Blanchette Rockefeller Neurosciences Institute
West Virginia University

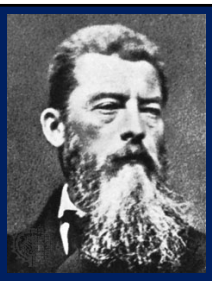
Consiglio Nazionale delle Ricerche
Istituto di Scienze Neurologiche

Università degli Studi del Molise

Digestion & Absorption-Beyond Nutrition: A journey into the gut microcosm

Prof. Giovanni Scapagnini, MD, PhD

Anti-Ageing Conference London 2010



n an essay entitled *Concerning Spiritualism and Materialism* (1863)
Ludwig Andreas Feuerbach wrote:
"Der Mensch ist, was er ißt."
That translates into English as
'man is what he eats'.

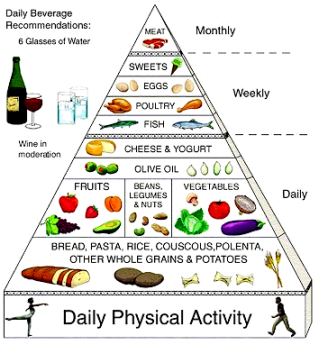
NUTRIGENOMICS

FOOD

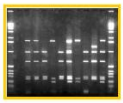
Health maintenance ⟵ ⟶ Diseases

It's about food, who we are, and our health

The Traditional Healthy Mediterranean Diet Pyramid



DNA - genes



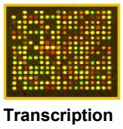
genomics

Health ⟷ Disease

Food

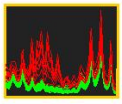
RNA

Metabolites

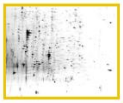


Transcription

Proteins

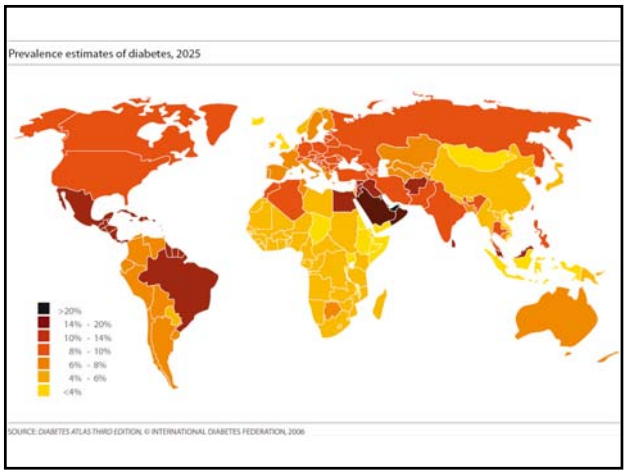


Metabolomics

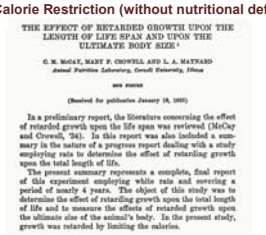


Proteomics

Genes – Lifestyle – Calories



Calorie Restriction (without nutritional deficiency) extends life in all species tested


THE EFFECT OF RETARDED GROWTH UPON THE LENGTH OF LIFE SPAN AND UPON THE ULTIMATE BODY SIZE[1]

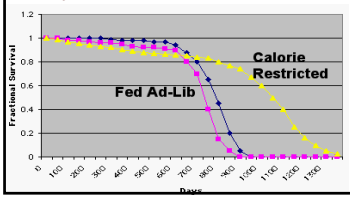
C. M. McCAY, MARY F. CROWELL AND L. A. MAYNARD
Animal Nutrition Laboratory, Cornell University, Ithaca

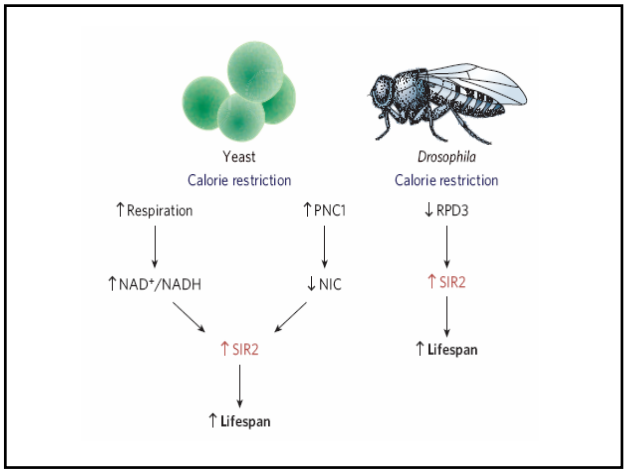
(Received for publication January 18, 1935)

In a preliminary report, the literature concerning the effect of retarded growth upon the life span was reviewed (McCay and Crowell, '34). In this report was also included a summary in the nature of a progress report dealing with a study employing rats to determine the effect of retarding growth upon the total length of life.

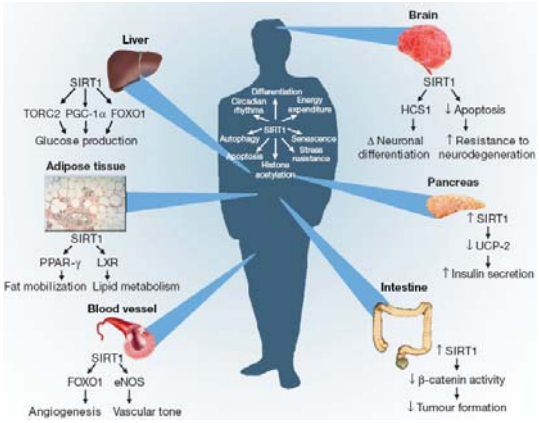
The present summary represents a complete, final report of this experiment employing white rats and covering a period of nearly 4 years. The object of this study was to determine the effect of retarding growth upon the total length of life and to measure the effects of retarded growth upon the ultimate size of the animal's body. In the present study, growth was retarded by limiting the calories.

Eakin F & Witten M Experimental Gerontology 1995, 30: 33-64
McCay CM et al. J. Nutrition 1935, 10: 63-79

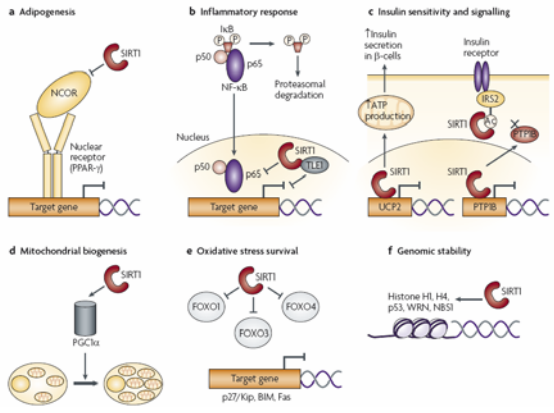




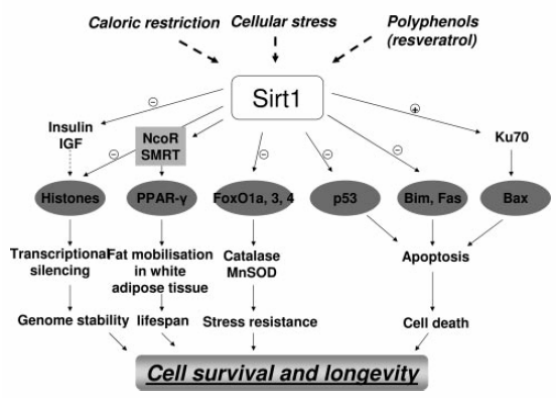
Sirtuin	Disease area	Therapeutic strategy	Substrates/ interactors	Overexpression/knockout model summary
SIRT1	Metabolic, neurological, cardiovascular, renal, cancer, mitochondrial	Activation	p53, FOXO1, FOXO4, COUP-TF, CTIP2, NF-κB-p65, NCOR, histone H1, histone H4, KU70, p300, BCL11A, Tat, PGC-1α, MEF2, eNOS, ACS1, E2F1, AR, p73, SMAD7, NBS1, RB, TLE1, IRS2, LXR, AROS, SUV39H1, WRN, DBC1, TORC2	• Efficacy observed in preclinical models of diabetes with small-molecule SIRT1 activators[34] • Transgenic overexpression of SIRT1 is cardioprotective against oxidative stress and heart ageing[37] • *Sirt1*-overexpressing mice show some phenotypes of calorie-restricted mice[7] • SIRT1 overexpression shows beneficial effects in Alzheimer's disease and Huntington's disease models[58,63] • Knockout mice have genomic instability and developmental defects[61,64] • SIRT1 activates PGC-1α by deacetylation and is involved in mitochondrial biogenesis[65]
SIRT2	Neurological, metabolic, cancer	Inhibition/ activation?	Tubulin, HOXA10, FOXO, histone H4, 14-3-3 protein	• Efficacy observed in a cellular and *Drosophila melanogaster* model of Parkinson's disease with small-molecule SIRT2 inhibitors[62]
SIRT3	Metabolic, mitochondrial	Activation	ACS2	• *Sirt3*-knockout mice have hyperacetylated proteins in mitochondria[60]
SIRT4	Metabolic, mitochondrial	Inhibition?	GDH, IDE, ANT2, ANT3	• *Sirt4*-knockout mice are viable and fertile; pancreatic mitochondrial lysates from knockout animals show higher GDH activity[35]
SIRT5	Neurological	Unknown	Unknown	• Increased expression of Sirt5 observed in frontal cortex of brains from serotonin receptor knockout mice[66]
SIRT6	Cancer	Activation	Histone H3	• Knockout mice have genomic instability, premature ageing phenotype and predisposition to developing cancer[67]
SIRT7	Cardiovascular	Activation	RNA polymerase I, p53	• Knockout mice have decreased lifespan with inflammatory cardiac hypertrophy[56]



Finkel et al. Nature 2009, 460:587-591



Lavu et al. Nature Reviews 2008; 7:841-853

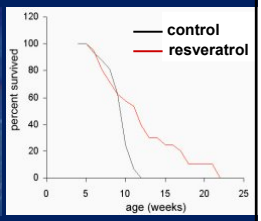


Sedding et al. Circ. Res. 2007;100;1396-1398

Valenzano DR, Cellerino A.

Resveratrol and the pharmacology of aging: a new vertebrate model to validate an old molecule.

Cell Cycle. 2006 May;5(10):1027-32.



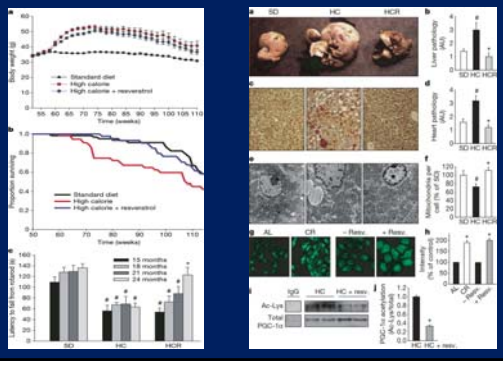
Resveratrol treatment prolonged lifespan and delayed the onset of age-related dysfunctions in this fish.

