECAL MICROBIOTA OF ANIMALS WITH COLITIS INDUCED BY DEXTRAN- SODIUM SULFATE

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Objective: The aim of this work was to evaluate the effect of a probiotic fermented soy beverage (fermented with Enterococcus faecium CRL183 and Lactobacillus helveticus 416 with addition of Bifidobacterium longum ATTC15707) and sulfassalazine on the fecal microbiota of rats with induced ulcerative colitis.
Methods: Four experimental groups (n=10) of rats receive water (C: control); DSS 4% in the drinking water for 7 days (CL); DSS plus probiotic beverage (CLP: 10^8 CFU, starting one week before colitis induction) and DSS plus sulfassalazine (CLS: 100mg/kg/day, starting after colitis induction), for 30 days with basal diet. Fecal samples were collected weekly during the study and fecal microbiota was characterized by enumerating the *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Enterobacterium* spp., *Clostridium* spp. and Bacteroides population.
Results: At the end of the protocol, the group that received the probiotic fermented product (CLF) exhibited the greatest increase in population of *Lactobacillus* spp. (0.84 $\log_{10}$ CFU/ g) and *Bifidobacterium* spp.(1.35 $\log_{10}$ CFU). The animals treated with sulfassalazine presented reduction on *Bifidobacterium* spp. population in the same period (0.93 $\log_{10}$ CFU/ g). The other groups of microorganism showed no significant alteration during the experimental protocol.
Conclusion: The results revealed that regular ingestion of the probiotic product resulted in an increase of *Lactobacillus* spp. and *Bifidobacterium* spp. population, microorganisms considered important for maintaining the integrity of the epithelial cells of the colon. The positive modulation of microbiota may be related to the protective effect of probiotc product on colitis development, verified in others studies.

LACTOBACILLUS PARACASEI F19 MODULATES THE INFLAMMATORY RESPONSE IN AN EX VIVO/IN VITRO MODEL OF BIOPSIES SAMPLES FROM DIARRHEA-PREDOMINAT IBS PATIENTS

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Objective: Mounting evidence suggests that the gut microbiota-related low-grade mucosal inflammation and local immune activation may play a key role in the pathogenesis of irritable bowel syndrome (IBS). This hypothesis opens the way to new treatment strategies that target the gut microbiota, including probiotics. Aim of this study was to to evaluate the effect of *Lactobacillus paracasei* F19 in modulating the TLRs-mediated inflammatory immune-response in diarrhea-predominant IBS.

Methods: We enrolled 4 outpatients diagnosed as IBS, according to Rome III criteria. Patients underwent lower endoscopy with mucosal biopsies that were placed in organ culture dishes and treated with $4x10^6$ probiotic bacteria (Lactobacillus paracasei F19) for 6 and 24h. Semi-quantitative RT-PCR was performed for the evaluation of the transcriptional levels of TLR4, TLR2, MYD88, p-65, IL-10, IL-4, TGF-beta baseline and after 6h of probiotic treatment. Protein levels of p-65 was evaluated by western blot baseline and after 24h of probiotic treatment.

Results: *Lactobacillus paracasei* F19 treatment reduced transcriptional levels of TLR4, Myd-88 and p-65 and increased TGF-beta and IL-4 with respect to no-treated biopsies. However only the variation of IL-4 and p-65 reached a significant p-value (p=0,01 and p=0,05, respectively). At protein levels, probiotic treatment reduced p-65 expression with respect to no-treated samples. Finally, *Lactobacillus paracasei* F19 treatment did not induce differences of TLR2 and IL-10 expression in respect to basal condition.

Conclusions: Our preliminary results demonstrated that in an *ex-vivo* organ culture model of IBS the anti-inflammatory *Lactobacillus paracasei* F19 may modulate the inflammatory response. Further analysis are needed to confirm this data.

THE PROBIOTIC STRAIN B. ANIMALIS SUBSP. LACTIS BI07 DIFFERENTLY MODULATES THE INFLAMMATION DEPENDENT UNBALANCES OF THE MUCOSA-ASSOCIATED INTESTINAL MICROBIOTA OF BREAST-FED INFANTS AND ADULTS

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Objective: This study aims to highlight the inflammation-dependent unbalances of the mucosal-adherent fraction of the Gut Microbiota (GM) in healthy breast-fed infants and adults, evaluating the potential of the probiotic strain *Bifidobacterium animalis* subsp. *lactis* BI07 to recover a mutualistic profile.
Methods: Faecal stools from 30-year-old donors were allowed to interact in adhesion assays with non-stimulated and TNF-alfa-stimulated HT29 cells, the latter in the presence or absence of *B. animalis* subsp. *lactis* BI07. The GM of breast-fed infants and adults was analyzed by pyrosequencing. Slurries and adherent microbial communities were characterized by qPCR and HTF-Microbi.Array
Results: Breast-fed infants and adults possess phylogenetically and functionally different GM mucosa-associated fraction. While in adults the mucosal fraction is distorted by the inflammatory stimulus, in breast-fed infants it remains unchanged. *B. animalis* subsp. *lactis* BI07 was effective in partially re-addressing the inflammation-dependent dysbiosis of the GM mucosa-associated fraction in adults.
Conclusions: Our *ex vivo* system can be used in non-invasive GM case-control screenings to assess the potential of probiotics to modulate different disease-associated deviations from a healthy mucosa-associated GM profile, either in adults or infants.

EFFECT OF TWO PROBIOTICS AND DATE PALM EXTRACT ON SKIN AND GUT GENE EXPRESSION OF GILTHEAD SEABREAM (SPARUS AURATA, L.)

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Objective: To know the effects of two probiotics and date palm extracts, separately or in combination, on the expression of four immune-relevant genes on two mucosal tissues (skin and gut) of gilthead seabream (*Sparus aurata* L.).
Methods: Seabream specimens were fed with: commercial diet (control diet), diet enriched with *Shewanella putrefaciens* (10^9cfu/g of diet), with *Bacillus* HM117830 10^9cfu/g of diet), with *Phoenix dactylifera* extract at 0.4g/ml or with a combination of the three components. Expression of beta-defensin, immunoglobulin M, major histocompatibility complex II and interleukin 1-beta was evaluated by real-time polymerase chain reaction after for 15 and 30 days of treatment.
Results: In general, gene expression in skin increased after 15 days but decreased after 30 days of treatment, compared to the expression found in control fish. The expression of IL-1β gene increase in gut mainly after 30 days.
Conclusions: The expression of each one of the studied genes depends on the diet, administration time and tissue examined. The skin gene expression was most susceptible to the effects of the diets than gut gene expression. This work contributes to know the molecular mechanisms by which probiotics exert their beneficial effects on the mucosal immunity.

DEVELOPMENT OF INTESTINAL FLORA IN NEWBORNS BORN TO MOTHERS WHO RECEIVED INTRAPARTUM ANTIBIOTIC PROPHYLAXIS

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Objective: The development of gut flora begins during the birth process; it can be influenced by intrapartum antibiotic prophylaxis (IAP), which is the most effective preventative method for group B streptococcal (GBS) neonatal disease.
The aim of this study is to evaluate the effect of IAP on the development of neonatal gut flora.
Methods: Term newborns were recruited on day 2 of life. Inclusion criteria were: 1) vaginal deliver; 2) birth weight appropriate for gestational age (AGA); 3) maternal GBS-positive vaginal swab with adequate IAP. Term, AGA, vaginally delivered newborns born to GBS-negative mothers who did not receive IAP were recruited as controls. For each infant two fecal samples, collected on day 7 and 30, were analyzed by real-time PCR.
Results: Fifty-five newborns were recruited (32 GBS/IAP-positive and 23 controls).
On day 7, a significantly lower count of *Bifidobacteria* was detected in the GBS/IAP-positive group, while no difference was detected on day 30. A significant increase in the count of *Bifidobacteria* was detected on day 30 in both groups, compared to day 7. On day 7, exclusively breast-fed infants had a significantly higher count of *Bifidobacteria* compared to those who also received formula.
Conclusions: IAP seems to influence the development of the neonatal gut flora, by reducing the colonization with *Bifidobacteria*. In breast-fed infants, this effect is transient, as the *Bifidobacteria* count normalizes by day 30. Further studies are needed to evaluate the impact of IAP on the development of gut flora in formula-fed newborns.

CORRELATION BETWEEN SPECIFIC BACTERIAL GROUPS IN THE ORAL CAVITY AND THE SEVERITY OF HALITOSIS: ANY POSSIBLE BENEFICIAL ROLE FOR PROBIOTICS?

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Conflict of interest disclosure and declaration of funding sources:
L. Mogna is an employee of Biolab Research Srl. G. Mogna is an employee of Probiotical S.p.A. All other authors: nothing to declare.

Objective: Halitosis is a really widespread problem, typically attributable to the presence of specific volatile sulfur compounds (VSC) in the breath. The aim of this study was to first correlate halitosis with the presence of *Helicobacter pylori* and secondly to quantify particular bacterial groups in the oral cavity flora, thus correlating them with VSC concentrations and Proton Pump Inhibitors (PPIs) intake.
Methods: Specific bacterial groups were quantified in samples of saliva from 29 subjects taking PPIs compared with 36 control subjects. The amount of three VSC in the breath and the presence of *H. pylori* were determined. The subjects with a total bacterial concentration higher than 10^5 cells/ml will be selected for an intervention study with lactobacilli.
Results: No significant correlation was found between the presence of *H. pylori* and halitosis as well as with PPIs intake. The baseline bacterial groups quantification ($\log_{10}$ CFU/ml of saliva, PPI group vs. control) showed: total bacteria 8.44 vs. 4.47 ($p<0.001$); total coliforms 4.95 vs. 2.82 ($p<0.001$); sulphite-reducing bacteria 5.47 vs. 2.58 ($p=0.052$). No statistically significant differences were found in VSC concentrations in the two groups.
Conclusions: The intake of PPIs directly correlated with the overgrowth of specific bacterial groups in the oral cavity, but there was no correlation with *H. pylori* or with VSC concentration in the breath. Consistently with VSC results, no significant differences were found in sulphite-reducing bacteria concentrations. In conclusion, is it possible to postulate any possible beneficial role for probiotics?

CORRELATION BETWEEN CHRONIC TREATMENT WITH PROTON PUMP INHIBITORS (PPIs) AND BACTERIAL OVERGROWTH IN THE STOMACH: ANY POSSIBLE BENEFICIAL ROLE FOR PROBIOTICS?

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Conflict of interest disclosure and declaration of funding sources:
L. Mogna is an employee of Biolab Research S.r.l. G. Mogna is an employee of Probiotical S.p.A. All other authors: nothing to declare.

Objective: The inhibition of physiological gastric acid secretion induced by the Proton Pump Inhibitors (PPIs), the most used drugs in the world, may cause a significant bacterial overgrowth in the GI tract as side effect. This study was undertaken to correlate PPIs intake with concentration of specific bacterial groups in the stomach as well as possible *Helicobacter pylori* infection.
Methods: Total bacteria, sulphite-reducing bacteria, total coliforms and total lactobacilli were quantified in samples of gastric juice from 29 subjects taking PPIs since at least 3 months compared with 36 control subjects. The presence of *H. pylori* was also assessed. The subjects with a concentration of total bacteria higher than 10^5 cells/ml will be selected for the intervention study with specific lactobacilli.
Results: No significant correlation was found between the presence of *H. pylori* and PPIs intake. The baseline quantification of bacterial groups ($\log_{10}$ CFU/ml of gastric juice, PPI group vs. control) showed: total bacteria 8.35 vs. 3.95 ($p<0.001$); total coliforms 4.98 vs. 2.35 ($p<0.001$); sulphite-reducing bacteria 5.71 vs. 2.28 ($p=0.065$); total lactobacilli 3.85 vs. 2.20 ($p=0.005$).
Conclusions: A significant impairment of intragastric acidity is sufficient to induce a relevant bacterial overgrowth. This fact can contribute to increasing the risk of infections and intestinal diseases. It could be crucial to restore the physiological "gastric barrier". Is it possible to hypothesize a beneficial role for probiotics?

