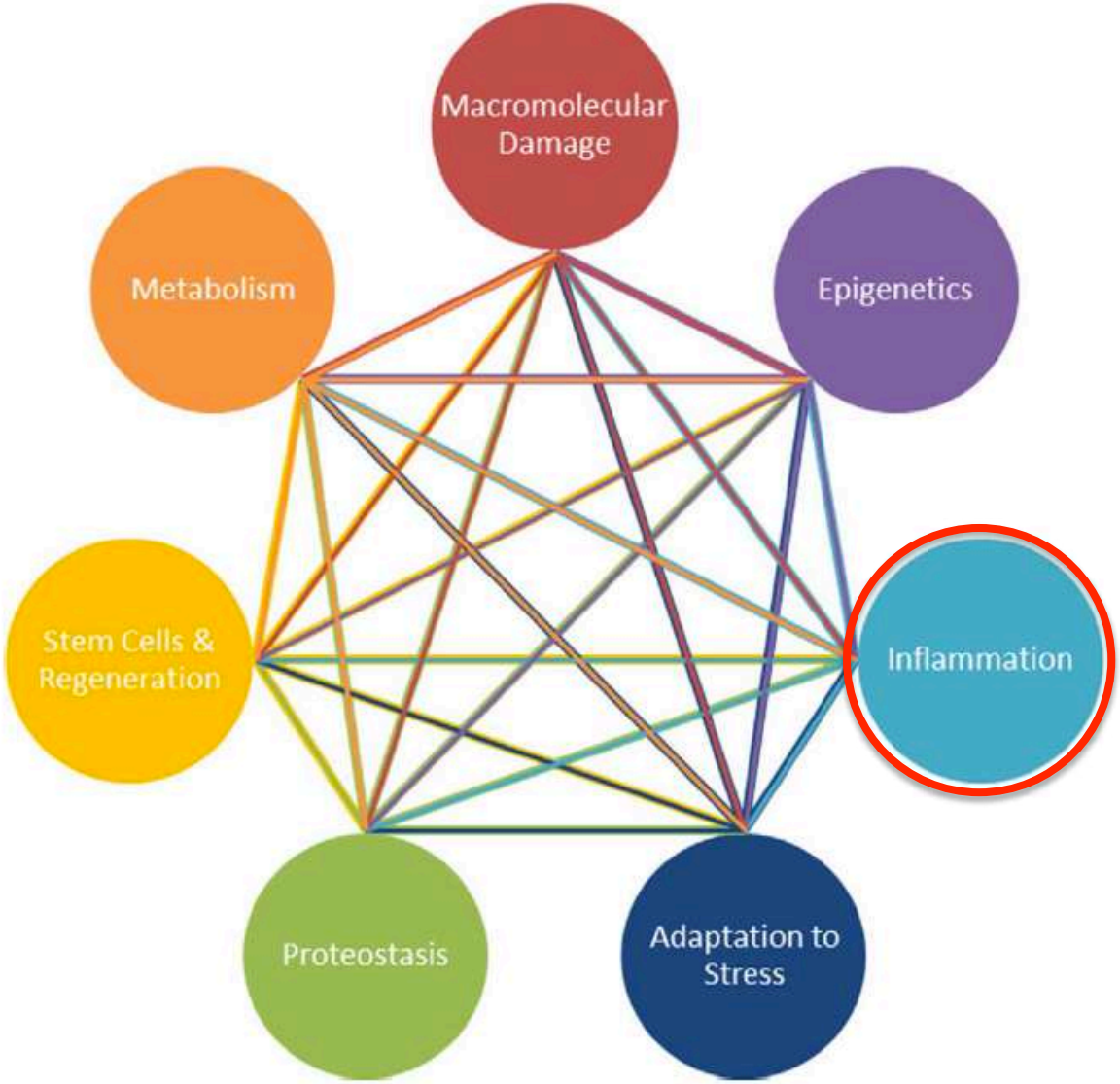
Mitokines and Volatile Organic Compounds (VOCs) in human aging and extreme longevity

