New dietary strategies for healthy aging in Europe: the NU-AGE project

22 November, 2016 / London, United Kingdom
Food Matters Live 2016
New dietary strategies addressing the specific needs of the elderly population for healthy aging in Europe

Coordinator: Prof. Claudio Franceschi, University of Bologna
Scientific Manager: Dr. Aurelia Santoro, University of Bologna
Start-End: May 2011 - April 2016
Fund: 9 million €

www.nu-age.eu
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)…

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Scientific Manager: Dr. Aurelia Santoro, University of Bologna
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Fund: 9 million €
NU-AGE

16 EU countries
30 Partners

15 Research institutions (nutritionists, biogerontologists, 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"
Spectrum of outcome measures

- **Primary**: inflammatory status
- **Secondary**: biochemical and functional markers, genetics, gut microbiome
- **Subsample**: Epigenetic and OMIC analyses

**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)
- **FRAIL SUBJECTS**

**1250 RANDOMIZED SUBJECTS**

- 625 **WHOLE DIET**
- 625 **CONTROLS**

- **60 NF SUBJECTS**
- **60 PF SUBJECTS**

Epigenetic & OMIC analyses

Genetics
Inflammation
Nutritional Status
Cognitive functions
Anthropometry
Physical functioning
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 inflammamageing 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>Jildaou 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>
</table>
Frailty phenotype based on the following criteria (Fried et al., 2001):

- Unintentional weight loss (4.5kg in past year)
- Self-reported exhaustion
- Weakness (grip strength)
- Slow walking speed
- Low physical activity

Pre-frail: presence of 1 or 2 criteria
A total of 1294 volunteers aged 65-79 years were included in the dietary intervention trial: 644 MedDiet + 650 Controls

% of Volunteers per:
- **FRAILTY STATUS**
- **GENDER**
- **AGE GROUP**

<table>
<thead>
<tr>
<th>Pre-frail</th>
<th>Netherlands (%)</th>
<th>UK (%)</th>
<th>Italy (%)</th>
<th>France (%)</th>
<th>Poland (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>21</td>
<td>22</td>
<td>13</td>
<td>30</td>
<td>22</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Men</th>
<th>Netherlands (%)</th>
<th>UK (%)</th>
<th>Italy (%)</th>
<th>France (%)</th>
<th>Poland (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>36</td>
<td>49</td>
<td>50</td>
<td>43</td>
<td>44</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>65-72y</th>
<th>Netherlands (%)</th>
<th>UK (%)</th>
<th>Italy (%)</th>
<th>France (%)</th>
<th>Poland (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>74</td>
<td>54</td>
<td>71</td>
<td>58</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>
Fig.4. The NuAge modified Food Guide 65+ Pyramid for the elderly has a narrower base (to reflect a decrease in energy needs), while emphasizing nutrient-dense foods, fibre and water. In addition, nutrient-specific supplements are appropriate for many older people.

**Targets:**
The dietary advice is aiming to meet the NU-AGE **quantitative requirements*** by means of NU-AGE **Food Based Dietary Guidelines*** (NU-AGE FBDGs)

*Based on existing FBDGs and RDA’s
NU-AGE DIETARY INTERVENTION

Starting points for guidance

- **Existing Nutrient Reference Values:**

- **Food Based Dietary Guidelines of the 5 involved countries:**

<table>
<thead>
<tr>
<th>NU-AGE pooled nutrient reference values</th>
<th>Individual req +/- 0.5 MJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>15-20</td>
</tr>
<tr>
<td>Protein (En%)</td>
<td>15-20</td>
</tr>
<tr>
<td>Carbohydrates (En%)</td>
<td>50-60</td>
</tr>
<tr>
<td>Fat total (En%)</td>
<td>25-30</td>
</tr>
<tr>
<td>Sat fat (En%)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Trans FA (En%)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>30-40</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>&lt;10-20#</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>1500</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2000</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1200-1300</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>15</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>400</td>
</tr>
<tr>
<td>Vitamin B12 (µg)</td>
<td>5</td>
</tr>
</tbody>
</table>