ACT AND NOT REACT: PROPHYLACTIC USE OF PROBIOTIC IN COLIC, REGURGITATION AND FUNCTIONAL CONSTIPATION, CLINICAL AND SOCIO-ECONOMIC IMPACT

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Objective: To investigate if oral supplementation with L. Reuteri DSM 17938 during the first three months of life can reduce the onset of colic, GER and constipation in term newborns and thereby reduce the socio-economic impact of these conditions

Methods: A multicentric double blind randomized clinical trial was performed in 589 term newborn aged one week born in 9 different Neonatology Unit all over Italy between September 1, 2010, and October 30, 2012 . Infants were randomly allocated to receive L. reuteri DSM 17938 or placebo. Parents were asked to record in a structured diary number of episodes per day of regurgitation, inconsolable crying episodes (minutes per day as already described in literature), and the number of evacuations per day, number of visits, feeding changes, hospitalization, access to first aid stations, and loss of working days and use of drugs.

Results: At three months of life, crying time (37,7 vs 70,9 $p < 0.01$) number of regurgitation (2,9 vs 4,6 $p < 0.01$) and the number of evacuations (4,2 vs 3,6 $p < 0,01$) per day were significantly different between the two groups. Use of the probiotic resulted in an estimated mean saving per patient of €88 for the family and an additional € 104 for the community.

Conclusions: Prophylactic use of L. Reuteri DSM 17938 during the first three month of life reduce di onset of Functional Gastrointestinal Disorders and reduce the cost for the family and the society in the management of this condition.

INHIBITORY EFFECT OF POTENTIAL BACILLUS PROBIOTIC STRAINS AGAINST PATHOGENIC BACTERIA AND YEAST ISOLATED FROM ORAL CAVITY

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Objective: The presence of resistant bacteria in the oral cavity can be the major cause of dental antibiotic prophylaxis failure. Multidrug efflux has been described for many organisms, including bacteria and fungi as part of their drugs resistance strategy. The potential use of probiotic bacteria can be considered as a new alternative in the prevention or cure of oral cavity diseases.
Methods: In this study, different Bacillus strains isolated from the environment were isolated and characterized using biochemical and molecular procedures. The inhibitory activity against different pathogenic bacteria and yeast strains was tested using diffusion agar assay method.
Results: Our data revealed that the tested strains have an antimicrobial effect against the pathogenic strains such as Streptoccus mutans. The inhibitory affect was variable depending from the probiotic and pathogenic strains.
Conclusions: The obtained result demonstrated that Bacillus can be used as a potential candidate's probiotic and help in the prevention and treatment of oral infections, including dental caries, periodontal disease and halitosis. Our data, partly encourage the use of probiotic strains because they don't produce a lot of acid which can contribute to faster installation decay and these are spore-forming bacteria that can withstand the stress of the oral cavity (acids, alkalis and salty foods).

INFLUENCE OF THE MICROBIAL COMMUNITY IN KEFIR GRAINS ON THE SURVIVAL AND GROWTH OF STAPHYLOCOCCUS AUREUS IN MILK

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Objective: According to literature Kefir contains live active cultures of strong strains of microorganisms that help to overtake pathogenic organisms. Regular kefir consumption can help relieve all intestinal disorders, create a healthier digestive system and possesses antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria and against some fungi. *Staphylococcus aureus* is an important potential pathogen that can cause severe hospital-acquired infections. The objective of our study was to investigate the influence of the microbiota in Kefir grains on the survival and growth of *Staphylococcus aureus.*

Methods: Different concentrations of *Staphylococcus aureus* cultures were inoculated into 20 mL of milk and 20 mL of milk with 1 g of Kefir grains. The samples were incubated at 20°C for 48 hours. Serial ten-fold dilutions were plated on different nutrient and selective media. The results were expressed as colony forming units (cfu).

Results: Milk with 1 g of Kefir grains had a total count of aerobic microbes $5{,}0x10^5$ cfu/mL after 48 hour incubation. The initial concentrations of *S. aureus* inoculated in milk and milk with Kefir grains were $1.0x10^{16}$-$6.88x10^{12}$ cfu/mL. Concentrations of *S. aureus* in milk and milk with Kefir grains after 48 hour incubation were $2.3x10^{10}$-$6.5x10^5$ cfu/mL and $3.7x10^5$-$4.0x10^0$ cfu/mL respectively.

Conclusions: Although the concentration of *S. aureus* in milk was decreased after 48 hour incubation for 6 log steps, the decrease in milk with Kefir grains was much higher at 11 log steps. Therefore, the microbiota in Kefir grains successfully inhibited the growth of *S. aureus* in milk.

EVALUATION OF PROBIOTICS AND DATE PALM EXTRACT IN INNATE DEFENCE PRESENT IN EPIDERMAL MUCUS OF GILTHEAD SEABREAM (Sparus aurata L.)

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Objective: The purpose of the present study was to evaluate and know the effect of two probiotics: Pdp11 (*Shewanella putrefaciens*) and *Bacillus* (HM117830) and extracts of date palm (*Phoenix dactylifera* L.), separately or in combination in the innate immune components in fish skin mucus of gilthead seabream (*Sparus aurata* L) after 15 and 30 days of oral dietary administration.
Methods: Probiotics were cultured in TSB medium and bacteria number was adjusted at 10^9cfu g^{-1} feed. Date palm was collected in Tunisia and the extract was obtained at 0.4g ml^{-1} in distilled water. Five groups were established: a) control group; b) Pdp11, c) *Bacillus*, d) date palm extract and e) mix group. The level of total IgM antibodies, the activities of lysozyme and protease, as well as the antibacterial activity against opportunist pathogens (*Vibrio harveyi, V. angillarum, Photobacterium damselae*) were evaluated in fish skin mucus.
Results: This study showed an increase of IgM levels and lysozyme in all groups between 15 and 30 days after oral dietary administration. Nevertheless, the activity of protease showed statistically significant differences in skin mucus of date palm extract and mix groups at 15 days with respect control group. In addition, skin mucus revealed an increase of antibacterial activity in date palm extract and mix groups and a decrease in Pdp11 and bacillus groups against tested bacteria at 15 days compared to control group.
Conclusions: This preliminary information could be useful in better understanding the effects of probiotics and date palm extract in mucosal immunity.

ORAL PROBIOTIC CAPSULES AS SUPPLEMENTATION OF THE STANDARD ANTIBIOTIC THERAPY TO PATIENTS WITH BACTERIAL VAGINOSIS OR AEROBIC VAGINITIS: RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

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Objective: To demonstrate whether the use of the probiotic preparation (prOVag®) containing three *Lactobacillus* strains together with a standard antibiotic treatment could reduce the recurrence rate of bacterial vaginosis (BV) or aerobic vaginitis (AV).
Methods: Design: a multicenter, randomized, double-blind, placebo-controlled trial. Setting: Out-patient private gynaecological clinics in South-West Poland during the period March 2009 until February 2012. Population: A total of 594 women with a history of recurrent symptomatic bacterial vaginosis (BV) and/or aerobic vaginitis (AV). Study participation included five visits. At first visit all eligible participants were assigned either to a control group receiving placebo and metronidazole or to a group receiving the tested probiotic preparation and metronidazole. Cases showing no effect of metronidazole were then treated with selected targeted antibiotic. From visit two to five, time to recurrence of BV/AV was measured based on evaluation of clinical (Amsel's criteria) and bacteriological assessment: Nugent score, quantitative and qualitative cultures of vaginal swabs for vaginal pathogens and lactobacilli.
Results: The tested probiotic preparation, compared to placebo, lengthened the time to relapse of clinical symptoms of BV/AV by as much as 51% ($p<0.05$). Moreover, the use of probiotic bacteria reduced and maintained the low vaginal pH and Nugent score, and increased vaginal *Lactobacillus* counts after the standard treatment.
Conclusions: The study demonstrated that prOVag preparation lengthened the remission period in patients with recurrent BV/AV and contributed to the improvement of clinical and microbiological parameters.

PREBIOTIC AND PROBIOTIC SUPPLEMENTATION PREVENTS HUMAN RHINOVIRUS INFECTIONS IN PRETERM INFANTS: A RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Background: Simple and safe strategies for the prevention of viral respiratory tract infections (RTIs) are needed. We hypothesized that early prebiotic or probiotic supplementation would reduce the risk of virus-associated RTIs during the first year of life in a cohort of preterm infants.
Methods: In this randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov, number NCT00167700), preterm infants (gestational age ≥32+0 and ≤36+6 weeks and birth weight >1500g) treated in Turku University Hospital, Finland, were allocated to receive orally prebiotics (galacto-oligosaccharide and polydextrose, mixture 1:1), probiotic (*Lactobacillus rhamnosus* GG, ATCC 53103), or placebo (microcrystalline cellulose) between days 3 and 60 of life. The primary outcome was the incidence of clinically defined virus-associated RTI episodes, confirmed from nasal swabs using nucleic acid testing. Secondary outcomes were the severity and duration of RTIs.
Findings: Altogether 94 infants (31, 31, and 32 infants receiving prebiotics, probiotic or placebo) were randomly assigned to the study groups and 68 (23, 21, and 24 respectively) completed the trial. A significantly higher incidence of RTIs was detected in infants receiving placebo compared to those receiving either prebiotics, RR 4·1 (95% CI 2·1–8·1); p< 0·001 or probiotic, RR 2·0 (95% CI 1·1–3·6); p=0·022. Also, the incidence of human rhinovirus (HRV) –induced episodes, comprising 80% of all RTI episodes, was found to be significantly higher in the placebo compared to the prebiotic, RR 3·3 (95% CI 1·5–7·0); p=0·003 and probiotic group, RR 2·0 (95% CI 1·0–4·1); p=0·051. No differences emerged among the study groups in HRV RNA load during infections, duration of HRV RNA shedding, duration or severity of HRV infections, or the occurrence of subclinical HRV infections.
Interpretation: Gut microbiota modification with prebiotics and probiotics may offer a novel and cost-effective means to reduce the risk of HRV infections.

