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Winterschool: Role of the GI tract for development of allergy and obesity

DHI & ØFN, Copenhagen
09-12-2008

Interplay between the gut flora and the immune system in the development of chronic inflammation

LIFE, University of Copenhagen
ActiFoods ApS
Aff. Professor, PhD
Peter Olesen

ActiFoods

Vækstforum Hovedstaden

ØRESUND FOOD NETWORK

Ministry of Science Technology and Innovation

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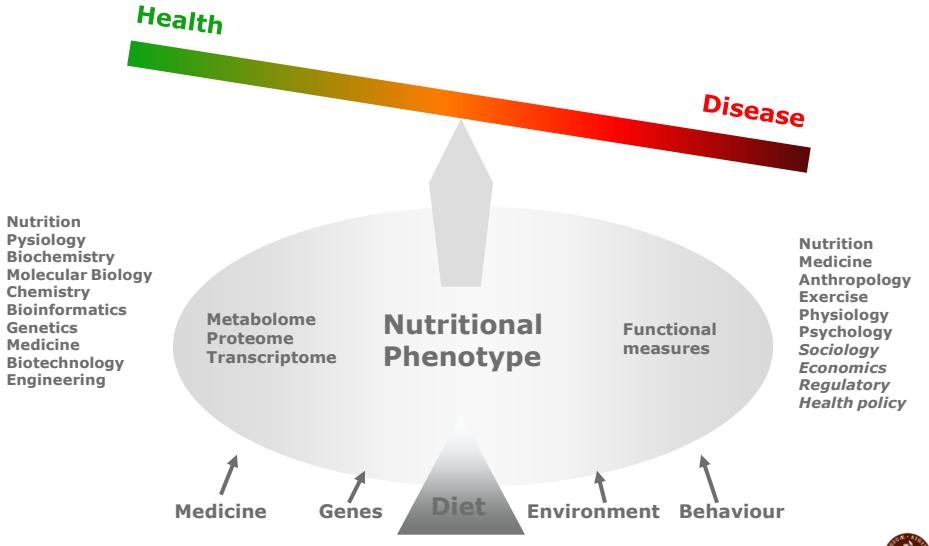
A complexity and multidisciplinary issue



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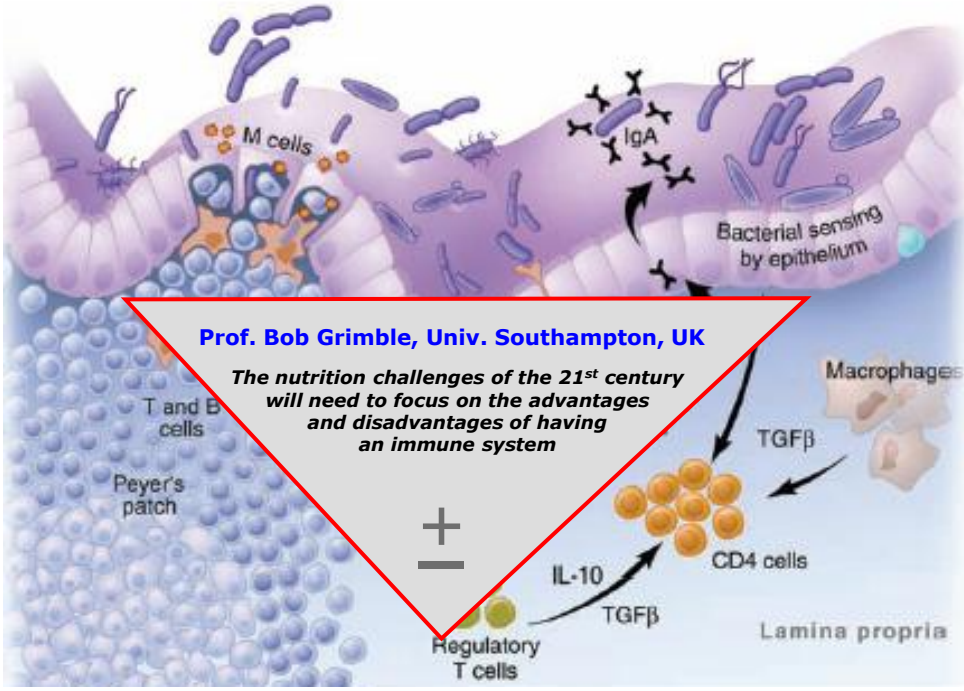
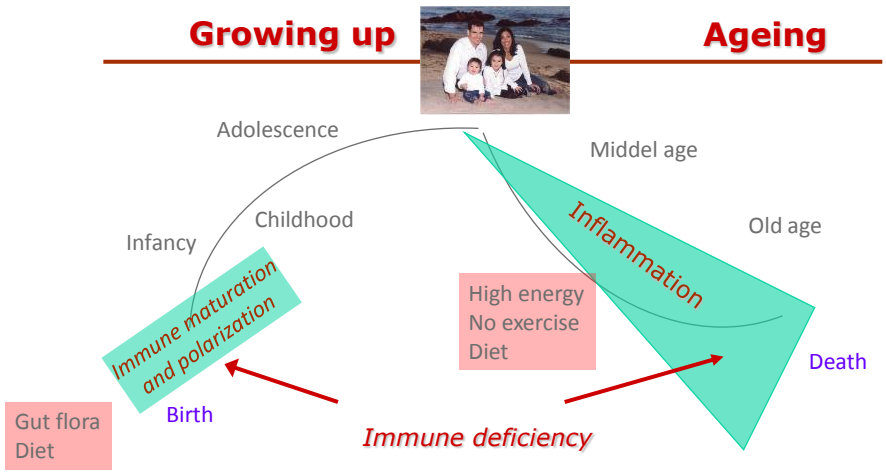
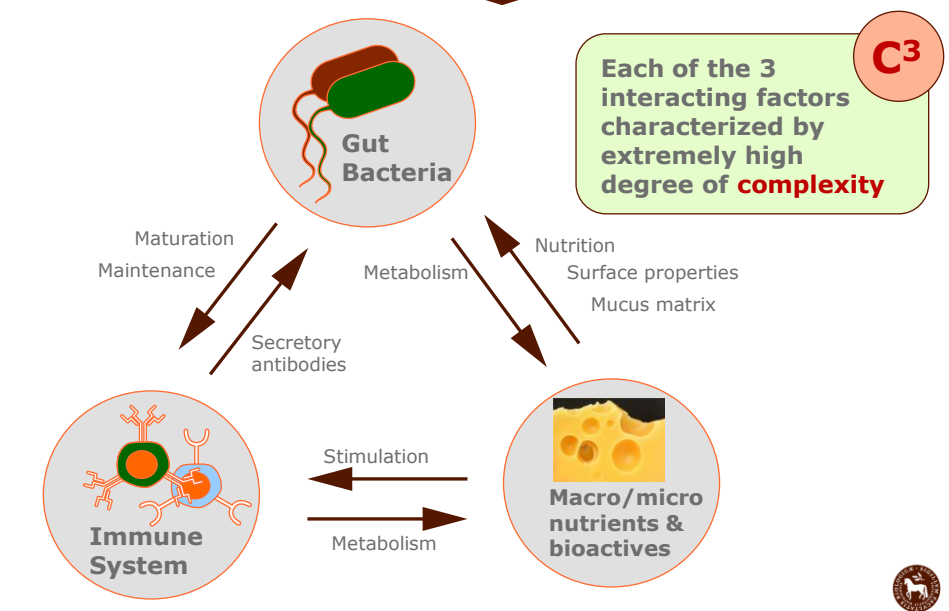
From: Steve Zeizel et al., 2005