* * Average requirement per day
# * max. 1 serving/day for women, 2 servings/ for men

Berendsen et al., MAD 2014
**The NU-AGE diet: an elderly tailored MedDiet**

<table>
<thead>
<tr>
<th>Food group</th>
<th>Amount</th>
<th>Food group</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grains</td>
<td>4-6 servings/day</td>
<td>Potatoes and pasta/rice</td>
<td>150 gram/day</td>
</tr>
<tr>
<td>Fruits</td>
<td>At least 2 servings/day</td>
<td>Eggs</td>
<td>2-4 times/week</td>
</tr>
<tr>
<td>Vegetables and legumes</td>
<td>At least 300 gram/day, 200 gram legumes/week</td>
<td>Oil/fat</td>
<td>Oil 20 gram/day, margarine 30 gram/day</td>
</tr>
<tr>
<td>Dairy and cheese</td>
<td>500 ml dairy/day, of which 30 gram cheese/day</td>
<td>Alcohol</td>
<td>Max. 1-2 glasses/day for men and 1 glass/day for women</td>
</tr>
<tr>
<td>Fish and other seafood</td>
<td>2 times 125 gram/week</td>
<td>Fluid</td>
<td>1,5 litre/day</td>
</tr>
<tr>
<td>Meat and poultry</td>
<td>4 times per 125 gram/week</td>
<td>Salt</td>
<td>5 grams /day</td>
</tr>
<tr>
<td>Nuts</td>
<td>2 times 20 gram/week</td>
<td>Sweets</td>
<td>Limited use</td>
</tr>
</tbody>
</table>

Berendsen et al., MAD 2014
NU-AGE dietary intervention

How?

✔ Control: leaflet

✔ Intervention: dieticians to support and motivate
  • counsel and give tailored dietary advice
  • provide nutrient-rich products
  • provide vitamin D supplement
### NU-AGE DIET COMPLIANCE (Italian cohort)

<table>
<thead>
<tr>
<th></th>
<th>Compliance</th>
<th>T0 Median (min-max)</th>
<th>T1 Median (min-max)</th>
<th>P (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CTR</strong></td>
<td>ALL</td>
<td>85.2 (37.8-116.1)</td>
<td>85.2 (42.3-120.2)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MALES</td>
<td>82.9 (37.8-116.1)</td>
<td>83.8 (42.3-120.2)</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>FEMALES</td>
<td>88.1 (52.1-108.5)</td>
<td>87.8 (56.0-107.4)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>DIET</strong></td>
<td>ALL</td>
<td>81.4 (36.0-117.2)</td>
<td>95.8 (55.8-112.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>MALES</td>
<td>81.0 (49.6-116.1)</td>
<td>94.6 (60.7-122.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>FEMALES</td>
<td>81.5 (36.0-117.2)</td>
<td>95.8 (55.8-121.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Compliance, absolute variation (T1-T0)**

- **ALL**
  - CTR: -25 to 25
  - DIET: -25 to 25

- **MALES**
  - CTR: -25 to 25
  - DIET: -25 to 25

- **FEMALES**
  - CTR: -25 to 25
  - DIET: -25 to 25

**P-values**:
- ns: Not significant
- <0.001: Highly significant
Daily total energy intake

Comparison of absolute variation between CTR and DIET group (Mann-Whitney test)
Daily intake of whole grain cereals

Whole grain cereals (g)

CTR | DIET
---|---
T0 | T1

Whole grain cereals intake (g), absolute variation (T1-T0)
Daily intake of fish

Fish (g)

CTR | DIET
---|---

Fish intake (absolute variation, T1-T0)
Daily intake of saturated fatty acid and cholesterol

Saturated fatty acid (g)

Cholesterol (mg)

** T0

** T1
Daily intake of salt and sodium

**Sodium (mg)**

- **CTR**
- **DIET**

**Salt (mg)**

- **CTR**
- **DIET**

* T0

* T1
Daily intake of fluid (water, tea and fruit juice) and total water (including liquid from foods)

