Probiotics
What We Know and Emerging Science

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CRN Day of Science

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Human Microbiome Project

- The NIH **Human Microbiome Project (HMP)** program (FY2007-2015) aims to develop tools and datasets for the research community for studying the role of these microbes in human health and disease.
- The first phase of HMP (FY2007-2012) characterized the composition and diversity of microbial communities which inhabit major mucosal surfaces of the human body, including nasal passages, oral cavities, skin, gastrointestinal tract, and urogenital tract, and evaluated the genetic metabolic potential of these communities.
- The current phase of HMP (FY2013-2015) is focused on the creation of the first integrated dataset of biological properties from both the microbiome and host from cohort studies of microbiome-associated diseases.

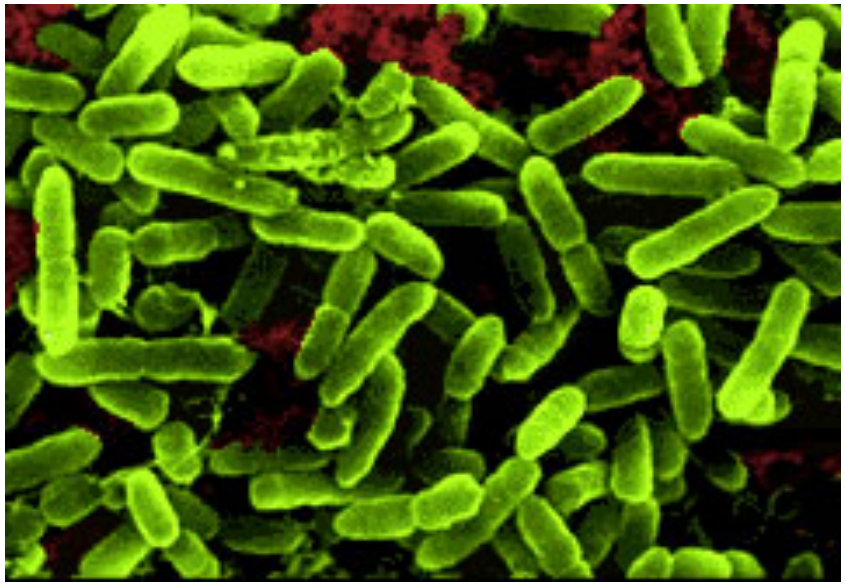
Human Microbiome
"Richness" and Metabolic Markers

- Human gut microbial composition was studied in 123 non-obese and 169 obese Danish individuals
- Individuals with "low bacterial richness" (23% of the population studied) were characterized by more:
  - Overall adiposity
  - Insulin resistance
  - Dyslipidemia
  - A more pronounced inflammatory phenotype