STEFANO SALVIOLI

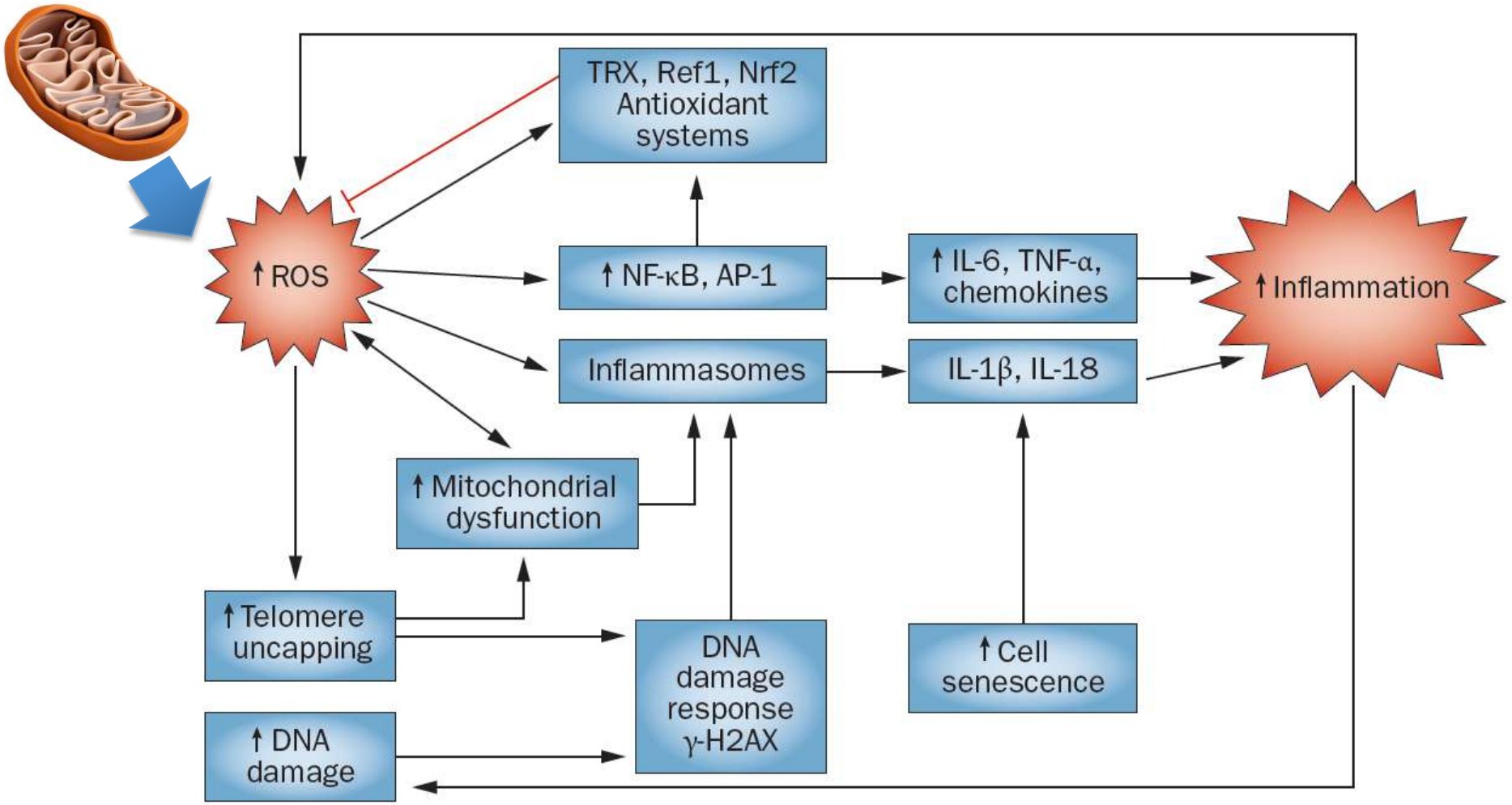
Dept. of Experimental, Diagnostic and Specialty Medicine
University of Bologna, Bologna, Italy

The “seven pillars” of aging



Adapted from Kennedy et al., 2014

Is inflammaging (at least in part) linked to mitochondrial dysfunction?