---

**Fluid (g)**

- CTR
- DIET

**Water (g)**

- CTR
- DIET

**Legend**

- T0
- T1
Daily intake of iron and zinc

**Iron (mg)**

CTR

DIET

**Zinc (mg)**

CTR

DIET

**Iron intake (mg), absolute variation (T1-T0)**

**Zinc intake (mg), absolute variation (T1-T0)**

T0

T1
Anthropometric measures

Comparison of absolute variation between CTR and DIET group (Mann-Withney)

- Weight (kg), absolute variation (T1-T0)
- BMI (kg/m²), absolute variation (T1-T0)
- Waist circumference (cm), absolute variation (T1-T0)
...BUT there are some important GENDER DIFFERENCES!
Comparison of nutrients intake normalized for total energy intake between men and women at T0

- **Calcium**
- **Fiber**
- **Fluid**
- **Folate**
- **Zinc**
Comparison of nutrients and food intake normalized for total energy intake between men and women at T0

- ω3 PUFA
- ω6/ω3 PUFA
- Saturated Fatty acids
- Alcoholic beverages
- Vegetables
- Wholegrain cereals
The NU-AGE volunteers are different in the 5 recruiting countries

DIFFERENCES per COUNTRY EMERGED FOR:

1. GENETIC STRUCTURE
2. BODY COMPOSITION
3. COMPLIANCE
4. DIET RESPONSE
5. BLOOD MEASUREMENTS
6. CYTOMEGALOVIRUS (CMV) POSITIVITY
7. INFLAMMATORY PARAMETERS

...
NU-AGE COMPLIANCE

Baseline NU-AGE score

<table>
<thead>
<tr>
<th>Country</th>
<th>Score (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>~80</td>
</tr>
<tr>
<td>UK</td>
<td>~70</td>
</tr>
<tr>
<td>Netherlands</td>
<td>~60</td>
</tr>
<tr>
<td>Poland</td>
<td>~75</td>
</tr>
<tr>
<td>France</td>
<td>~90</td>
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</table>

NU-AGE score

<table>
<thead>
<tr>
<th>Country</th>
<th>Score (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>~10</td>
</tr>
<tr>
<td>UK</td>
<td>~15</td>
</tr>
<tr>
<td>Netherlands</td>
<td>~20</td>
</tr>
<tr>
<td>Poland</td>
<td>~25</td>
</tr>
<tr>
<td>France</td>
<td>~40</td>
</tr>
</tbody>
</table>

* indicates statistical significance.
N. 1178 volunteers aged 65-79 years were genotyped by the Illumina OmniExpress Beadchip containing 713,014 SNPs.
NU-AGE POPULATION
GENETIC STRUCTURE

1: BO
2: UK
3: THE NETHERLAND
4: POLAND
5: FRANCE

PCA
Removed each SNP that has an R2 value greater than 0.1 with any other SNP within a 50-SNP sliding window (advanced by 10 SNPs each time)

After filtering (81530 SNPs)
5 GROUPS EMERGED BY COUNTRY OF ORIGIN, SIMILAR TO THE GENETIC STRUCTURE OF THE POPULATION
NU-AGE and Inflammm-aging

BASIC HYPOTHESIS & RATIONALE

Appropriate WHOLE DIET (an ad hoc fortified “Mediterranean Diet”) can decrease the level of the chronic, subclinical, low grade inflammatory process characteristic of old age. We have proposed to call INFLAMM-AGING (Franceschi et al., 2000)
Appropriate MedDiet for the elderly + Gut microbiota Metabolome → inflammaging → age-related diseases
Why the primary target of NU-AGE is to slow down inflammaging?
The network and the remodeling theories of aging: historical background and new perspectives

C. Franceschi\textsuperscript{a,b,*,1}, S. Valensin\textsuperscript{a,1}, M. Bonafè\textsuperscript{a,1}, G. Paolisso\textsuperscript{c}, A.I. Yashin\textsuperscript{d}, D. Monti\textsuperscript{e}, G. De Benedictis\textsuperscript{f}