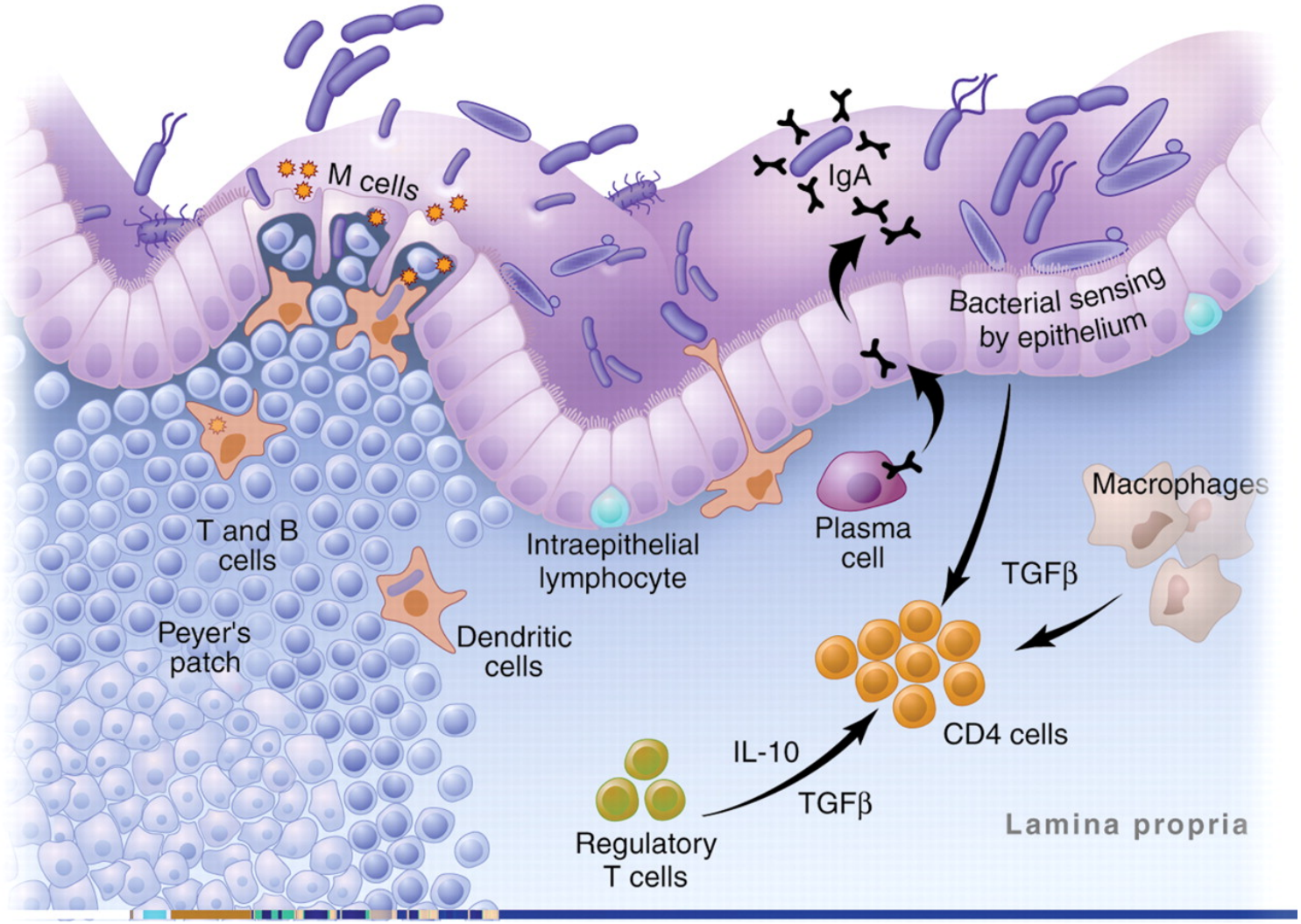
Nature 2013;500:541-6.

Probiotics

- Revised FAO/WHO Definition (ISAPP consensus Oct. 13, 2013):
  - "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."



Probiotics and the Immune System

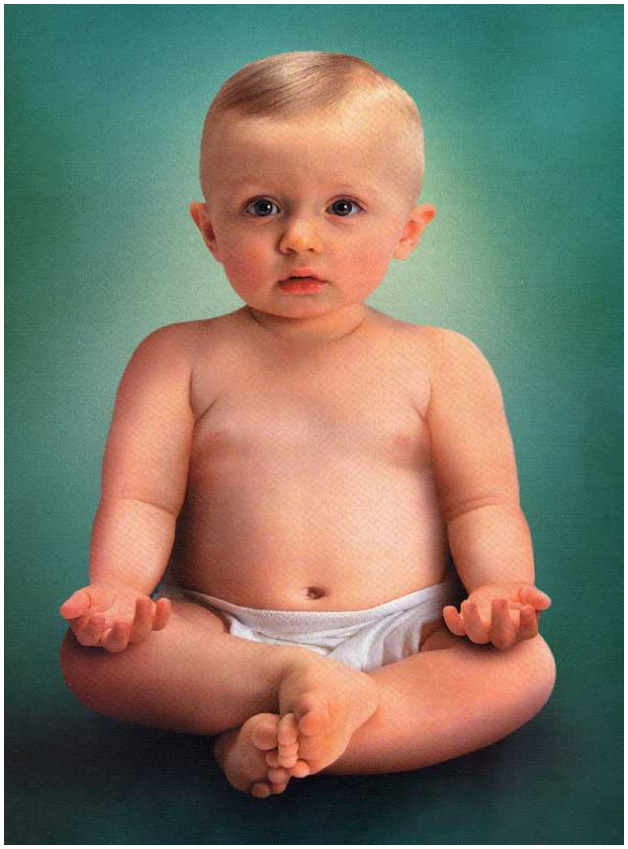


Immunomodulatory Effects

- Some probiotic strains have been shown *in vitro* induce Th1 production and downregulate some proinflammatory cytokines and Th2-mediated allergic responses (IgE). Implications for allergic rhinitis, atopic dermatitis and ulcerative colitis?
- Effect on toll-like receptors – essential to innate defense against pathogens and trigger adaptive immune responses.
- Additionally, by influencing cell adhesion and cell-cell signaling in the gut, probiotics appears to strengthen the integrity of the gut mucosa.