GOOD SELECTION OF BIFIDOBACTERIUM LONGUM STRAINS PREDICT THEIR EFFICACY IN PREVENTION OF COLITIS IN MICE EXPERIMENTAL MODEL

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Objective: Bifidobacteria comprise a dominant microbial population in intestine with potential probiotic activity. The aim of the study was to find bifidobacteria with immunomodulative properties which will protect mice against dextran sulfate sodium (DSS)-induced colitis.
Methods: Formalin-inactivated bacteria (10 strains) were cultivated with splenocytes and cytokines were determined by ELISA. Two *B. longum* ssp. *longum* (BL) strains with different patterns of cytokine induction were analyzed in vitro using bone marrow-derived dendritic cells (DC) and human embryonic kidney cells transfected by PRR. *In vivo* mice received BL strains or saline by intragastric gavages before and during 2.5% DSS solution treatment. Macroscopic signs of intestinal inflammation, histological and immunohistochemical evaluation and cytokines were determined.
Results: We found that cytokine induction in spleen cells by bifidobacteria is strain dependent. Strain BL367 compared to strain BL372 stimulated lower levels of IFN-gamma, TNF-alpha and IL-10 in naive splenocytes or DC; the induction of co-stimulatory molecules was also less pronounced. Both strains engaged strongly TLR2 receptor but BL372 signalization through NOD2 was stronger compared to BL367. In DSS-model of ulcerative colitis, BL367 strain reduced macroscopic and histological signs of intestinal inflammation and increased tight junction proteins (ZO-1, occludin).
Conclusions: The *in vitro* less immunogenic strain BL367 was able to protect from the development of DSS-colitis in mice contrary to the strain 372. Probiotic strains inducing less pronounced immune responses might be good candidates for IBD prevention/ treatment.
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LACTOBACILLUS STRAINS AS A POTENTIAL PROBIOTIC TREATMENT FOR GNOTOBIOTIC ARTEMIA CULTURE

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Objective: Bacterial diseases are probably the major cause of mortality in aquaculture. The use of probiotics is one of the solutions to manage bacterial infections by preventing the colonization and exclusion of pathogenic bacteria. Their use is gaining a priority in the developed countries with the aim of replacing conventional drugs.
Methods: To evaluate the potential probiotic effect of two *Lactobacillus plantarum* isolated from live prey and identified by sequencing of 16s rRNA toward *Artemia* cultured in different gnotobiotic conditions, antibacterial and adherence assays were investigated and challenge tests with *Artemia* nauplii were performed. The anti bacterial activity was tested in vitro against reference strain of *Vibrio* using agar well diffusion assay and in vivo conditions by challenge tests on *Artemia* culture. Bacterial adhesion onto the inner surface of the test tubes and on an abiotic surface was determined using a semi-quantitative adherence assay.
Results: Lactobacillus strains formed the biofilm layer on internal walls of the glass tubes. While adherence assay into polystyrene plates revealed that these potential probiotic bacteria are low adherent, with a values ranging from 0.16 to 0.24 at 595 nm. Antagonism assay revealed that these strains have an inhibitory effect against tested pathogenic bacteria. The in vivo challenge tests demonstrate that the tested strains have no negative effect on the survival of axenic *Artemia* culture and enhance protection against pathogenic *Vibrio*.
Conclusions: Based on the obtained results, these strains can be used as a potentiel probiotic candidate for *Artemia* culture.

PUBLIC HEALTH AND BUDGET IMPACT OF PROBIOTICS IN CONTROLLING UPPER RESPIRATORY TRACT INFECTIONS IN FRANCE

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Objective: Meta-analyses (York Health Economics Consortium [YHEC]; Cochrane) demonstrated that probiotics are effective in reducing duration (-0.77 days) and frequency (0.58x) of common respiratory tract infections (CRTI). Antibiotic use also decreased (0.67x). We analysed the potential public health impact of probiotic consumption on CRTI in France.
Methods: A 1/1,000 virtual age- and gender-standardized population was generated using a Markov model. CRTI risk factors were age, active/passive smoking, living in a community setting. Incidence rates came from a national general practitioner (GP) network over the 2011-2012 flu season and the analysis was limited to patients visiting a GP. Economic perspectives were society, national health system (NHS) and family. Data on resource utilization came from the GP network. Outcomes included numbers of CRTIs days and episodes, antibiotics courses, sick leaves, medical and indirect costs.
Results: Based on YHEC, probiotics saved 2.85million CRTI-days, 330,000 antibiotics courses and 653,000 sick leave days. Applying the Cochrane data, reductions were 7.1million CRTI days, 536,000 antibiotics courses and 1.3million sick days. The economic impact of probiotics was about 95million Euro saved from the Society perspective based on YHEC (Family: -21.7million Euro; NHS: -15.4million Euro) and 229.1million Euro based on Cochrane (Family: -130.4million Euro; NHS: -34.6million Euro). More savings were observed in children, active smokers and people with more human contacts.
Conclusions: Public health and budget impact of probiotics are substantial, whether they reduce CRTI episodes frequency or duration. Noteworthy, the 2011-12 winter CRTI incidence was low and this analysis focused on the 1% CRTI patients accessing the NHS.

SELECTION OF A PROBIOTIC STRAIN POTENTIATING THE IMMUNOMODULATORY PROPERTIES OF AN ELDERBERRY EXTRACT (EE)

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Objective: There is growing evidence for associating plant extracts with probiotics to enhance immune responses. We previously identified an immunomodulating EE. As bacterial strains have disparate effects in stimulating the immune system, the aim of this work was to select a probiotic strain able to potentiate the properties of EE.
Methods: Peripheral blood mononuclear cell (PBMC) from healthy subjects were stimulated 24h with live bacterial cells at different ratios bacteria:PBMC. Cytokines in supernatant were quantified using the Luminex technology.
Results: Seven strains were screened for their IL-6 production. The three *Bifidobacterium* spp tested were the strongest inducers of IL-6. We choose to conduct further studies with EE with the four *Lactobacillus* spp strains. Upon addition of EE, only *L. rhamnosus* GG was found to significantly potentiate IL-6 secretion. Cytokine profiles showed *L. rhamnosus* GG did not stimulate the production of IL-2, -4, -5, -9, -13, 15-, 17A, typically produced by T cells. In contrast, substantial amounts of inflammatory (IL-1beta, IL-12p70, TNF--alpha, IFN-gamma and GM-CSF) and anti-inflammatory (IL-1RA and IL-10) cytokines and chemokines (CCL2, CCL3, CCL4, CCL22, CXCL8 and CXCL10) were induced. In the presence of EE, the production of 6 cytokines was significantly potentiated, while the production of CXCL10 was antagonised.
Conclusions: *L. rhamnosus* GG did not stimulate T cells. In contrast, it upregulated the production of cytokines involved in the recruitment and activation of immune cells, such as monocytes/macrophages, neutrophils and lymphocytes. Based on these properties and its strain-specific interactions with EE, *L. rhamnosus* GG was selected.

PROBIOTICS FOR THE TREATMENT OF IRRITABLE BOWEL SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS OF EVIDENCE IN CHILDREN AND ADULTS

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Objective: Probiotics may have a role in reducing the severity of symptoms in IBS. The objective of the study was to systematically review the evidence on the treatment with probiotics in children and adults with IBS, and to perform a meta-analysis of the major clinical outcomes comparing the effect of intervention in different age ranges.
Design: Medline and CENTRAL databases were searched for randomized controlled trials on the use of probiotics in children and adults with IBS (defined according to age-specific Rome criteria). Trials scoring $\geq$3 according to the Linde Internal Validity Scale (LIVS) were included in meta-analysis.
Results: Thirty-one trials (4 on children and 27 on adults) were included in the qualitative analysis and 23 in the meta-analysis. Differently from adults, children treated with probiotics had a significant improvement in severity (SMD -0.25, 95%CI -0.45 to -0.05, p<0.001) and frequency (SMD -1.05, 95%CI -1.44 to -0.66, p<0.001) of abdominal pain. A significant reduction of abdominal distention was demonstrated both in adults (SMD -0.21, 95%CI -0.35 to -0.08, p=0.002) and children (SMD -2.15, 95%CI -2.80 to -1.50). However, standardized IBS symptoms scores (SMD -0.15, 95%CI -0.92 to 0.62, p=0.70) and quality of life scores (SMD -0.10, 95%CI -0.28 to 0.08, p=0.26) were not significantly affected by probiotic in adult population.
Conclusions: Probiotics may have a slight efficacy in reducing IBS-related symptoms, mainly abdominal pain and distention. This effect seems to be more evident in children rather than adults. However, the high heterogeneity among studies may partially affect interpretation of results.

INTERACTION OF LACTOBACILLUS FERMENTUM BGHI14 WITH RAT COLONIC MUCOSA – IMPLICATIONS FOR COLITIS INDUCTION

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Objective: The research aimed to test the response of colon mucosa of healthy and colitic rats to oral application of *Lactobacillus fermentum* BGHI14. Considering potential antigenic nature of administered bacteria, we tested mucosal reaction after different BGHI14-treatment durations and impact of treatment in defined time point of colitis induction.
Methods: Healthy rats were orally gavaged with BGHI14 for 16 or 28 days. Rats from colitic group were pretreated for 16 days with BGHI14 before intrarectal TNBS administration and sacrificed after 48 hours. Control vehicle-treated groups were set for each BGHI14-treated group. Treatment effects were assessed by weight measurements during the experiment and by H&E staining and qPCR analysis of colonic IL-1beta, TNFalpha, IL-17F, Hsp70 and Tjp1 mRNA expression. DGGE was employed for intestinal microflora profile analysis.
Results: Treatment with BGHI14 for 16 days stimulated healthy mucosa which was apparant from histological scores, elevated TNFalpha mRNA synthesis and transient body mass decrease. 28-days BGHI14-treated rats didn't show immune reaction histologically although TNFalpha and IL-1beta mRNA levels were increased. BGHI4 pretreatment elevated Hsp70 mRNA synthesis without affecting other genes expression nor histological scores of colitic rats. No microflora perturbation was detected by DGGE after BGHI14-treatment.

SURVIVAL AND RETENTION OF THE PROBIOTIC PROPERTIES OF BACILLUS SP. STRAINS UNDER MARINE STRESS STARVATION CONDITIONS AND THEIR POTENTIAL USE AS A PROBIOTIC IN ARTEMIA CULTURE

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Objective: Aquaculture is the world's fastest growing food-production sector. However, one of the most serious problems regarding the culture of marine fishes is the mortality associated with pathogenic bacteria that occurs in the critical phases of larval development. Conventional approaches, such as the use of antimicrobial drugs to control diseases, have had limited success in the prevention or cure of aquatic diseases. Promising alternatives to antibiotics are probiotics, which are food supplements consisting of live microorganisms that benefit the host organism.
Methods: In the search for more effective and environmentally friendly treatments with probionts against pathogenic species in shrimp larval culture, the probiotic properties of *Bacillus* strains isolated from *Artemia* culture such as antibacterial activity, adhesion, pathogenicity, toxicity and the effect of marine stress on viability and survival were investigated, as well as the changes occurring in their properties.
Results: Analyses showed that these bacteria corresponded to the genus *Bacillus* sp. Antagonism and adherence assays revealed that these strains have an inhibitory effect against pathogenic bacteria in vitro and in vivo conditions and are fairly adherent. Challenge tests performed with *Artemia* larvae provided evidence that the tested *Bacillus* strains were neither pathogenic nor toxic to the host. The tested strains maintained their viability and their probiotic properties during the period of study.
Conclusions: The results suggest that the tested strains have suffered changes allowing them to survive in seawater in the absence of nutrients and outside their natural host, identifying them as potential probiotic candidates for *Artemia* culture.

ON THE INTERACTION OF LISTERIA MONOCYTOGENES AND TUNISIAN PROBIOTIC LACTOBACILLUS PLANTARUM EXTRACTS

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Objective: A wide range of lactic acid bacteria produce bacteriocins, which were essentially active against the food-borne pathogen. This study aims to evaluate a possible bioprotective activity of selected *Lactobacillus plantarum* species to prevent *Listeria monocytogenes* attachment.
Methods: Lactobacilli species were isolated from traditional Tunisian fermented food and then characterized for its ability to inhibit *Listeria monocytogenes* growth. Antagonistic effect of *Lactobacillus plantarum* on *Listeria monocytogenes* strains was tested in soft artisanal cheese as well as the anti-biofilm activity of Lactobacillus extracts.
Results: Moreover, a potential anti-biofilm effect of *L. plantarum* extracts with BIC_{50} values ranging from 5% to 15% for *L. monocytogenes* ATCC 19115 was demonstrated. Although probiotic lactic acid bacteria extracts were able to eradicate significantly a preformed *L. monocytogenes* biofilm ($P < 0.05$) and make *L. monocytogenes* bacteria less adhesive to Vero cells. Growth inhibition of preformed biofilm was more difficult to achieve then planktonic bacteria.
Conclusions: *Lactobacillus plantarum* strains could be used as a bioprotective culture to prevent *Listeria* growth. Moreover *L. plantarum* neutralized extract could reduce cell attachment ability.