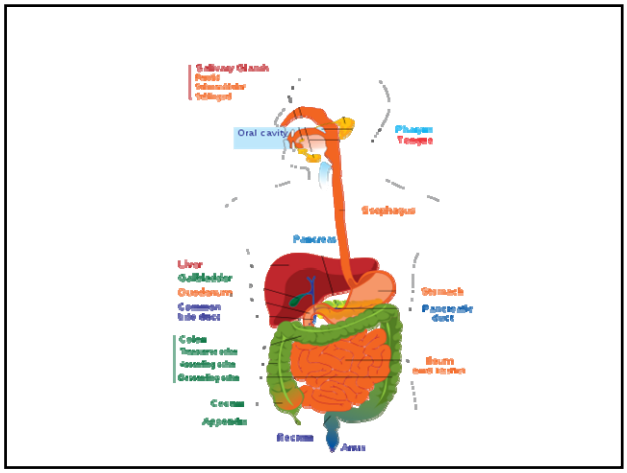
Baur JA, de Cabo R and Sinclair DA

Resveratrol improves health and survival of mice on a high-calorie diet

Nature advance online publication 1 November 2006

Department of Pathology, Paul F. Glenn Laboratories for the Biological Mechanisms of Aging, Harvard Medical School





BEYOND DIGESTION

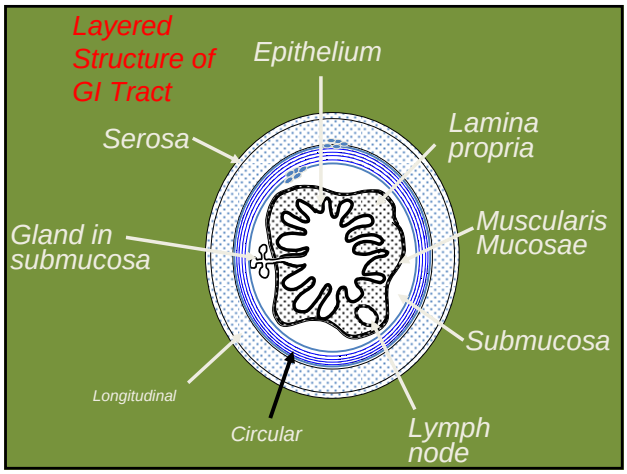
- The gut is a sensory organ. Protozoa know their environments by ingestion.
- The gut is a neuroendocrine organ. Every CNS neurotransmitter is present and active here.
- The gut has a brain of its own, an intact and independent nervous system.
- The gut is the largest organ of immune function in the body; 70% of our lymphocytes live here.

BEYOND DIGESTION

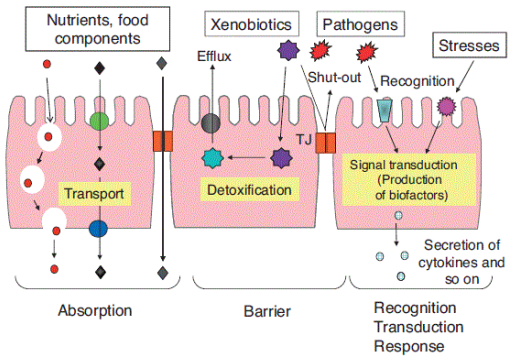
- The gut contents are an inner world that is “outside” the cellular body. Its surface is a frontier of 100 square meters and a thickness of one cell
- Gut flora are an organ that contains as many microbial cells as the cellular body has mammalian cells (100 trillion)
  - -Over 500 species
  - -Over 90% are anaerobic

BEYOND DIGESTION

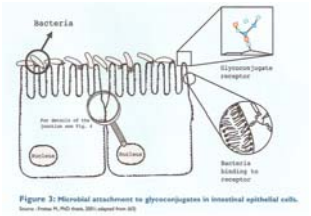
- The normal intestinal microflora constitute a huge chemical factory that alters our food and our GI secretions
- The normal intestinal microflora present our immune systems with a mass of antigens that are partially absorbed



Three major functions of the Intestinal epithelial cell



Intestinal Epithelium



- The surface of the Epithelium consists of intestinal glycoconjugates made up of mucus gel. These mucins and glycoconjugates play a key role in the barrier effect.

Digestion and Absorption

- A typical meal contains carbohydrates, proteins, lipids, water, electrolytes, and vitamins. The digestive system handles each component differently. Large organic molecules must be broken down by digestion before absorption can occur. Water, electrolytes, and vitamins can be absorbed without preliminary processing, but special transport mechanisms may be involved.

The Processing and Absorption of Nutrients

- Food contains large organic molecules, many of them insoluble. The digestive system first breaks down the physical structure of the ingested material and then proceeds to disassemble the component molecules into smaller fragments.
- The molecules released into the bloodstream are absorbed by cells and either (1) broken down to provide energy for the synthesis of ATP or (2) used to synthesize carbohydrates, proteins, and lipids.
- Digestive enzymes break the bonds between the component molecules of carbohydrates, proteins, lipids, and nucleic acids in a process called hydrolysis.
- The classes of digestive enzymes differ with respect to their targets. Carbohydrases break the bonds between simple sugars, proteases split the linkages between amino acids, and lipases separate fatty acids from glycerides.

The Processing and Absorption of Nutrients

•Digestive enzymes secreted by the salivary glands, tongue, stomach, and pancreas are mixed into the ingested material as it passes along the digestive tract. These enzymes break down large carbohydrates, proteins, lipids, and nucleic acids into smaller fragments, which in turn must typically be broken down further before absorption can occur.

•The final enzymatic steps involve brush border enzymes, which are attached to the exposed surfaces of microvilli. Nucleic acids are broken down into their component nucleotides. Brush border enzymes digest these nucleotides into sugars, phosphates, and nitrogenous bases that are absorbed by active transport.

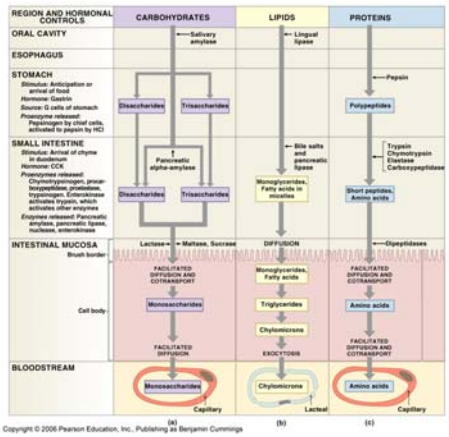


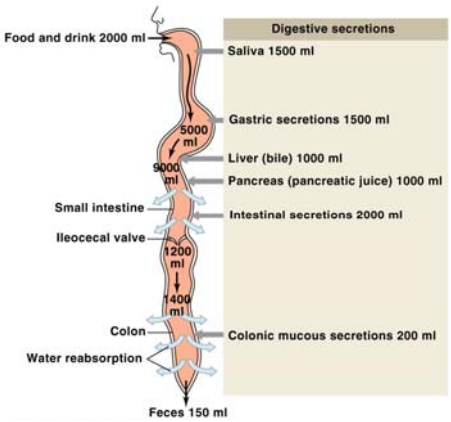
Fig. 24-26, p. 903

Water Absorption

•Cells cannot actively absorb or secrete water. All movement of water across the lining of the digestive tract, as well as the production of glandular secretions, involves passive water flow down osmotic gradients.

Ion Absorption

- Osmosis does not distinguish among solutes; all that matters is the total concentration of solutes. To maintain homeostasis, however, the concentrations of specific ions must be closely regulated.
- The rate of sodium ion absorption by the digestive tract is increased by aldosterone, a steroid hormone from the adrenal cortex. Calcium ion absorption involves active transport at the epithelial surface. The rate of transport is accelerated by parathyroid hormone (PTH) and calcitriol.
- As other solutes move out of the lumen, the concentration of potassium ions increases. These ions can diffuse into the epithelial cells along the concentration gradient.
- The absorption of magnesium, iron and other cations involves specific carrier proteins; the cell must use ATP to obtain and transport these ions to interstitial fluid.
- The anions chloride iodide bicarbonate and nitrate are absorbed by diffusion or carrier-mediated transport. Phosphate and sulfate ions enter epithelial cells only by active transport.



Vitamin Absorption

- Vitamins are organic compounds required in very small quantities. There are two major groups of vitamins: fat soluble vitamins and water-soluble vitamins. Vitamins A, D, E, and K are fat-soluble vitamins; their structure allows them to dissolve in lipids. The nine water-soluble vitamins include the B vitamins, common in milk and meats, and vitamin C, found in citrus fruits.
- All but one of the water-soluble vitamins are easily absorbed by diffusion across the digestive epithelium. Vitamins cannot be absorbed by the intestinal mucosa in normal amounts, unless this vitamin has been bound to intrinsic factor, a glycoprotein secreted by the parietal cells of the stomach.

Three Components of the GI Ecosystem

- **Diet**
- **Microbial flora**
- **Mucosa**
  - Epithelium
  - Mucus layer
  - Immune cells
  - Blood vessels
  - Nerve endings

GI regulatory molecules

ENDOCRINE:	Gastrin, CCK, motilin, somatostatin, secretin, *GIP
NEUROCRINE:	ACh, VIP, substance P, NO, CCK, serotonin, somatostatin, **CGRP
IMMUNE/JUXTRA-CRINE:	Histamine, cytokines, adenosine, reactive oxygen species

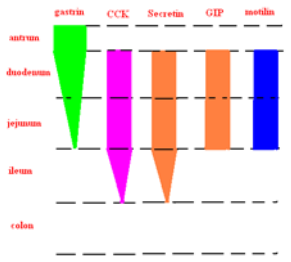
*Gastric inhibitory peptide, a.k.a. glucose-dependent insulinotropic peptide
**Calcitonin gene-related peptide

GASTROINTESTINAL HORMONES

produced by cells in	names of hormones
stomach	gastrin, somatostatin
duodenum, jejunum	secretin, CCK, GIP, somatostatin, motilin
pancreatic islets	insulin, glucagon, somatostatin, pancreatic polypeptide
ileum, colon	enteroglucagon, peptide YY, neurotensin, somatostatin

underlined hormones are ones we will emphasize

Sites of GI hormone production



Intestinal Hormones

- The intestinal tract secretes a variety of peptide hormones with similar chemical structures. Many of these hormones have multiple effects in several regions of the digestive tract, and in the accessory glandular organs as well.

Intestinal Hormones

- Duodenal enteroendocrine cells produce the following hormones known to coordinate digestive functions:
  - Secretin
  - Cholecystokinin (CCK)
  - Gastric inhibitory peptide (GIP)
  - Vasoactive intestinal peptide (VIP)
  - Gastrin
  - Enterocrinin

Intestinal Hormones

- Secretin is released when chyme arrives in the duodenum. Secretin's primary effect is an increase in the secretion of bile and buffers by the liver and pancreas.

Intestinal Hormones

- Gastric inhibitory peptide (GIP) is secreted when fats and carbohydrates—especially glucose—enter the small intestine.

Intestinal Hormones

- Vasoactive intestinal peptide (VIP) stimulates the secretion of intestinal glands, dilates regional capillaries, and inhibits acid production in the stomach.

Intestinal Hormones

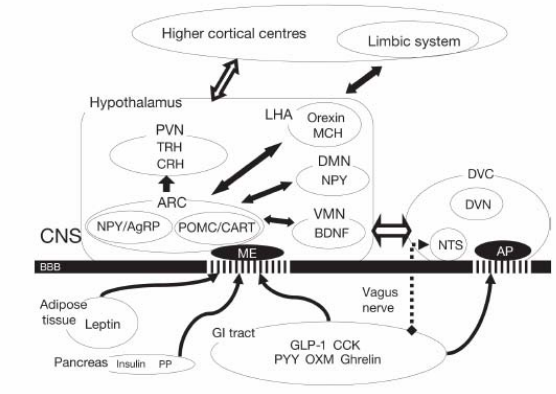
- Gastrin is secreted by G cells in the duodenum when they are exposed to large quantities of incompletely digested proteins. The functions of gastrin include promoting increased stomach motility and stimulating the production of acids and enzymes.

Intestinal Hormones

- Enterocrinin, a hormone released when chyme enters the small intestine, stimulates mucin production by the submucosal glands of the duodenum.

Intestinal Hormones

- Cholecystokinin (CCK) is secreted when chyme arrives in the duodenum, especially when the chyme contains lipids and partially digested proteins.
  - In the pancreas, CCK accelerates the production and secretion of all types of digestive enzymes.
  - It also causes a relaxation of the hepatopancreatic sphincter and contraction of the gallbladder, resulting in the ejection of bile and pancreatic juice into the duodenum.



CCK was the first gut hormone shown to modulate food intake.

