METAFLAMMATION & INFLAMMAGING

International KBBE Forum
Health for all: understanding the ageing process
Villa Guastavillani, Bologna 1-3 October 2014
At present the pillars to combat aging are:

• NUTRITION
• PHYSICAL ACTIVITY
• HEALTHY LIFESTYLE including emotional stress and sleep
Nutritional stimuli

Epigenetic changes
Gut microbiota - Brain Immune system
INFLAMMATION

Long term effects on health and longevity
Nutritional Re-Programming of the aging process is possible/doable and should be pursued
Ottaviani E, Ventura N, Mandrioli M, Candela M, Franchini A, Franceschi C.

*Gut microbiota as a candidate for lifespan extension: an ecological/evolutionary perspective targeted on living organisms as metaorganisms.*

Biogerontology 2011 Dec;12(6):599-609
New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe

Coordinator: Professor Claudio Franceschi, University of Bologna
Start-End: May 2011- April 2016
All epidemiological data show that Mediterranean diet is beneficial for preventing age-related diseases...but we do not WHY? We do not know the mechanism(s)...

Coordinator: Professor Claudio Franceschi, University of Bologna
Start-End: May 2011- April 2016
NU-AGE and Inflammaging

BASIC HYPOTHESIS & RATIONALE

Appropriate WHOLE DIET (an *ad hoc* fortified “Mediterranean diet”) can decrease the level of the chronic, sub-clinical, low grade inflammatory process characteristic of old age we have proposed to call INFLAMMAGING (Franceschi et al., 2000)
NU-AGE

16 European countries
30 Partners

15 Research institutions
(nutritionists, bio-gerontologists, geriatricians, immunologists, expert in intestinal ecology and microbiology, bioinformaticians, statisticians, and mathematical modelers, among others)

9 SMEs (8 Food SMEs and 1 Biotech SME)

3 Large Food Industries

3 Stakeholders: “portatori di interesse”
NU-AGE PROJECT

INCLUSION CRITERIA
(healthy, free-living, independent subjects aged 65-79)

FRAILTY ASSESSMENT (Fried et al., 2001)

625 NON FRAIL SUBJECTS (NF)

625 PRE-FRAIL SUBJECTS (PF)

1250 RANDOMIZED SUBJECTS

625 WHOLE DIET

625 CONTROLS

60 NF SUBJECTS

60 PF SUBJECTS

OMICS: Epigenetic, Metabolomics, Metagenomics, Transcriptomics, Glycomics...

Genetics
Inflammation
Nutritional Status
Cognitive functions
Anthropometry
Physical functioning

NOT ADMITTED
RECRUITMENT OF VOLUNTEERS

Inclusion

Baseline session

Nutritional intervention during one year: control vs intervention group

M0

M4

M8

M12

Months

Follow-up questionnaire

Phone contact for both groups if necessary

- Clinical investigation, body composition
- Physical functioning
- Cognitive status
- Health status
- **Dietary and nutritional evaluation**
- Biological collections (blood, urine, faeces)
Biological samples

- **Blood** (Plasma-Serum-PBMC)
- **Urine**
- **Feces**

**genetic and non-genetic analysis IN ALL SUBJECTS**

- **ON DNA a GWAS (750,000 SNP, Illumina 750K)**
- **ON FECES a phylogenetic analysis of gut microbiome before and after one year diet**
NU-AGE “OMICS” before and after dietary intervention in a representative subgroup of subjects (half treated – half control)

In depth immunology (in plasma/serum)

- Transcriptomics (PBMC, immune function and inflammation)
- Oxido-lipidomics (in plasma)
- Metabolomics (in plasma and urine)
- Shot gun microbiome (in feces)
- Epigenetics (DNA from PBMC whole genome Illumina 450K BeadArray)
NU-AGE generates high dimensionality data (nutritional, clinical, OMIC data...) stored in an *ad hoc* database set up according to advanced guidelines, and analysed by the most advanced bioinformatic and statistical tools.
NU-AGE will built a mathematical model of inflammaging and Mediterranean diet capable of integrating all risk factors, processes, mechanisms (pathways), modulators (specific nutrients)
Could a Mediterranean diet, rich in olive oil, fish and fresh fruit, lead to a healthy microbiome in old age?

