SYNBIOTIC EFFECTS DO THEY MAKE A DIFFERENCE?

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Objectives

- Discuss rationale for synbiotic supplementation
- Present evidence for support of synbiotics and associated outcomes

SPECIAL ISSUE

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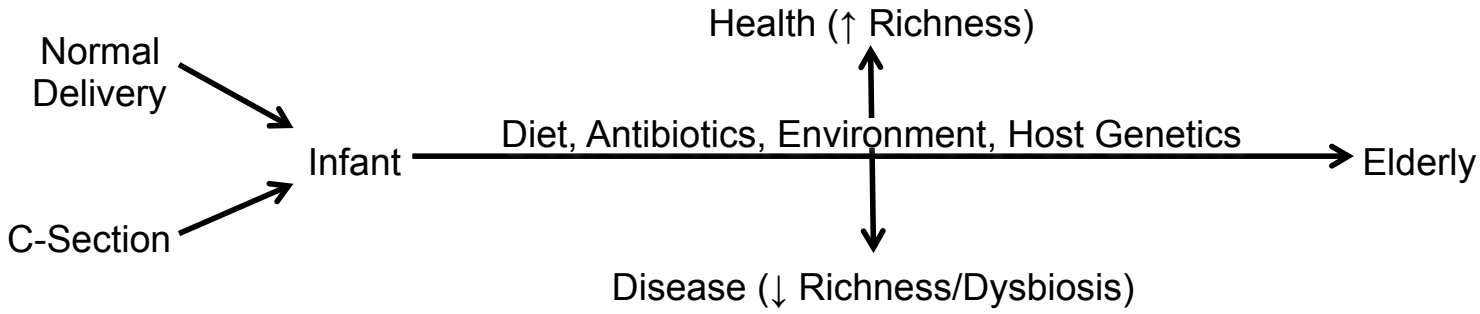
The Gut Microbiome in Health and Disease.

- Concepts in Mammalian Gut Microbiome
- The Gut Microbiome and Disease
- Therapeutic Modification of the Gut Microbiome



Altering Gut Microbiota Strategies

- Probiotics
  - Limitations:
    - Viability
    - Fermentation substrate lacking
    - Transient colonization
- Prebiotics
  - Limitations:
    - Bacterial target depleted
- Fecal Microbiota Transplant
  - Limitations:
    - FDA regulated (except for *C. difficile* colonization)
    - Expensive with minimal reimbursement
    - Donor recruitment and screening (Biosafety level 1 and/or 2)
    - Aesthetic

Synbiotic

- Combination of probiotic and prebiotic
  - Meets criteria of probiotic and prebiotic
  - The prebiotic selectively supports the growth of the probiotic component
- General Aim:
  - Support the probiotic and other indigenous beneficial organisms by providing a preferred carbon and energy source to promote its growth
  - Provide substrate for optimal or desired fermentation byproducts of probiotic

Types of Synbiotic Approaches

- Complementary
  - Probiotic chosen based on specific desired beneficial effects on the host
  - Prebiotic independently chosen to selectively increase concentrations of beneficial microbiota components
    - Indirectly promotes growth and activity of probiotic
- Synergistic
  - Probiotic is chosen based on specific desired beneficial effects on the host
  - Prebiotic is selected to have higher affinity for probiotic
    - Chosen to improve probiotic survival and growth in host
    - May also increase levels of beneficial host GI microbiota
      - Primary target is ingested probiotic

What is the evidence?

- Limited studies for synbiotic therapy
- Areas studied:
  - Irritable bowel syndrome
    - Very few studies <5
    - Disease etiology unknown
    - Target IBS symptoms?
    - Limited understanding of microbiota composition associated with IBS
  - Colon cancer risk
    - Main evidence animal studies of tumorigenesis, transgenic animals, chemically induced models of mutagenesis, *in vitro* cell line models
  - Glycemia, insulin, lipid parameters in obesity, overweight
  - Surgical Patients
  - Inflammatory bowel disease