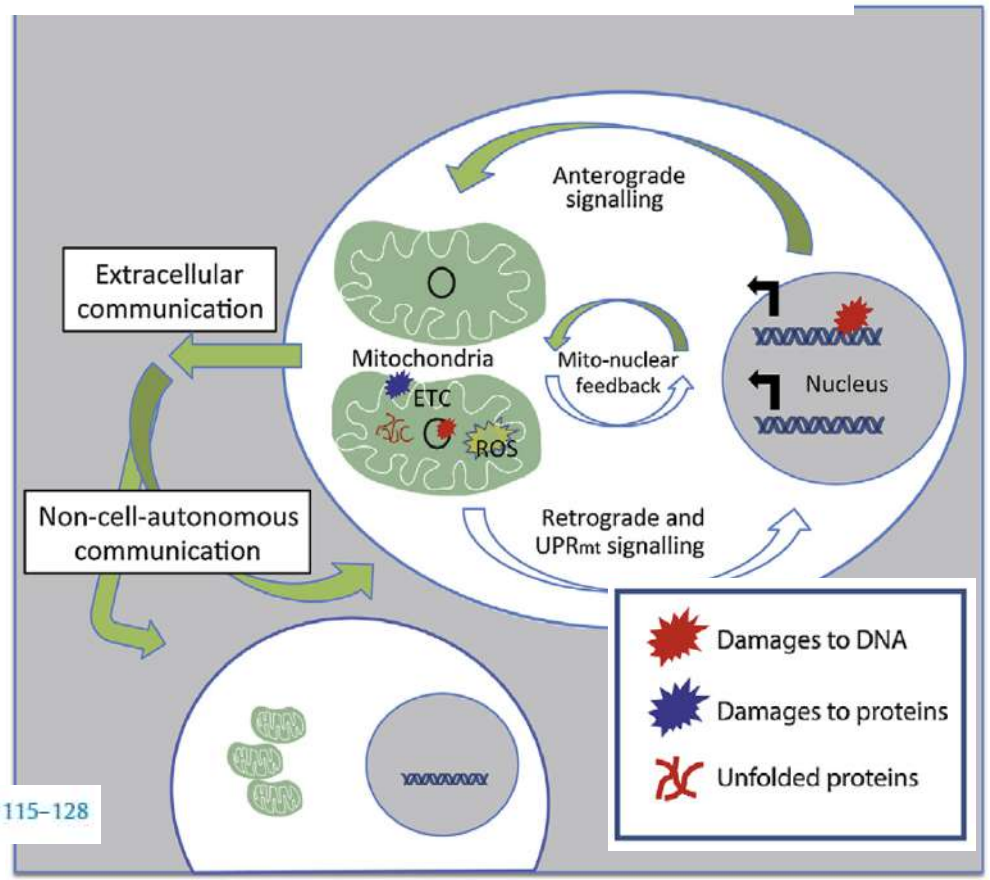
Vitale, Salvioli and Franceschi, Nat Rev Endocrinol, 2013

However, mitochondria can be determinants of longevity as a mild dysfunction can trigger a number of stress responses (ISR, UPR^{mt}) and the positive effects of these responses can spread through the organism via soluble mediators indicated as mitokines

Mitochondria and mitochondria-induced signalling molecules as longevity determinants

Giuseppina Rose[a], Aurelia Santoro[b,c,*], Stefano Salvioli[b,c]

[a] Department of Biology, Ecology and Earth Science, University of Calabria, Rende (CS), Italy
[b] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Via San Giacomo 12, 40126 Bologna, Italy
[c] Interdepartmental Centre "L. Galvani" (CIG) University of Bologna, Via San Giacomo 12, 40126 Bologna, Italy