**Microbiome**

**Cultural differences**

*Studies of gut bacteria are beginning to untangle how diet affects health in old age — but determining cause and effect is tricky.*

*BY VIRGINIA HUGHES*
Franceschi, O’Toole and two dozen other academic and industry groups are now part of a €9 million project called NU-AGE, which includes 1,250 older individuals from France, Italy, Poland, the Netherlands and the United Kingdom. For one year, half will be given the Mediterranean diet, half will remain on their normal diet, and the NU-AGE researchers will measure how their health changes. O’Toole’s team will sequence the participants’ gut microbiota before and after the dietary intervention, while other researchers will look at genetic, epigenetic and metabolic signatures in their blood. Each of these biological levels might give insight on how the diet changes the microbiome.

NU-AGE is exactly the kind of large, longitudinal study that scientists the world over are clamouring for.
Special Issue

Mediterranean Diet and Inflammaging in the elderly: The European project NU-AGE

Guest Editors:
Aurelia Santoro, Patrizia Brigidi, Stathis Gonos, Vilhelm A. Bohr, and Claudio Franceschi
A collection of 15 papers dedicated to the main topics envisaged by the NU-AGE project

<table>
<thead>
<tr>
<th>N°</th>
<th>Article</th>
<th>Corresponding Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Combating inflammaging through a Mediterranean whole diet approach: the NU-AGE project's conceptual framework and design</td>
<td>Aurelia Santoro-UNIBO</td>
</tr>
<tr>
<td>2</td>
<td>A parallel randomized trial on the effect of a healthful diet on inflammaging and its consequences in European elderly people: design of the NU-AGE dietary intervention study</td>
<td>Agneta Maria Berendsen-WU</td>
</tr>
<tr>
<td>3</td>
<td>Iron status in the elderly</td>
<td>Susan Fairweather-Tait-UEA</td>
</tr>
<tr>
<td>4</td>
<td>Micronutrient-gene interactions related to inflammatory/immune response and antioxidant activity in ageing and inflammation. A systematic review.</td>
<td>Eugenio Mocchegiani-invited</td>
</tr>
<tr>
<td>5</td>
<td>Water-loss dehydration and aging</td>
<td>Lee Hooper-UEA</td>
</tr>
<tr>
<td>6</td>
<td>Cognitive Decline, Dietary Factors and Gut-Brain Interactions</td>
<td>Barbara Caracciolo-KIARC</td>
</tr>
<tr>
<td>7</td>
<td>Maintenance of a healthy trajectory of the intestinal microbiome during aging: a dietary approach</td>
<td>Marco Candela-UNIBO</td>
</tr>
<tr>
<td>8</td>
<td>Nutrition and protein energy homeostasis in elderly</td>
<td>Noel Jose Cano-INRA</td>
</tr>
<tr>
<td>9</td>
<td>Effect of resistance-type exercise training with or without protein supplementation on cognitive functioning in frail and pre-frail elderly</td>
<td>Ondine van de Rest-WU</td>
</tr>
<tr>
<td>10</td>
<td>Musculoskeletal system in the old age and the demand for healthy ageing biomarkers</td>
<td>Sebastiano Collino-NESTEC</td>
</tr>
<tr>
<td>11</td>
<td>Present and future of anti-ageing epigenetic diets</td>
<td>Paolo Garagnani-UNIBO</td>
</tr>
<tr>
<td>12</td>
<td>Nutrition, diet and immunosenescence</td>
<td>Simon Carding-IFR</td>
</tr>
<tr>
<td>13</td>
<td>Adipose tissue, diet and aging</td>
<td>Mauro Zamboni-invited</td>
</tr>
<tr>
<td>14</td>
<td>The role of low-grade inflammation and metabolic flexibility in aging and nutritional modulation thereof: a systems biology approach</td>
<td>Jildau Bouwman-TNO</td>
</tr>
<tr>
<td>15</td>
<td>Healthy aging diets other than the Mediterranean: A Focus on the Okinawan Diet</td>
<td>Bradley Willcox-invited</td>
</tr>
</tbody>
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The European project NU-AGE

Why the primary target of NU-AGE is to slow down inflammaging?
Appropriate DIET for the elderly

Gut microbiota  Imm Syst Metabolome Epigenome

+ inflammaging
- age-related diseases
The Inflammatory Theory of Aging (an example of adaptation/remodelling)

Inflamm-aging

An Evolutionary Perspective on Immunosenescence

CLAUDIO FRANCESCHI, a,b,e MASSIMILIANO BONAFÈ, a SILVANA VALENSIN, a
FABIOLA OLIVIERI, b MARIA DE LUCA, d ENZO OTTAVIANI, c AND
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“chronic”, “low grade”, “sterile”