CCK is secreted postprandially from the I cell of the small intestine into the circulation with a plasma half-life of a few minutes. CCK levels rise rapidly reaching a peak within 15 minutes after a meal. It is also reported to reduce food intake in humans and rodents. There are two CCK receptor subtypes: CCK1 and CCK2, both receptors being widely distributed in the brain including the brainstem and hypothalamus. The anorectic action appears to be mostly mediated through CCK1R on vagal afferents.

Although intermittent CCK infusion to rats at the onset of each meal reduces meal size, it is compensated for by an increase in meal frequency. Long-acting CCK analogs have produced sustained reductions in food intake across days, increasing the possibility that sustained CCK receptor activation could sustain reductions in food intake sufficient to produce weight loss. Such an idea was recently put to the test in a large clinical trial

Peptide tyrosine tyrosine (PYY)

PYY is a member of the PP-fold family. This family also includes NPY and pancreatic polypeptide (PP). PP-fold peptides act via G protein-coupled receptors: Y1, Y2, Y4,Y5 and Y6.

Two circulating forms of PYY are released by L cells in the distal gut. PYY (3-36), the major circulating form, is produced by cleavage of the N-terminal Tyrosine-Proline residues from PYY (1-36) by the enzyme dipeptidyl-peptidase IV (DPPIV).

PYY (3-36) binds with highest affinity to the hypothalamic Y2R causing
a reduction in food intake.

Circulating PYY concentrations are low in the fasted state and rapidly increase following a meal, peaking at 1-2 hours and remaining elevated for several hours. Ingestion of fat results in greater release of PYY than observed with ingestion of carbohydrate or protein meals with a similar caloric content.

Pancreatic polypeptide (PP)

- PP is secreted from PP cells in the pancreatic islets of Langerhans and is thought to reduce food intake directly through the Y4R in the brainstem and hypothalamus.
- It may also act via the vagus nerve to reduce food intake since the anorectic effects of PP are abolished by vagotomy in rodents.
- Circulating PP concentrations rise after a meal in proportion to the calorific load.
- In mice, acute and chronic peripheral administration of PP reduces food intake. Although differences in circulating levels of PP between lean and obese people have been conflicting, some studies have demonstrated significantly lower levels in obese subjects.

Glucagon-like peptide-1

- GLP-1 is a proglucagon derived peptide. GLP-1 is co-secreted with PYY from L cells in the intestine and has a potent incretin effect by stimulating insulin secretion in a glucose-dependent manner. In addition, GLP-1 possesses trophic effects on pancreatic β cells. DPPIV degradation and renal clearance rapidly inactivate and remove GLP-1 from plasma circulation, resulting in a halflife of 1-2 minutes.
- GLP-1 exerts its effect at the GLP-1R to stimulate adenylyl cyclase activity and cAMP production. GLP-1R expression is widely distributed particularly in the brain, GI tract and pancreas.
- Circulating GLP-1 levels rise after a meal and fall in the fasted state. GLP-1 reduces food intake, suppresses glucagon secretion and delays gastric emptying.
- Intravenous infusion of GLP-1 results in a dose-dependent reduction in food intake in both normal weight and obese subjects although obese subjects have a blunted postprandial GLP-1 response compared to lean subjects

Exendin-4, a naturally occurring peptide from the saliva of the Gila monster lizard, is a DPPIV-resistant GLP-1R agonist . It has been licenced for the treatment of type 2 diabetes and has been shown to reduce food intake and body weight, and improve glycaemic control.

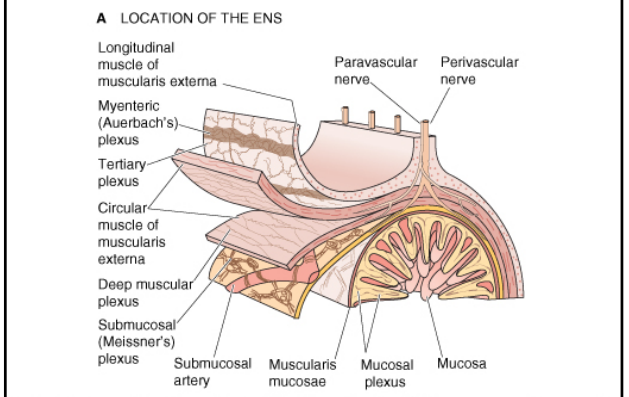
Ghrelin

- Ghrelin, a 28-amino acid peptide primarily produced by gastric endocrine cells, is the only known orexigenic gut hormone.
- It was initially identified as an endogenous ligand for GH secretagogue receptor (GHS-R) in rat stomach. However the GHS-R is also expressed in the hypothalamic ARC, and levels of circulating ghrelin have been noted to increase before meals and fall rapidly after eating.
- Both CNS and peripheral administration of ghrelin increases food intake and body weight with a reduction in fat utilization in rodents. The role of ghrelin in short-term energy balance has been supported in multiple studies, including those with humans. More recent findings suggest that ghrelin is more than just an energy related signal and also mediates changes in the motivational aspects of feeding, including reward and memory.
- A ghrelin-neutralizing RNA Spiegelmer (a single stranded mirror image oligonucleotide), NOX-B11, that attaches to the active form of ghrelin and blocks its ability to bind to its receptor has been investigated in preclinical experiments.
- YIL-870 and YIL- 781, two piperidine-substituted quinazolinone-derived compounds, are potentGHSRantagonists that vary in their ability to

INNERVATION OF THE GI TRACT

✓ **Autonomic nervous system (extrinsic innervation)**
- **Sympathetic**
- **Parasympathetic**

✓ **Enteric nervous system (intrinsic innervation)**
- **Myenteric plexus**
- **Submucosal plexus**



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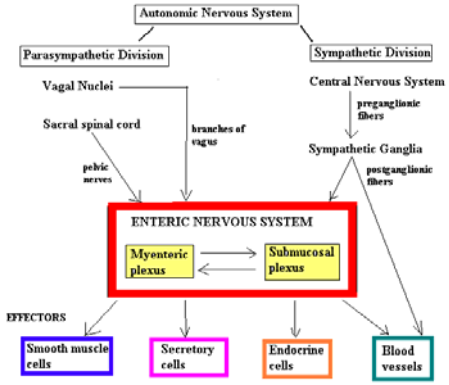
NEURAL CONTROL OF GI TRACT



THE GI TRACT HAS A BUILT-IN BRAIN: *THE ENTERIC NERVOUS SYSTEM*

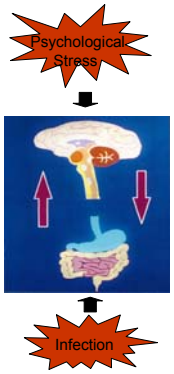
✓ **10^8 neurons in the enteric nervous system!**

✓ **Several plexuses without cell bodies are present**

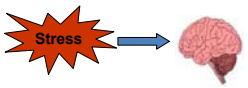
✓ **Two major ganglionated plexus: submucosal and myenteric plexuses that contain**
- **Sensory neurons**
- **Effector (motor) neurons**
- **Interneurons**

Bi-directional communication

- Bi-directional brain-gut interactions play an important role in the regulation of many vital functions in health and disease.
- In health-regulation of digestive processes (including appetite and food intake), and the gut immune system.
- In disease, altered brain-gut interactions may underlie the symptom generation in functional GI disorders (FGIDs), and in the pathophysiology of various eating disorders.
- Routes of communication include:
  - neural (physical connection)
  - immune system
  - hormonal system



Stress and the gut



Activation of the HPA axis
(ie↑ CRF, ACTH, cortisol)

- Increase in gut permeability
- Increase in mucosal inflammation
- Changes in GI motor function
- Changes in gut flora

THERE ARE THOUSANDS OF NEURONS IN ONE CENTIMETER OF GUT AND THEY MAKE A VARIETY OF NEUROTRANSMITTERS

Number and types of neurons in one centimeter length of guinea-pig small intestine

	myenteric plexus	submucosal plexus
Total number	10,000	7,200
Substance P	350	820
VIP	240	3,060
Somatostatin	470	1,260
Enkephalin	2,450	0
5-HT	200	0
Amine handling	50	850

These compounds are important in CNS: "gut-brain peptides"

Some are transmitters, some are modulators

Established and probable neurotransmitters

Substance	*Location and function*
Acetylcholine (ACh)	Excitatory transmitter to smooth muscle, intestinal epithelial cells, parietal cells, certain endocrine cells, and at neuro-neuronal synapses
Adenosine triphosphate (ATP)	Inhibitory transmitter to smooth muscle
Calcitonin gene-related peptide (CGRP)	Released by enteric sensory neurons onto interneurons in enteric ganglia and central ganglia
Gastrin-releasing peptide	Released by secretomotor neurons onto G cells
Nitric oxide (NO)	Inhibitory transmitter to smooth muscle cells
Substance P (and other tachykinins	Excitatory transmitter to smooth muscle cells
Vasoactive intestinal peptide (VIP)	Inhibitory transmitter to smooth muscle cells, excitatory secretomotor transmitter to epithelial and gland cells, vasodilator transmitter

Present in enteric neurons
Transmitter function not established

Substance	*Location and possible function*
Cholecytsokinin (CCK)	Present in some secretomotor neurons and interneurons, may contribute to excitation
Dynorphin and related peptides	Present in some secretomotor neurons, interneurons, and motor neurons to muscle
Enkephalins and related peptides	Present in some interneurons and in motor neur to smooth muscle
Galanin	Present in some secretomotor neurons, interneurons, and inhibitory motor neurons t smooth muscle
Glutamate	May be an excitatory transmitter at synapses between enteric neurons
γ-amino butyric acid (GABA)	Present, but transmitter role is not known
Neuropeptide Y	May inhibit secretion of electrolytes and wat
Serotonin (5-HT)	May be excitatory transmitter at synapses betw enteric neurons
Somatostatin	Present in numerous enteric neurons, but transmitter role is not established

Types of neurons in enteric nervous system

Type of neuron	*Function*
Motor neurons	
Motor neurons to muscle cells	
Excitatory	Promote contraction of smooth muscle
Inhibitory	Inhibit contraction of smooth muscle
Motor neurons to blood vessels	Vasodilator neurons
Motor neurons to epithelial cells	Promote secretion of electrolytes and water
Motor neurons to gland cells	Promote secretion of specific substances
Motor neurons to endocrine cells	Promote secretion of hormones
Sensory neurons	Respond to stretch or to chemical stimuli
Associative neurons	Interneurons in motor, secretomotor, and vasomotor pathways
Intestinofugal neurons	Neurons with cell bodies in enteric ganglia and nerve terminals in sympathetic ganglia

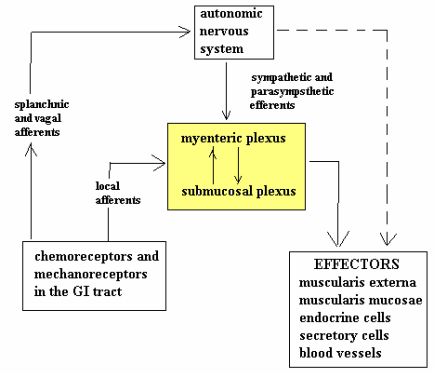
NEURONS OF MYENTERIC PLEXUS

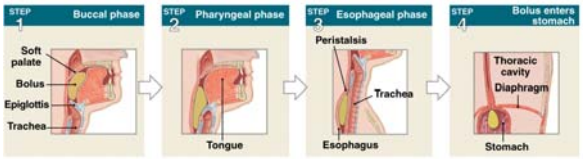
- **Excitatory motor neurons to muscle** — **ACh (muscarinic)**, **Substance P**
- **Inhibitory motor neurons to muscle** — **VIP**, **NO**
- **Sensory neurons (mechanoreptors)** — **CGRP**
- **Interneuron** — **ACh (nicotinic)**

NEURONS OF SUBMUCOSAL PLEXUS

Stimulatory secretomotor neurons	ACh (muscarinic)
	VIP
Sensory neurons	Stretch, touch, and chemoreceptors
	CGRP
Vasodilator neurons	ACh, VIP
Interneurons	ACh (nicotinic)

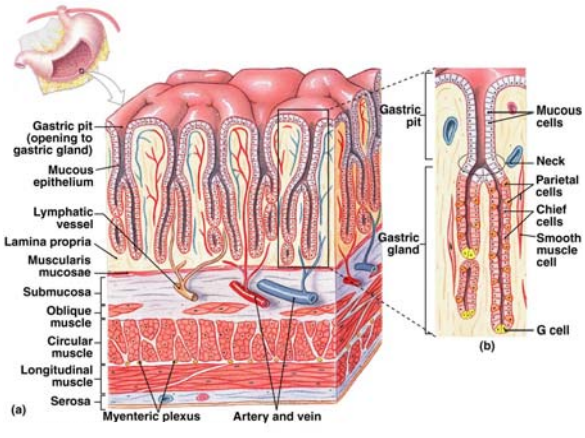
LAYERS OF REFLEX CONTROL





The Stomach

- The stomach performs four major functions: (1) storage of ingested food,
- (2) mechanical breakdown of ingested food,
- (3) disruption of chemical bonds in food material through the action of acids and enzymes,
- (4) production of intrinsic factor, a glycoprotein whose presence in the digestive tract is required for the absorption of vitamin B12 in the small intestine.