Nutrition Reviews 2006;64:1-14.

Probiotics and Pediatric Health



Development of Gut Microflora

- The human gut is germ free at birth. Microbial colonization begins at birth when the infant swallows microflora form the vaginal fluid at delivery.
- Development of GALT begins shortly after birth
- Factors influencing the gut flora of an infant include:
  - Mode of delivery – vaginal vs. caesarean
  - Breast fed vs. Formula fed
  - Genetics

Development of Gut Microflora, cont.

- Canadian study compared gut flora in infants 4 months after birth:
  - The profiles were generally dominated by Actinobacteria (mainly the genus Bifidobacterium) and Firmicutes (with diverse representation from numerous genera).
  - Infants born by cesarean delivery had particularly low bacterial richness and diversity.
  - Compared with breastfed infants, formula-fed infants had decreased richness of species, with over-representation of *Clostridium difficile*.

CMAJ 2013.DOI:10.1503/cmaj.121189.

Probiotics – Prenatal and Postnatal
Atopy and Asthma

- Meta-analysis of 25 studies with a total of 4,031 participants.[1]
  - Probiotics were effective in reducing total IgE and the reduction was more pronounced with longer follow-up.
  - Probiotics significantly reduced risk of atopic sensitization when administered prenatally and postnatally.
  - Probiotics did not significantly reduce asthma/wheeze
- Another meta-analysis from Canada found no evidence to support a protective association between perinatal use of probiotics and asthma or wheeze.[2]

1. *Pediatrics* 2013 Sep;132(3):e666-76.
2. *BMJ* 2013 Dec 4;347:f6471. doi: 10.1136/bmj.f6471.

Probiotics
Antibiotics and GI Indications

Antibiotics (AAD, CDAD)

IBS

Human Intestinal Microbiota
Influence of Antibiotics

- Ciproflaxin caused rapid and profound change in gut microbiota in 3-4 days. By one week, there was a partial return to the initial state. However, by 10 months, the return was incomplete in some cases.[1]
- Clarithromycin and metronidazole cause a rapid and profound change in 1 week. Some recovered over time but, in some cases, perturbation of gut microbiota for up to 4 yrs post treatment.[2]

1. *PNAS* 2011;108(suppl 1):4554-61.
2. *PLoS One* 2010;5:e9836.

Can Probiotics Prevent Disruption?

- Small double-blind, placebo-controlled, trial (DBPCT) with 30 patients being treated for *H. pylori.* All subjects received 7 days of triple therapy (amoxycillin, metronidazole, lansoprazole) and were randomized to one of three groups:
  - Group 1 – Placebo from days 1 to 15
  - Group 2 – Placebo days 1 to 7 and probiotic* days 8 to 15
  - Group 3 – Probiotic days 1 to 15
- Stool samples were collected at days 1, 7, 12, 17, 27. The following was found:
  - Group 1 – facultative anaerobes were elevated at day 27
  - Group 2 – elevation from days 1 to 7 but decreased significantly between days 7 to 27
  - Group 3 – remained stable throughout

**Lactobacillus acidophilus*, *B. bifidum* - 2.5 x 10^{10} cfu/capsule

International Immunopharmacol 2005;5:1091-7.

AAD Prevention

- **Meta-Analyses**
  - *J Pharmacol* 2002;16:1461-7. [7 Studies]
  - *BMJ* 2002;324:1361-6. [9 Studies]
  - *Aliment Pharmacol Ther* 2005;22:365-72. [5 Studies]
  - *Lancet Infect Dis* 2006;6:374-82. [19 Studies]
  - *Am J Gastroenterol* 2006;101:812-22. [25 Studies]

Probiotics reduce the incidence of AAD by about 50%

Pediatric AAD
Probiotics for Prevention

- Meta-analysis includes 16 studies with 3,342 children.
- The incidence of AAD in the probiotic group was 9% compared to 18% in the control group but ITT analysis was non-significant overall
- An *a priori* analysis suggests that higher doses of probiotics (> 5 billion cfu/day) is more effective than lower doses (< 5 billion cfu/day) [p = 0.010]. In the high dose group, the NNT to prevent one case of diarrhea is seven.
- Reviewers call for greater attention to drop-out rates and subjects lost to follow-up in future studies as well as greater standardization of organisms used.