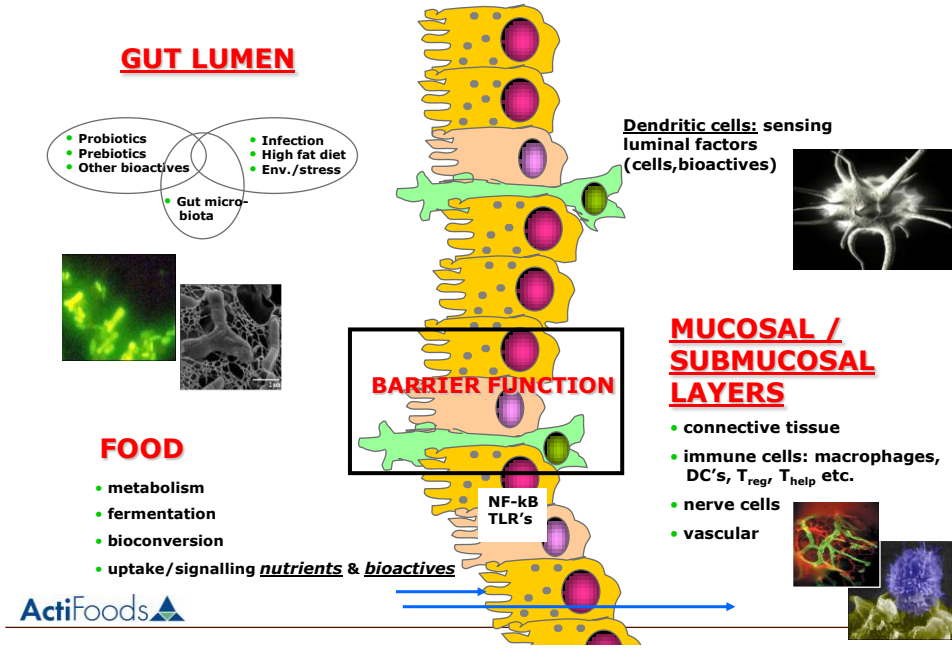
Immune maturation and inflammation





Courtesy of Dr. Tine R. Licht, Food-DTU

FOCUS: epithelial barrier & immune response



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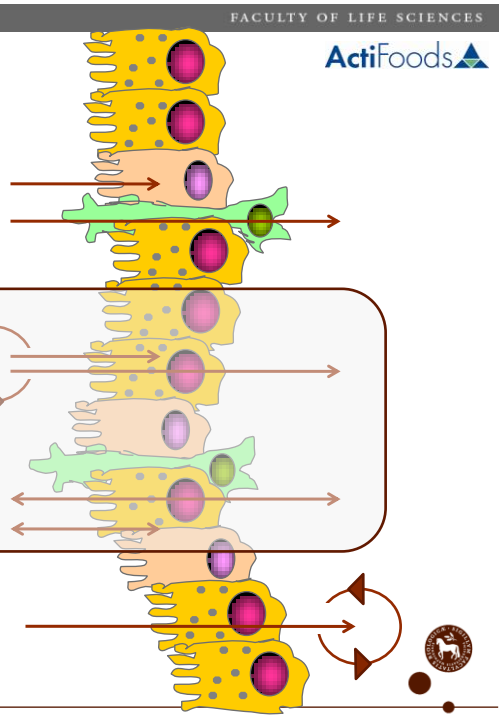
Modes of action for food bioactives

Direct action
- as ingested or bioconverted in gut
  ex: milk peptides
  ex: resveratrol
  ex: antioxidative food

Microbiota
- diversity and dynamics
  ex: chicken flora
  ex: probiotic effects
  ex: prebiotic / synbiotic effects

Complex communications
- bioactives/microbiota/host immune cells
  ex: microbial symbiosis factor
  ex: epithelial response factor

Drug interference
- bioactive improving antibiotic function
  ex: flavor compounds and TB therapy



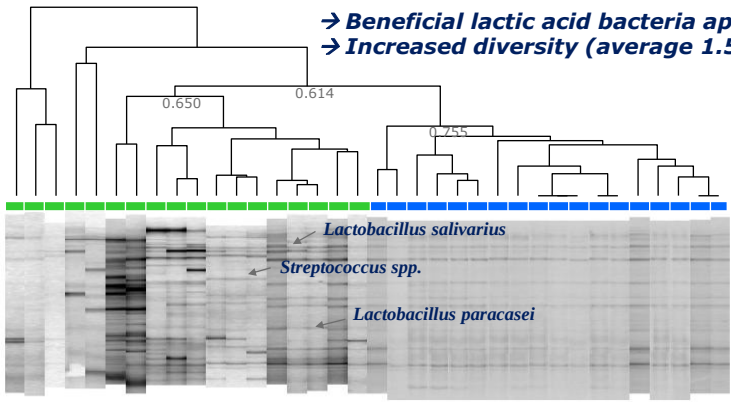
CHR. HANSEN

Feeding GalliPro (*Bacillus subtilis*) to broiler chicken alters the bacterial community in the ileum and enhances growth