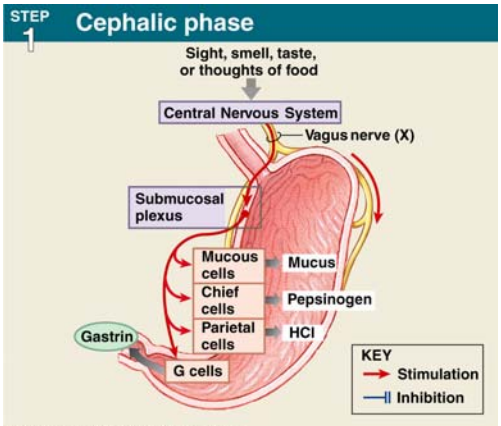
Regulation of Gastric Activity

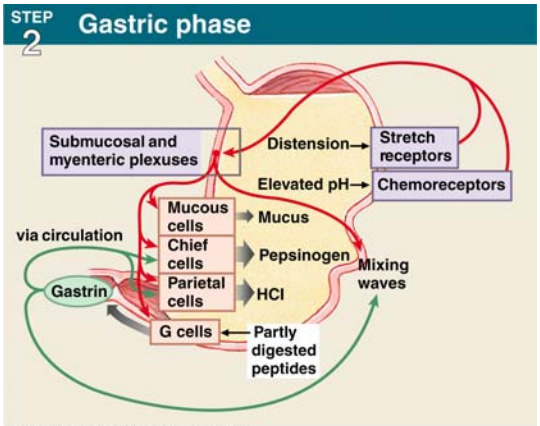
The production of acid and enzymes by the gastric mucosa can be:

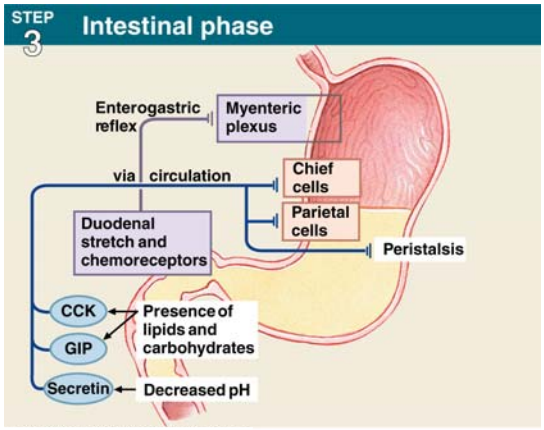
(1) controlled by the CNS,

(2) regulated by short reflexes of the enteric nervous system, coordinated in the wall of the stomach,

(3) regulated by hormones of the digestive tract.







Digestion and Adsorption in the Stomach

- The stomach performs preliminary digestion of proteins by pepsin and, for a variable period, permits the digestion of carbohydrates and lipids by salivary amylase and lingual lipase.
- As the stomach contents become more fluid and the pH approaches 2.0, pepsin activity increases and protein disassembly begins.
- Although digestion occurs in the stomach, nutrients are not absorbed there.

Stomach

- The stomach is a storage site that provides time for the physical breakdown of food that must precede chemical digestion.
- Protein digestion begins in the acid environment of the stomach through the action of pepsin.
- Carbohydrate digestion, which began with the release of salivary amylase by the salivary glands before swallowing, continues for a variable period after food arrives in the stomach.

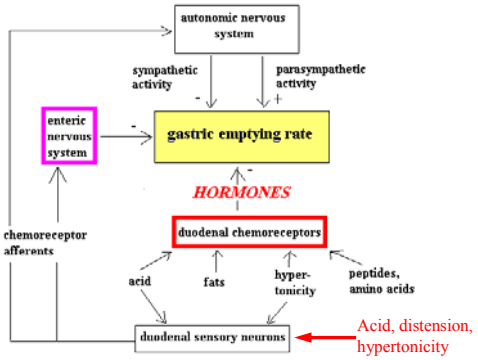
The Coordination of Secretion and Absorption

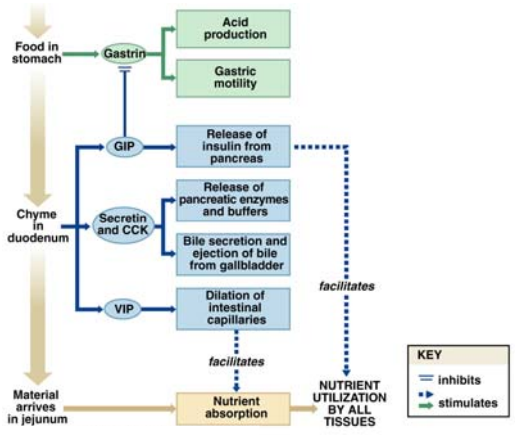
- A combination of neural and hormonal mechanisms coordinates the activities of the digestive glands. These regulatory mechanisms are centered around the duodenum, where acids must be neutralized and the appropriate enzymes added.
- Neural mechanisms involving the CNS (1) prepare the digestive tract for activity (parasympathetic innervation) or inhibit gastrointestinal activity (sympathetic innervation) and (2) coordinate the movement of materials along the length of the digestive tract (the enterogastric, gastroenteric, and gastroileal reflexes).
- In addition, motor neurons synapsing in the digestive tract release a variety of neurotransmitters.

How hormones regulate gastric emptying

- ❑ CCK, gastrin, GIP, and secretin all promote constriction of pyloric sphincter
- ❑ Secretin and GIP decrease force of antral contractions
- ❑ Gastrin and CCK increase force of antral contractions. Under most circumstances the effect on the pyloric sphincter predominates
- ❑ All of these tend to slow gastric emptying

DUODENAL CONTROL OF GASTRIC EMPTYING





Intestinal Absorption

- On average, it takes about five hours for materials to pass from the duodenum to the end of the ileum, so the first of the materials to enter the duodenum after you eat breakfast may leave the small intestine at lunchtime.
  - Along the way, the organ's absorptive effectiveness is enhanced by the fact that so much of the mucosa is movable.
  - The microvilli can be moved by their supporting microfilaments, the individual villi by smooth muscle cells, groups of villi by the muscularis mucosae, and the plicae by the muscularis mucosae and the muscularis externa.
  - These movements stir and mix the intestinal contents, changing the environment around each epithelial cell from moment to moment.

COLON

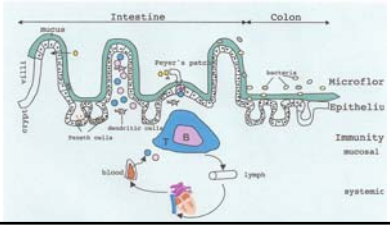
- ✓ **Receives 500 to 1500 ml of fluid from ileum/day**
- ✓ **Absorbs all but 50 to 100 ml/day**
- ✓ **Longitudinal smooth muscle in taenia coli**
- ✓ **Parasympathetic supply from vagal branches down to transverse colon**
- ✓ **Descending and sigmoid colon, rectum, anal canal from pelvic nerves from sacral spinal cord**
- ✓ **Parasympathetics stimulate motility, sympathetics inhibit motility**

DEFECATION

- ✓ Urge elicited by distension of the rectum
- ✓ Urge subsides unless external sphincter relaxed
- ✓ Both reflex and voluntary; sacral spinal cord is integrating center with input from higher centers
- ✓ Puborectalis and internal anal sphincter relax
- ✓ Intra-abdominal pressure elevated dramatically by forced inspiration, then contraction of both respiratory and abdominal muscles
- ✓ (Intra-abdominal pressure may reach 200 cm water = 150 mmHg) [what will this do to venous return?]
- ✓ Contraction of rectum and anal canal

Gut's Defense System

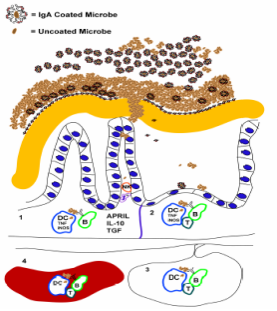
- The defense system in the gut can be split into three lines:
  - the gut flora
  - the gut mucosa and epithelium
  - the related immune system



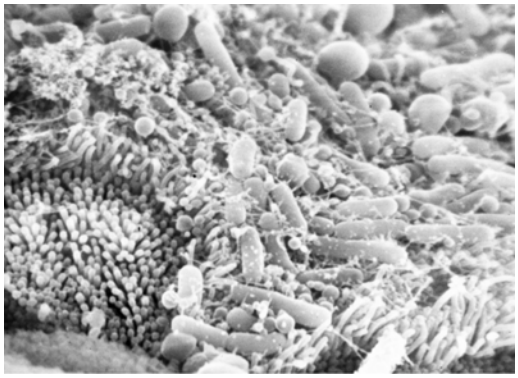
The GI Immune System

- **Mucosa-associated lymphoid tissue (CTS/Phys)**
- **GALT = gut-associated lymphoid tissue**
- **The mass of lymphoid cells in the GI tract is equal to that in the rest of the body!**
- **Immunocytes reside in Peyer's patches and in other sites in mucosa and submucosa**
- **Immunocytes include B and T lymphocytes, plasma cells, mast cells, macrophages, eosinophils**
- **Immunocytes respond to lumenal and mucosal antigens and secrete inflammatory mediators (histamine, prostaglandins, leukotrienes, cytokines) that mostly promote GI motility and secretion of salts and water**
- **Hyperactivity of GI immune system in celiac disease, inflammatory bowel disease (IBD), Crohn's disease**

Host-microbial interactions affected by IgA are modulated as result of stimulation of the immune system at different sites



Secretory IgA, antimicrobial peptides, and mucus are among the factors that comprise the mucosal barrier. The ability of different microorganisms to develop resistance to these factors, or to degrade mucus, will contribute to greater microbial pattern recognition receptor-mediated signaling in epithelial cells, dendritic cells (DCs),



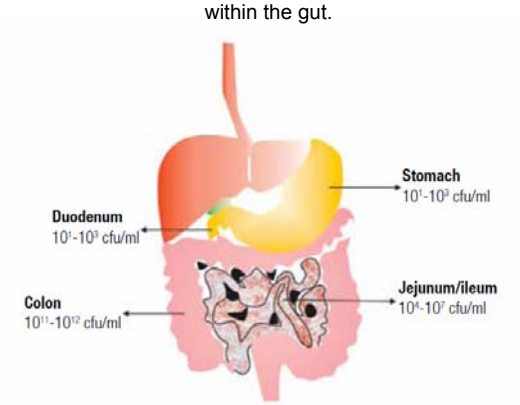
Some Background

- Present in immense numbers of in the gut:~1 x 10^{14} bacteria.
- 30-40 species compose of 99% of the population.
- Mutualistic/symbiotic relationship with hosts. In humans, they serve several key roles:
  - Metabolism and fermenting unused energy substrates.
  - Training the immune system
  - Production of vitamins.
  - And competitive inhibition of harmful species.
- Very significant for health:
  - Obesity
  - Disease
  - Aging

Gut Microflora

- **Microbiologically, the gut has three principal regions: the stomach, small intestine, and colon.**
- **The stomach has very low bacterial numbers**
- **Facultative anaerobes such as lactobacilli, streptococci, and yeast are present at ;100 colony forming units (CFU) per millilitre due to the low environmental pH .**
- **The small intestine has a larger bacterial load that consists of facultative anaerobes such as lactobacilli, streptococci, and enterobacteria as well as anaerobes such as Bifidobacterium spp., Bacteroides spp., and clostridia at levels of ;10^4–10^8 CFU/ml.**
- **However, the colon, has a total population of 10^{11}–10^{12} CFU/ml of contents**

Relative concentrations of bacteria at various locations within the gut.