\textsuperscript{a}Department of Experimental Pathology, University of Bologna, Bologna, Italy  
\textsuperscript{b}Department of Gerontological Research, Italian National Research Center on Aging (INRCA), Ancona, Italy  
\textsuperscript{c}Department of Geriatric Medicine and Metabolic Diseases, Second University of Naples, Naples, Italy  
\textsuperscript{d}Max Plank Institute for Demographic Research, Rostock, Germany  
\textsuperscript{e}Department of Experimental Pathology and Oncology, University of Florence, Florence, Italy  
\textsuperscript{f}Department of Cell Biology, University of Calabria, Cosenza, Italy
An example of remodeling/adaptation: the Inflammatory Theory of Aging

Inflamm-aging

An Evolutionary Perspective on Immunosenescence

CLAUDIO FRANCESCHI, a,b,c MASSIMILIANO BONAFÈ, a SILVANA VALENSIN, a
FABIOLA OLIVIERI, b MARIA DE LUCA, d ENZO OTTAVIANI, c AND
GIOVANNA DE BENEDITTIS d

a Department of Experimental Pathology, University of Bologna, Bologna, Italy
b Department of Gerontological Research, Italian National Research Center on Aging (INRCA), Ancona, Italy
c Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy
d Department of Cell Biology, University of Calabria, Calabria, Italy

“chronic”, “low grade”, “sterile”

Ann. N.Y. Acad. Sci., 908, 244-254, 2000

Inflammaging is based on studies on the evolution of immune response and stress from invertebrates to mammals
An example of remodeling/adaptation: the biological role inflammation lifelong

Inflammation is a most fundamental, beneficial biological response, conserved throughout evolution, critical for development and survival until age of reproduction, that turns detrimental in the post-reproductive period of life, because of the persistent increased production of inflammatory stimuli which produce a chronic activation of innate immunity (macrophages).

Thus, Inflammaging fits/complements the two major theories of aging:

• Antagonistic Pleiotropy (George Williams)
• Quasi-programmed (Mikhail Blagosklonny)
Inflammageing is macrophage-centered

The cell firstly described by Mechnikov & highly conserved in evolution from invertebrates to humans

where inflammation, stress response and innate immunity converge

Franceschi et al., 2000
We soon realized that pro- and anti-inflammatory cytokines changed together and proposed that increasing levels of **pro-inflammatory** molecules with age would stimulate a corresponding increase in **anti-inflammatory** molecules.
Advances in Geroscience: Impact on Healthspan and Chronic Disease Perspective

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

Claudio Franceschi$^{1,2}$ and Judith Campisi$^{3,4}$

$^1$DIMES, Department of Experimental, Diagnostic and Specialty Medicine and CIG, Interdepartmental Center “Luigi Galvani”, University of Bologna, Italy.
$^2$IRCCS Institute of Neurological Sciences, and CNR-ISOF, Bologna, Italy.
$^3$Buck Institute for Research on Aging, Novato, California.
$^4$Life Sciences Division, Lawrence Berkeley National Laboratory, California.

Address correspondence to Claudio Franceschi, MD, DIMES, Department of Experimental, Diagnostic and Specialty Medicine and CIG, Interdepartmental Center “Luigi Galvani”, University of Bologna, Via S. Giacomo 12, 40126 Bologna, Italy. Email: claudio.franceschi@unibo.it

The enemy from within
The Inflammaging Theory of Aging

- Metabolic syndrome
- Type 2 Diabetes
- Cancer
- Alzheimer
- PD
- PO Delirium
- COPD
- Cardiovascular diseases
- Sarcopenia
- Frailty
- OA
- Depression
Review

Inflammaging and Cancer: A Challenge for the Mediterranean Diet

Rita Ostan 1, Catia Lanzarini 1,2, Elisa Pini 1, Maria Scurti 1, Dario Vianello 1,
Claudia Bertarelli 1, Cristina Fabbri 1, Massimo Izzi 2, Giustina Palmas 2, Fiammetta Biondi 2,
Morena Martucci 1, Elena Bellavista 1,2, Stefano Salvioli 1,2, Miriam Capri 1,2,
Claudio Franceschi 1,3,4 and Aurelia Santoro 1,*