Mechanisms of Ageing and Development 165 (2017) 115–128

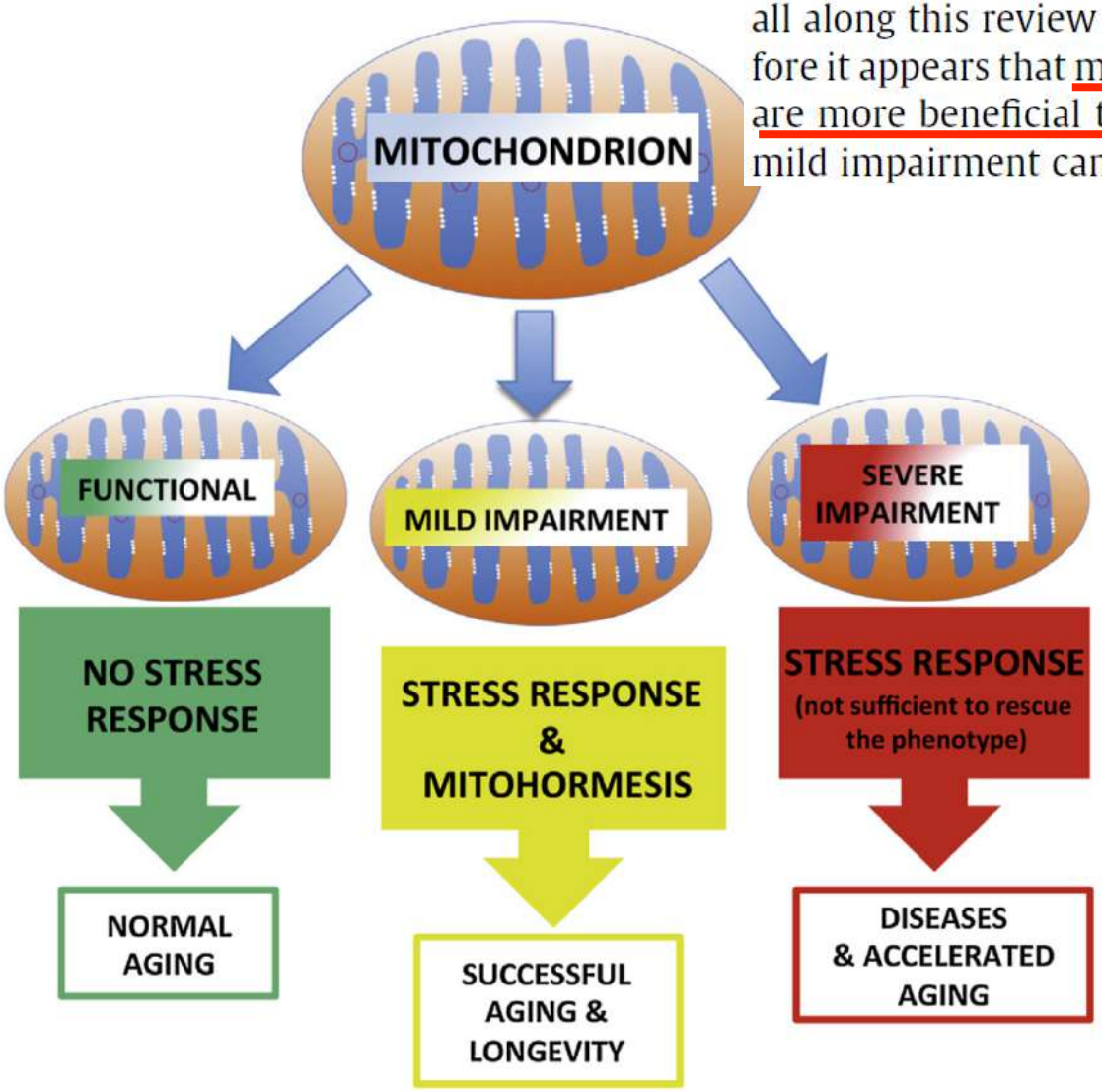
The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity

Jenni Durieux,[1] Suzanne Wolff,[2] and Andrew Dillin[1,*]
[1]The Howard Hughes Medical Institute, The Glenn Center for Aging Research, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[2]The Scripps Research Institute, Department of Molecular and Experimental Medicine, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: dillin@salk.edu
DOI 10.1016/j.cell.2010.12.016

"We report that the alteration of mitochondrial function in key tissues is essential for establishing and maintaining a prolongevity cue.
We find that regulators of mitochondrial stress responses are essential and specific genetic requirements for the electron transport chain (ETC) longevity pathway. Strikingly, we find that mitochondrial perturbation in one tissue is perceived and acted upon by the mitochondrial stress response pathway in a distal tissue."

[…] Because this signal is the product of perceived mitochondrial stress that results in increased survival, we have termed this cell-non-autonomous signal a "mitokine."

more recent evidence suggests that a certain level of mitochondrial impairment seems to be functional to attain longevity in animal models, as it effectively activates a stress response able to modulate a series of rescue mechanisms and promote survival as discussed all along this review and summarised at a glance in Fig. 2. Therefore it appears that mitochondria with mild functional impairment are more beneficial than perfectly working mitochondria, as this mild impairment can act as a hormetic signal.