PRODUCTION OF NEW OAT GRAINS PROBIOTIC YOGHURT

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Abstract: Food industry companies place rather high expectations in food products made to meet the consumers' demand for a healthy life style. In this context, 'Functional Foods' play a special role. In many of the studies that have involved examination of the use of probiotics and prebiotics in infants, the primary health outcomes have been microbiological in nature. This study aimed to develop probiotic yoghurt inclusion of oat which attends to the human's energy and nutritional demands, as well as functional because of its high content of soluble fiber, which are highly beneficial to humans' health .The results indicated that: the effect of probiotic lactic acid bacteria strain tested with and without oat for their ability to assimilation of Cholesterol *In Vivo* and *In Vitro* and their effect on feeding mice on general health indicators, hematological parameters and their ability to reduce the cholesterol level in blood serum; the strain with oat effect on reduce the level of cholesterol in the blood serum. The formulae had presented excellent microbiological quality and the sensory evaluation through multiple comparisons had pointed out that the samples did not differ between themselves, and that they had satisfactory acceptance by the consumers.
Keywords: Functional food, probiotic, prebiotic, oats.

GLYCAEMIC RESPONSE TO FOUR DIFFERENT SWEETENERS IN TIPE II DIABETES (SUCROSE IN THREE DIFFERENT PREPARATION VERSUS ACALORIC SWEETENER)

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Objective: The underlying hypothesis was that sucrose in a different preparation with respect to the purified one could remain inside in the gut in order not to augment glycaemia and arrive to the place of GLP1 production and stimulate it.
Methods: In 64 patients (age 70.76±8.05 years, M 44, F 20, HbA1c 7.05±0.72, BMI 28.9±4.7) with Type 2 diabetes mellitus we compared the glycaemic response to the intake of a brioche prepared with different sweeteners. Sucrose was used in different preparations: purified sucrose, molasses of beet, or pulp of beet, tested vs. acaloric erythritol plus sucralose. The four brioches (≈50 gr.) were consumed in double blind and randomized order; after 30, 60, 90, 120 min capillary glucose sample was taken using a Bayer glucometer.
Results: A systematic glucose response to brioche intake was observed in all cases, with blood glucose increasing from 130.29±24.04 mg/dL at time 0' to;157.03±28.83;170.19±28.32;161.61±29.13;144.49±26.63 during the observation period; with no significant differences according to the type of sweetener (3.5-4.0 g of sucrose inside in each preparation).
Conclusions: We conclude that glucose response is mainly driven by carbohydrates (15 g) in the flour (mainly manitoba flour), not by added sweeteners.

PROTECTIVE EFFECTS OF LACTOBACILLUS ACIDOPHILUS CRL 1014 AND YACON (SMALLANTHUS SONCHIFOLIUS) ON DMH-INDUCED COLON CARCINOGENESIS IN WISTAR RATS

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Objective: In this study, the *Lactobacillus acidophilus* CRL 1014 and yacon (*Smallanthus sonchifolius*) were used to compare their protective potential against 1,2 dimethylhydrazine dihydrochloride (DMH)-induced chemical colon carcinogenesis in Wistar rats.
Methods: The rats (n= 25) were divided into 5 groups. Colon cancer was induced by the application of DMH twice a week in a dose of 40 mg/kg s.c. in groups G2–G5. Before 2 weeks of DMH-initiation, groups were fed basal diet (G1 and G2) or basal diet + *Lactobacillus acidophilus* CRL 1014 at 10^8 CFU/mL (G3), basal diet + yacon (G4), basal diet + *Lactobacillus acidophilus* CRL 1014 at 10^8 CFU/mL and yacon (G5) for 8 weeks. Stool samples were collected, every 15 day during the experimental protocol for the pH, ammonium, short chain fatty acids (SCFA) analysis and intestinal microbiota composition. For count of Aberrant Crypts Foci (ACF), the proximal, medial and distal colons were stained in Leishman (Merck ®) for 2 min.
Results: The pH was significantly lower in the group fed probiotic. All groups showed increased in ammonium ion. A significantly increase in SCFA contents was observed in the group 5. The group 5 showed the significantly increase in *Lactobacillus* spp. and *Bifidobacterium* spp. and *Clostridium* spp. was not significant difference. A significant reduction in number of ACF was observed in groups 3, 4 and 5, however the greater reduction was observed in group 5.
Conclusions: This study indicates that yacon plus *L. acidophilus* CRL 1014 intake may reduce the development of chemically-induced colon cancer.

DOES EARLY OLIGOSACCHARIDES CONSUMPTION AFFECT PANCREAS MATURATION?

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Galacto-oligosaccharides (GOS) are commonly added to infants formulae to better mimic maternal milk. However their delayed impacts on adult health are poorly characterized. Since GOS are known to stimulate colonic fermentation and GLP-1 production by L-cells, while GLP-1 stimulates proliferation and neogenesis of beta-cells, we hypothesized that early GOS consumption could modulate endocrine pancreas maturation and possibly adult metabolism.
Suckling rat pups were supplemented with GOS/inulin (3.2 $g.kg^{-1}$), or control solution from days 5 to 15, half of the CTL animals being injected with Exendin-4 (3 $\mu g.kg^{-1}$) as a reference. Half of the total pups were sacrificed at PND8, while the others were weaned at PND21 to standard chow and followed-up until PND146. Caecocolonic concentration of fermentation products, portal concentration of total GLP-1, density of colonic GLP-1 producing L-cells, endocrine pancreas anatomy and response to oral glucose load were analyzed.
At PND8, GOS supplementation increased caecocolonic concentration of fermentation products (medians±interquartiles: 683±611 $\mu moles.g^{-1}$ *vs* 494±721 for CTL) but did not significantly affect the portal concentration of GLP-1 (267±75 *vs* 328±246 pM for CTL) nor the number of GLP-1-positive L-cells (75±35 *vs* 90±35 cells per cm^2). Endocrine pancreas proliferation was stimulated by Ex4 but not by GOS/In. Preliminary long-term data suggest that none of the treatments significantly altered the metabolic response to oral glucose load nor growth or body composition.
We concluded that very early GOS supplementation does not modify endocrine pancreas maturation and adult metabolism in rat. Whether this result is also true in human would deserve further investigation.

CAPABILITY OF THE TWO MICROORGANISMS *BIFIDOBACTERIUM BREVE* B632 AND *BIFIDOBACTERIUM BREVE* BR03 TO COLONIZE THE INTESTINAL MICROBIOTA OF CHILDREN: THEIR PROSPECTIVE USE IN COLICKY INFANTS.

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Conflict of interest disclosure and declaration of funding sources:
L. Mogna is an employee of Biolab Research Srl. G. Mogna is an employee of Probiotical SpA. All other authors: nothing to declare.
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Objective: The total number of bacteria present in the gut microbiota of a newborn is consistently lower than the average found in adults, with the extent of this difference being directly related to body weight and age. It could be assumed that a lower number of viable probiotic cells is necessary to achieve a significant gut colonization in infants and children. This study assessed the capability of *Bifidobacterium breve* B632 (DSM 24706) and *Bifidobacterium breve* BR03 (DSM 16604), two strains able to significantly *in vitro* inhibit some Gram-negative bacteria and with a prospective use in colicky infants, to integrate into the intestinal microbiota of children.
Methods: Ten healthy children aged an average of 5.7 ± 2.6 were given a probiotic oily suspension containing *B. breve* B632 and *B. breve* BR03 for 21 consecutive days. The daily dose was 100 million live cells of each strain. Faecal specimens were collected and analyzed at the beginning (T0) and at the end of the study (T21). Total faecal bifidobacteria and coliforms have been quantified by microbiological plate counts.
Results: A significant increase in total faecal bifidobacteria (from 8.99 to 9.47 $\log_{10}$ CFU/g, p=0.0422) and a parallel decrease in total coliforms (from 8.60 to 7.93 $\log_{10}$ CFU/g, p=0.0475) was recorded, thus suggesting an effective gut colonization.
Conclusions: In addition to gut colonization in healthy children, *B. breve* B632 and *B. breve* BR03 were able to decrease the total faecal coliforms, therefore confirming their potential specific use in colicky infants.

DECAFFEINATED COFFEE RESTORES GUT PERMEABILITY IN A RAT MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Objective: Exposure to bacterial products of intestinal origin, leads to liver inflammation and hepatic injury. Impaired gut epithelial integrity due to alterations in tight junction (TJ) proteins may be the pathological mechanism underlying bacterial translocation. The gut permeability plays a crucial role in the pathogenesis of non-alcoholic steatohepatitis (NASH) and some nutrients may modulate intestinal TJ. Studies demonstrate that coffee beverage reduces liver damage caused by high fat diet (HFD). Aims of the study were to evaluate the effect of coffee on the HFD-induced damage of intestinal mucosal barrier and on pro-inflammatory TLR-4 in a rat model of NASH.
Methods: Male Wistar rats were fed with HFD for 5 months and divided into a group drunk water while another group drunk water added with 1.2 mL of decaffeinated coffee/die starting from the 4^{th} month. Contemporarily, another group of rats fed with standard diet were used as control. Protein and mRNA expression levels of TLR-4, Occludin and ZO-1 were examined from proximal jejunum of rats fed with standard diet, HFD+water and HFD+coffee.
Results: A significant reduction of TJ proteins Occludin and Zo-1 in HFD fed rats was observed; it was partially reverted by the addition of coffee to the HFD (0.83 ± 0.27 vs 0.14 ± 0.07, $p<0.05$ and 0.85 ± 0.12 vs 0.57 ± 0.14, $p<0.05$ respectively). Coffee also reduced TLR-4 expression up-regulated in the HFD+water group.
Conclusions: These preliminary data confirmed that HFD impairs the intestinal TJ barrier integrity and that coffee is able to partially revert it by increasing ZO-1 and Occludin and reducing TLR4 expression.

MECHANISM OF PROLONGEVITY INDUCED BY LACTOBACILLUS GASSERI SBT2055 IN C. ELEGANS

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Objective: Recently, effects of lactic acid bacteria to extend the lifespan were shown using some model animals, but the mechanisms are not entirely understood. In this study, we evaluated the effects of *Lactobacillus gasseri* SBT2055 (SBT2055) to extend life span and prevent senescence, and analyzed these mechanisms in *Caenorhabditis elegans* (*C. elegans*).
Methods: We analyzed the life span by administration of SBT2055 or control E.coli in wild type N2, *daf-16* mutant or *daf-2* mutant of *C. elegans*. In addition, senescence was evaluated by lipofuscin accumulation. Furthermore, expression of critical genes for insulin like signaling, and oxidative stress response was determined using qPCR.
Results: SBT2055 administration showed an approximately 29% increase in mean life span and prevented senescence in *C. elegans*. These effects were observed in even loss-of-function mutants, *daf-16* and *daf-2*. Furthermore, SBT2055 administrated N2 became highly resistant to oxidative stress by paraquat treatment. In addition, mRNA levels of *skn-1* and *sod-1* genes were much higher in SBT2055 administrated N2 than in control worms.
Conclusions: SBT2055 administration was effective to extend life span and inhibit senescence in *C.elegans*. These effects were suggested to depend on the induction of oxidative stress resistance genes, but not on the regulation of insulin like signaling.

PRELIMINARY RESULTS ON CLINICAL EFFECTS OF THE PROBIOTIC L.SALIVARIUS LS01 IN CHILDREN AFFECTED BY ATOPIC DERMATITIS

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Introduction: Atopic dermatitis (AD) is an inflammatory skin disease. Probiotics have been reported to modulate immune responses and thus are now being suggested as a potential approach to the prevention and manipulation of allergic diseases such as atopic dermatitis (AD). However, the influence of different bacterial strains and their immunomodulating capacities is still largely unknown.