Factors affecting the intestinal micro ecosystem

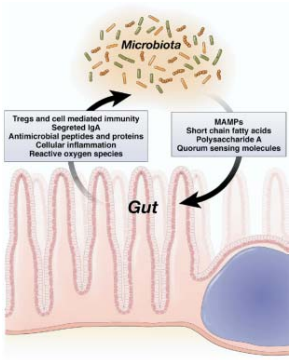
- Any action taken to kill 'bad' bacteria essentially kills 'good' bacteria as well.

- **Antibiotics and other drugs intake**
- **Microbial infections**
- **Diet (highly processed, low-fiber foods)**
- **Chronic diarrhea**
- **Stress**

Imbalance of intestinal micro flora results in:

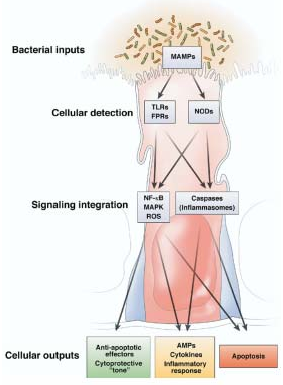
- **Poor nutritional response**
- **Reduced efficacy of medications**
- **Physiological dysfunction**
- **Accelerated aging**
- **Cancer**
- **Deficient immune response**
- **Susceptibility to infection**
- **Physical discomfort**

Mechanisms of microbiota and gut crosstalk

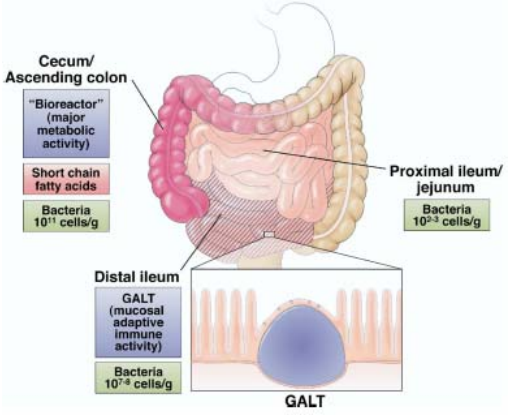


Both parties in the symbiotic dyad possess means to alter and shape each other, resulting in a "negotiated settlement" at equilibrium. A breakdown on this crosstalk may result in a "dysbiotic" microbiota and clinical consequences.

Cellular consequences to bacterial stimuli



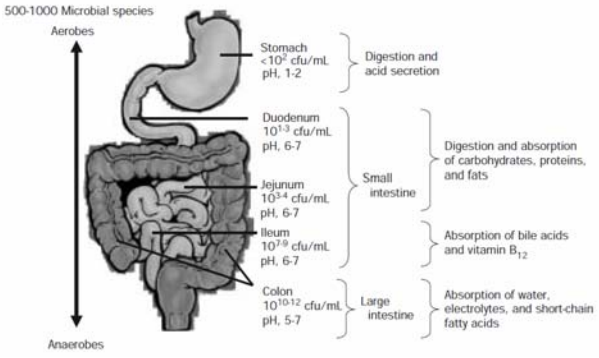
Preferred sites of commensal/probiotic interaction with the gut



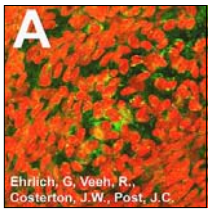
Influences of the Microbiota

1. Metabolic/nutritional/energy utilization
   - Vitamin synthesis
   - SCFA as energy source—role in obesity
2. Innate Immune Regulation
   - Dampening of inflammatory responses
3. Adaptive immune Regulation.
   - Induction of Immunosuppressive T cells (Tregs)
4. Epithelial development and survival
   - Stimulation of proliferation, angiogenesis, epithelial restitution
   - Cytoprotective effects of PRR signaling
5. Competitive exclusion of pathogens

Key physiologic and microbiological features of the gut



Phylogenetic tree representing the groups of bacteria most frequently detected in human faeces using 16S rRNA gene sequencing.



Metagenomics can theoretically access 100% of an environment

Direct isolation of DNA from the environment

Isolation and laboratory cultivation

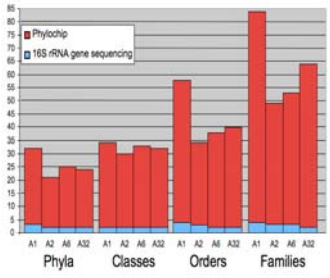
DNA

Traditional cultivation and genomics can at best access between 1-10% of an environment

Traditional genomics approaches

Hybridization-based approaches

- Version 2.0 of the Affymetrix PhyloChip targets over 30,000 unique 16S rRNA sequences, totaling almost 9,000 distinct taxonomic groups



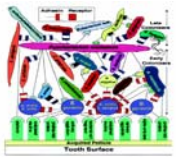
The Human Microbial Metagenome

Microbiome

A multi-genus/species community of bacteria that exists within a defined environmental domain

Human Microbiome

The community of bacteria that live on/in the human host (mucosal surfaces, skin, tooth surface, etc.) Beneficial/neutral/adversari



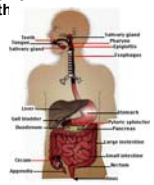
Metagenomics

The culture-independent study of the genomes of many organisms simultaneously in order to understand microbial communities as intact systems

The Human Microbial Metagenome

Humans are born without any microorganisms

Colonization of skin, oral/respiratory tract, genitourinary system and gastrointestinal tract begins immediately at birth



Our adult bodies contain 10 times more microbial cells than human cells

Human colon contains up to 100 trillion bacteria

Numerous studies have suggested that shifts in the populations of microbial communities may be associated with a number of important acute and chronic diseases: inflammatory bowel disease, obesity, cardiovascular disease, eczema and other skin diseases, vaginal infections

This presents an opportunity to develop new approaches to therapy as a means of maintaining health

Metagenomic Analysis of the Human Distal Gut Microbiome

Steven R. Gill,[1*‡] Mihai Pop,[1†] Robert T. DeBoy,[1] Paul B. Eckburg,[2,3,4] Peter J. Turnbaugh,[5] Buck S. Samuel,[5] Jeffrey I. Gordon,[5] David A. Relman,[2,3,4] Claire M. Fraser-Liggett,[1,6] Karen E. Nelson[1]

- 28 year-old female; 37 year-old male, one a vegetarian; no antibiotics in the previous year
- 65,959 and 74,462 reads from random libraries of fecal DNA

Science 312, 1355–1359 (2006)

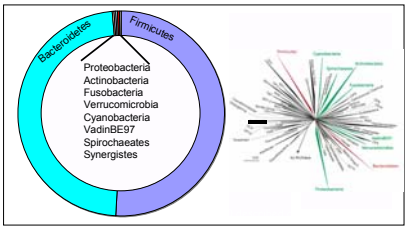
The Human Microbial Metagenome

Four environments on the human body are the most densely populated with microorganisms:

-gastrointestinal tract (800 phylotypes)
-oral cavity (500 phylotypes)
-vagina (200 phylotypes)
-skin (100 phylotypes)

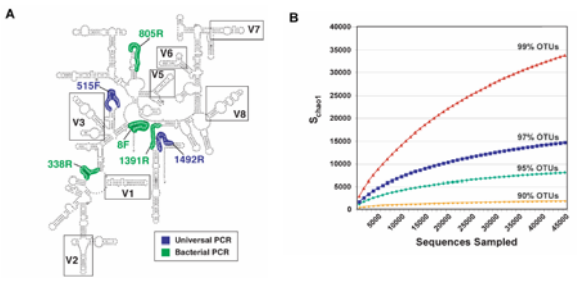
Setting The Stage

- 'metagenome' is a composite of *Homo sapiens* genes and genes present in the genomes of the trillions of microbes that colonize our adult bodies



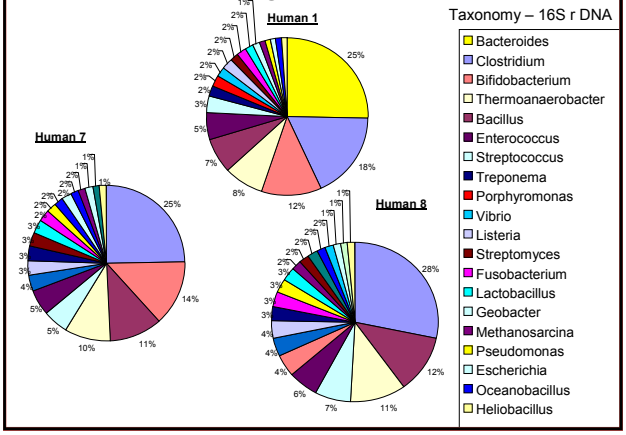
16S rRNA sequence-based enumeration of the adult human distal gut microbiota
Eckburg *et al.* (11,831 sequences)
Ley and Gordon (19,653 sequences)

Bacterial SSU rRNA gene-based surveys of the gut microbiota



(**A**) Cartoon of the general structure of the bacterial 16S rRNA gene, showing conserve and variable regions (based on http://www.rna.ccsb.utexas.edu/)
(**B**) Analysis of diversity in the human gut microbial community. Collector's curves of observed and estimated richness are shown. Richness is estimated to be 18,000 (genus-level

16S rRNA Sequencing – Human Gut Microbiome



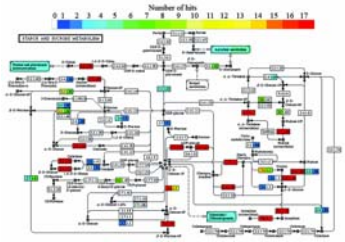
Metagenomics of the Human Colon Microbiome

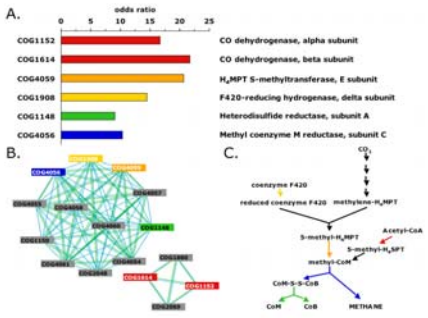
Glycan metabolism

The plant polysaccharides we consume are rich in xylan-, pectin- and arabinose-containing carbohydrate structures. The human genome lacks most of the enzymes required for degrading these glycans.

At least twenty six different glycoside hydrolase families are encoded in the microbiome, many of which are not present in the human glycobiome.

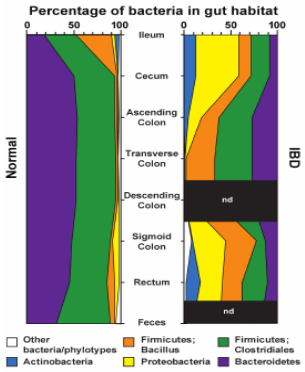
Enrichment for genes in the starch metabolism pathway in the human colonic microbiome. The left and right sides of each boxed EC number indicate whether the microbial gene product is present in human colonic samples 7 and 8, respectively, and to what extent (color scale: white no hits; red ≥17 hits).