Rose et al., 2016

What about centenarians?

Based on this assumption and on data on inflammation, it could be hypothesised that they are characterized by mildly defective mitochondria

www.impactaging.com AGING, April 2014, Vol. 6, No 4

Research Paper

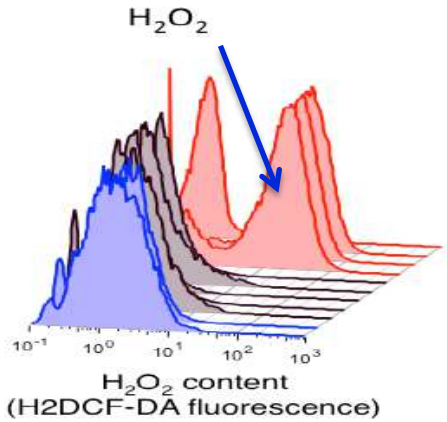
Mitochondria hyperfusion and elevated autophagic activity are key mechanisms for cellular bioenergetic preservation in centenarians

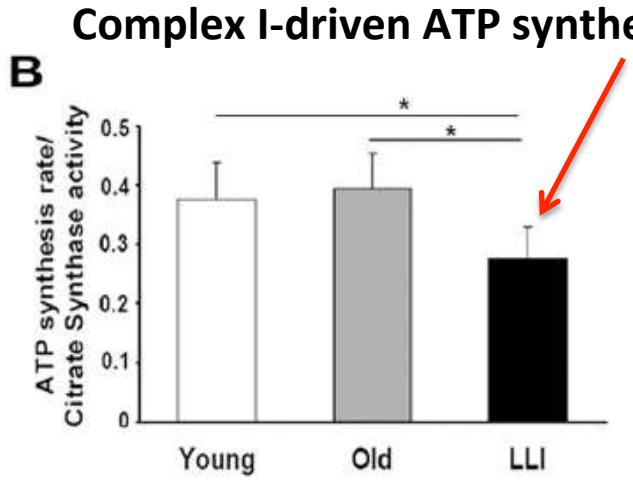
Gianluca Sgarbi[1*], Paola Matarrese[2,3*], Marcello Pinti[4], Catia Lanzarini[5], Barbara Ascione[2], Lara Gibellini[4], Emi Dika[5], Annalisa Patrizi[5], Chiara Tommasino[2], Miriam Capri[5,6], Andrea Cossarizza[7], Alessandra Baracca[1], Giorgio Lenaz[1], Giancarlo Solaini[1], Claudio Franceschi[5,6,8,9], Walter Malorni[2,10], and Stefano Salvioli[5,6]