A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity

Study–year	Country	Supplement	Study design/ quality score	Population	Mean BMI (kg/m^2)	Sex	Age (years)	Sample size (n)	Intervention – daily dose	Control – daily dose	Duration (days)	Results (Intervention vs control – after supplementation)
Asemi et al., 2014 [21]	Iran	Synbiotic	Cross-over RCT/EPHPP:1 CONSORT:23	Excess weight with type 2 diabetes	Intervention 29.9 ± 5.2 Control 29.6 ± 4.5	M/F	35–70	62	*L. sporogenes* (27×10^7 CFU) + 1.08 g of inulin ($n = 62$)	Control food without synbiotc ($n = 62$)	62	↓ Fasting insulin (−1.98 μU/mL) ↔ HDL-c ↔ LDL-c ↔ Fasting glucose ↔ Total cholesterol ↔ Triglycerides
Malaguarnera et al., 2012 [27]	Italy	Synbiotic	Parallel RCT/ EPHPP: 1 CONSORT: 26	Excess weight with non alcoholic steatohepatitis	Intervention: 27.3 ± 1.4 Control: 27.2 ± 1.3	M/F	30–65	66	*B. longum* W11 (5×10^9 CFU) + 2.5 g of FOS ($n = 34$)	Placebo – 2.5 g ($n = 32$)	168	↓ LDL-c (−21.7 mg/dL) ↔ Fasting glucose ↔ Fasting insulin ↔ Total cholesterol ↔ HDL-c ↔ Triglycerides
Moroti et al., 2012 [28]	Brazil	Synbiotic	Parallel RCT/ EPHPP: 2 CONSORT: 26	Excess weight with dyslipidemia and type 2 diabetes	Intervention: 27.7 ± 0.8 Control: 28.2 ± 0.9	F	50–65	20	200 mL of shake containing *L. acidophilus* (8×10^8 CFU) + *B. bifidum* (8×10^8 CFU) + FOS – 2 g ($n = 10$)	200 mL of shake without synbiotic ($n = 10$)	30	↔ Total cholesterol ↔ Triglycerides ↔ Fasting glucose. ↔ HDL-c
Eslamparast et al., 2014 [24]	Iran	Synbiotic	Parallel RCT/ EPHPP: 1 CONSORT: 29	Obesity with nonalcoholic fatty liver disease	Intervention: 32.1 ± 2.4 Control: 31.3 ± 2.3	M/F	≥18[b]	52	*L. casei* + *L. rhamnosus* + *S. thermophilus* + *B. breve* + *L. acidophilus* + *B. longum* + *L. bulgaricus* (4×10^8) + FOS ($n = 26$)	Maltodextrin ($n = 26$)	196	↓ Fasting insulin (−1.2 μU/mL) ↓ Fasting glucose (−6.7 mg/dL)

Beserra BT et al. *Clin Nutr* 2014; Published online: October 20, 2014

Results of 13 trials;

513 adults with BMI > 25 kg/m^2

Synbiotic vs. Placebo

NO DIFFERENCE:

- Total Cholesterol
- LDL-Cholesterol
- HDL-Cholesterol

Beserra BT et al. *Clin Nutr* 2014; Published online: October 20, 2014

Results (cont)

Synbiotic vs. Placebo

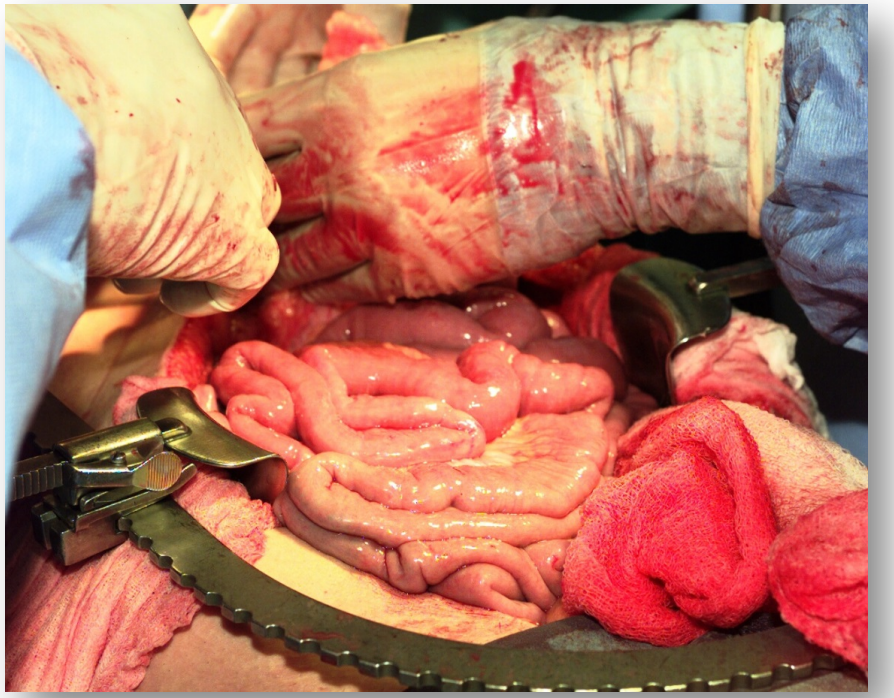
Significant reduction in:

- Triglyceride concentrations
- Fasting Insulin

Beserra BT et al. *Clin Nutr* 2014; Published online: October 20, 2014

Surgical Patients

- Transplant patients, Post-operative patients
  - Sepsis
  - Nosocomial infections
  - Gut permeability
  - Gut dysbiosis



Surgical Synbiotic Studies

- Results encouraging
- Commercially available products studied
- Multi-strain probiotics
- Lack of placebo or proper controls
  - Unable to elucidate mechanisms

Study-year	Study Design	Patient Population	Intervention	Outcomes
Anderson et al, 2004	Randomized DB, Placebo-controlled	N=137, elective laparotomy	Supplement 3x/day: $4x10^9$ CFU *L. acidophilus* La5, *L. bulgaricus, B. lactis* Bb12, *S. thermophilus* +16 g oligofructos 2x/day Supplemented 1-2 weeks preop until hospital discharge	No difference in bacterial translocation and colonization, systemic inflammation, septic complications
Rayes et al, 2005	Randomized DB, no placebo	N=66, liver transplant	Synbiotic 2000 vs fiber only – added to enteral formula x14 days post-op	Lower post-op infections in Synbiotic (3%) vs fiber (48%); less antibiotic duration required
Rayes et al, 2005	Randomized DB, no placebo	N=89; Pylorus preserving pancreatoduod enectomy	Synbiotic 2000 vs fiber only: 1 day preop + 8 days post-op	Lower post-op infections in Synbiotic (12.5%) vs fiber (40%)
Sugawara et al, 2006	Randomized, controlled	N=101; high-risk hepatobiliary resection	Post-op only vs 2 wk Pre-op +2 wk post-op: $4x10^{10}$ *L. casei* Shirota, 10^{10} *B. breve,* 15 g GOS – delivered orally/daily preop; 10^8 CFU *L. casei,* 10^8 CFU *B. breve* + 15 g GOS	Periop treatment resulted in decreased infections, WBC counts, CRP; Both probiotics detected in feces

Synbiotic 2000 [10^{10} CFU of each *Pediacoccus pentoseceus, Leuconostoc mesenteroides, L. casei* spp. *paracasei* F1977:1, *L. plantarum* 2362 + 2.5 g each of β-glucans, resistant starch, inulin, pectin]

Inflammatory Bowel Disease
Crohn's, Ulcerative Colitis, Pouchitis

- No cure – limited to maintenance of remission
- Current therapies: anti-inflammatory and immunomodulating drugs, nutritional support, surgery
- Disease etiology unknown
  - Believed in part due to altered tolerance to normal gut microbiota or disturbed microbiota
  - Likely caused by complex combination of genetics, environmental factors, immune system
- Probiotic against UC and pouchitis studied
  - Several encouraging reports
  - Multi-strain better than single strain products
- Limited studies with synbiotics and IBD

Synbiotics in IBD

- Difficult to design a synbiotic against a disease of unknown etiology
- Development of immune biomarkers as potential targets for synbiotic development