COG analysis reveals enrichment for archaeal metabolism.
(A) Five archaeal COGs are significantly enriched (p<1e-5).
(B) STRING protein map of COGs (colored) and predicted interactors (grey).
(C) Location and role of each enzyme in methanogenesis. STRING lines indicate neighborhood (green) and co-occurence (blue) connections.

Distribution of predominant bacterial phylotypes in the human intestinal tract



IBD: samples from patients with either Crohn's disease or ulcerative colitis (Frank et al., 2007); **Normal:** data from healthy young adults from Eckburg et al (2005) plus the non-IBD controls reported in Frank et al. (2007)

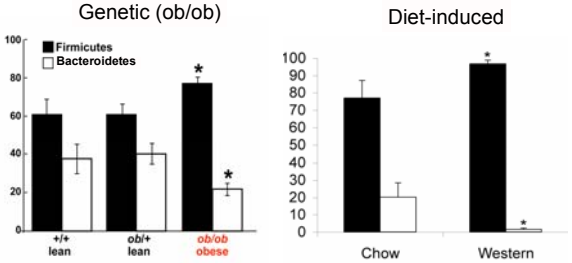
Human Microbial Metagenomics: Grand Challenges

- Do we all share an identifiable core 'microbiome' surrounded by a shell of diversity? Is this best defined by species, gene content, or functional capabilities?
- Should differences in our microbiome be viewed, along with our immune and nervous systems, as features of our biology that are profoundly affected by both our genotypes and by our individual environmental exposures?
- How is the human microbiome evolving (within and between individuals) over varying time scales as a function of age, diets, disease, lifestyle, and biosphere?
- Are changes in community composition the cause of disease or a read-out of a disease process?

Evidence for a link between gut microbial communities and adiposity

- Colonization of adult germ-free mice with a gut microbial community from conventionally-raised mice produces a rapid and marked increase in adiposity without an increase in food consumption
- Equivalent response in males and females from several inbred lines
- Does not require a functional innate or adaptive immune system
- Mechanism: increased fermentation of otherwise indigestible dietary polysaccharides; microbial regulation of host genes that regulate storage of extracted calories in adipocytes

Identification of a linkage between adiposity and microbial ecology in mice

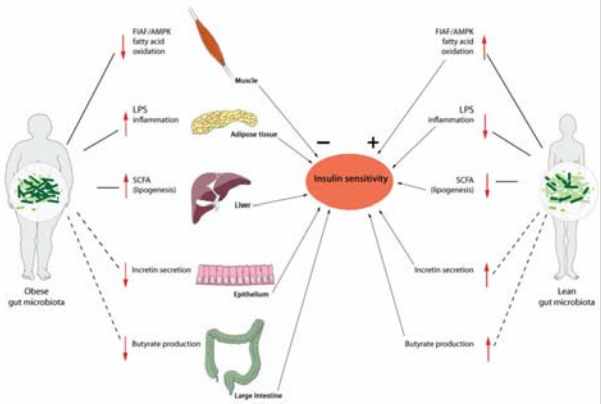


Genetic and diet-induced mouse models of obesity both exhibit increased abundance of Firmicutes

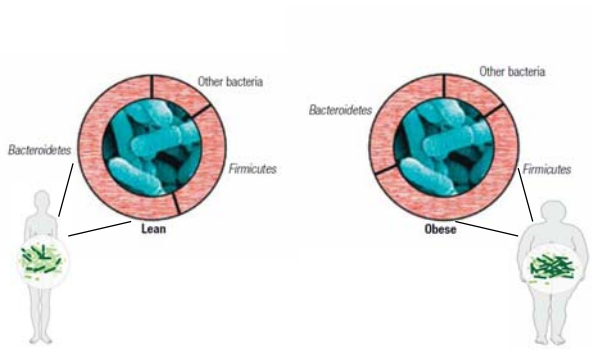
Linkage between gut ecology and adiposity in humans

- Following an initial loss of ~5% body weight, there is a progressive, statistically significant, division-wide shift towards more Bacteroidetes and fewer Firmicutes as more weight is lost
- Changes occur independent of diet

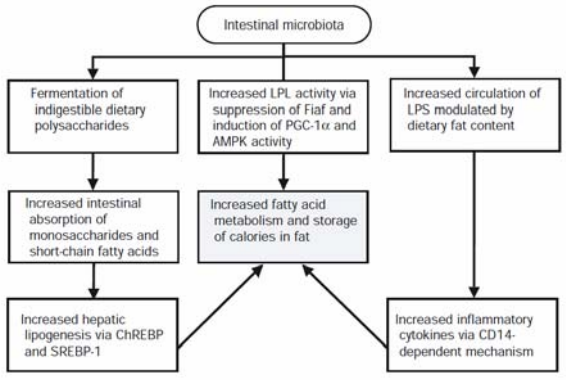
Possible links between the gut microbiota and metabolism.



Relative proportion of firmicutes and bacteroidetes in lean and obese mice.



Mechanisms by which the intestinal microbiota may contribute to obesity.



Probiotic Concept

- **Probiotic (Greek Language) " for life" .**
- **It was first used by Lilly and Stillwell in 1965 to describe "substances secreted by one microorganism which stimulates the growth of another".**
- **Parker was the first to use the term *probiotic* in the sense that it is used today "organisms and substances which contribute to intestinal microbial balance".**
- **In 1989, Fuller attempted to improve Parker's definition of probiotic with the following distinction: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance."**

History of Health Claims

- **Persian version of the Old Testament (Genesis 18:8) states " Abraham owed his longevity to the consumption of sour milk."**
- **In 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis .**
- **Metchnikoff claimed that the intake of yogurt containing lactobacilli results in a reduction of toxin-producing bacteria in the gut and this increases the longevity of the host.**

The probiotic concept:

- **effects exerted by viable microorganisms**
- **applicable independent of the site of action and route of administration.**
- **include sites such as the oral cavity, the intestine, the vagina, and the skin.**
- **In the case of probiotic foods, the health effect is usually based on alteration of the gastrointestinal micro flora and, therefore, based on survival during gastrointestinal transit.**

Selection of probiotic organism

- **Safety**
- **Origin**
- **Functional aspects**
- **Survival**
- **Adherence, colonisation**
- **Anti-microbial products**
- **Immune stimulation**
- **Genetic stable**
- **Prevention of pathogens**

(Mullan, 2002)

Major pre-requisite properties for a microbe to be accepted as a probiotic are:

- **It should be non-pathogenic, non-toxic and non-allergic.**
- **It should be capable of surviving and metabolizing in upper G.I. tract secretion in the gut environment e.g. Resistant to low pH, organic acids, bile juice, saliva and gastric acid.**
- **It should be human in origin, genetically stable and capable of remaining viable for long periods in field condition.**
- **It should be able to modulate immune response and provide resistance to disease through improved immunity or by the production of antimicrobial substance in the guts.**

Contd….

- **It should have a good adhesion/ colonization to human intestinal tract and influence on gut mucosal permeability.**
- **It should be antagonistic against carcinogenic/ pathogenic organism.**
- **It should posses clinically proven health benefit, e.g. gastrointestinal disorders, persistant diarrhoea, clostridium difficle colitis, antibiotics associated diarrhoea, acute infantile gastroenteritis.**
- **It should have technologic properties for commercial viability such as stability of desired characteristics during processing, storage and transportation.**

Established effects of probiotics

- **Aid in lactose digestion**
- **Resistance to enteric pathogens**
- **Anti-colon cancer effect**
- **Anti-hypertensive effect**
- **Small bowel bacterial overgrowth**
- **Immune system modulation**
- **Blood lipids, Heart disease**
- **Urogenital infections**
- **Hepatic encephalopathy**

(Roberfroid, 2000)

Mechanism for the benefit of Probiotics:

- **Adherence and colonization of the gut**
- **Suppression of growth or epithelial binding/invasion by pathogenic bacteria and production of antimicrobial substances**
- **Improvement of intestinal barrier function**
- **Controlled transfer of dietary antigens**
- **Stimulation of mucosal and systemic host immunity**

(Harish and Varghese, 2006)

Advantages of Probiotics

- **Produce lactic acid- lowers the pH of intestines and inhibiting bacterial villains such as *Clostridium, Salmonella, Shigella*, *E. coli,* etc.**
- **Decreases the production of a variety of toxic or carcinogenic metabolites.**
- **Aid absorption of minerals, especially calcium, due to increased intestinal acidity.**
- **Production of β- D- galactosidase enzymes that break down lactose .**

Contd….

- **Produce a wide range of antimicrobial substances - acidophilin and bacteriocin etc. help to control pathogenic bacteria .**
- **Produce vitamins (especially Vitamin B and vitamin K)**
- **Act as barriers to prevent harmful bacteria from colonizing the intestines**

(Roberfroid, 2000)

Effects of probiotics on pathogenic bacteria

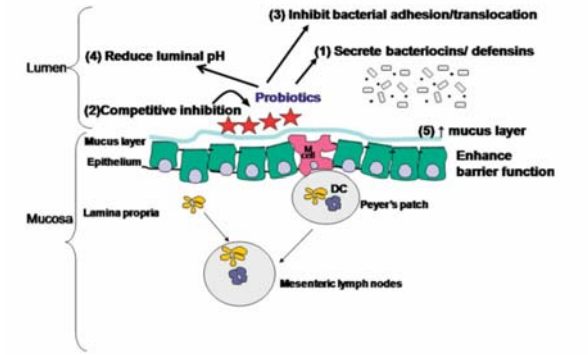
- **Probiotics reduce plasma levels of bacterial endotoxin concentrations, by inhibiting translocation of bacteria across the GI lumen into the bloodstream.**
- **Decreases in translocation of bacteria may occur as a result of the ability of probiotics to tighten the mucosal barrier.**
- **There are several ways probiotic microflora can prevent pathogenic bacteria from adhering and colonizing gut mucosa.**
- **Probiotics disallow colonization by disease-provoking bacteria through competition for nutrients, immune system up-regulation, production of antitoxins, and up-regulation of intestinal mucin genes.**

contd…

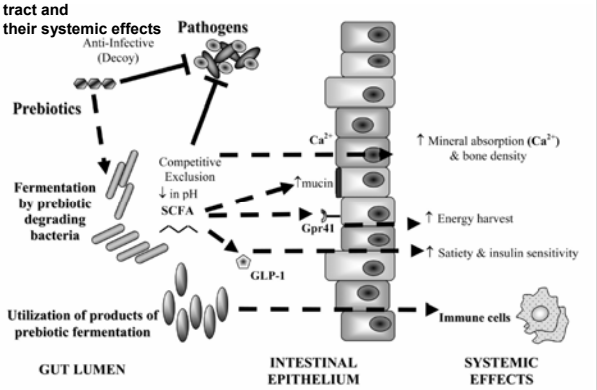
- **Probiotics lower colon luminal pH and foster growth of non-pathogenic commensal bacteria by SCFA (Short Chain Fatty Acid) production. One SCFA, acetic acid, has antimicrobial activity against molds, yeasts, and bacteria.**
- **Probiotics exert protective effects through production of hydrogen peroxide and benzoic acid, which inhibit many pathogenic, acid-sensitive bacteria .**

(Sanders, 2003)

Inhibition of enteric bacteria and enhancement of barrier function by probiotic bacteria.