Cochrane Database Syst Rev 2011;11:CD004827.

Clostridium difficile-Associated Diarrhea
Probiotics for Prevention in Adults and Children

- Meta-analysis – 23 clinical trials including 4,213 participants (Strains included *S. boulardii, LGG, L. acidophilus* CL 1285 as well as others)
- Probiotics reduced incidence of CDAD by 64%
- In a population with a 5% incidence of antibiotic-associated CDAD (median control group risk), probiotic prophylaxis would prevent 33 episodes per 1,000 persons.
- "Moderate quality evidence" for prevention of CDAD

Cochrane Database Syst Rev 2013. May 31;5:CD006095. doi: 10.1002/14651858.CD006095.pub3.
Ann Intern Med 2012;157:878-88.

Dysbiosis in IBS and IBD

- Small intestinal bacterial overgrowth (SIBO) postulated to be a causative factor in IBS. 75% improvement in IBS after eradication of SIBO. (*JAMA* 2004;292:852-8)
- Colonic microflora of 57 patients with active IBD was compared to 46 healthy controls. Diversity of bacterial microflora significantly less in the IBD patients with notable loss of normal anaerobic bacteria such as *Lactobacillus* spp. (*Gut* 2004;53:685-93)

Irritable Bowel Syndrome
Meta-Analysis of Probiotic Research

- 14 randomized DBPCT identified as sufficient for review (4-26 weeks in duration); two were pediatric studies. Number of strains varied from 1 to 8.
- With the exception of one trial, the data suggests a modest improvement in overall symptoms. Seven trials found a significant reduction in abdominal pain; five for flatulence and four for bloating.
- Authors suggest future trial focus on the type and optimal dose of probiotics and subgroups of patients most likely to respond.

BMC Gastroenterol 2009;9:15 (doi: 10.1186/1471-230X-9-15)

Probiotics and Female GU Health

Lactobacilli
Disruption of Urogenital Biofilms

- Uropathogenic *E. coli* and pathogens associated with bacterial vaginosis (*G. vaginalis, Atopobium vaginae*) form dense biofilms, permitting evasion of the host's immune system and impending antimicrobial access
- An *in vitro* study found that *L. rhamnosus* GR-1 and *L. reuteri* RC-14 are able to incorporate themselves into the pathogenic bioflims and cause disruption and some killing of the bacteria
- Metronidazole produced holes in *G. vaginalis* and *A. vaginae* biofilms but did not eradicate the organisms

Colloids Surfaces B: Biointerfaces 2011;86:58-64.

Probiotics vs. Antibiotics
Prevention of UTIs

- 252 postmenopausal women with at least 3 self-reported UTIs in the past year were randomized receive antibiotic (TMP-SMX, 480 mg/day) or probiotics (*L. rhamnosus* GR-1 and *L. reuteri* RC-14 - 1 x 10^9 cfu of each strain per day).
- After 12 months, the mean number of symptomatic UTIs (clinical recurrences) was 2.9 in the antibiotic group and 3.3 in the probiotic group. The median time to a recurrence was 3 months in the probiotic group and 6 in the antibiotic group.
- While the probiotic group showed no increase in antibiotic resistance, the antibiotic group had a more than two-fold increase in resistance to both TMP-SMX and amoxicillin.

Arch Intern Med 2012;172:704-12.

Prevention of Recurrent UTI
L. crispatus

- RDBPCT - 100 women (median age 21 years) treated for acute UTI were randomized to receive either a vaginal capsule with *L. crispatus* CTV-05 (10^8 cfu/mL; Lactin-V) or placebo daily for 5 days after UTI treatment 7-10 days) and then once weekly for 10 weeks.
- In the probiotic group, the rate of culture-confirmed UTI was 15% compared to 27% for the placebo group (RR, O.5; 95% CI. 0.2-1.2).
- Women in the probiotic group that achieved a high level of *L. crispatus* colonization had a significant reduction in UTI recurrence compared to those who did not ($p < 0.01$).

CID 2011;52:1212-7.