Nutrients 2015, 7, 2589-2621
AGING
CVD, T2D, CANCER
NEURODEGENERATION

Ostan et al., 2015
Trends in Endocrinology & Metabolism

Review

Inflammaging and ‘Garb-aging’

Claudio Franceschi, Paolo Garagnani, Giovanni Vitale, Miriam Capri, and Stefano Salvioli
‘Inflammaging’ refers to the chronic, low-grade inflammation that characterizes aging. Inflammaging is macrophage centered, involves several tissues and organs, including the gut microbiota, and is characterized by a complex balance between pro- and anti-inflammatory responses. Based on literature data, we argue that the major source of inflammatory stimuli is represented by endogenous/self, misplaced, or altered molecules resulting from damaged and/or dead cells and organelles (cell debris), recognized by receptors of the innate immune system. While their production is physiological and increases with age, their disposal by the proteasome via autophagy and/or mitophagy progressively declines. This ‘autoreactive/autoimmune’ process fuels the onset or progression of chronic diseases that can accelerate and propagate the aging process locally and systemically. Consequently, inflammaging can be considered a major target for antiaging strategies.
SELF GARBAGE
CELL DEBRIS - MISPLACED CELL COMPONENTS
recognized as DAMPs (Damage Associated Molecular Patterns)

INFLAMMAGING

NUTRIENTS (EXCESS) METABOLITES

MICROBIOMES VIROMES
Normal cell components that are recognized as DAMPs when misplaced

<table>
<thead>
<tr>
<th>DAMPs</th>
<th>Origin</th>
<th>Engaged receptors</th>
<th>REF</th>
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</thead>
<tbody>
<tr>
<td>mtDNA</td>
<td>Mitochondria</td>
<td>TLR9, NLRPs</td>
<td>[53,54,58]</td>
</tr>
<tr>
<td>N-formyl peptides</td>
<td>Mitochondria</td>
<td>Formyl peptide receptor-1, NLRPs</td>
<td>[106]</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>Mitochondria</td>
<td>NLRPs</td>
<td>[56]</td>
</tr>
<tr>
<td>Histones</td>
<td>Nucleus</td>
<td>TLRs</td>
<td>[107]</td>
</tr>
<tr>
<td>High Mobility Group Box 1 protein (HMGB1)</td>
<td>Nucleus</td>
<td>RAGE, NLRPs, TLR4</td>
<td>[57,74]</td>
</tr>
<tr>
<td>Nuclear DNA (CpGs)</td>
<td>Nucleus</td>
<td>TLR9</td>
<td>[108]</td>
</tr>
<tr>
<td>Heat Shock Proteins (e.g. HSPA1A, HSP90AA1); ER chaperons (CRT, ERp57, GP96)</td>
<td>Cytoplasm, mitochondria, Endoplasmic reticulum</td>
<td>TLR2, TLR4, NLRPs</td>
<td>[45,109,110]</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td>Lysosomes</td>
<td>NLRPs</td>
<td>[58]</td>
</tr>
<tr>
<td>Triphosphate nucleotides (ATP, UTP)</td>
<td>Cytoplasm</td>
<td>NLRPs</td>
<td>[45]</td>
</tr>
<tr>
<td>S100 proteins (including S100a8, a9 and a12)</td>
<td>Cytoplasm – granules (neutrophils)</td>
<td>RAGE, TLR4, TLR9</td>
<td>[57,75]</td>
</tr>
<tr>
<td>Lipids (fatty acids, ceramides)</td>
<td>Cytoplasm, membranes</td>
<td>TLR4, NLRPs</td>
<td>[58,111]</td>
</tr>
<tr>
<td>Crystals (e.g. monosodium urate, cholesterol crystals, calcium pyrophosphate dihydrate)</td>
<td>Cytoplasm</td>
<td>NLRPs, TLR2, TLR4, CD14</td>
<td>[45,57]</td>
</tr>
<tr>
<td>Hyaluronans</td>
<td>Extracellular matrix</td>
<td>NLRPs</td>
<td>[45]</td>
</tr>
<tr>
<td>Altered N-glycans</td>
<td>Serum proteins</td>
<td>DC-SIGN, MBR</td>
<td>[112]</td>
</tr>
</tbody>
</table>
PRODUCTION & PROPAGATION OF INFLAMMAGING