Ann. N.Y. Acad. Sci., 908, 244-254, 2000
Inflammmaging and anti-inflammmaging: A systemic perspective on aging and longevity emerged from studies in humans

Claudio Franceschi, Miriam Capri, Daniela Monti, Sergio Giunta, Fabiola Olivieri, Federica Sevini, Maria Panagiota Panourgia, Laura Invidia, Laura Celani, Maria Scurti, Elisa Cevenini, Gastone C. Castellani, Stefano Salvioli

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High levels of circulating anti-inflammatory molecules in centenarians: TGFbeta, Cortisol, IL-1RA, Adiponectin
pro-inflammatory agents

- coagulation factors
- PG, LT
- HSP70
- TNF-α
- CRP
- IL-6

anti-inflammatory agents

- cortisol
- TGF-β
- IL-10
- LPX

gut microbiota
circulating mtDNA & inflamma-miR
N-glycans

LONGEVITY and the balance between Inflamm-aging & anti-Inflamm-aging

protective genes
some eicosanoids

high resistance to infectious diseases
susceptibility to infectious diseases

early life survival
inflammaging
inflammatory diseases
unsuccessful aging

anti-inflammaging
longevity
late life survival

Franceschi et al., MAD 2007
The Inflammmaging Theory of Aging

Metabolic syndrome
Type 2 Diabetes

Cancer
Alzheimer
PD
COPD
Depression

Cardiovascular diseases
Sarcopenia
Frailty
OA
Recently, the Summit, *Advances in Geroscience: Impact on Healthspan and Chronic Disease* was held on the NIH campus in Bethesda in late 2013.
AGING IS THE MAJOR RISK FACTOR for the most common chronic age-related diseases.

Aging and major age-related diseases share the same basic molecular and cellular mechanisms.
The New Geroscience

After a keynote address by the NIH Director, Dr. Francis Collins, seven sessions were focused on the most likely mechanisms driving aging and enabling disease onset.
This integrated perspective indicates that

the postponement/prevention of chronic diseases

appears to be DOABLE

by targeting aging in order to

TO COMBAT AGE-RELATED DISEASES AS A WHOLE AND NOT ONE BY ONE
Introduction

Claudio Franceschi, M.D.
Professor of Immunology
Alma Mater Studiorum, Università di Bologna, Italy

Franceschi C. & Campisi J.
Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases.
Advances in Geroscience: Impact on Healthspan and Chronic Disease Perspective

Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases

Claudio Franceschi¹,² and Judith Campisi³,⁴

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²IRCCS Institute of Neurological Sciences, and CNR-ISOF, Bologna, Italy.
³Buck Institute for Research on Aging, Novato, California.
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“GARBAGING” and INFLAMMAGING

- INFLAMMAGING is triggered by DAMAGE/GARbage, i.e. by a variety of "danger signals“ which can be exogenous or PAMPs (viruses such as CMV or HIV, bacteria including the gut microbiota and its products) or endogenous/self or DAMPs (damaged and SENESCENT CELLS, dysfunctional organelles such as mitochondria, cell debris, altered/modified proteins, lipids and N-glycans, mtDNA, ROS, ATP, uric acid, AGE, ceramides, cardiolipin, HMGB1);

- Thus, “garbage” and garbage disposal plays a major role in triggering inflammation and inflammaging

Franceschi & Campisi, 2014
This perspective assumes that there is a CONTINUUM between young and old bodies (as well as between physiology and pathology). Inflammaging would represent the progressive impairment with age of such a physiological garbage disposal.

Franceschi & Campisi, 2014
Inflammaging would be fostered by:

- Increased exposure to exogenous PAMPs and "danger" signals (e.g., gut microbiota, CMV)
- Increased generation of endogenous/self DAMPs and "danger" signals (increased number of senescent cells and cell debris produced by dying cells, dysfunctional mitochondria and altered/displaced molecules, i.e., aggregated/dysplaced proteins, lipids, mtDNA, N-glycans, ROS, ATP, uric acid, AGE, ceramides, cardiolipin, HMGB1...)
- Decreased garbage disposal, including the decreased efficiency of UPS/proteasomes, auto- and mito-phagy
- Increased activation of NF-kB and inflammasomes

Franceschi & Campisi, 2014
Basic molecular and cellular mechanisms fuelling inflammaging