Probiotics and Prevention of URTIs

URTIs in Children
Prevention with Probiotics

- 326 children (3-5 years of age) were randomized to receive either *L. acidophilus* (1 x 10^{10} cfu/day; n = 110), *L. acidophilus NCFM* combined with *B. animalis subsp lactis* Bi07 (1 x 10^{10} cfu/day; n = 112), or placebo (n = 104) for 6 months.
- Compared to the placebo group, the following was found:
  - Reduced fever incidence – single probiotic (53%; p = 0.0085); combination (72.7%; p = 0.0009)
  - Reduced coughing incidence – single (41.4%; p = .027); combination (62.1%; p = 0.005)
  - Reduced rhinorrhea – single (28.2%; p = 0.68) combination (58.8%; p = 0.03)
  - Fever, coughing, and rhinorrhea duration was decreased significantly by 32% in the single strain group (p = 0.0023) and 48% in the combination (p < 0.001)

URTIs in Children
Prevention with Probiotics, cont.

- Incidence of antibiotic use was reduced by 68.4% in the single strain group ($p = 0.0002$) and 84.2% in the combination group ($p < 0.0001$)
- There was a significant reduction in absences from day care in both probiotic groups compared to the placebo group—31.8% in the single strain group ($p = 0.002$) and 27.7% in the combination group ($p < 0.001$)

Pediatrics 2009;124:e172-e179.

Prevention of URTIs in Adults

- 310 adults ages 18 to 60 yrs received either *B. lactis* BI-04 (2 x 10^9 cfu/day) for 150 days
- The risk of a URTI was significantly lower in the BI-04 group ($p = 0.022$) and a delay in the time in getting sick compared to the placebo group
- This equated to a reduced risk of 27% in the BI-04 group

Clin Nutr 2014;33:581-7.

Emerging Science!

Probiotics and Hyperlipidemia

- Various studies have found equivocal effects on lowering LDL-C with probiotic-enriched fermented milk or yogurt
- In a RDBPCT with127 subjects, *L. reuteri* NCIMB 30242 (2×10^9 cfu/day) was consumed in capsules for 9 weeks following a 2-week wash out period and 2-week run-in period.
- Compared to placebo, significant reductions in the probiotic group were seen for LDL-C (11.64%; $p < 0.001$), total cholesterol (9.14%; $p < 0.001$), apoB-100 (13.39%; $p = 0.006$), and apoA-1 (9%; $p = 0.026$). Triglycerides and HDL-C remained unchanged.
- The probiotic group also had significant reductions in high sensitivity C-reactive protein and fibrinogen compared to placebo.

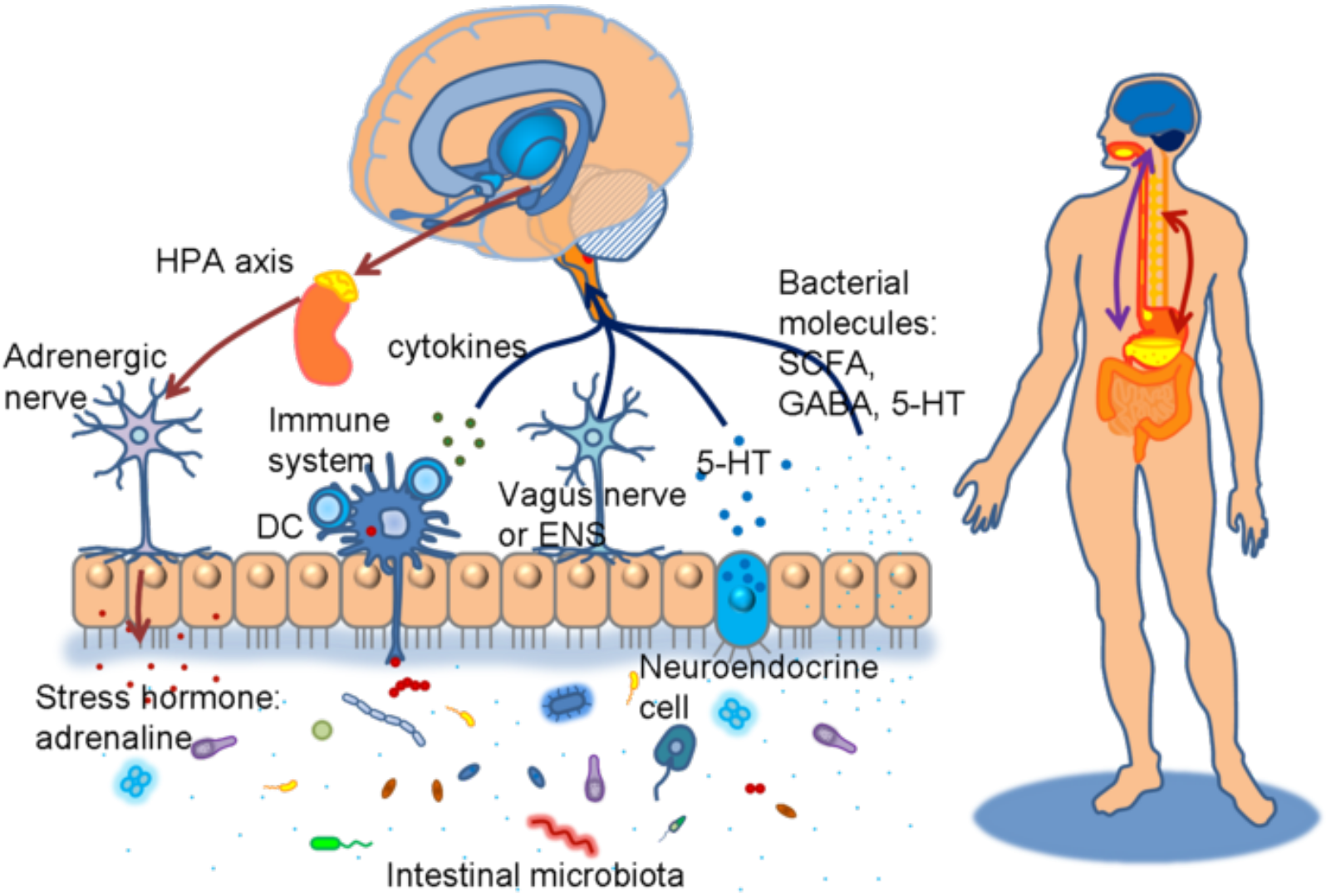
Eur J Clin Nutr 2012;66:1234-41.