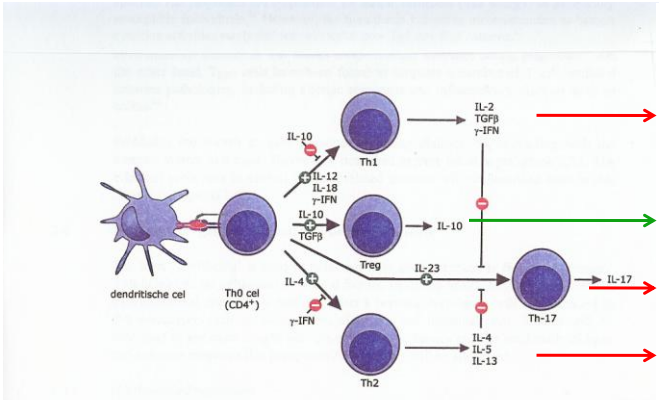
N Milora et al. 2008 J Prob Preb Res (in press):

Dice coefficient analysis of the ileal profiles from control birds and birds fed GalliPro resulted in a grouping associated with the dietary treatment

→ ***Beneficial lactic acid bacteria appeared***
→ ***Increased diversity (average 1.5 – 2X)***



PCR-DGGE



Involved in pathogenesis of autoimmune disorders, incl. gut inflammatory diseases.

Control of immune responses to self- and foreign antigens - i.e., balance immunity / tolerance.

Pro-inflammatory in e.g., induction of experimental colitis

Allergen-specific responses involved in atopic disorders, incl. many allergies.

Generic model of the immune system: differentiation of T-cells after activation by maturing dendritic cells (DC's)

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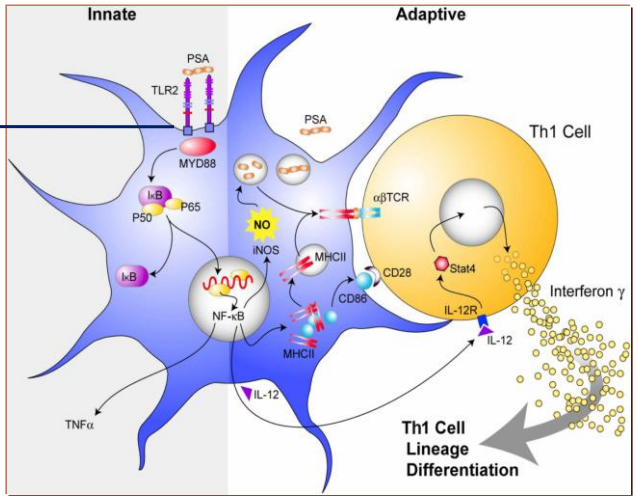
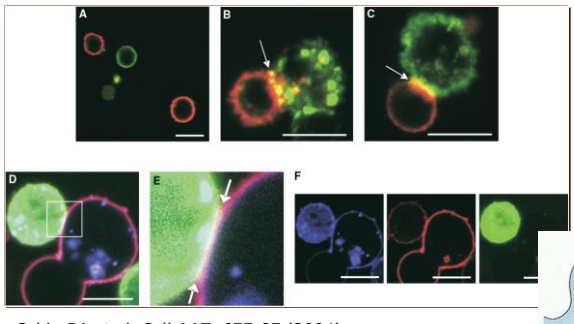
Toll-like receptors (TLR's) are involved in sensing the microbes present and initiation of immunity/tolerance responses.

TLR's recognize highly conserved structural motifs like LPS endotoxin, peptidoglycans, PSA on pathogens as well as commensals



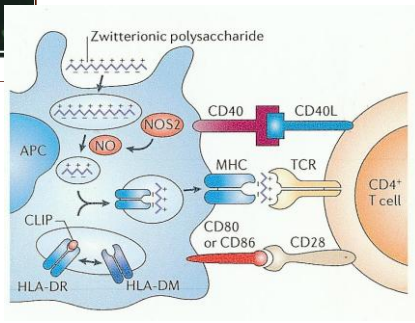
Wang, Q et al. (2003) J.Exp.Med. 203 (13) 2853-63.

The Immunological Synapse concept



Cobb, BA et al. Cell **117**, 677-87 (2004).

- APC's = red cells
- T-cells = green cells
- Synapses = yellow structures (antigen + MHCII)



Mazmanian SK & Kasper DL. Nature Rev. Immunol.**6**, 849-58 (2006).

Increase in Bifidobacteria may help prevent inflammation and development of metabolic syndrome

High fat diet	High fat diet + oligofructose
↑ Endotoxeamia (plasma LPS)	↓ Endotoxeamia (plasma LPS)
↓ Caecal counts - Gram positives - Bifidobacteria	↑ Caecal counts - Gram positives - Bifidobacteria

Both probiotic and prebiotic (synbiotic?) effects

Selective Increases of Bifidobacteria in Gut Microflora Improve High-Fat-Diet-Induced Diabetes in Mice Through a Mechanism Associated with Endotoxaemia
Cani PD et al. 2007, Diabetologia, in press

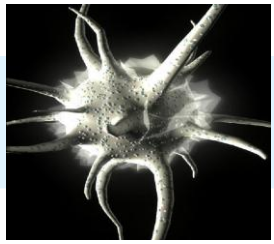
Modifying the gut microbiota in favour of Bifidobacteria may prevent deleterious effects of high-fat-diet-induced metabolic diseases...