Aim of study: The aim of this study was to evaluate the efficacy of the oral administration of *L.salivarius* LS01 on the clinical course and the quality of life of children affected by moderate and severe atopic dermatitis (AD).

Material and methods: Forty-six pediatric patients aged from 0 to 10 years (M/F ratio 1:1) with AD were recruited at Pediatric Allergology Unit of the Hospital of Spoleto and Foligno, and at Pediatric clinics adhering to the current study. The enrollment was carried out from 1° December 2012 to 31 January 2013. The severity of atopic dermatitis was evaluated by mean of SCORAD index. Subjects with the following characteristics were excluded from the study:

- Rhino conjunctivitis and/or acute asthma;
- Chronic diseases;
- Treatment in the last month with probiotics, systemic steroids, systemic antihistamines, immunomodulatory drugs;
- Infectious diseases in progress;
- Hypersensitivity to some components contained in the sachets administrated.

Probiotic treatment: Patients took sachets contained a lyophilized form of *L.salivarius* LS01 (10^9 CFU/sachet) b.i.d for 8 weeks and s.i.d for the following 8 weeks. Subjects could use emollients and only if strictly needed they could be subjected to topical steroid therapy according to the specialist's advices. Patients were visited at the beginning of the study (T0), and every 4 weeks (at T4, T8, T12, T16, T20 during the treatment and at T20 after the end of treatment).

Preliminary results: Withdrawn patients: a total of 12 drop-outs has been recorded (5 patients at T4, 1 at T8, 2 at T12, 1 at T16, 3 at T20). No adverse event was reported.

The SCORAD and the objective SCORAD values were evaluated until the 16° week of therapy (T16) and the following 4^{th} weeks after the end of therapy (T20).

Here reported the results obtained at T0 and T20:

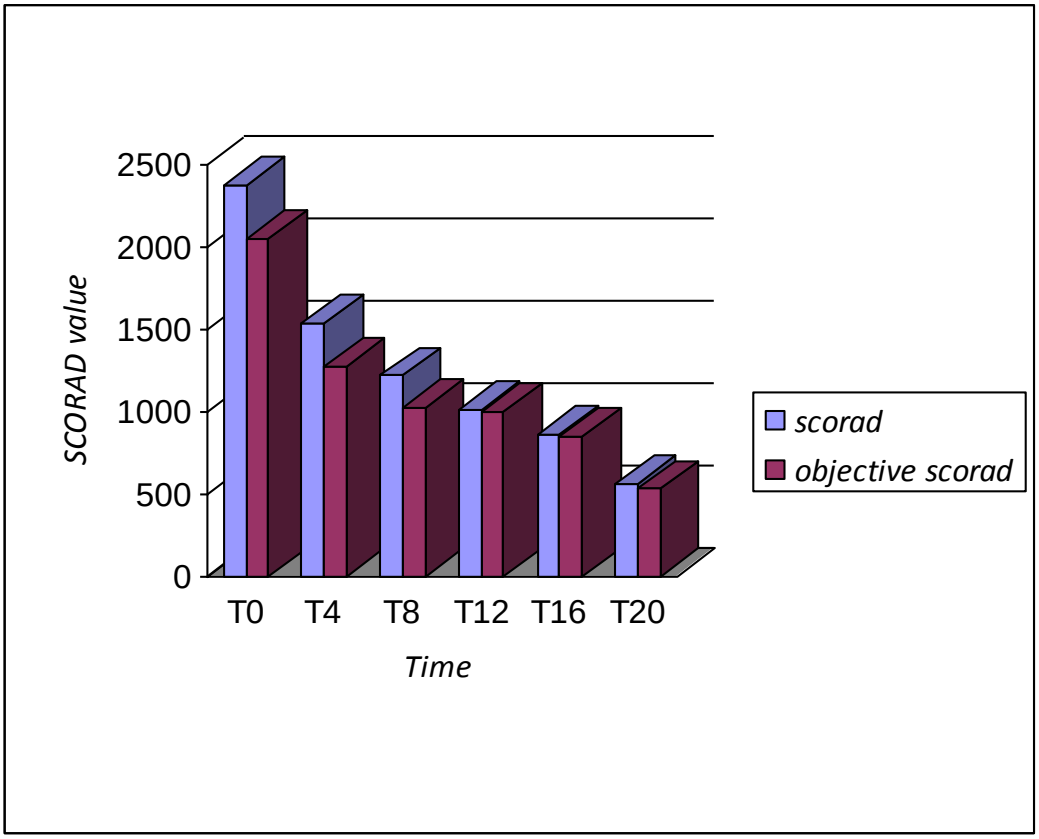
- SCORAD maximum value at T0: 2384.2
- SCORAD minimum value at T20: 564.7
- Objective SCORAD maximum value at T0: 2050.7
- Objective SCORAD minimum value at T20: 527.7

Figure 1 shows the overall SCORAD values obtained during the treatments.

Conclusions: These preliminary data showed a remarkable efficacy of probiotic *L.salivarius* LS01 treatment on children affected by AD. Indeed, pruritus and the extension of cutaneous lesions have been observed to decrease during probiotic treatment, as well as the SCORAD and objective SCORAD values (Figure 1). Moreover, a significant improvement of skin hydration was observed in many patients during the study.

A double-blind study needs to be performed to confirm these results and to deeply investigate the clinical, immunological and microbiological effects of the treatment with probiotic *L.salivarius* LS01 on pediatric patients affected by atopic dermatitis.

Figure 1. Decrease in SCORAD and objective SCORAD value during the study



CAPABILITY OF EXOPOLYSACCHARIDE-PRODUCING LACTOBACILLUS PARAPLANTARUM BGCG11 TO COUNTERACT THE EFFECT OF ENTEROPATHOGENS ON THE INTESTINAL CELL LINE HT29-MTX

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Objective: The study of the protective role of exopolysaccharide (EPS-CG11) produced by *Lactobacillus paraplantarum* BGCG11 upon HT29-MTX cell monolayers challenged with opportunistic pathogens common inhabitants of the intestinal microbiota.
Methods: HT29-MTX monolayers were incubated for 3 h with seven pathogens (10^7 cells: 10^8 CFU), or with combinations of each pathogen and three lactobacilli, or pathogens and purified EPS-CG11 (1 mg/ml). The BGCG11 derivative NB1 that has lost the EPS-CG11 production, probiotic strain *Lactobacillus rhamnosus* LMG18243 (named GG) were used as controls. The cellular response was evaluated by measuring the production of IL-8 (ELISA test), mucin (by fluorescence-conjugated lectin) and determining the cellular cytolysis (by LDH release).
Results: The presence lactobacilli in combination with some pathogens increased the production of IL-8 with respect to the pathogen alone ($p<0.05$ for *Clostridium difficile* LMG21717, *Listeria monocytogenes* LMG13305, *Shigella sonnei* LMG10473 and *Yersinia enterocolitica* LMG7899). The IL-8 production induced by lactobacilli is a positive trait - this chemokine could increase the recruitment of neutrophils. Furthermore, the production of mucin was also slightly improved in the presence of lactobacilli ($p<0.05$ for *C. difficile*, *L. monocytogenes* and *Y. enterocolitica*) and the cytolysis of HT29-MTX cells was significantly reduced when *C. difficile* was co-incubated with the lactobacilli or the purified EPS-CG11.
Conclusions: *Lactobacillus paraplantarum* BGCG11 and its derivative showed an *in vitro* probiotic potential since both were able to induce the synthesis of defense molecules by the epithelial cells challenged with opportunistic pathogens. Therefore, we propose that these lactobacilli could act by reinforcing the innate mucosal barrier.

LIVE LACTOBACILLUS GG COUNTERACTS THE EFFECTS INDUCED BY GLIADIN ON THE CELLULAR POLYAMINE CONTENT AND INTESTINAL EPITHELIAL PARACELLULAR PERMEABILITY OF CACO-2 CELLS

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Objective: The study aimed at establishing in epithelial Caco-2 cells whether i) gliadin affects the cellular polyamine content along with paracellular permeability and ii) concomitant administration of live Lactobacillus rhamnosus GG (L.GG) could counteract the gliadin induced alterations. The effects of L.GG on TJ protein expression were tested in presence or absence of polyamines.
Methods: Caco-2 cells were treated with gliadin (1 mg/ml) alone or in combination with viable L.GG (108 CFU/ml) for 6h. The resistance of the cell monolayer was measured using a Millicell-ERS volt-ohm meter and lactulose flux by HPAEC. Zonulin release was quantified by an ELISA kit. Polyamine levels were evaluated by HPLC. Finally, ZO-1, Claudin-1 and Occludin gene expression was performed by RT-real-time PCR.
Results: Gliadin administration significantly increased (+36%) the polyamine content and altered paracellular permeability as indicated by the reduction in transepithelial resistance accompanied by a three-fold increase in zonulin release and lactulose paracellular transport. When L.GG was added to gliadin, the cellular polyamine levels lowered (-21%) and barrier functions were restored. Besides, ZO-1, Claudin-1 and Occludin mRNAs increased significantly compared to that in controls or cells treated with gliadin alone. These modifications were not observed when cells were deprived in polyamines by DFMO.
Conclusions: Gliadin significantly increases the polyamine content and alters the intestinal paracellular permeability in Caco-2 cells. Concomitant administration of L.GG is able to counteract these effects. Interestingly, the presence of cellular polyamines is necessary for L.GG to restore paracellular permeability by affecting also the expression of TJ proteins.

EFFECTS OF MULTISTRAIN PROBIOTIC PREPARATION ON THE CYTOKINE PROFILE IN RAT LIVER AND PANCREAS UPON LONG-TERM GASTRIC HYPOCHLORHYDRIA

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Objective: Long-term gastric hypochlorhydria (LTGH) can lead to digestive disorders, dysbiosis and inflammation development in gastrointestinal tract, including liver and pancreas. Probiotics are widely used for restoration of gastrointestinal homeostasis by virtue of a broad spectrum of biological activities.
Cytokine profile is a useful indicator of inflammation upon different diseases. So, the aim of study was to determine the cytokine profile in rat liver and pancreas upon LTGH and administration of multistrain probiotic (MSP).
Methods: Experiments were performed on white non-strain rats. LTGH was induced by 28-day long abdominal omeprazole injection (14 mg/kg per day). The second animal group was simultaneously orally treated with multiprobiotic preparation containing 18 strains. The levels of interleukins (IL) and TNF-alpha were determined in liver and pancreatic homogenates by immunochemiluminometry.
Results: The increased levels of IL-6, IL-8 and TNF-alpha (in 8, 1,2 and 1,3 times, respectively) and decreased concentration of IL-10 (in 1,3 times) in rat liver in comparison to control upon LTGH were observed. Moreover, levels of IL-6, IL-8 and TNF-alpha were higher than control in 1,5, 4,5 and 3 times, respectively, while the concentration of IL-10 was 1,8 times lower. Joint administration of MSP with omeprazole was associated with normalization of studied parameters in liver and pancreatic tissues.
Conclusions: The increase in pro-inflammatory cytokines (IL-6, IL-8, TNF-alpha) and decrease in anti-inflammatory cytokine IL-10 indicate the inflammation development in rat liver and pancreas upon LTGH. MSP ameliorates endogenous intoxication and promotes immunity correction of hypochlorhydria-related systemic consequences, probably through tolerated microbiota restoration.