Cells produce pro-inflammatory molecules as a consequence of:

1. DYSFUNCTIONAL MITOCHONDRIA
2. DEFECTIVE AUTOPHAGY/MITOPHAGY (disposal of dysfunctional organelles)
3. ENDOPLASMIC RETICULUM (ER) STRESS
4. ACTIVATION OF INFLAMMASOME by cell debris and misplaced self molecules
5. DEFECTIVE UBIQUITIN/PROTEASOME SYSTEM (misfolded/oxidized proteins)
6. ACTIVATION OF DNA DAMAGE RESPONSE & INDUCTION OF CELL SENESCENCE

EV= Extracellular Vesicles

Franceschi et al., Trends in Endocrinology and Metabolism, 2016 in press
"TRAINED IMMUNITY": Corpse Engulfment Generates a Molecular Memory that Primes the Macrophage Inflammatory Response

The remarkable plasticity and capacity for innate immune memory places macrophages as key therapeutic targets for treatment of inflammatory disorders.

Weavers et al., Cell 2016 Jun 16; 165(7): 1658–1671
"TRAINED IMMUNITY": Corpse Engulfment Generates a Molecular Memory that Primes the Macrophage Inflammatory Response

The remarkable plasticity and capacity for innate immune memory places macrophages as key therapeutic targets for treatment of inflammatory disorders.

Weavers et al., Cell 2016 Jun 16; 165(7): 1658–1671
Aging and the blurring of the distinction between self and not self.

INFLAMMAGING and AGE-RELATED DISEASES can be conceptualized as the result of a low-grade, systemic AUTO-INFLAMMATORY PROCESS driven by a peculiar, chronic stimulation/activation of the Innate Immune System favoring the functional decline of the adaptive immune responses (immunosenescence).
Age-related disease likely contribute to propagate and accelerate the aging process.
The complex, systemic nature of INFLAMMAGING

Cevenini et al., Curr Opin Clin Nutr Metab Care 2012
- The ECOSYSTEM of the human gut consists of trillions of bacteria forming a bioreactor that is fueled by dietary macronutrients to produce bioactive compounds.
- There are more microbial cells in the gut as human cells in the body.
- Approximately 1,200 different bacterial species have been identified in the human gut microbiota.
- Each individual is host to a distinct set of at least 160 species in the gut.
- The collective microbial genome (microbiome) encodes 200-500 times more genes than the human genome.
Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians

Elena Biagi, Lotta Nylund, Marco Candela, Rita Ostan, Laura Bucci, Elisa Pini, Janne Nikkila, Daniela Monti, Reetta Satokari, Claudio Franceschi, Patrizia Brigidi, Willem De Vos

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
Butyrate producers

Pathobionts

Changes in the microbiota composition can be caused by and contribute to inflammaging.

8.9% of the total variability of the GM is correlated with the pattern of pro-inflammatory cytokines.

MICROBIOTA AND INFLAMMAGING

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>S</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>61.4 ± 18.9</td>
<td>24.6 ± 6.2</td>
<td>20.9 ± 4.3</td>
</tr>
<tr>
<td>IL-8</td>
<td>30.4 ± 7.8</td>
<td>22.0 ± 4.2</td>
<td>21.9 ± 4.1</td>
</tr>
</tbody>
</table>

MCP, P = 0.01
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.
Gut microbiota & Aging

**↓ BIODIVERSITY**

**↓** SCFA producers, such as *Clostridium* cluster XIVa and *Clostridium* cluster IV

**↓** Bifidobacteria

**↑** Facultative anaerobes, including Bacilli and Proteobacteria ("pathobionts")

**↑** microbial genes that utilize tryptophan
SCFA Butirate contributes to the maintainance of intestinal immunological homeostasis:

• Acts as a **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-β secretion** by epithelial cells
• Triggers the production of cytoprotective cytokine **IL-10 and retinoic acid production** by dendritic cells and macrophages
• Suppresses the proliferation of cancerous epithelial cells
Inflamming and tryptophan metabolism

• In the gut of old people there is an increased number of bacteria consuming tryptophan, which reduces its bioavailability within the host.