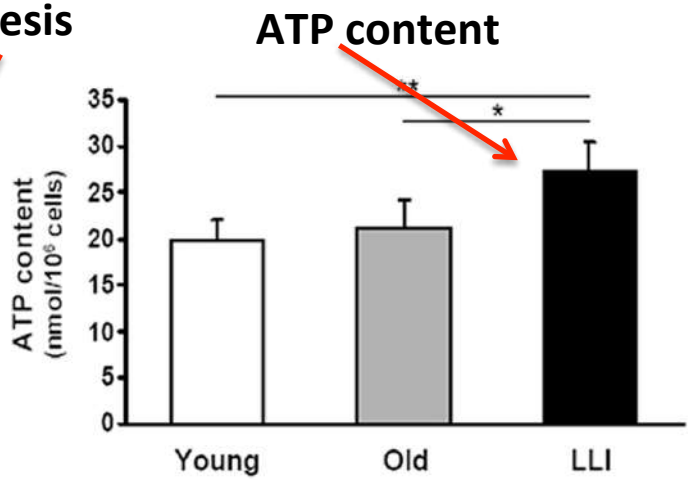
REMODELING OF MITOCHONDRIAL BIOENERGETICS IN 100+

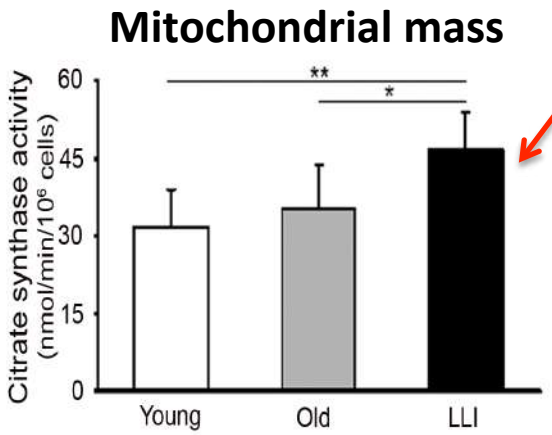
Mitochondria from fibroblasts of 100+ displayed **lower complex I-driven ATP synthesis** and **higher H2O2 production**, but **maintain energetic competence** (**ATP production**) due to **compensatory mechanisms** (maintained **supercomplexes organization**, **increased mitochondrial mass** and **autophagy,** with **hyperfused elongated/networked mitochondria.**

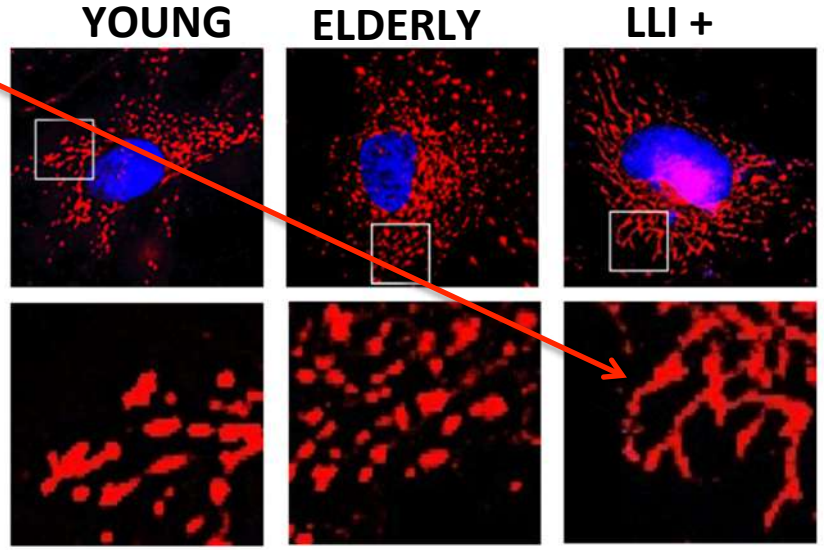
Mitochondria from DFs of centenarians (LLI) produce more ROS (H_2O_2) and are defective in Complex I-driven ATP synthesis, but maintain global energetic competence by upregulating mitochondrial mass and forming large networks.