Dendritic cells (DC's) are sensing and responding to gut microbes *in vitro*

Courtecy of Dr. Hans van Noort TNO, NL

Receptors

cytokine receptors
chemokine receptors
growth factor receptors
toll-like receptors, TLR's
DC-SIGN
DEC 205
…



Molecular signals to the immune system

cytokines
chemokines
growth factors
MHC molecules
Co-stimulatory and adhesion molecules
…

DC's are crucial messengers of immune-regulatory signals and central in defining strength and quality of immune responses

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Human DC's show differential *in vitro* responses to different probiotic bacteria

Courtecy of Dr. Hans van Noort TNO, NL

LPS
L. acidophilus NCFM
L. acidophilus La-5
B. lactis Bb-12
B. lactis BI-07

- activation of pattern-recognition receptors on DC's incl. TLR's
- powerful induction of DC maturation
- early cytokine & chemokine responses are often similar, indicating common activation of NF-κB
- other cytokine responses are quantitatively very different
- late DC responses diverge and indicate differential induction by bacteria of many growth and differentiation factors

The in vitro response of human DC's to probiotics involve secretion of several immune regulatory and/or health-promoting factors

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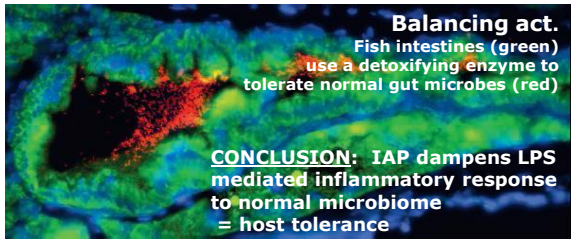
Microbial signals can elicit host responses involved in maitaining a healthy balance

A 'fishy' story about Intestinal Alkaline Phosphatase, IAP

Source:
Guillemen K et al. 2007: *Cell Host & Microbe* (Dec 13, 2007) & commentary *Science* 318: 1853 (Dec 21, 2007).



FINDINGS:

- No IAP activity and no neutrophils in germ free fish
- + IAP and neutrophils when microflora or LPS added
- IAP dephosphorylates LPS
- Chemically/genetically abolished IAP activity leads to boost in neutrophils and LPS-induced death

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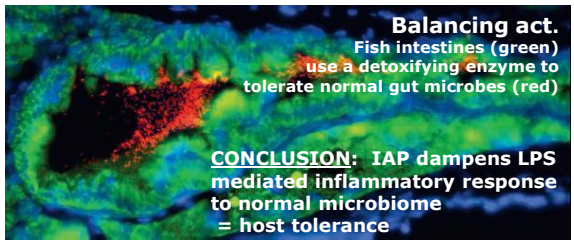

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QUESTIONS:

- **May pathogens 'fool' the IAP response by high LPS loads or interference with regulation of the IAP response?**
- **May 'unhealthy' microbiota (e.g. diet-induced obesigenic composition) disturb the IAP/LPS mechanism and lead to chronic endotoxaemia, inflammation and MS?**
- **May emphasise role of LPS in cross-talk between microflora , immune system and inflammation?**

ActiFoods

Microbial signals can elicit host responses involved in maitaining a healthy balance

Intestinal inflammation in mice can be tamed by bacterial sugars

Source:
Mazmanian K et al. 2008: *Nature* Vol 453/29 May 2008, 620-25.
A microbial symbiosis factor prevents intestinal inflammatory disease

Experimental colitis induced by *Helicobacter hepaticus* (a murine commensal) can be abolished by a single microbial molecule (polysaccharide A, PSA) produced by e.g. *Bacteroides fragilis* (a human commensal).

- PSA seems to suppress pro-inflammatory IL-17 production in intestinal immune cells a.o. *in vitro* cell cultures
- PSA protection is based upon a functional requirement for IL-10 producing CD4+ T-cells.
- Germ-free mice have defects in CD4+ T-cell development and human B fragilis can correct the deficiency through PSA expression

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WORKING HYPOTHESIS

FOOD

Based upon in vitro and animal models, it is anticipated that the microbiota (incl. probiotics a.o. 'intruders') are constantly monitored by the immune system via DC's a.o. antigen presenting cells of the immune system

HEALTH

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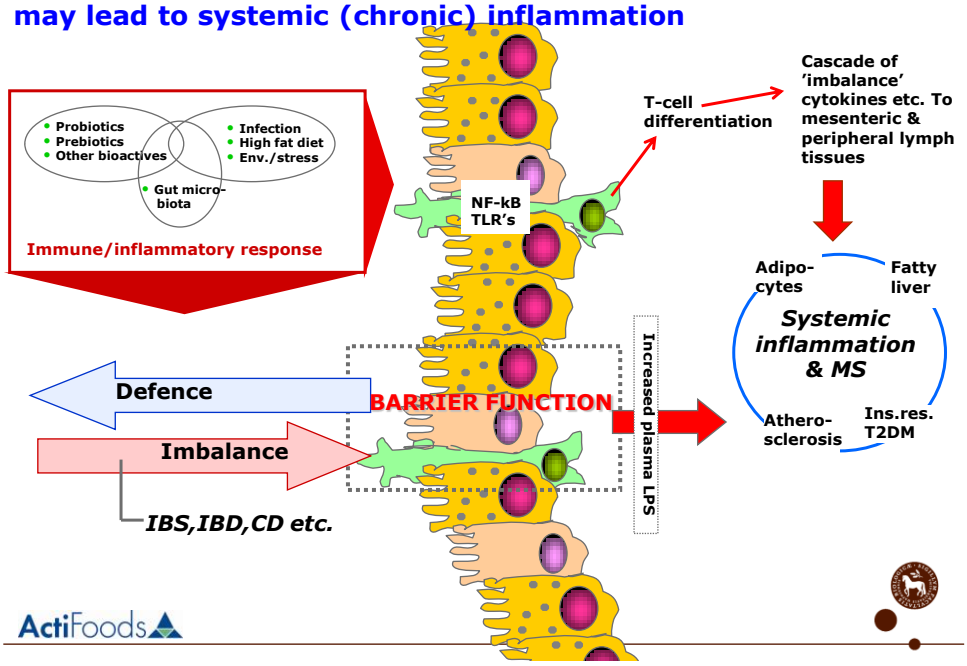
Imbalances in microbiota and immune responses may lead to systemic (chronic) inflammation



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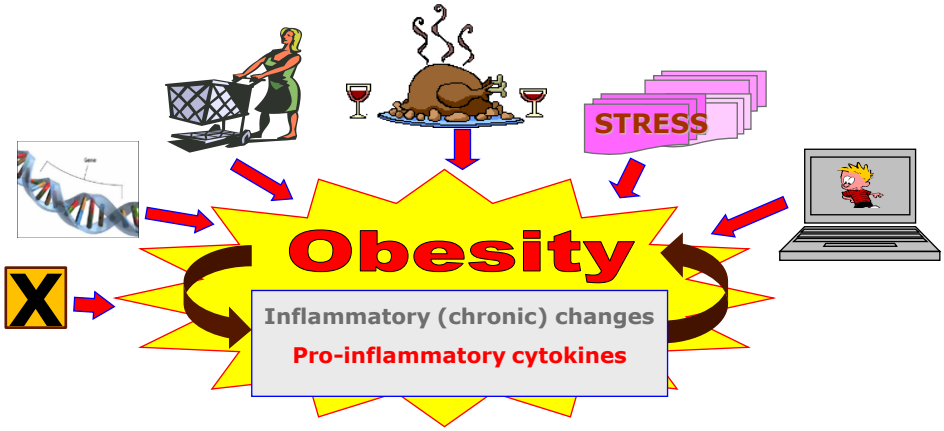
Lifestyle and metabolic diseases