Study-year	Study Design	Patient Population	Intervention	Outcomes
Furrie et al, 2005	Randomized, controlled, pilot	N=18, UC patients	2x1011 CFU *B. longum* + prebiotic (6g inulin/ oligofructose) 2x/day for 4-weeks; placebo : starch + 6g maltodextrose	Synbiotic group decreased TNFα, IL1α, antimicrobial human β-defensin peptides; increase mucosal Bifidobacteria
Osman et al, 2006	Sprague-Dawley rats, controls	Dextran sulfate sodium (DSS) - induced colitis	*B. Infantis* DSM 15158 or *B. infantis* DSM 15159, alone or with prebiotic (6g inulin/ oligofructose); pretreated for 7 days (single probiotic +/- prebiotic) – then treatments for 7 days continued	All treatments reduced disease activity indices [bacterial translocation, SCFA, cytokine production, myeloperoxidase, malondialdehyde]; additive effect with prebiotic (increased succinate levels); *B. infantis* DSM 15159 better in reducing malondialdehyde levels
Ishikawa et al, 2011	Randomized, placebo	N=41, Ulcerative colitis	*B. Breve* Yakult + galactooligosaccharide, for 1 year	Improved endoscopic score, Matt's classification
Fujimori et al, 2007	Open label study, no controls	N=10, active Crohn's disease	3x1011 CFU *B. breve,* 3x1011 CFU *L. casei,* 1.5x1010 CFU *B. longum* daily + prebiotic (3.3g pysilium 2x/day) 10 months	Improved symptom scores (n=7)
Chermesh et al, 2007	Randomized, placebo	N=30, ileal resection Crohn's disease patients	Synbiotic 2000 – 24 months	No effect on remission or disease scores
Steed et al, 2010	Randomized, DB, placebo	N=35, active Crohn's patients	*B. Longum* + prebiotic (6g inulin/oligofructose)	Decreased disease activity indices and histological scorese; decreased TNFα at 3 mo (not 6 mo); ? Mucosal bifidobacteria

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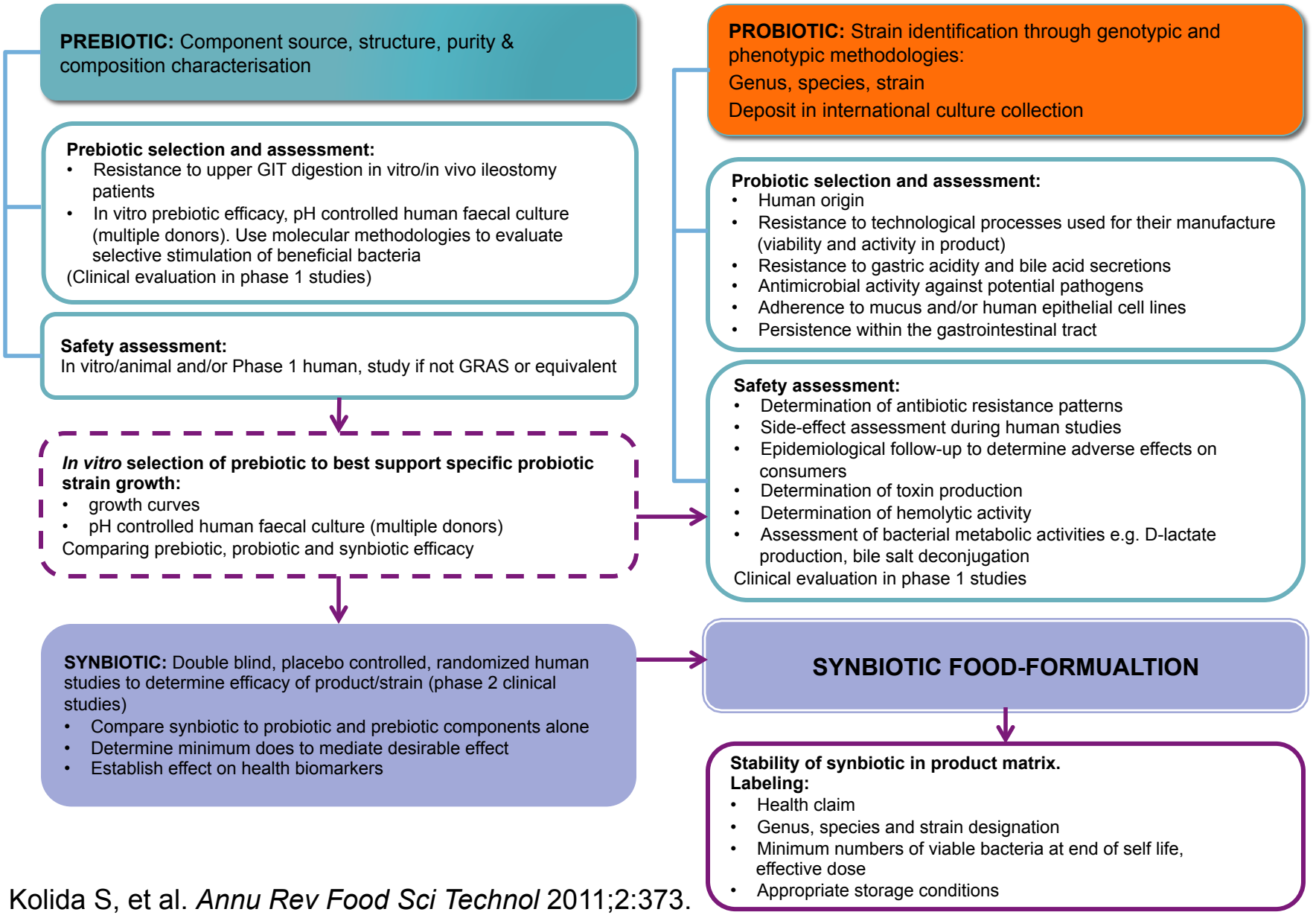
Suggested Steps for Establishing a Synbiotic Formulation