EFFECTS OF EARLY PREBIOTIC AND PROBIOTIC INTERVENTION ON DEVELOPMENT OF GUT MICROBIOTA AND CRYING IN PRETERM INFANTS - A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Objective: The aim of this study was to evaluate the impact of early pre- and probiotic intervention on preterm infants' crying and microbiological programming.
Methods: 94 preterm infants (gestational age 32-36 weeks and birth weight >1500g) who were randomized to receive prebiotics (a mixture of galacto-oligosaccharide and polydextrose 1:1), probiotics (*Lactobacillus rhamnosus* GG) or placebo during the first 2 months of life were followed up for 1 year. Infants were categorized into two groups based on their crying amount during the first two months of life. Their gut microbiota were investigated by FISH (n=66) and qPCR (n=63).
Results: During the first two months of life 27/94 (29%) infants were classified as excessive crying infants. The infants receiving pre- or probiotics manifested significantly less frequently excessive crying (19% in the both groups) than the infants receiving placebo (47%, p=0.02). The placebo group manifested higher proportion of *Clostridium Histolyticum* counts to total bacterial counts than the probiotic group (13.9% vs 8.9%; p= 0.05 respectively). No adverse events related to the supplementations were reported.
Conclusions: Both pre- and probiotic supplementation decreased early crying in preterm infants. This novel observation calls for further studies to reveal the exact mechanism behind this interconnection.

IMPACT OF THE PROBIOTIC LACTOBACILLUS PLANTARUM TENSIA (DSM 21380) COMPRISING YOGHURT ON BLOOD INDICES AND BLOOD PRESSURE OF HEALTHY VOLUNTEERS

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A substantial body of evidence has implicated that the dietary approaches are become more and more relevant for prevention of metabolic syndrome, incl. high blood pressure. The blood pressure lowering effect of dairy products helps to lower the risk of CVD.
We have previously reported (6th Probiotics, Prebiotics, New Food, OC2.11) the antihypertensive effect of *L. plantarum* Tensia containing cheese.

Objective: To evaluate the blood pressure lowering effect of probiotic *L. plantarum* Tensia yoghurt applying the same study design.
Material and methods: We conducted a double-blind placebo-controlled cross-over study with 68 healthy Estonian adults consuming test yoghurt or control yoghurt for 3 weeks. Blood samples were collected and blood pressure was measured at the beginning and at the end of the trial.
Blood samples were collected after an overnight fast and tested for inflammatory and metabolic (plasma glycose and lipids) markers and also osteoprodegerin (OPG).
Results: No significant changes in inflammatory and metabolic markers and BMI were found.
There were significant differences in blood pressure in probiotic group: median change (baseline *vs* after probiotic consumption) in SBP -3.5 mmHg, $p<0.005$ and DBP -0.8 mmHg, $p<0.05$**).**
Median change in OPG values during 3-week probiotic consumption: -0.15 pmol/L ($p<0.05$).
Conclusions: Our preliminary study showed that 3-week consumption of probiotic *L. plantarum* Tensia yoghurt has the SBP and DBP pressure lowering effect and has also reduces the stiffness of small blood vessels. Indicator of OPG influences the stiffness of small blood vessels (capillars), which could be a possible mechanism of maintain the health of cardiovascular system.

IDENTIFICATION OF LACTOBACILLUS SPP. STRAINS BY MALDI BIOTYPER

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Introduction: Classical phenotypic/biochemical characterization of bacteria remains important for preliminary identification, although it is not always very efficient in the case of lactobacilli and is expensive. Molecular methods, though reliable, are also expensive and time consuming.
A novel fast, procedurally simple and sensitive chemotaxonomical method with a high discriminatory power, the Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF MS) is gaining popularity in identification of clinical isolates. However, little information is available concerning this method in identification of lactobacilli.
Objective: To assess MALDI TOF MS profiling as a tool for reliable and fast identification of lactobacilli.
Methods: Altogether 726 *Lactobacillus* spp. strains isolated from intestinal tract of healthy Estonian and Swedish adults were identified using MALDI Biotyper (Bruker Daltonik). Of them, 470 strains were identified additionally by API 50 CHL (BioMérieux, Marcy-l'Etoile, France).
Results: Using MALDI Biotyper, 667 / 726 (92%) were successfully identified during the first attempt. Additionally 41 strains were identified during next 2 attempts and 18 strains (2%) remained unidentified.
The identified strains belonged to 17 species, most numerous were *L. paracasei* (203 strains), *L. gasseri* (163), *L. acidophilus* (124), *L. fermentum* (44), *L. ruminis* (36) and *L. rhamnosus* (35).
When comparing the chemotaxonomical (MALDI Biotyper) and biochemical (API 50 CHL) identification method, we revealed full agreement only for 37% of strains since the *Lactobacillus* species database is significantly smaller in case of API method than MALDI TOF MS method (18 vs 107 species). However, the agreement in fermentation group level was 99.4%.
The majority of discrepancies between the two systems tested occurred for the *L. acidophilus* group while the most coinciding results were seen in case of *L. plantarum, L. paracasei* and *L. fermentum.*
Conclusion: MALDI TOF MS is a rapid and reliable method for identification of lactobacilli.

PRODUCTION OF ANTIMICROBIAL SUBSTANCES IN MICROORGANISMS PROBIOTICS

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Objective: Some lactic acid bacteria produce antimicrobial substances such as, organic acids, hydrogen peroxide and bacteriocins, capable of inhibiting the growth of microorganisms contaminants of food, that are potentially pathogenic for humans. The aim of this work was to evaluate the production of antimicrobial substances by probiotic strains of *Enterococcus faecium* CRL 183 and *Lactobacillus acidophilus* CRL 1014.
Methods: Antimicrobial activity was determined by the spot-on-the-lawn assay, using *Listeria monocytogenes* IAL 628, *Escherichia coli* IAL 339 and *Salmonella enterica* subsp. *enterica* ser. *typhimurium* IAL 2431 as indicator strains. Isolates of *Enterococcus faecium* CRL 183 or *Lactobacillus acidophilus* CRL1014 (2 µl) was spotted on trypticase soy agar (TSA) supplemented with 0.6% yeast extract. (TSAYE) and incubated at 37 °C for 24 hours. Finally, the plate was overlaid with 8.0 ml of soft BHI agar (0.5% agar) seeded with 1% of a suspension of 10^7CFU/mL of each indicator organism separately, and incubated under anaerobic conditions for 24 hours at 37°C. The antagonism was detected by formation of an inhibition zone (clear zone) around the tested microorganism.
Results: The results showed that the both probiotic strains evaluated were not able to produce antimicrobial substances against the tested microorganism, since no clear zone was observed after incubation period.
Conclusions: Despite the well known health properties, *Enterococcus faecium* CRL 183 and *Lactobacillus acidophilus* CRL1014 could not be used to prevent the food contamination by the strains evaluated, since no antimicrobial substances production were detected.

EPIDEMIOLOGICAL FACTORS AND FOOD: WHICH IS THE ROLE IN HELICOBACTER PYLORI RE-INFECTION IN PEDIATRIC AGE?

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Background: *Helicobacter pylori* (Hp) infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis and gastric cancer. Its acquisition is related with poor socioeconomic conditions while the relationship of nutrition and Hp is still a question.
AIM To analyzed if socioeconomic factors and dietary contribute to Hp re-infection in pediatric age
Patients and Methods: 150 patients (92 males; age range 5-16 years) with Hp infection treated and eradicated in the past. 55 patients with Hp re-infection and 95 patients not re-infected.
We interviewed the children with questionnaire about socioeconomics factors, hygiene, living conditions and their dietary habits.
Results: A lower frequency of fermented dairy food, fruits and vegetable consumption was registred among children with Hp re-infection as compared to not been re-infected.
Among persons with Hp re-infection were noted low socio-economic markers such as croweded living conditions, a large number of siblings and unclean water.
Conclusions: Might decrease the risk of Hp re-infection the use of probiotic, vitamin C, antioxidants contained in fruit and vegetables.
Risk factors for Hp re-infection are low socioeconomics factors, hygiene and living conditions.

ROLE OF FERMENTED MILK WITH B. BIFIDUM ASSOCIATED LB.ACIDOPHILUS IN THE ANTI-DIARRHEAL LUTE

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Objective: Role of fermented milk with *B. bifidum* associated *Lb.acidophilus* in the anti-diarrheal lute

Methods: In this study, seven strains of bifidobacteria have been used, and a strain of *E. coli*, enteropathogenic (EPEC) O111:. B4. The strain of *B. bifidum* was selected based on its characters acidotolérance quite significant. In addition, it seems to produce an active antibacterial substance to EPEC

The antagonistic effect *in vivo* of *B. bifidum* associated with *Lb. acidophilus* to EPEC, focused on four lots holoxenic mouse with 4 mice each. After provoking diarrhea in mice, treatment of the latter was made. It consists of the daily administration of 1 ml of adapted milk containing 10^9 CFU / ml for *B. Bifidum and Lb. acidophilus* alone or associated with hydrolyzable of casein (prtébiotique). Lot of witnesses was provided (EPEC alone, only *B. bifidum* associated with *Lb. acidophilus*, *B. bifidum* associated with *Lb. acidophilus* + hydrolyzable casein)

Results: A fermented milk with *B. bifidum* associated with *Lb. acidophilus* allows greater decrease *E.coli* only when the milk is fermented with one or *B. bifidum* enriched casein hydrolyzate (bifidigéne factor). Indeed, the evolution of the number of *E. coli* in the feces of mice were given two treatments: preventive treatment (before contamination with *E. coli*) and therapeutic (after contamination with *E. coli* showed better results when it comes to treatment preventive and antagonize significantly higher in the presence of both species combined with a decrease rate of *E. coli* (90%), and the third day of treatment. However, a reduction rate of 23% only, was obtained with the casein hydrolyzate, and no reduction was observed when *B. bifidum* alone. In the therapeutic treatment, a percentage reduction of 94.9% was noted after taking the fermented milk with *B. bifidum* mixed culture. A mortality rate of 50% was noted for the lot that received *E.. coli*. results of Audit of the intestinal flora after dissection mice show the ability of both species to survive many in their gastric transit to exert an antagonistic effect towards the establishment of *E. coli enteropathogenic* responsible for childhood diarrhea.

GELENTERUM AMELIORATES MURINE COLITIS WHILE MODULATING GUT MICROBIOTA AND INTESTINAL MUCUS LAYER

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Objective: Gelatine Tannate (Gelenterum), a gelatin powder containing Tannic Acids, is commonly used for diarrhea in children. Few information exist on its mechanisms of action, involving gel formation and bacterial toxin sequestration, mostly obtained by in vitro studies.
Aim of this study was to evaluate the effect and mechanisms of action of Gelenterum in the murine model of acute colitis by DSS.
Methods: C57BL/6 mice receiving 2,5% DSS in drinking water ad libitum for 8 days.
At day 4, 5, 6 and 7, were treated with gelenterum 1 mg or 10 mg in 200ml of tap water, or saline solution, given orally by gavage starting day.
Body weight, occult blood test and stool consistency were measured every other day to calculate Disease Activity Index (DAI), as assessment of severity of colitis.
Mice were sacrificed at day 9, serum samples were collected, colon length measured for histology assessment by Rachmilewitz score.
To characterize gut microbiota modulation, stools were collected at day 0, 5 and 9 and cultured in selective media.
RT-PCR was also performed on fecal as well as on intestinal samples.
Colonic mucus layer were analysed at confocal microscopy at 2 photon and atomic force microscope.
Finally, LPS and peptidoglycan were evaluated by ELISA test in peripheral blood.
Results: Gelenterum reduced DAI and body weight loss in treated mice, being 10 mg more efficacious than 1 mg dose.
Gelenterum treated mice displayed a longer colon. In fecal culture, Acinetobacteria, Enterobacteria, Enterococci and Lactobacilli grew from stool as well as from intestinal culture.
Treated mice showed a lower concentration of Enterobacteria and Enterococci, and higher concentration of lactobacilli.
At confocal microscopy, intestinal samples from healthy and treated mice displayed a similar structure in mucus layer thickness and composition, while samples from placebo group had no mucus layer or thinner stratus.
Atomic force microscopy confirmed these findings, suggesting that mucus in treated mice could derive from 2 different sources.
Serum LPS levels were lower in non colitic mice as well as in Gelenterum treated mice, while no significant variation in Peptidoglycan serum levels was observed.
Conclusions: Gelenterum decreased the severity of colitis in mice and re-establishes gut homeostasis by modulating microbiota composition and by maintaining mucus layer, and probably reducing gut permeability to LPS. Further analysis are required to better define mechanisms of action underlying these findings and more indication for gelenterum could be developed following specific studies.