1. **Cell senescence** and its pro-inflammatory senescent associated secretory phenotype (SASP)

2. **DNA damage**, including telomere shortening, by ROS and by a variety of other agents, which in turn triggers a **DNA Damage Response** and the production of pro-inflammatory compounds

3. **Mitochondrial dysfunction**

4. **Activation of inflammasomes and NFkB**
ROS and INFLAMMAGING

- ↑ ROS
- TRX, Ref1, Nrf2 Antioxidant systems
- ↑ NF-κB, AP-1
- Inflammasomes
- ↑ IL-6, TNF-α, chemokines
- IL-1β, IL-18
- ↑ Inflammation
- ↑ Mitochondrial dysfunction
- ↑ DNA damage response γ-H2AX
- ↑ Telomere uncapping
- ↑ DNA damage
- ↑ Cell senescence

Vitale, Salvioli and Franceschi, Nat Rev Endocrinol, 2013
“Danger Signals”

Microbes – Infection
Self-Garbage
Gut microbiota and its products
  Urate Crystals
  Amyloid
  ROS/mtDNA
  Extracellular ATP
  Cytosolic DNA
  Ceramides, Free Cholesterol
  Lipid Peroxidation
  Glycation end products

“INFLAMMAGING”

Immune Sensors

Pattern recognition receptors
  - TLRs
  - RLRs
  -- NLRs (Inflammasomes)

Antigen Receptors
  TCR
  BCR

Mediators

Effectors

TNFα
IL-6
IL-17
IFN
IL-18
IL-1β

Courtesy of V. Dixit
Il'ja Il'ič Mečnikov, 1845-1916
Nobel Prize winner for Immunology in 1908
and his concept of “physiological inflammation”
Inflammaging would recapitulate and represent the **point of convergence of other types of "sterile" inflammations** that have been conceptualized by other authors in different contexts, such as "**para-flammation**" (Medzhitov 2008) and "**metaflammation**" (Gregor and Hotamisligil 2011).
The concept of "METAFLAMMATION" (metabolic inflammation) has been proposed, to point out the systemic and pervasive state of inflammation observed in response to excess of nutrients and energy.

- **Nutrient excess** can engage the classical pathogen sensing or immune-response pathways, such as TLRs present in most cells of the body and recognized as antigens & inflammatory stimuli.

- **High levels of free fatty acids and glucose** induce a stress in the pancreatic islets and in adipose tissue, liver and muscle inducing the local release of cytokines, chemokines and adipokines.
Aging

Metabolic Danger signals (DAMP)
Ceramides, free cholesterol, uric acid, lipid peroxidation

Mitochondrial Dysfunction
PAMPs: Infections, gut microbiota

META-FLAMMAGING

Sarcopenia Diabetes Dementia/AD Immune Dysfunction Cancer CVD Bone Loss

Functional Decline & Reduced Healthspan

Courtesy of V. Dixit
Inflammation and Diabetes

Obesity and diabetes affect more than half a billion individuals worldwide. Interestingly, the two conditions do not always coincide and the molecular determinants of "healthy" versus "unhealthy" obesity remain ill-defined. Chronic metabolic inflammation (metaflammation) is believed to be pivotal.
Inflammation and Diabetes

- Inflammation of (omental) adipose tissue might play a role in the etiology of insulin resistance independent of body weight.
- This suggests that targeting inflammatory pathways might prevent the development of insulin resistance and diabetes.
- A recent study shows that patients using anti-inflammatory drugs against rheumatoid arthritis or psoriasis had a significantly lower risk of development of diabetes.
A PROPAGATING VIEW OF THE AGING PROCESS
“inflammaging is a fire”

There is evidence that:
• The aging phenotype is maintained by cell autonomous mechanisms (the aged micro/macros environment).
• Inflammaging can propagate from cells to cells and from organs to organs.
• Thus, local and systemic inflammation trigger, sustain and reinforce each other, in a (vicious) circle where it is difficult to identify priorities (A ->B->C)
• Within such a complex circuitry the classical “chicken-egg” problem could be a “wrong” question (the need of a new concept of “CAUSALITY” adequate for complex systems)
• In the cancer field the propagation to bystander cells of DNA damage, DNA damage response and inflammation, has been conceptualized as "para-flammation" (Martin et al., 2011);
The model of heterochronic parabiosis
The ageing systemic milieu negatively regulates neurogenesis and cognitive function