Kolida S, et al. *Annu Rev Food Sci Technol* 2011;2:373.

Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trail

- Synbiotic development
  - Screened 19 *Bifidobacterium* isolates for suitability as probiotics
    - 10 isolated from healthy colonic mucosa, 5 healthy feces, 4 culture collections
    - Tested for aerotolerance, acid tolerance, bile-salt resistance, adhesion to epithelial cells, ability to survive freeze drying and long-term storage
    - Ability to metabolize FOS as energy source determined
    - Ability to reduce proinflammatory cytokine production tested (*in vitro*)
  - B. longum isolated from healthy rectal mucosa and selected for further study
- Human pilot study (n=16)
  - 8 ingested $2x10^{11}$ viable, freeze-dried *B. longum* in gelatin capsule + 6 gm prebiotic (inulin/oligofructose) 2x/d for 4 weeks vs control (starch + maltodextran)
  - Sigmoidoscopy scores, TNFα, IL1α, antimicrobial human β defensin, mucosal bifidobacteria start/end of treatment
- Improvements in parameters in Synbiotic group

Furrie E et al. *Gut* 2005;54:242-249

Synbiotic consumption changes the metabolism and composition of the gut microbiota in older people and modifies inflammatory processes: a randomised, double-blind, placebo-controlled crossover study

- Synbiotic vs placebo in Healthy volunteers (n=43)
  - Placebo vs *B. longum* ($2x10^{11}$)+ prebiotic (inulin/oligofructose [DP2-60] – 6 g) twice daily (after breakfast, following evening meal)
  - 12 wks: 4 wk ⇒ 4 wk washout ⇒ 4 wk
- Outcomes:
  - Increase fecal bifidobacteria
  - Improvements in colonic bacterial composition, inflammatory markers linked to aging, bowel habit, health status

Macfarlane S et al. *Aliment Pharmacol Ther* 2013;38:804-816

Results

Macfarlane S et al. *Aliment Pharmacol Ther* 2013;38:804-816

Bowel habit and general mood of volunteers throughout the study*

	Synbiotic			Placebo		
	Baseline	Week 2	Week 4	Baseline	Week 2	Week 4
Abdominal pain	1.7 ± 3.4	1.4 ± 3.1	1.2 ± 3.3	1.2 ± 3.1	2.0 ± 3.8	1.6 ± 3.6
No of stools	11.7 ± 8.1	11.6 ± 7.3	11.7 ± 5.3	10.5 ± 5.6	10.9 ± 6.0	11.4 ± 6.2
Stool consistency	14.5 ± 4.3	14.7 ± 4.7	15.4 ± 4.3	14.6 ± 4.8	15.0 ± 4.7	14.0 ± 5.0
Bowel movement frequency			0.3 ± 0.9			0.3 ± 1.3
Well-being			0.1 ± 0.7			0.2 ± 0.9
Values are means ± s.d (*n* = 43)						