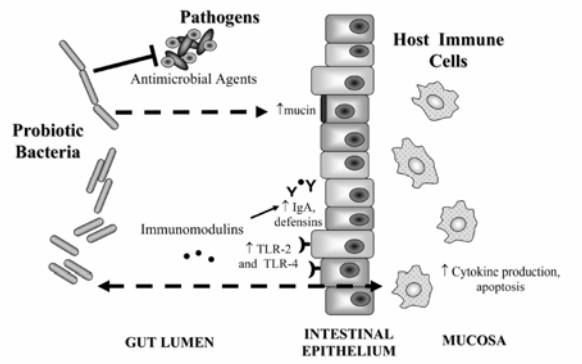


Depiction of the beneficial roles of prebiotics in the mammalian gastrointestinal tract and their systemic effects



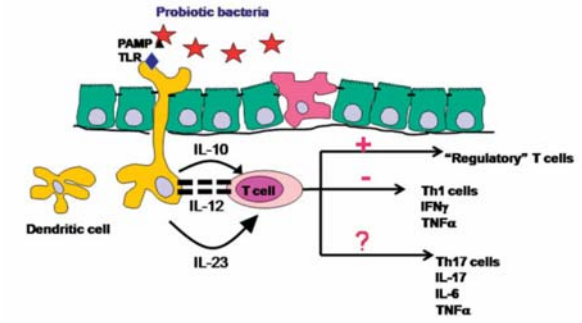
Ca2+: Calcium; GLP-1: Glucagon-Like Peptide-1; Gpr41: G-protein coupled receptor 41;

Depiction of the beneficial roles of probiotics in the mammalian gastrointestinal tract



IgA: Immunoglobulin A; TLR: Toll-like receptor

Modulation of mucosal immune response by probiotic bacteria



Cytokines produced following the interaction of probiotics with the intestinal epithelium

Organism	Cytokines/ Chemokines	Cells	References
L. sakei	IL-1β, IL-8, TNF-α (pro-inflammatory, apoptosis inducer)	Caco-2	(Hütter et al., 2000)
L. johnsonii	TGF-β (pro-inflammatory Th17 inductor)	Caco-2	(Hütter et al., 2000)
E. coli Nissle 1917	IL-8 (Pro-inflammatory, neutrophiles stimulator, bactericid activity by oxygen activation)	T-84, HT-29	(Lammers et al., 2002); (Otte and Podolsky, 2004)
L. reuteri	NGF (nerve growth factor)	T84, HT-29	(Ma et al., 2004)
B. lactis Bb12	IL-6 (Pro-inflammatory, growth factor of B cells, support the production of blood plates)	Primary intestinal epithelial cells, Mode-k	(Ruiz et al., 2005)
L. rhamnosus GG	IL-8	Caco-2	(Zhang et al., 2006)
L. casei DN-114 001	CXCL1, CXCL2, CCL20 (attract macrophages)	Caco-2	(Ten et al., 2006)
L. casei CRL 431	IL-8	Primary intestinal epithelial cells	(Vinderola et al., 2005)
L. helveticus R389	IL-8	Primary intestinal epithelial cells	(Vinderola et al., 2005)
L. casei subsp. *casei*	IL-15 (NK cells activation)	Caco-2	(Ogawa et al., 2006)

Probiotic influence on different immune functions

Immune system effect	Organism	References
Increased phagocytosis capacity	*L. acidophilus (johnsonii) La1* *L. casei* *B. lactis Bb12* *B. lactis HN019* *L. rhamnosus GG* *L. rhamnosus HN001*	(Arunachalam et al., 2000; Donnet-Hughes et al., 1999; Pelto et al., 1998; Perdigon et al., 1986; Schiffrin, 1994; Schiffrin et al., 1997)
Increased NK cell activity	*L. rhamnosus HN001* *B. lactis HN109* *L. casei* subsp. *casei* + dextran	(Gill et al., 2001a; Ogawa et al., 2006; Sheih et al., 2001)
Stimulation of IgA production	*B. bifidum* *L. acidophilus (johnsonii) La1* *L. casei rhamnosus GG* *B. lactis Bb12*	(Fukushima et al., 1998; Ibnou-Zekri et al., 2003; Isolauri et al., 1995; Kaila et al., 1995; Link-Amster et al., 1994; Maiamaa et al., 1995; Park et al., 2002)
Suppression of lymphocyte proliferation Induction of apoptosis	*L. rhamnosus GG* *L. casei GG* *B. lactis* *L. acidophilus* *L. delbrueckii* subsp. *bulgaricus* *S. thermophilus* *L. paracasei* *E. coli Nissle 1917*	(Carol et al., 2006; Pessi et al., 1999; Sturm et al., 2005; von der Weid et al., 2001)
Increased cell-mediated immunity	*L. casei Shirota*	(de Waard et al., 2003)

Some probiotics and their effects on Dendritic Cells maturation

Organism	Effect	Reference
L. rhamnosus	↓ proliferation and activation of T cells	(Braat et al., 2004)
L. reuteri	↓ IL-12, IL-6, TNF-α, inhibits the expression of B7.2, induces regulatory T cell differentiation	(Christensen et al., 2002; Smits et al., 2005)
L. casei subsp. *Alactus*	↑ IL-12, IL-6, TNF-a	(Christensen et al., 2002)
VSL #3	↑ DC maturation, ↓ lymphocyte proliferation, ↓ IL-12, ↑ IL-10, ↓ Th1	(Drakes et al., 2004; Hart et al., 2004)
B. longum	↑ IL-10, IL-12	(Rigby et al., 2005)
L. casei	Induces regulatory T cell differentiation	(Smits et al., 2005)
L. gasseri, *L. johnsonii* and *L. reuteri*	↑IL-12 and IL-18, but not IL-10	(Mohamadzadeh et al., 2005)
L. casei	↑IL-12 via macrophages stimulation	(Shida et al., 2006)

Analysis of findings on the capacity of probiotic strain to alter Th1/Th2 balance

Reference	Organism	Effect
(Pochard et al., 2002)	*L. plantarum*	↑ Th1
(Pochard et al., 2002)	*L. lactis*	↑ Th1
(Pochard et al., 2002)	*L. casei*	↑ Th1
(Pochard et al., 2002)	*L. rhamnosus* GG	↑ Th1
(Shida et al., 2002)	*L. casei* Shirota	↑ Th1
(Shida et al., 2002)	*L. johnsonii*	NIL
(Pohjavuori et al., 2004)	*L. rhamnosus* GG	↑ Th1
(Sutas et al., 1996a)	*L. rhamnosus* GG	↑ Th1
(Sheil et al., 2004)	*L. salivarius*	↓ Th1
(McCarthy et al., 2003)	*B. infantis*	↓ Th1
(Pena et al., 2005)	*L. reuteri*	↓ Th1
(Sturm et al., 2005)	*E. coli* Nissle 1917	↓ Th1
(Kato et al., 1996)	*L. casei* Shirota	↓ Th1
(Perdigon et al., 2002)	*L. casei*	↑ Th1
(Perdigon et al., 2002)	*L. delbrueckii* subsp. *bulgaricus*	↑ Th1
(Perdigon et al., 2002)	*L. acidophilus*	↑ Th1, ↑ Th2
(Cross et al., 2002)	*L. rhamnosus* HNOOI	↑ Th1, ↑ Th2

Antibiotic associated diarrhea

Disease → Antibiotic treatment → Disturbance of intestinal microbiota → *Clostridium* overgrowth produces toxin → Diarrhea → Disease

Antibiotic associated diarrhea

Disease → Antibiotic treatment → Disturbance of intestinal microbiota → *Clostridium* overgrowth produces toxin → Microbiota in balance

Probiotics

Probiotics and Cancer

- Enzymes (Glycosidase, B- glucuronidase , azoreductase , and nitroreductase) of the intestinal flora convert the precarcinogens to active carcinogens

- Probiotics reduce:
  - Faecal concentrations of enzymes
  - Secondary bile salts
  - Reduce absorption of harmful mutagens that may contribute to colon carcinogenesis.

- Activity of L. *acidophilus and* L. *casei* supplementation in humans helped to decrease levels of these enzymes

- Several mechanisms have been proposed as to how lactic acid bacteria may inhibit colon cancer:
  - Enhancing the host's immune response
  - Altering the metabolic activity of the intestinal microflora
  - Binding and degrading carcinogens
  - Producing antimutagenic compounds
  - Altering the physiochemical conditions in the colon

(Harish and Varghese, 2006)

Hepatic Diseases

Mechanisms by which probiotics may treat Hepatic Encephalopathy:

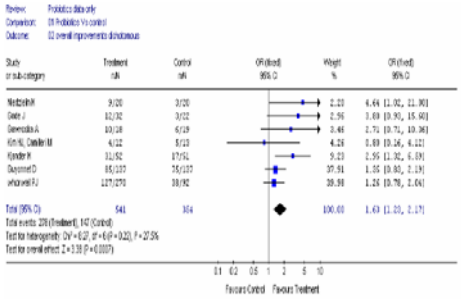
- Decreased portal blood ammonia by reduced bacterial urease activity
- Decreased pH due to less ammonia absorption
- Less intestinal permeability and improved gut epithelium
- Decreased inflammation and oxidative stress due to reduced ammonia toxins
- Reduced uptake of other toxins

Helicobacter pylori Infections

- It the most common chronic bacterial infection in humans and causes :
  - Chronic gastritis
  - Peptic ulcers
  - Gastric adenocarcinoma
  - Lymphoma and a number of non-gastrointestinal disorders.
- In vitro studies have suggested that lactic acid bacteria may inhibit or kill *H. pylori* by acting as a bactericide.
- Bifidobacteria and *B. subtilis* may inhibit the growth or attachment of *H. pylori.*
- Possible mechanisms by which *L. salivarius* eradicates *H. pylori* include the ability of the former to bind to gastric epithelial cells, to produce a high quantity of lactic acid, and to proliferate rapidly.

A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome

Hoveyda N et al. **BMC Gastroenterology** 2009, **9**:15



Forest plot of improvement in overall symptoms in patients with IBS treated with probiotics compared to placebo.

Use of probiotics beyond GIT

- Allergy: Probiotics have the potential:
  - To modify the structure of antigens
  - Reduce their immunogenicity
  - Reduce intestinal permeability
  - Generation of proinflammatory cytokines that are elevated in patients with a variety of allergic disorders

Probiotics in Pregnancy:

- **Bacterial vaginosis , increases the risk of preterm labour and infant mortality .**
- **Probiotics decrease the risk of bacterial vaginosis and maintain normal lactobacilli vaginal flora**
- **Studies using *L. rhamnosus* GG and *B. lactis* BB12 have shown that atopic dermatitis, a condition that causes severe skin rashes in up to 15% of babies, can be prevented in 50% of cases if mothers ingest probiotics during pregnancy and newborns ingest them during the first 6 months of life**

Probiotic strains currently used

Lactobacillus species

- ***L. acidophilus***
- ***L. plantarum***
- ***L. casei subspecies rhamnosus***
- ***L. brevis***
- ***L. delbreuckii subspecies bulgaricus***

Bifidobacterium species

- ***B. adolescentis***
- ***B. bifidum***
- ***B. longum***
- ***B. infantis***
- ***B. breve***

Others

- ***Streptococcus salivarius* ssp. *thermophilus***
- ***Lactococcus lactis* ssp. *lactis***
- ***Lactococcus lactis s* ssp. *cremoris***
- ***Enterococcus faecium***
- ***Leuconostoc mesenteroides* ssp. *dextranicum***
- ***Propionibacterium freudenreichii***
- ***Pediococcus acidilactici***
- ***Saccharomyces boulardii***

Strains of bacteria one should look for in a probiotic:

- **It is important to choose a high quality formula that contains the right strains of bacteria for optimum results .**
- **It is best to choose a probiotic that contains the entire family of lactobacillus bacteria.**
- **The family members are *: L. acidophilus, L. delbrueckii, L. casei, L. bulgaricus, L. caucasicus, L. fermenti, L. plantarum, L. brevis, L. helveticus, L. leichmannii, L. lactis, L. bifidus, and L.sporogenes.***

- **It is important that the complete family of 13 strains is present. Most people don't realize this family works in harmony only if the complete family is present.**
- **A good probiotic should have most of these strains:**
- ***Bifidobacterium bifidum* - Protects the body against invasive pathogens, salmonella and rotavirus. Helps suppress tumors and reduce inflammation. Helps protect against diarrhea and intestinal infections and strengthens immune system.**