Probiotics and Hypertension

- A meta-analysis of 9 clinical trials was completed to determine the effect of probiotics on blood pressure (BP). The total number of participants was 543 and the included studies were all parallel, randomized, controlled trials, with 7 studies reporting a double-blind design.
- Consuming probiotics may have a modest effect on blood pressure (BP), especially in persons with BP > 130/85 mm Hg and when taken for ≥ 8 weeks as a multi-strain product at a potency of ≥ 10^{11} colony forming units per day.

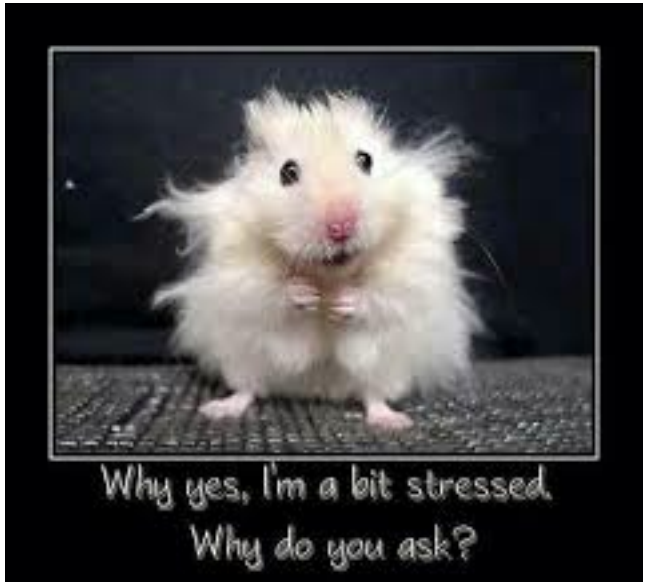
Hypertension 2014;doi: 10.1161/HYPERTENSIONAHA.114.03469.

Microbiota-Gut-Brain Axis



Microbiota-Gut-Brain Axis

Campylobacter jejuni
Citrobacter rodentium
Antibiotics
Lean Beef



Probiotics
"Regular" Chow Diet

Gut Bacteria and Obesity

Probiotic Strains
Checklist

- Important issues in strain selection
  - Viability (including stomach acid and bile resistance)
  - Intestinal adherence
  - No translocation
  - No transferable antibiotic resistance
  - Clinical evidence of safety and efficacy
  - Commercial – stability and shelf-life - potency to time of the expiration date!!

Questions?