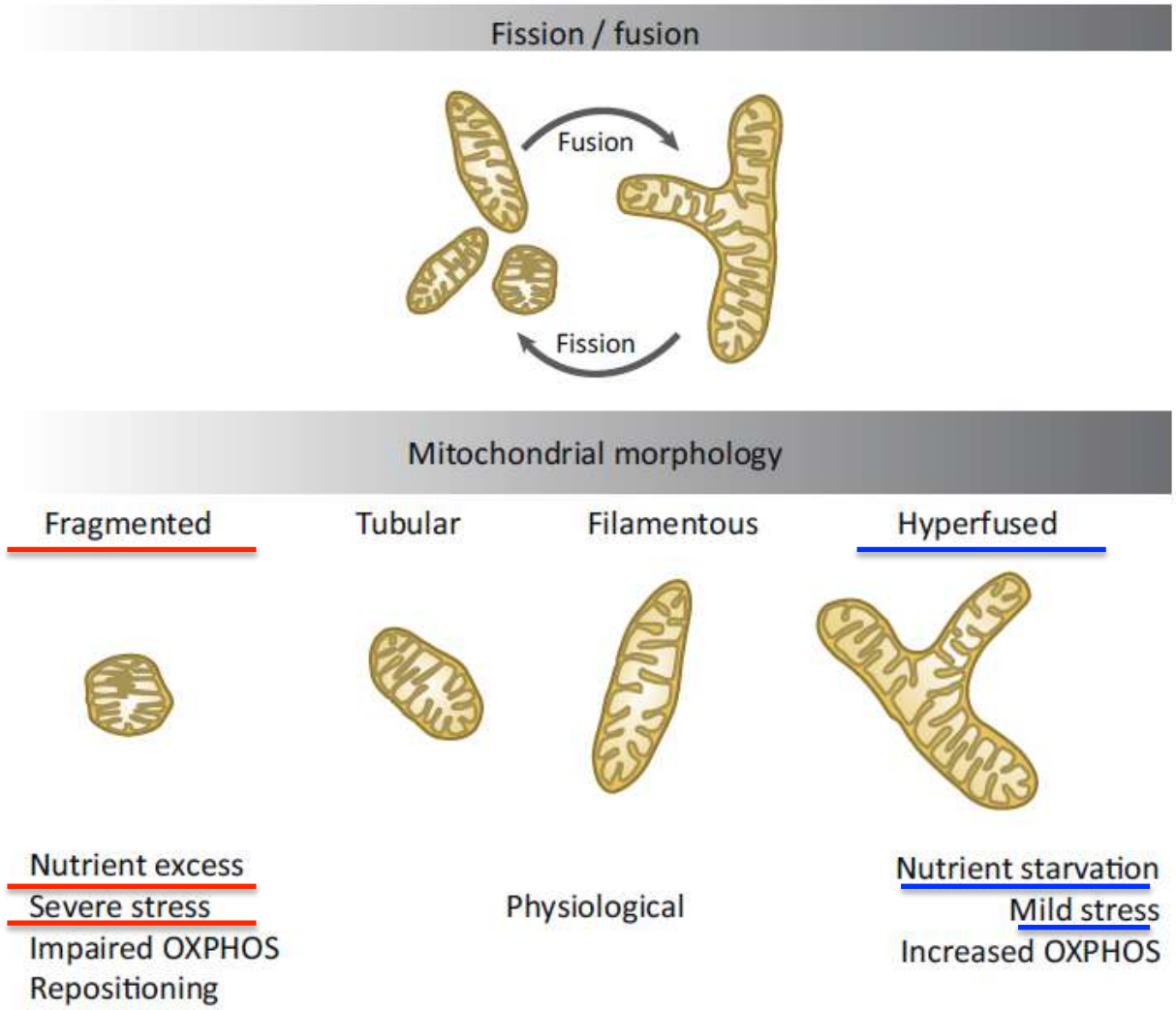






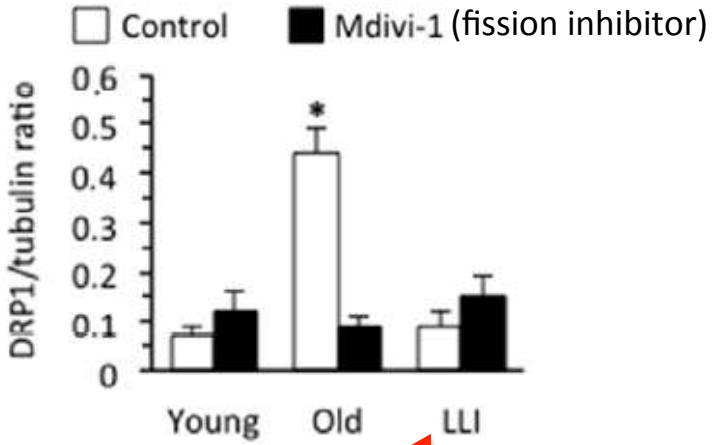
Sgarbi et al., Aging 2014

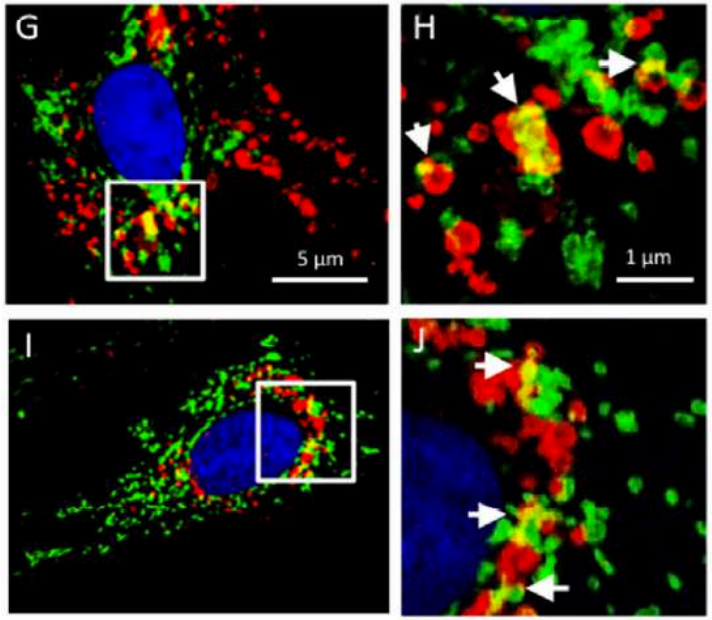
Mitochondrial Morphology Is Linked to the Cellular Metabolic State