CLOSTRIDIUM BOTULINUM AND LISTERIA MONOCYTOGENES: SINGLE SPECIES OR CONSORTIA OF PROBIOTICS TO LIMIT THE PATHOGENS RISK?

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Objective: *Clostridium botulinum* and *Listeria monocytogenes* are two of the current microbiological hazards associated with foods, as both have been implicated in intestinal infection diseases.
Probiotics used in some foods and supplements have a potential role for microbial risk control.
The purpose of this study was to determine whether some associations of different probiotic species (*consortia*) may be more effective in the inactivation of the above pathogens, compared to the same probiotic species used individually.
Methods: The study had therefore two steps:
1) analysis of reciprocal inhibition of different probiotic species commonly used in the *consortia* and selection of the combinations with less reciprocal inhibition;
2) evaluation of the antimicrobial activity of the different selected probiotic *consortia vs C. botulinum* and *L. monocytogenes*, compared to the activity of the single probiotic species.
Specifically, we tested the reciprocal antibacterial activity of eighteen single probiotic species belonging to five different genera. On the bases of the obtained results we selected three probiotic *consortia* to be tested. The inhibitory activity was evaluated using the agar spot test by measurement of the inhibition area around spots.
Results: Most of the single probiotic strains used in our consortia showed a higher antimicrobial activity *vs* the two pathogens, compared to the selected probiotic *consortia*.
Conclusions: In conclusion, we have identified some strains of probiotics that have a low reciprocal inhibition, but a considerable inhibitory activity toward *C. botulinum* and *L. monocytogenes.* These strains, preferably used individually, still retain a significant activity also in *consortia*.

LACTOBACILLUS PLANTARUM AND BIFIDOBACTERIUM LONGUM ENHANCE INTESTINAL ABSORPTION OF NUTRIENTS

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Objective: There is a growing evidence of beneficial effects of probiotics, especially if used in combination with some therapies. This is also due to a direct action of probiotics to the intestinal mucosa, which is responsible of digestion and absorption of several molecules and sugars such as inositols.
The main part accountable for the molecules' absorption, is the small intestine, where only a few probiotics grow and act; in particular two bacteria, Bifidobacterium longum and Lactobacillus plantarum, are known for their action to small intestine. Thus, they could improve Pharmaceutical and nutritional drugs absorption and activity
Methods: It has been made a review of literature, regarding the use and application of these probiotics. The main issue was the in vitro/in vivo evaluation of beneficial action to the intestinal activity
Results: The administration of Bifidobacterium longum induces a greater enzyme activity and intestinal absorption of nutrients. In particular, it has been shown a relation with the decrease of blood serum levels of glucose, mineral and lipids.
Lactobacillus plantarum, has shown a strong action to keep the integrity of intestinal barrier, restoring junctions and promoting physiological activity of intestine
Conclusions: Even if the importance of probiotics for human health is well known, there is still little evidence showing a direct action of these bacteria to intestinal digestion and drugs absorption. Recently, there was a growing interest on probiotics, and some of these, such as Bifidobacterium longum and Lactobacillus plantarum, have shown to be especially useful in improving the intestinal absorption of molecules.

N-(1-CARBAMOYL-2-PHENYL-ETHYL) BUTYRAMIDE, A NEW SYNTHETIC BUTYRATE DERIVATIVE, REDUCES INTESTINAL INFLAMMATION IN DEXTRAN SODIUM SULPHATE-INDUCED COLITIS

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Objective: In this study we comparatively evaluated the effects of a new synthetic butyrate derivate, n-(1-carbamoyl-2-phenyl-ethyl) butyramide (FBA), and of the equimolecular dose of sodium butyrate (Butyrate) in dextran sulfate sodium (DSS)-induced colitis.
Methods: Experimental colitis was induced in male BALB/c mice by 2.5% DSS in drinking water for 5 days. The oral treatment with Butyrate (20 mg/kg/d) or FBA (42.5 mg/kg/d), started 7 days before DSS challenge and continued for all experimental period (20 days).
Results: DSS induced a significant reduction in animal weight, intestinal and colonic length. Hematoxylin and eosin staining reveled that in DSS-treated mice the normal architecture of the mucosa was lost. In these animals, the inflammatory status of the mucosa was assessed by the increase of COX-2, iNOS and cytokines TNF-α and IL-6. DSS increased neutrophil infiltration and reduced occludin and IL-10 mRNA levels in the colon mucosa with a reduction in serum adiponectin. Butyrate and FBA showed protective effects on weight loss, oxidative damage and structural integrity of colonic mucosa. In addition Butyrate, and in particular FBA, were able to limit mucosal inflammation, decreasing COX-2, iNOS, TNF-α and IL-6. A similar effect was observed on reducing myeloperoxidase and partially restoring adiponectin and IL-10 levels.
Conclusions: Butyrate is able to limit early molecular events underlying inflammatory process and intestinal damage, suggesting its potential clinical utility as preventive and therapeutic strategy for UC. Since FBA does not have the characteristic odor of rancid cheese, this derivative may represent a viable therapeutic alternative to Butyrate, favoring a better compliance and a greater effectiveness.

PROBIOTIC CHEESE WITH CARDIO-PROTECTIVE EFFECT

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Cardiovascular disease is the most frequent cause of mortality and morbidity in elderly. Long term exposure to elevated blood pressure (BP) in younger subjects also leads to partly irreversible risk situation, shortening otherwise longer life expectancy.
Previously (6th Probiotics, Prebiotics, New Food, OC2.11) we reported that 3-week consumption of *L.plantarum* TENSIA® (DSM 21380) containing cheese helps to maintain normal BP.

Objective: To demonstrate sustained effect of continuous consumption of the probiotic over longer period (8-weeks) as suggested by EFSA (2011).
Methods: A randomized, blind, controlled, parallel-designed 2-armed intervention (ISRCTN29105501) was carried out. Clinically healthy adults (n=206; 43.6±11.1yrs) consumed 50g of control or probiotic cheese for 4+4 weeks (daily dose of TENSIA 10.0 logCFU).
Primary outcome: significant ($p<0.05$) SBP reduction. Secondary outcome measure: significant DBP reduction. Additional markers: changes in oxidised-LDL, total peroxide count (TPX), total antioxidant capacity (TAC), oxidative stress index (OSI).
Results: Median change during 4-week and 8-week probiotic consumption:
SBP (-5.28mmHg and -7.97mmHg, p=0.0002, p=<0.0001, respectively),
DPB (-5.65mmHg and -7.92mmHg, p=0.0001, p=<0.0001, respectively),
OSI (-0.4 and -3.5, p=0.050, p=<0.001, respectively),
TPX (-5.0 and -32.0mol/L, p=0.193, p=<0.001, respectively),
TAC (0.02 and 0.08µmol Trolox equivalent/L, p=0.135, p=0.046, respectively),
Ox-LDL (-2.1 and -4.2U/L, p=0.050, p=<0.001, respectively).
Conclusions: The probiotic cheese containing *L.plantarum* TENSIA has cardio-protective effect through lowering blood pressure and reduction of some subclinical oxidative stress markers.

EFFECTS OF A SPECIFIC MIXTURE OF SHORT-CHAIN GALACTOOLIGOSACCHARIDES AND LONG-CHAIN FRUCTOOLIGOSACCHARIDES ON STOOL FREQUENCY AND STOOL CONSISTENCY

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Objective: A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS) in a ratio of 9:1 has been shown to beneficially affect the composition of the intestinal microbiota and to reduce the incidence of infections and the risk of allergies in healthy or at-risk infants. Additionally, prebiotic oligosaccharides may improve digestive comfort by affecting faecal characteristics such as stool consistency and stool frequency. Here, we aim to review the evidence on the described effects of this specific mixture of scGOS/lcFOS (ratio 9:1) on stool consistency and stool frequency.
Methods: All studies that were done with a specific mixture of scGOS/lcFOS (ratio 9:1) in healthy term or preterm infants, using regular infant milk formulas, were screened on key words that are related to stool characteristics.
Results: Seven studies addressed effects of scGOS/lcFOS (ratio 9:1) on stool frequency, and six of these also addressed effects on stool consistency or stool viscosity. Stool frequency was significantly increased in the prebiotic group when compared to a standard group in four out of seven studies. Stool consistency was significantly softer in the prebiotic group compared to a standard group in five out of six studies.
Conclusions: Supplementation of infant milk formulas with a specific mixture of the prebiotic oligosaccharides scGOS/lcFOS (ratio 9:1) was shown to decrease stool consistency and/or to increase stool frequency in healthy (pre)term infants in the majority of the reported studies. This illustrates that the addition of scGOS/lcFOS (ratio 9:1) to infant milk formulas may improve digestive comfort in healthy infants.

PROBIOTIC SUPPLEMENTATION MODULATES INTESTINAL MICROFLORA AND IMPROVE NK CYTOTOXIC ACTIVITY IN PEDIATRIC PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Conclusions: Combined prescription of multiprobiotic Symbiter with broad-spectrum antibiotics, e.g. beta-lactam antibiotics, is effective to prevent development of hepatic and colonic dysfunctions.
Objective: In this study we evaluated, in pediatric HSCT recipients, the role of probiotic supplementation in preventing infections and intestinal complications and in improving microbiological and immune reconstitution. We characterized intestinal microflora and analysed the probiotic modulation of innate immunity in recipients and controls without probiotic supplementation.
Methods: 2 patients were supplemented with *L. paracasei* I 1688, *L. salivarius* I 1794 and one patients was supplemented with *B. bifidum* W23,*B. lactis* W52, *Lactococcus lactis* W58, since 1-2 month after HSCT. Gut microflora was evaluated through gel electrophoresis in denaturing gradient (DGGE), following RT-PCR and innate antiviral response was detected through NK cytotoxicity assay. NK cells population was characterized in flow cytometry.
Results: Conditioning therapy for HSCT induced intestinal dysbiosis, with an increasing of *Enterococcus faecium*, while probiotic supplementation restore the normal heterogeneity of microbiological pattern, with an improvement of *Ruminococcus* species. Probiotic supplementation in patients improve NK activity, not detected in the control group. No patients developed bacterial infections during follow up. One patient, presenting a IV degree acute intestinal GVHD unresponsive to therapy, showed resolution of inflammatory lesions and reduction to II degree GVHD after probiotic supplementation.
Conclusions: This data may suggest that probiotics can be useful to stimulate motility through the restoration of normal intestinal microflora. Probiotics stimulate proliferation and activation of NK cells, underlying a positive effect on antiviral response. Data on GvHD complications show an improvement in two patients' prognosis, suggesting a possible role of probiotics in the improvement of immunological reconstitution and patients' follow up.