Changes in bacterial populations in healthy older people during 4-week synbiotic or placebo consumption, as determined by fluorescent *in situ* hybridisation*

	Synbiotic			Placebo		
	Baseline	Week 2	Week 4	Baseline	Week 2	Week 4
Total bacteria	10.2 ± 0.5	10.4 ± 0.5	10.5 ± 0.6	10.4 ± 0.6	10.3 ± 0.5	10.3 ± 0.5
Firmicutes	9.5 ± 0.7	10.2 ± 0.6^{ao}	10.3 ± 0.6^{go}	9.8 ± 0.7	9.7 ± 0.5^{a}	9.7 ± 0.6^{g}
Bacteroidetes	9.4 ± 0.6	9.7 ± 0.4	9.5 ± 0.6	9.6 ± 0.4	9.5 ± 0.4	9.5 ± 0.5
Proteobacteria	7.9 ± 1.4	7.9 ± 1.4^{b}	6.9 ± 1.2^{ho}	8.0 ± 1.2	8.3 ± 1.2^{b}	7.9 ± 0.9^{h}
Actinobacteria	9.3 ± 0.5	9.8 ± 0.4^{c}	9.9 ± 0.2^{i}	9.3 ± 0.2	9.4 ± 0.4^{c}	9.1 ± 0.3^{i}
Total bifidobacteria	8.7 ± 0.4	9.5 ± 0.3^{do}	9.9 ± 0.4^{jo}	8.6 ± 0.3	8.7 ± 0.4^{d}	8.5 ± 0.4^{j}
B. adolescentis	8.2 ± 0.3	8.4 ± 0.3	9.2 ± 0.4^{ko}	8.0 ± 0.6	8.1 ± 0.5	7.9 ± 0.2^{k}
B. angulatum	8.0 ± 0.5	8.5 ± 0.5^{eo}	9.1 ± 0.5^{lo}	8.2 ± 0.6	8.0 ± 0.4^{e}	8.3 ± 0.5^{l}
B. bifidum	7.8 ± 0.2	8.4 ± 0.3^{o}	8.6 ± 0.5^{mo}	8.1 ± 0.3	8.3 ± 0.4	8.2 ± 0.5^{m}
B. breve	ND	ND	ND	7.9 ± 1.8	ND	ND
B. catenulatum/pseudocatenulatum	ND	7.9 ± 0.8	7.8 ± 0.9	ND	ND	ND
B. longum	8.1 ± 0.4	8.6 ± 0.2^{fo}				
Values are mean $\log_{10}$ cells/g of faeces ± s.d (*n* = 43)						

	Synbiotic			Placebo		
	Baseline	Week 2	Week 4	Baseline	Week 2	Week 4
Acetate	58.9	58.7	60.4	61.3	60.7	62.6
Propionate	22.3	19.5	18.1	19.6	19.2	18.7
Butyrate	18.8	21.8	21.5	19.1	20.1	18.7

↓TNFα

↑ Firmicutes/Bacteroidetes ratio 1.3 to 6.6

Conclusions & Future Directions

- Scope is broadening for synbiotics in health and disease
- Term “synbiotic” has been used loosely
  - Little rational selection of the prebiotic/probiotic combinations
- Research lacking in proper controls to confirm/deny synergistic or additive effect
- Several studies use fibers not recognized as prebiotics
- Little attempt made to confirm growth of the probiotic on the prebiotic questioning the nature of the effect
- Formulation of successful synbiotic is complex
- Future studies should include
  - Minimum effective dose to mediate desirable effect in absence of side effects
  - Rational selection of pre/probiotic with appropriate targeted biomarkers

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