Saul A. Villeda1,2, Jian Luo1, Kira I. Mosher1,2, Bende Zou3, Markus Britschgi1, Gregor Bieri1,4, Trisha M. Stan1,5, Nina Fainberg1, Zhaoqing Ding1,5, Alexander Eggel1, Kurt M. Lucin1, Eva Czirr1, Jeong–Soo Park1, Sebastien Couillard–Després6, Ludwig Aigner6, Ge Li7, Elaine R. Peskind7,8, Jeffrey A. Kaye9, Joseph F. Quinn9, Douglas R. Galasko10, Xinmin S. Xie3, Thomas A. Rando1,11,12 & Tony Wyss–Coray1,2,5,11

Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice

Saul A Villeda1–6, Kristopher E Plambeck1,2,10, Jinte Middeldorp6,10, Joseph M Castellano6,10, Kira I Mosher6,7,10, Jian Luo6, Lucas K Smith1,2, Gregor Bieri1,2,6,7, Karin Lin1–3, Daniela Berdnik6, Rafael Wabl6, Joe Udeochu1,2,4, Elizabeth G Wheatley1,2,5, Bende Zou8, Danielle A Simmons6, Xinmin S Xie8, Frank M Longo6 & Tony Wyss–Coray6,7,9
Growth differentiation factor 11 (GDF11) is a member of the TGFbeta family and decreases with age in the blood. C-C chemokine 11 (CCL11) blood levels increase with age.

Bitto & Kaeberlein, 2014
Proliferation of human satellite cells (muscle stem cells) from old donors (mean age: 83 yrs) is significantly increased in serum from young donors (mean age: 30 years)

Barberi et al, 2013
The impaired differentiation (expression of MyHC) of satellite cells from old donors is partially rescued in serum from young donors

Barberi et al, 2013

(MyHC) (nuclei stained with Hoechst)
A PROPAGATING VIEW OF AGING & INFLAMMAGING

- Candidate stimuli present in the blood (circulating) which could maintain and propagate aging and inflammaging:
  - inflamma-miR
  - lipid metabolites
  - agalattosylated N-glycans
  - circulating mtDNA
  - gut microbiota products and metabolites
The complex, systemic nature of INFLAMMAGING

Cevenini et al., Curr Opin Clin Nutr Metab Care 2012
Beneficial functions of a healthy gut microbiota

Studies with Patrizia Brigidi’s team
Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians

Elena Biagi¹*, Lotta Nylund²,³, Marco Candela¹, Rita Ostan⁴, Laura Bucci⁴, Elisa Pini⁴, Janne Nikkila³, Daniela Monti⁵, Reetta Satokari², Claudio Franceschi⁴, Patrizia Brigidî¹, Willem De Vos³,⁶

¹ Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy, ² Functional Foods Forum, University of Turku, Turku, Finland, ³ Division of Microbiology and Epidemiology, Department of Basic Veterinary Medicine, University of Helsinki, Helsinki, Finland, ⁴ Department of Experimental Pathology and CIG-Interdepartmental Center L. Galvani, University of Bologna, Bologna, Italy, ⁵ Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy, ⁶ Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands
**MAJOR PHYLOGROUPS WHICH CHANGE WITH AGE**

- **Verrucomicrobia**
- **Uncultured Clostridiales**
- **Proteobacteria**
- **Clostridium cluster XV**
- **Clostridium cluster XIVa**
- **Clostridium cluster XI**
- **Clostridium cluster IX**
- **Clostridium cluster IV**
- **Clostridium cluster III**
- **Bacteroidetes**
- **Bacilli**
- **Actinobacteria**

* qPCR analysis: rearrangement in the composition of this cluster
The fecal microbiota of Centenarians

Decreased biodiversity
↓ *Clostridium* cluster XIVa*
↓ Bifidobacteria
Rearrangement of *Clostridium* cluster IV*
↑ Facultative anaerobes, including Bacilli and Proteobacteria (“pathobionts”)

* butyrate producer
Changes in the microbiota composition may be caused by and/or contribute to the age-related inflammatory status.
By Illumina shotgun sequencing of the fecal microbial DNA from the centenarians, elderly and young people, we generated a total of 214.6 million paired-end reads, with an average of 23.841 million (± 0.067 SD) reads per subject.
The genera *Escherichia* and *Ruminococcus* were over-represented in centenarians, whereas *Faecalibacterium*, *Eubacterium* and *Bifidobacterium* were more abundant in elderly.