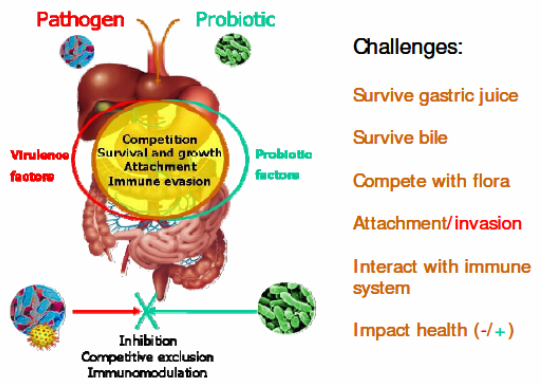
- ***Bifidobacterium breve* - Helps prevent rotavirus-induced diarrhea and activates your immune system. Also helps IBS.**
- ***Bifidobacteria infantis* - Inhibitory effect on some strains of E. coli. Protects against gastroenteritis (inflammation of the stomach and bowels) and is useful in the prevention and therapy of solid tumors (breast tumors).**
- ***Bifidobacteria lactis* - Enhances resistance to oral salmonella typhimurium. Enhances natural immune function. Helps alleviate constipation, prevents diarrhea and decreases chronic inflammation of the colon.**

- *Bifidobacterium longum* - **Able to eliminate harmful nitrates commonly found in foods. Inhibits the development and growth of colon, liver and breast cancers in laboratory animals. Helps prevent diarrhea caused by antibiotic use, helps constipation and reduces fecal odor.**
- *Lactococcus lactis* - **A natural antibiotic that reduces the ability of pathogenic microbes to grow and cause infection.**
- *Lactobacillus salivarius* - **Produces a high amount of lactic acid, thus completely inhibiting the growth of Helicobacter pylori (H. pylori) bacteria, which are now known to be the major cause of peptic ulcers. It is also effective against Salmonella typhimurium**

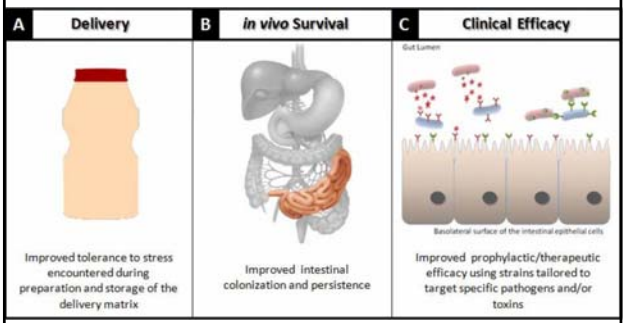
- *Lactobacillus casei-* **Provides protective activity against Listeria bacteria which have been found to cause the inflammation and infection of the brain and spinal cord (Spinal Meningitis). Inhibits activity in tumor cells and stimulates beneficial activity in normal cells.**
- *Lactobacillus reuteri* - **Controls Cryptosporidium parvum (C. Parvum), a parasite that survives the chlorination process, sometimes found in springs or water wells, infection causing nausea, diarrhea, fever and abdominal cramps. Beneficial in the treatment of diarrhea associated with rotavirus, E. coli and Salmonella.**
- *Lactobacillus sporogenes* – **Can reduce LDL (harmful) cholesterol levels, while increasing HDL (healthy).**
- *Streptococcus thermophilus* -**Its antioxidant activity scavenges the body for free radicals. Effective in maintaining vaginal and intestinal health.**

List of selected food products marketed in the USA containing probiotics and prebiotics

Product	Functional Additive (Quantity per Serving when indicated)	Health Claim
Infants and Children:		
GOOD START® NATURAL CULTURES™ Infant Formula	*B. lactis*	Increases levels of key antibodies, and promote natural protective barrier in the digestive tract
Early Advance™ Infant Formula	Galacto-oligosaccharides	Help stimulate growth of healthy bacteria, which support developing immune system
BiogaiaAB Reuteri Drops	*L. reuteri* Protectis DSM 17938 (100 million)	Reduces infantile colic
DanActive™ Dairy Drink	*L. casei* DN-114001 Immunitas™	Strengthens body's defenses
Danimals® Yogurt	*L. rhamnosus* GG™	Positive effects on gastrointestinal and immune function, and oral health
Stonyfield Farm® Yo-Baby Yogurts	*L. bulgaricus, S. thermophilus, L. acidophilus, bifidobacteria, L. casei,* and *L. rhamnosus*	Helps aid in digestion and supports immune system
BiogaiaAB Probiotic Straw and LifeTop Cup	*L. reuteri* DSM 17938 (100 million)	Maintain overall gut health
Adults and Elderly:		
Activia® Yogurt	*B. animalis* DN-173 010 Regularis™	Regulates the digestive system by helping reduce long intestinal transit time
BiogaiaAB Probiotic LifeTop Cup	*L. reuteri* DSM 17938	Maintain overall gut health
BiogaiaAB Prodontis Chewing Gum and Lozenges	*L. reuteri* DSM 17938 and *L. reuteri* Prodontis ATCC PTA 5289	Reduces gingivitis
DanActive™ Dairy Drink	*L. casei* DN-114001	Helps strengthen body's defenses
LiveActive™ Cheese	*B. lactis* and *L. rhamnosus*	Replenish live cultures in digestive system, promoting digestive health
LiveActive™ Cottage Cheese	Inulin (3 g)	Serves as food source for probiotic bacteria
Silk plus for Bone Health Soy Milk	Fructan (1 g)	Helps promotes bone health
LiveActive™ cereals	Inulin (3 g)	Helps promote digestive health
Stonyfield Farm® Yogurts and Smoothies	*L. bulgaricus, S. thermophilus, L. acidophilus, bifidobacteria, L. casei,* and *L. rhamnosus*	Helps aid in digestion and supports immune system
Yo-Plus™ Yogurt	*B. lactis* Bb-12™ and Inulin	Help maintain healthy balance of friendly bacteria in digestive system



Recent advances in the design of more effective probiotic cultures



Nontraditional safety-related considerations in the use of probiotics

Unpredictable behavior of naturally occurring microorganisms

Unpredictable behavior of genetically altered microorganisms

Unexpected interactions of bacteria within the specific local environment of the human host

Unexpected release of novel bacteria into the (external) environment

RR Sharp et al. **Helping Patients Make Informed Choices About Probiotics: A Need For Research.** *Am J Gastroenterol*. 2009 April ; 104(4): 809–813

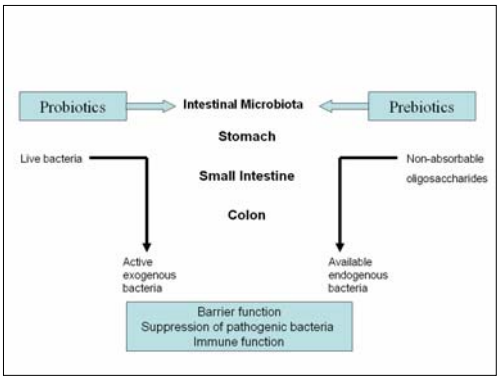
Prebiotics

- **The term prebiotic was introduced by Gibson and Roberfroid who exchanged "pro" for "pre," which means "before" or "for."**

  - **A non-digestible food ingredient**
  - **Beneficial effects**
  - **Selective stimulation**
  - **Improved host health**

- **Prebiotics acts as cofactors for probiotics.**
- **Complex carbohydrates pass through the small intestine to the lower gut where they become available for some colonic bacteria but are not utilized by the majority of the bacteria present in the colon.**
- **The main end products of carbohydrate metabolism are short-chained fatty acids, namely acetate, butyrate and propionate, which are further used by the host organism as an energy source.**

Concept of Probiotics and Prebiotics:



Harish and Varghese, 2006

Potential Candidate Prebiotic Substrates

Fructo-oligosaccharides*	Lactosucrose
Galacto-oligosaccharides*	Mannan oligosaccharides
Inulin*	Melibiose oligosaccharides
Lactulose*	N-acetylchito-oligosaccharides
Gentio-oligosaccharides	Oligodextrans
Germinated Barley foodstuff	Pectic oligosaccharides
Gluco-oligosaccharides	Polydextrose
Gluconic acid	Resistant starch
Hemicellulose rich substrate	Soybean Oligosaccharides
Isomalto-oligosaccharides	Sugar alcohols
Lactoferrin derived peptide	Xylo-oligosaccharides

*Proven prebiotics.

Proposed Benefits of Prebiotics to the Well-being and Health of the Animal Host

Microflora changes	Selective stimulation of beneficial members of gut flora
	Inhibition of epithelial adherence and invasion of microbial pathogens
	Blocking of epithelial sites for microbial pathogens
Intestinal barrier function	Improving intestinal permeability
	Augmentation of mucus production
SCFA	Increased production of short-chain fatty acids, as fermentation product
Immune system	Increasing anti-inflammatory cytokines
	Decreasing pro-inflammatory cytokines

Clinical Studies Testing Prebiotics or Synbiotics in the Treatment of Human IBD

Author	Patients	Treatment	Compared to	Duration	Outcome
Welters 2002 (n = 24)[86]	Pouchitis	Inulin	Placebo (cross-over)	3 wks	Reduction of inflammation
Furrie 2005 (n = 18)[88]	UC	Synbiotics	Placebo (RCT)	1 month	Reduction of inflammation
Lindsay 2006 (n = 10)[68]	CD	FOS	None (open label)	3 wks	Reduction of inflammation
Chermesh 2007 (n = 30)[89]	Postop. CD	Synbiotics	Placebo (RCT)	24 months	No prevention of relapse
Casellas 2007 (n = 19)[65]	UC	Synergy	Placebo (pilot RCT)	2 wks	Reduction of calprotectin leve

Characteristics of an ideal prebiotics

- **It should not be hydrolyzed or absorbed in the upper part of G.I. tract.**
- **It should be a selective substrate for one or a limited number of potentially bacterial commercial to the colon culture protagonist.**
- **It should be able to alter the colonic micro flora towards a healthier composition or selectively stimulates the growth and/or activity of intestinal bacteria associated with health and well being.**
- **It should help in increasing the absorption of certain minerals such as calcium and magnesium.**
- **It should or may have a favorable effect on the immune system and provide improved resistance against infection.**

Synbiotics

- **Synbiotic = Probiotic + Prebiotic**
  - **The concept of synbiotics has been proposed to characterize health-enhancing foods and supplements used as functional food ingredients in humans (Gibson, 2004).**
  - **Potential synergy between pro- & prebiotics**
  - **Improve survival in upper GIT**
  - **More efficient implantation**
  - **Stimulating effect of Probiotics**

Some of the major health benefits of synbiotics:

- **Improved survival of live bacteria in food products, prolonged shelf life,**
- **Increased number of ingested bacteria reaching the colon in a viable form**
- **Stimulation in the colon of the growth and implantation of both exogenous and endogenous bacteria**
- **Activation of metabolism of beneficial bacteria, antagonistic toward pathogenic bacteria**
- **Production of antimicrobial substances (bacteriocins , hydrogen peroxide, organic acids etc)**
- **Immunostimulation**
- **Anti-inflammatory, Anti-mutagenic, Anti-carcinogenic, and production of bioactive compounds (enzymes, vaccines, peptides etc)**

(Nagpal et al, 2007)

Products in the international market using synbiotic health foods concept.

Product	**Producer**
Actifit	Emmi, Switzerland
Probioplus	Migros, Switzerland
Symbalance	Tonilait, Switzerland
Proghurt	Ja naturlich naturprodukte, Austria
Fysiq	Mona, Netherlands
Vifit	Sudmilch / Stassano, Belgium, Germany, UK
Fyos	Nutricia, Belgium
"On Guard" (Liquid yoghurt)	LBL Foods, US
Impact	National Cancer Institute, USA
Orafti's synergy- 1	
Synbiotic supplement	

VSL#3®
The Living Shield

- *Streptococcus thermophilus*
- *Bifidobacterium breveBifidobacterium longum*
- *Bifidobacterium infantis*
- *Lactobacillus acidophilus*
- *Lactobacillus plantarum*
- *Lactobacillus paracasei*
- *Lactobacillus delbrueckii* subsp. *bulgaricus*