INTESTINE ANTI-INFLAMMATORY EFFECTS OF OLIGOSACCHARIDES DERIVED FROM LACTULOSE (OSLU) IN THE TNBS MODEL OF RAT COLITIS

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Objective: Oligosaccharides derived from lactulose (OsLu) are new bioactive carbohydrates synthesized to obtain a more slowly fermenting prebiotic. They would be able to reach distal colon without being hydrolyzed, thus avoiding the side effects ascribed to lactulose, like abdominal distension or flatulence among others. OsLu showed higher prebiotic index value than lactulose and is able to also stimulate bifidobacteria and lactobacilli growth. Lactulose has been reported to exert intestinal anti-inflammatory effect in experimental colitis. The aim of this study is to test the effects of OsLu in the trinitrobenzenesulphonic acid (TNBS) model of experimental rat colitis, and compare it with the effects of the parent compound lactulose.
Methods: Female Wistar rats were treated with both lactulose or OsLu, incorporated in the drinking water at the concentration of 2.5% (w/v) for three weeks. After this time, colitis was induced by intracolonic administration of 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v).
Results: Both Lactulose and OsLu, showed intestine anti-inflammatory effects, being this associated with an inhibition of different cytokines (IL-1β, IL-6, IL-17) and chemokines (CINC-1, MCP-1), as well as by the restoration of the colonic epithelial integrity evidenced by an increase in the expression of MUC-2, MUC-3 and TFF-3. The beneficial effect of OsLu was also associated with an inhibition of iNOS expression and a reduction of Th17 cell activity.
Conclusions: Both prebiotics were able to restore the ratio between beneficial and potential pathogen bacteria, which was modified in colitic rats.

PREVENTION AND TREATMENT OF DIARRHEA WITH *SACCHAROMYCES BOULARDII* IN CHILDREN WITH ACUTE LOWER RESPIRATORY TRACT INFECTIONS

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Objective: To determine whether *Saccharomyces boulardii (S. boulardii)* can prevent and treat diarrhea and antibiotic-associated diarrhea (AAD) in children.
Methods: A total of 333 children with acute lower respiratory tract infection were enrolled in an open randomized controlled trial. This was a 2-phase study. During the 1st phase all children received intravenous (IV) antibiotic treatment and were randomized in two groups: group A (*S. boulardii* + IV antibiotics, n =167) and group B (IV antibiotics alone, n =166). The children in group A received also 500 mg *S. boulardii* for the duration of the antibiotic treatment. All children were followed for 2 weeks. Analyses were based on allocated treatment and included data from 283 patients. In the 2nd phase of the study, patients in group B who developed diarrhea during the antibiotic treatment were randomized in two sub-groups: group B1 (*S. boulardii* + oral rehydration solution (ORS)) and Group B2 (ORS alone).
Results: Patients from Group A had a lower prevalence of diarrhea than group B [11/139 (7.9%) vs. 42/144 (29.2%); relative risk (RR): 0.27, 95% confidence interval (CI): 0.1–0.5]. *S. boulardii* reduced the risk of AAD compared with group B [6/139 (4.3%) vs. 28/144 (19.4%), RR: 0.22; 95% CI: 0.1–0.5]. When children in group B developed diarrhea (n:42), *S. boulardii* treatment during 5 days (group B1) resulted in lower stool frequency and better recovery than single ORS ($P<0.05$). After 5 days, the recovery rate in group B1 (91.3%) was significant higher than in group B2 (21.1%) ($\chi 2=21.3$，$P<0.001$). The mean duration of diarrhea in group B1 was shorter than in B2 (2.31 ± 0.95 vs 8.97 ± 1.07 days, $t=21.4$，$P<0.001$). No adverse events related to *S. boulardii* were observed.
Conclusion: *S. boulardii* is effective in the prevention of diarrhea and AAD in children treated with antibiotics because of an acute lower respiratory tract infection and the treatment of diarrhea developing in these children.

A DOUBLE BLIND RANDOMIZED TRIAL SHOWING PROBIOTICS TO BE INEFFECTIVE IN ACUTE DIARRHEA IN INDONESIAN CHILDREN

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Objective: To investigate the efficacy of probiotics added to oral rehydration solution and zinc in the treatment of acute infectious diarrhea in children in Indonesia.
Methods: A prospective randomized double blind placebo-controlled trial was performed to test the efficacy of a probiotic food supplement in 112 children aged 6-36 months with acute infectious diarrhea and moderate dehydration in the Kenari subdistrict, central Jakarta. The supplemented group was given standard therapy (oral rehydration solution and zinc) and the probiotic strains *Lactobacillus (L.) rhamnosus* R0011 $1.9x10^9$ colony forming units (cfu) and *L. acidophilus* R0052 $0.1x10^9$ cfu/day for 7 days, while the control group was given standard therapy and placebo.
Results: Median duration of diarrhea was 68.5 (range 13-165) hours in the supplemented and 61.5 (range 21-166) hours in the control group (P=0.596). Median daily frequency of defecation until diarrhea stopped was 5.0 in the supplemented versus 5.5 in the control group (P=0.795).
Conclusion: This probiotic food supplement tested did not reduce the duration of acute infectious diarrhea compared to oral rehydration and zinc.

CYSTIC FIBROSIS'S GUT MICROBIOTA UNVEILED BY COMBINED –OMIC APPROACHES: A TOOL FOR SYSTEMS MEDICINE

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Objective: Cystic fibrosis (CF), is a lethal hereditary disorder leading to respiratory infections, chronic inflammation, repeated antibiotic treatments and swallowing of infected respiratory mucus has known or suspected links to gut microbiota.
The aim of this work was to investigate the gut microbiota composition and modulation of CF patients by metagenomic and metabolomic combined analyses in relation with healthy children.
Methods: Thirty faecal samples from either CF patients and healthy children (HC) (age range 0-6 years), were collected. The metabolomics were performed by GC-MS/SPME and ^{1}H-NMR, while metagenomics was conduct by 454 pyrosequencing platform.
Results: About two hundred volatile organic compounds (VOCs) were detected and quantified by GC-MS/SPME. VOCs were grouped according to chemical classes. The inter-individual variability of VOCs levels resulted high. Compared to HC, the level of esters, alcohols and aldehydes were higher in CF patients. On the contrary, SCFAs were higher in HC than CF confirmed also by the ^{1}H-NMR analysis. Moreover the CF patients showed lower levels of aminoacids and uracil than HC. Metagenomic results on FC patients indicate *Firmicutes* as most abundant phyla, while *Bacteroidetes* and Proteobacteria varied according to the sample analyzed. This analysis identified a remarkable degree of variability of the OTUs (200-1000) confirming the high level of inter-individual metabolic variability.
Conclusions: By this integrated approach it's possible to generate personalized "omics" charts that can be used for the monitoring of the nutritional state of the child and for the evaluation of gut absorption in CF patients, hence provide a translational medicine tool.

IN VITRO IMMUNOMODULATORY EFFECTS OF THE PROBIOTIC LACTOBACILLUS FERMENTUM CECT5716

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Objective: *Lactobacillus fermentum CECT5716* is a probiotic with reported immunomodulatory properties. The aim of the present study was to evaluate the *in vitro* effects of probiotic *Lactobacillus fermentum CECT5716* (live or death) in two different cell types involved in the immune response: RAW 264.7 cells (macrophages) Caco-2 cells (intestine epithelial cells). In addition, we investigated the effects of these probiotics on the signaling pathways associated to mitogen-activated protein (MAP) kinases in Caco-2 cells.
Methods: Cells were incubated for 3 hours with each probiotic (live or death) (10^8 CFU/ml), and stimulated with LPS (100 ng/ml) or IL-1β (1ng/ml) for 30 minutes (western blot) or 24 h (IL-8 or nitrite determination). Western blots were performed with protein extracts to analyze phosphorylated or total forms of p42/44 ERK.
Results: *Lactobacillus fermentum CECT5716*, live or death, inhibited the stimulated production of either IL-8 (Caco-2 cells) and nitric oxide (RAW 264.7). In the epithelial cells, this inhibitory effect was associated with a reduced phosphorylation of MAP kinases evaluated (p42/44 ERK) when it compared with stimulated cells without probiotic.
Conclusions: The viability of *Lactobacillus fermentum CECT5716* was not essential to downregulate the stimulated immune response in both epithelial cells and macrophages. Moreover, in Caco-2 cells, this immunomodulatory effect was related with the inhibition of MAP kinase pathways.

EFFECTIVENESS OF AN ASSOCIATION OF A CRANBERRY DRIED EXTRACT, D-MANNOSE AND THE THREE MICROORGANISMS *L. PLANTARUM* LP01, *L. PARACASEI* LPC09 AND *S. THERMOPHILUS* ST10 IN WOMEN AFFECTED BY CYSTITIS: A PILOT STUDY.

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Conflict of interest disclosure and declaration of funding sources:
L. Mogna is an employee of Biolab Research Srl. G. Mogna is an employee of Probiotical S.p.A. All other authors: nothing to declare.

Objective: Urinary Tract Infections (UTIs) are the most common bacterial infection in women. Most UTIs are acute uncomplicated cystitis caused by *Escherichia coli* (86%). This study was undertaken to assess the effectiveness of an association of a cranberry dried extract, D-mannose and the three microorganisms *L. plantarum* LP01 (LMG P-21021), *L. paracasei* LPC09 (DSM 24243) and *S. thermophilus* ST10 (DSM 25246) in women affected by cystitis.
Methods: 33 premenopausal, non-pregnant women diagnosed with acute uncomplicated cystitis were enrolled in a pilot prospective study. Subjects were directed to take two doses per day during the first month, then to continue with 1 sachet per day until the sixtieth day. Nitrites and leukocyte esterase on urine dipstick testing were used as indicators of cystitis, with analysis performed at enrolment, after 30 and 60 days, and after one month of follow-up.
Results: Positive results for the presence of nitrites and leukocyte esterase were found in 14 and 20 subjects after 30 days and in 9 and 14 women after 60 days, respectively ($p<0.001$). At the end of the follow-up period relapses were recorded in only 4 out of 24 subjects (16.7%, $p=0.103$) with negative results after 60 days, therefore suggesting the long-term barrier effect exerted by the product. Typical symptoms of cystitis, like dysuria, frequent voiding and urgency were significantly improved as well.
Conclusions: A long-term ability of the association of cranberry, D-mannose and the three microorganisms tested to significantly improve the uncomfortable symptoms reported by women with acute cystitis has been suggested.

EFFECTIVENESS OF THE TWO MICROORGANISMS *L. FERMENTUM* LF15 AND *L. PLANTARUM* LP01, FORMULATED IN SLOW RELEASE VAGINAL TABLETS, IN WOMEN AFFECTED BY BACTERIAL VAGINOSIS (BV): A PILOT STUDY

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Conflict of interest disclosure and declaration of funding sources:
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Objective: Bacterial Vaginosis (BV) is a syndrome with a noteworthy incidence in reproductive age women and often associated with *Gardnerella vaginalis*. This study was undertaken to first select at least two lactobacilli able to *in vitro* antagonize *G. vaginalis* and *Escherichia coli*, and then to perform a human study in women affected by BV using the most promising bacteria.
Methods: The two lactobacilli *L. fermentum* LF15 (DSM 26955) and *L. plantarum* LP01 (LMG P-21021) were tested in the form of slow release tablets in a human intervention, double-blind, placebo-controlled, pilot trial involving 34 women with BV. A clinical examination was performed and the Nugent score was quantified for each patient at enrolment (d_0), after 28 days (d_{28}) and at the end of the second month of relapses prevention (d_{56}).
Results: The two lactobacilli significantly reduced the Nugent score below the threshold of 7 after 28 days in 22 patients out of 24 in the active group (91.7%, $p<0.001$). At the end of the second month, only 4 women registered a Nugent score higher than 7 and definable as BV (16.7%), therefore suggesting the long-term barrier effect exerted by the product. In the placebo no significant differences were recorded at any time.
Conclusions: The long-term ability of *L. fermentum* LF15 and *L. plantarum* LP01 to significantly improve the uncomfortable symptoms reported by women with BV has been demonstrated. In the light of the *in vitro* inhibitory activity against *E. coli*, their prospective use in aerobic vaginitis (AV) could prove interesting.