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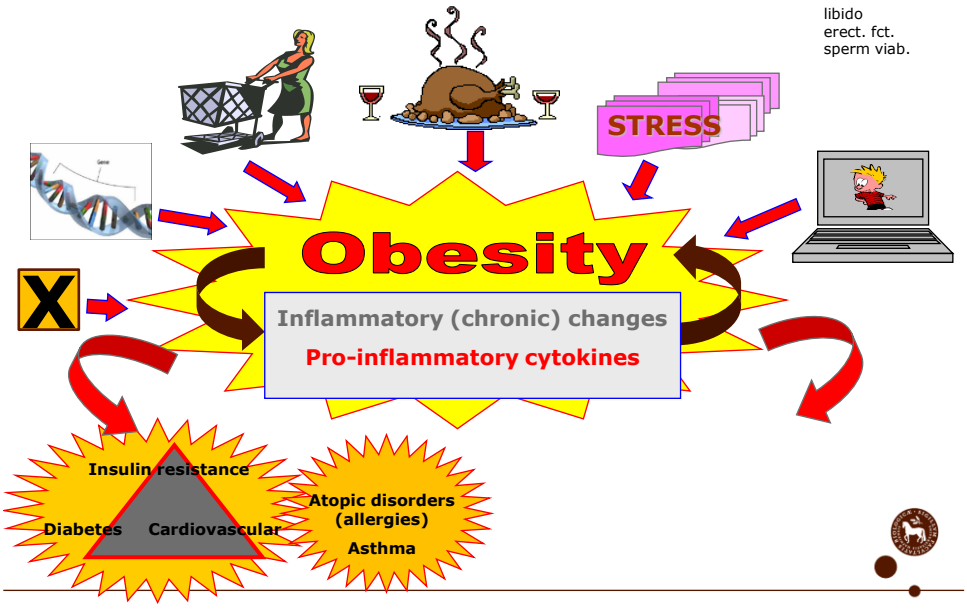
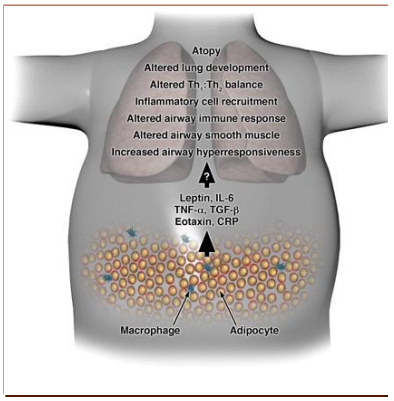
Lifestyle and metabolic diseases



Inflammation, IR, obesity and asthma

Conclusions (from Inter99 study)

- All considered obesity measures were associated with incident asthma in adults
- Insulin resistance is a risk factor for incident asthma symptoms in adults – the effect is independent of obesity
- Inflammatory pathways involved in insulin resistance may also contribute to the pathogenesis of asthma
- These inflammatory processes may be part of the underlying biological mechanism linking obesity to asthma



The Capital Region of Denmark

Allan Linneberg, MD, PhD
Lise-Lotte Husemoen, M.Sc., PhD
Lars-Georg Hersoug, M.Sc.
Betina H. Thuesen, M.Sc.

RESEARCH CENTRE FOR PREVENTION AND HEALTH

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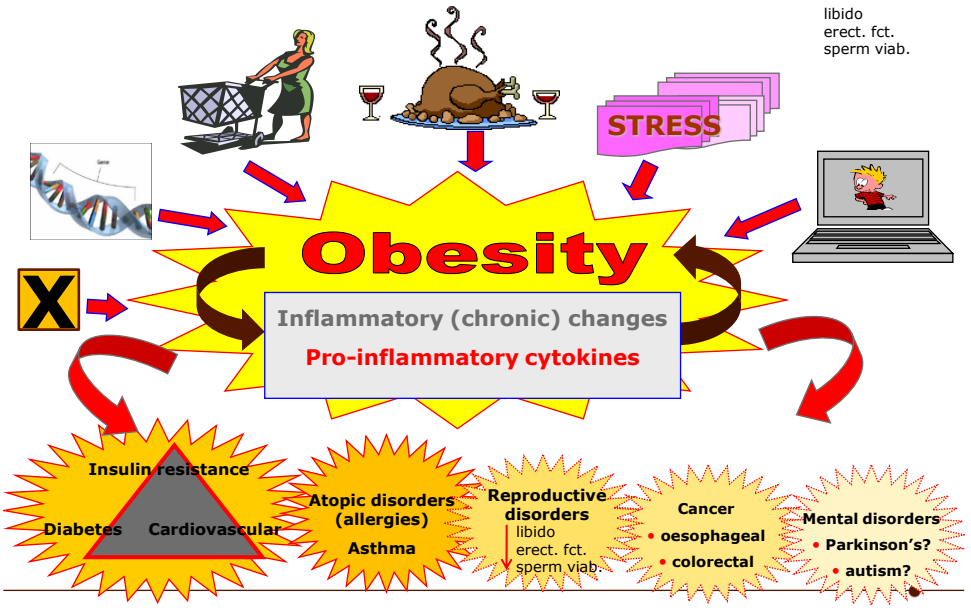
Lifestyle and metabolic diseases