• This observation is in agreement with the reduction of tryptophan in plasma of elderly.

• Reduced plasma tryptophan levels are related to an increase of immune activation which can nurture inflammaging.

Rampelli et al., AGING 2013
Schroeder et al., 2016

Signals from the gut microbiota to distant organs in physiology and disease

Brain
- Autism spectrum disorder
- Stress
- Stroke

Lung
- Allergic asthma

Liver
- NAFLD/NASH

Skin
- Atopic dermatitis

Adipose tissue
- Inflammation
- Obesity

Whole body
- Type 2 diabetes
- Systemic lupus erythematosus
- Undernourishment
- Atherosclerosis

The gut-brain axis in health and disease

Healthy CNS function → Abnormal CNS function

Healthy gut function → Abnormal gut function

The gut-joint axis
The gut-brain axis and serotonergic metabolism

TPH
Tryptophan hydroxylase

CNS Effects
- Motor control
- Circadian rhythm
- Cerebellar regulation
- Body temperature
- CNS vascular tone

AAAD
Aromatic amino acid decarboxylase

Behavourial Effects
- Visceral pain
- Emotion
- Stress response
- Appetite
- Addiction
- Sexuality

GI Effects
- Gastric secretion
- Gastrointestinal motility
- Intestinal secretions
- Colonic tone
- Pancreatic secretion

The kynurenine pathway of tryptophan metabolism

QUIN= quinolinic acic  IDO= indoleamine-2,3-dioxygenase
KYNA= kynurenic acid  TDO= tryptophan-2,3-dioxygenase

S.M. O'Mahony et al. / Behavioural Brain Research 277 (2015) 32–48
We reconstructed the longest available human microbiota trajectory (22-109 years) by analyzing persons >105 years old, compared to centenarians, elderly and adults.
The remodeling with age of the gut microbiota

Gut Microbiota and Extreme Longevity

Elena Biagi,1,* Claudio Franceschi,2,3,4 Simone Rampelli,1 Marco Severgnini,5 Rita Ostan,2,3 Silvia Turroni,1 Clarissa Consolandi,5 Sara Quercia,1 Maria Scurti,2,3 Daniela Monti,6 Miriam Capri,2,3 Patrizia Brigidi,1 and Marco Candela1,*

1Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
2DIMES-Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
3CIG-Interdepartmental Centre “L. Galvani,” Alma Mater Studiorum, University of Bologna, Bologna 40126, Italy
4IRCCS, Institute of Neurological Sciences of Bologna, Bologna 40139, Italy
5Institute of Biomedical Technologies, National Research Council (ITB-CNR), Segrate, Milan 20090, Italy
6Department of Clinical, Experimental and Biomedical Sciences, University of Florence, Florence 50134, Italy

*Corresponding author.  E-mail addresses: Elena.Biagi@unibo.it; Claudio.Franceschi@unibo.it; Simone.Rampelli@unibo.it; Marco.Severgnini@unibo.it; Rita.Ostan@unibo.it; Silvia.Turroni@unibo.it; Clarissa.Consolandi@unibo.it; Sara.Quercia@unibo.it; Maria.Scurti@unibo.it; Daniela.Monti@unibo.it; Miriam.Capri@unibo.it; Patrizia.Brigidi@unibo.it; Marco.Candela@unibo.it
Highlights

• **A core microbiota** of highly occurring bacterial groups (mostly belonging to *Ruminococcaceae, Lachnospiraceae and Bacteroidaceae* families) accompanies human life, **decreasing in abundance along with aging**;