Rampelli *et al.*, AGING 2013
Metagenome function analysis

![Color Key]

**Function_Spearman_Ward**

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<td>L</td>
<td>Replication, recombination and repair</td>
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<td>Posttranslational modification, protein turnover, chaperones</td>
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<td>D</td>
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Rampelli et al., AGING 2013
Metagenome function analysis

In GM of 100+ we found an age-related reduction of the pathways involved in SCFA production via proteolytic fermentation.

Pyruvate and butanoate metabolisms, containing genes involved in SCFA production, showed an inverse association with aging.

Rampelli et al., AGING 2013
Short Chain Fatty Acids (SCFA) contribute to intestinal homeostasis

SCFA such as acetate, n-propionate, and n-butyrate:
- are end products of bacterial anaerobic fermentation of dietary fibers;
- are secreted in high amounts by commensals bacteria (e.g., clusters IV and XIV of Clostridia);
- can be found at high concentrations in the large intestine (e.g., 20mM n-butyrate in colonic lumen);
- are an important energy source
- have strong anti-inflammatory properties
Butirate is mainly produced by clusters IV and XIV of Clostridia & contributes to the maintenance of intestinal immunological homeostasis:

- Acts as an energy source for normal colonic epithelial cells (throphic effect)
- Upregulates histone H3 acetylation at regulatory regions of Foxp3 gene facilitating differentiation of CD4+ T cells into Treg cells
- Induces TGF-beta secretion by epithelial cells
- Triggers the production of cytoprotective cytokine IL-18 and stimulate IL-10 and retinoic acid production by dendritic cells and macrophages
- Suppresses the proliferation of cancerous epithelial cells
Inflammaging and tryptophan metabolism

• In GM of 100+ we observed an age-related increase of genes involved in the tryptophan metabolism pathway.
• This observation is in agreement with the reduction of tryptophan in the plasma of 100+.
• Reduced plasma tryptophan levels are related to increase of immune activation.
• The increased consumption of tryptophan by the gut microbiota, affects its bioavailability within the host, and can nurture inflammaging.

Rampelli et al., AGING 2013
Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour

John F. Cryan\textsuperscript{1,2} and Timothy G. Dinan\textsuperscript{1,3}
The gut-brain axis

Healthy status
- Normal behaviour, cognition, emotion, nociception
- Healthy levels of inflammatory cells and/or mediators
- Normal gut microbiota

Stress/disease
- Alterations in behaviour, cognition, emotion, nociception
- Altered levels of inflammatory cells and/or mediators
- Intestinal dysbiosis
METABOLOMICS
with Sebastiano Collino

&

METAGENOMICS
with Patrizia Brigidi
A total of 457 individuals:
N= 143 centenarians
N= 220 offspring of centenarians
N= 73 offspring of non long-lived parents
N= 21 young subjects
Metabonomic and lipidomic biomarkers of human aging and longevity

Healthy Ageing is characterized by an unique capacity to adapt/respond to aging-induced accumulation of oxidative and chronic inflammatory conditions

- Decrease concentration of AA (Trp)
- Decrease concentration GPL (LPCs)
- Increase concentration of sphingolipids (SM)
- Different membrane FA composition/integrity (PC/PE ratio) with lower MUFA/PUFA ratio
- Increase proinflammatory synthesis (Leukotrienes)
- Activation anti-inflammatory mechanism (HETE, EET)
- Reduction of oxidative stress (9-HODE,9-oxoODE)

For the first time, a metabolic phenotype of centenarians is reported: a good reference model for human longevity and healthy ageing

Collino et al. PLOS ONE 2013
An emerging concept is that **inflammaging starts early in life, likely *in utero*,** and that early events (e.g. the type of birth natural or cesarean, early nutrition and exposure to infectious agents) may have long term (deleterious) effects in adult and old age, depending on the lifelong exposure (intensity and persistence) to the internal and external inflammatory stimuli.
The hypothesis of the fetal origin of adult diseases can be extended to include the entire lifespan: "IMMUNOBIOGRAPHY" lifelong exposure to infectious agents & self garbage → long term effects of "Inflammatory Memory"
Towards a liquid self: how time, geography, and life experiences reshape the biological identity

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The importance of context/immunobiography
Waddington landscape of self/non-self

immunological biography

- early immunological events
- host-pathogen interactions
- gut microbiome
- proteasomal splicing molecular mimicry
- glycosylation

molecule X

molecule’s immunological fate

Grignolio et al., 2014
“Personalized Inflammaging”
Thanks for your attention!