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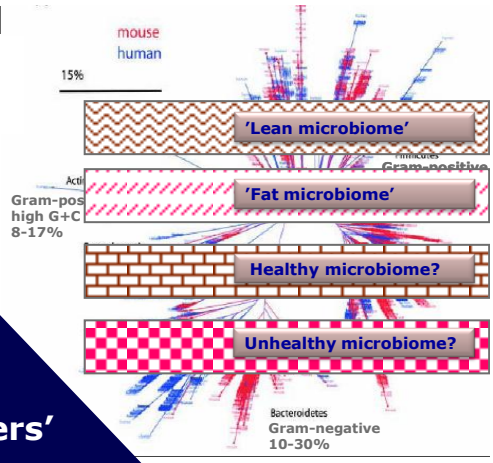
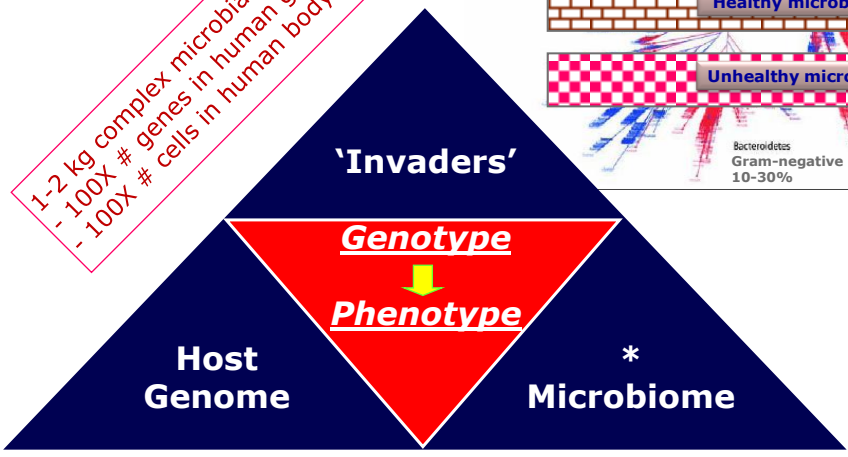
Genes, genomes and metagenomes…



1-2 kg complex microbial ecosystem
- 100X # genes in human genome
- 100X # cells in human body



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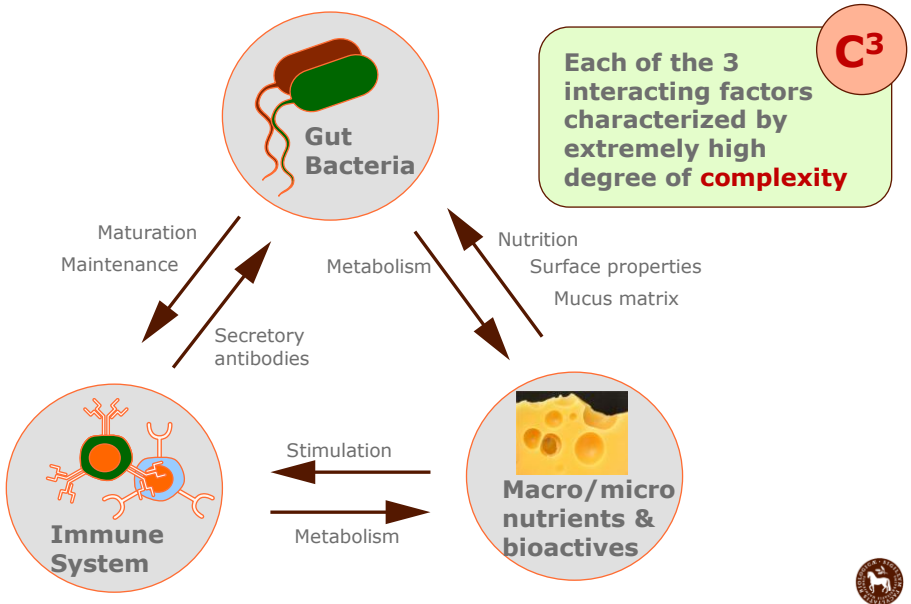

Courtesy of Dr. Tine R. Licht, Food-DTU

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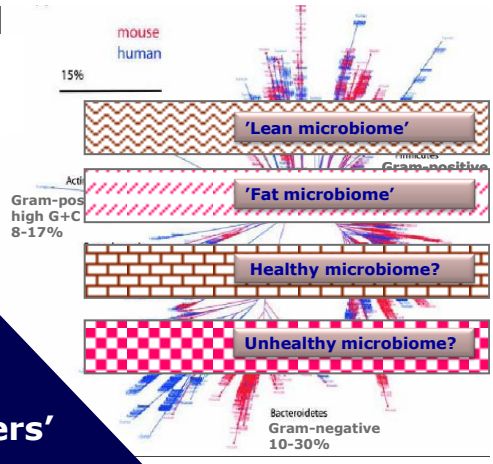
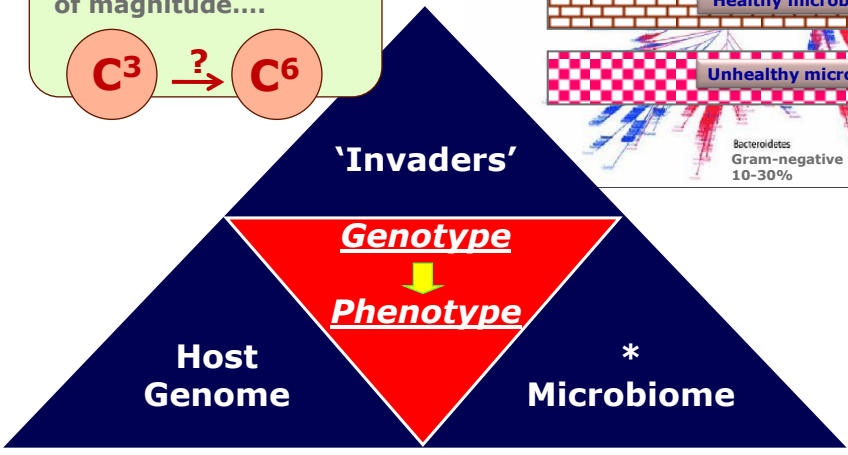
Genes, genomes and metagenomes...

Increases **complexity** with several orders of magnitude....

C^3 → ? → C^6





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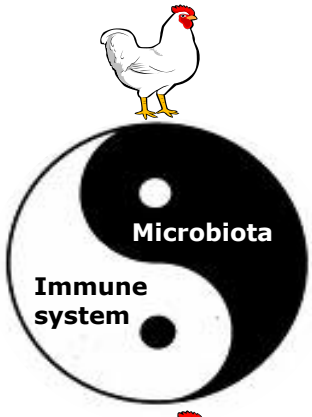

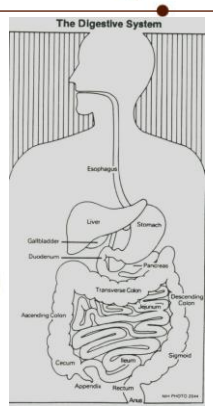

We only see bits and pieces (e.g., effects of added probiotics and single commensals) but the overall picture is still far away....