• In longevity, the age-related **enrichment of subdominant taxa** is boosted accommodating, along with pro-inflammatory species, also **health-associated taxa** such as *Akkermansia* and *Bifidobacterium*, known to promote immunomodulation, protect against inflammation, and promote a healthy metabolic homeostasis, that might support extreme aging and longevity;

• **“Longevity adaptation”** seems to involve enrichment in health-associated gut bacteria
The adaptive remodeling of gut microbiota with age

Biagi et al., *Curr Biol* 2016
Table 1. Age-related trajectory of bacterial groups contributing to the sample separation.

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Average relative abundance (%)</th>
<th>Trajectory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group Y</td>
<td>Group E</td>
</tr>
<tr>
<td><em>Coprooccus</em></td>
<td>8.4</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Roseburia</em></td>
<td>7.9</td>
<td>4.6</td>
</tr>
<tr>
<td><em>Faecalibacterium</em></td>
<td>8.6</td>
<td>7.6</td>
</tr>
<tr>
<td><em>Uncl. Lachnospiraceae</em></td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td><em>Oscillospira</em></td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td><em>Odoribacter</em></td>
<td>0.08</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Butyricimonas</em></td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Eggerthella</em></td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Akkermansia</em></td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Anaerotruncus</em></td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td><em>Bilophila</em></td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Christensenellaceae</em></td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Synergistaceae</em></td>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>
The Microbiota Affects Metabolic Syndrome via the Immune System

(A) *Firmicutes*↑  Bacteroidetes↓  (B) *Proteobacteria*↑

(C) *Akkermansia*

Mucus

Increased provision of calories to the host

Increased serum LPS

CD4 T cell

NKT cell

IFNγ

IL-13
TNFα
= insulin resistance

IL-1β
TNFα
= insulin resistance

↑Type 1 macrophages

↓Type 2 macrophages

IL-4
IL-13
IL-25
IL-33

Hand et al., 2016 in press  Trends in Endocrinology & Metabolism
Mediterranean Diet and Hormesis: a possible link to elucidate the benefits on healthspan?

Morena Martucci\textsuperscript{1,*}, Rita Ostan\textsuperscript{2,*}, Fiammetta Biondi\textsuperscript{1}, Elena Bellavista\textsuperscript{1}, Cristina Fabbri\textsuperscript{1}, Claudia Bertarelli\textsuperscript{1}, Stefano Salvioli\textsuperscript{1,2}, Miriam Capri\textsuperscript{1,2}, Claudio Franceschi\textsuperscript{3}, Aurelia Santoro\textsuperscript{1,2}

*Corresponding authors

*Authors contributed equally

Nutrition Reviews, 2016 under revision
Hormesis is a term used by toxicologists to refer to a biphasic dose response to an environmental agent characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect. In the fields of biology and medicine, hormesis is defined as an adaptive response of cells and organisms to a moderate (usually intermittent) stress. Examples include ischemic preconditioning, exercise, dietary energy restriction and exposures to low doses of certain phytochemicals.

Mattson MP
Hormesis

a short working definition of hormesis is:

“a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism”

Mattson MP
Hormesis

The concept that drugs exert their beneficial effects by hormetic mechanisms of action can be traced to the 16th century and a Swiss chemist and physician called Paracelsus who wrote: “All things are poison and nothing is without poison, only the dose permits something not to be poisonous”
Hormesis is a fundamental concept in evolutionary theory. From the beginning through the present time, life on earth has existed in harsh environments in which cells are often exposed to free radicals and toxic substances. To avoid extinction organisms have developed complex mechanisms to cope with the environmental hazards.

Mattson MP
Chemical Hormesis: An Explanation for the Health Benefits of Fruits, Vegetables and Some Drugs?
THANKS FOR ATTENTION

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Patrizia Leone
Valeria Genovesi

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Patrizia Brigidi
Marco Candela

Statistics-UNIBO:
Mario Mazzocchi

NU-AGE PROJECT CONSORTIUM
Thanks for your attention!