A classical biological conundrum in understanding metabolic homeostasis

Clinical efficacy a major shortcome

3-4 main categories of food bioactives known to have health-promoting (and disease prevention) functionalities, BUT.....

Probiotics & Prebiotics	***(Fermented) Milk Peptides***	***Resveratrol a.o. Plant Phenolics***
➢ GI stability	➢ Anti-hypertensive	➢ Anti-oxidant
➢ Inflammatory gut diseases	➢ Anti-arrhythmic	➢ Anti-inflammatory
➢ Anti-inflammatory	➢ Anti-cholesterolaemic	➢ Anti-diabetic
➢ Anti-infective	➢ Anti-atherosclerotic	➢ Anti-obesigenic
➢ Immune stimulating	➢ Anti-inflammatory	➢ Liver protecting
➢ Anti-allergic	➢ Satiating (anti-obesigenic)	➢ Energy (endurance)
➢ Satiating /anti-obesigenic)		➢ Anti-carcinogenic

COMMON FEATURES: small, additive, multiple/multifactorial effects
COMMON CHALLENGE: impressive laboratory and pre-clinical data – BUT human clinical trials often inconclusive

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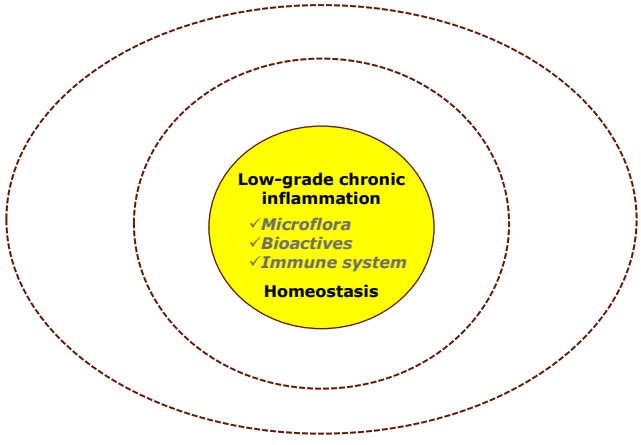
Documentation: cause – effect - efficacy

The biomarker challenge



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Documentation: cause – effect - efficacy

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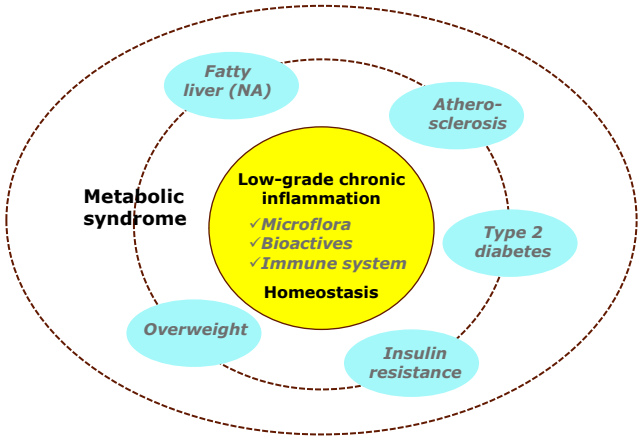

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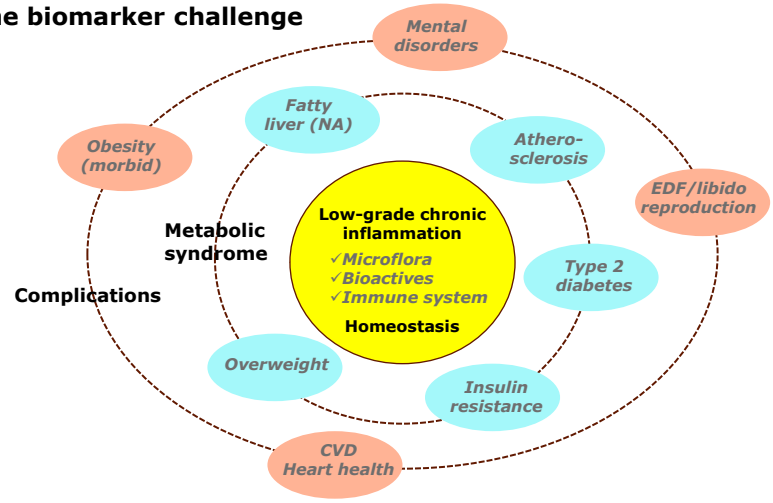
Documentation: cause – effect - efficacy

The biomarker challenge



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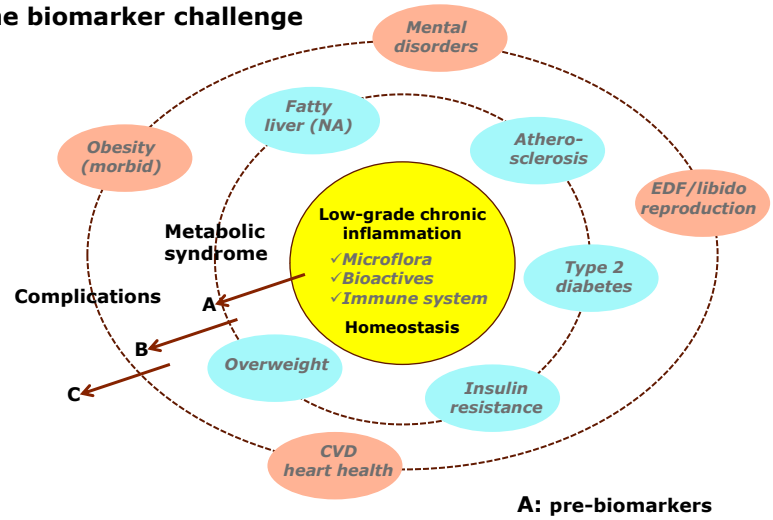
Documentation: "The Clinical Dogma"

The biomarker challenge



A: pre-biomarkers
B: surrogate endpoints (validated)
C: endpoints (clinical)

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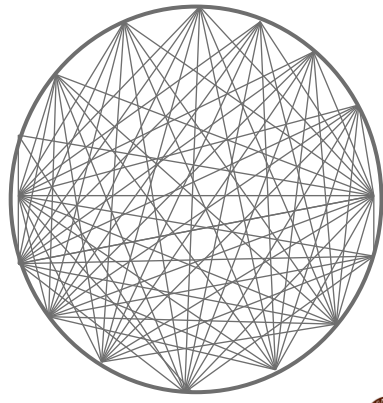
The omics ('holistic') approach

The genome

The transcriptome
- response to diet
- individuals
- metagenome(s)

The metabolome
- lipidome, proteome
- 'urinome'

Other 'omes'?
-
-



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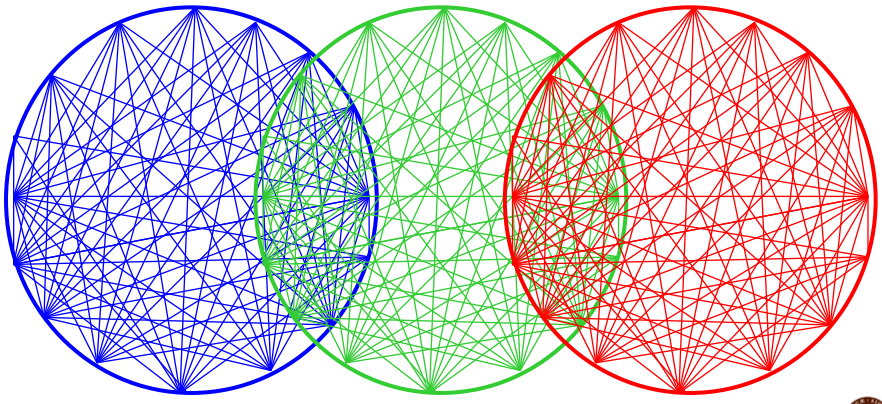
A (very) complicated pattern profiling



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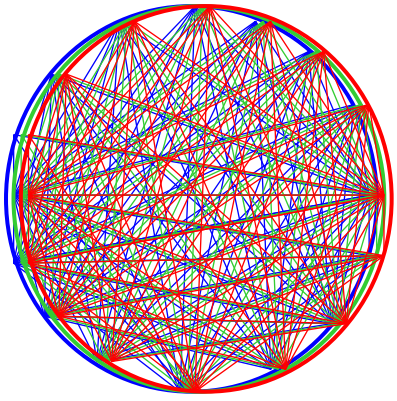


DOABLE?

Analytical chemistry

Bioinformatics

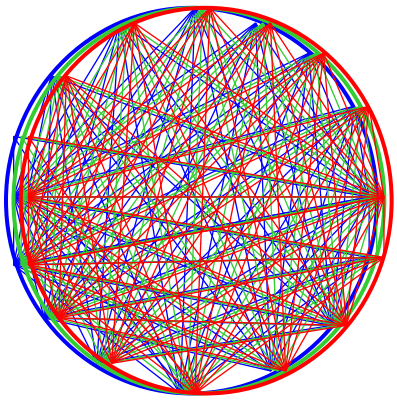
Chemometrics/PCA

New advanced technologies

-

-

A (very) complicated pattern profiling



DOABLE? - YES!

Analytical chemistry

Bioinformatics

Chemometrics/PCA

New advanced technologies

-

- Members of the microflora and the gut epithelial/immune cells do it all the time

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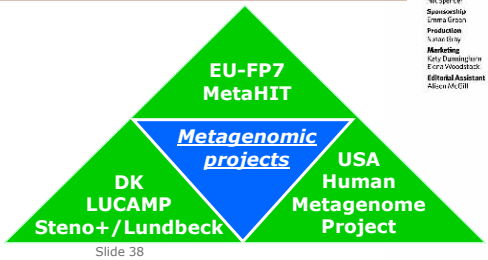
NEED: Metagenomic approaches to microbiota dynamics - the way to reduce complexity?

"Interactions between symbiotic or pathogenic microbes and the hosts they colonize are central to both health and disease.

This rapidly advancing field is now bearing the fruits of interdisciplinary efforts by microbiologists, immunologists, cell biologists, geneticists and ecologists"



HOST-MICROBE INTERACTIONS

nature insight

FEATURE

804 The Human Microbiome Project
P. J. Turnbaugh, R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight & J. I. Gordon

REVIEWS

811 Anecological and evolutionary perspective on human–microbe mutualism and disease
L. Dethlefsen, M. McFall-Ngai & D. A. Relman

819 Recognition of microorganisms and activation of the immune response
R. Medzhitov

827 Manipulation of host-cell pathways by bacterial pathogens
A. ?. Bhavsar, J. A. Guttman & B. B. Finlay

835 Bacterial pathogenomics
M. J. Pallen & B. W. Wren

nature insight

803

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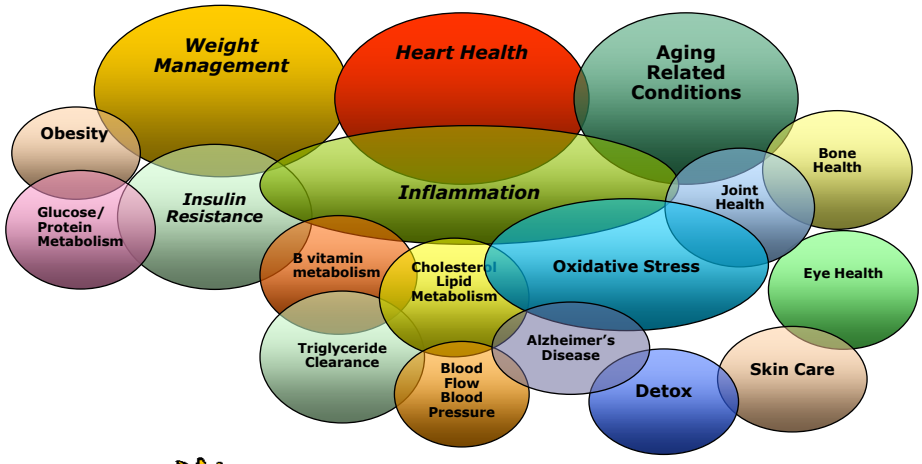
...and there is enough for everybody!

(Dr. Keith Grimaldi, Sciona)



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...and there is enough for everybody!

(Dr. Keith Grimaldi, Sciona)