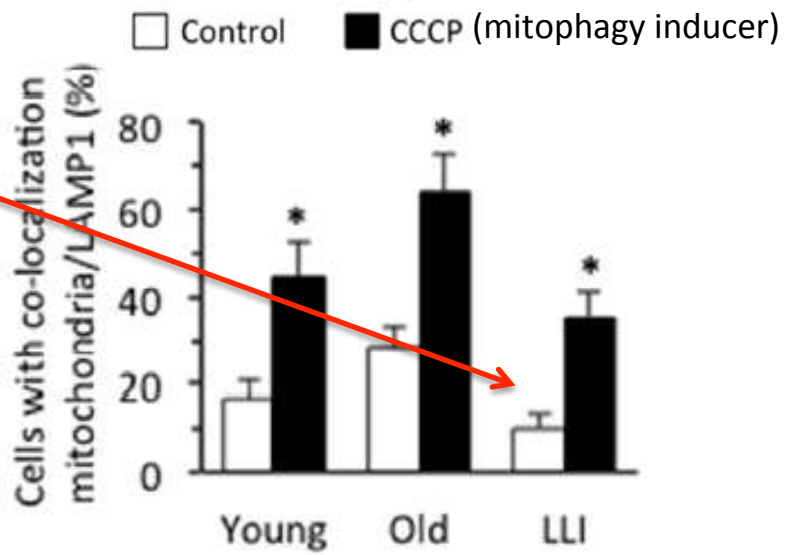
Rambold and Pearce 2017

Mitochondrial Fission





Centenarians (LLI) have low levels of mitochondrial fission and mitophagy



The phenotype of centenarians largely overlaps that of persons under calorie restriction, even though they never fasted during their life

(Franceschi, Ostan & Santoro Annu Rev Nutr. 2018)

Adaptation	Parameter	Calorie-restricted diet (people aged 21–60 years)	Centenarians (>100 years)	References
Concordant				
Glucose metabolism	Glucose	↓	↓	4, 10, 31, 33, 54, 69, 71, 100, 101, 108, 121, 136, 137, 139, 141
	Insulin	↓	↓	
	Insulin sensitivity	↑	↑	
Blood pressure	Systolic	↓	↓	47, 64, 108, 109, 114, 135, 139, 141, 151
	Diastolic	↓	↓	
Thyroid	T3	↓	↓	54, 66, 75, 137, 141
Lipid profile	Total cholesterol	↓	↓	1, 10, 33, 108, 121, 139–141
	LDL cholesterol	↓	↓	
	Triglycerides	↓	↓	
Body composition	BMI	↓	↓	1, 33, 101, 103, 108, 136, 137, 139–141, 145, 146
	Fat-free mass	↓	↓	
	Bone mineral density	↓	↓	
Metabolism	Cortisol	= or ↑	↑	4, 10, 18, 66, 122, 136, 141, 145
	Adiponectin	↑	↑	
	Leptin	↓	↓	
	Tryptophan	↓	↓	

- a number of mitokines have been identified. Some of them are nuclear encoded and are expressed in response to UPR^{mt}. These include Fibroblast Growth Factor 21 (FGF21) and Growth Differentiation Factor 15 (GDF15).
- **FGF21** is a hormone-like member of FGF family involved in metabolic processes, also described as stress hormone for its role as a potent longevity factor.
- **GDF15** is a member of the transforming growth factor (TGF)-β family, produced by several tissues. It is considered an indicator of mitochondrial dysfunction in muscle and it has cardioprotective and neuroprotective activity.
- In addition to nuclear encoded mitokines, a number of peptides encoded by mitochondrial DNA, including **Humanin (HN)**, are produced in response to mitochondrial stress and have anti-apoptotic and neuro- and cardio-protective effects. It also improves mitochondriogenesis and insulin sensitivity.

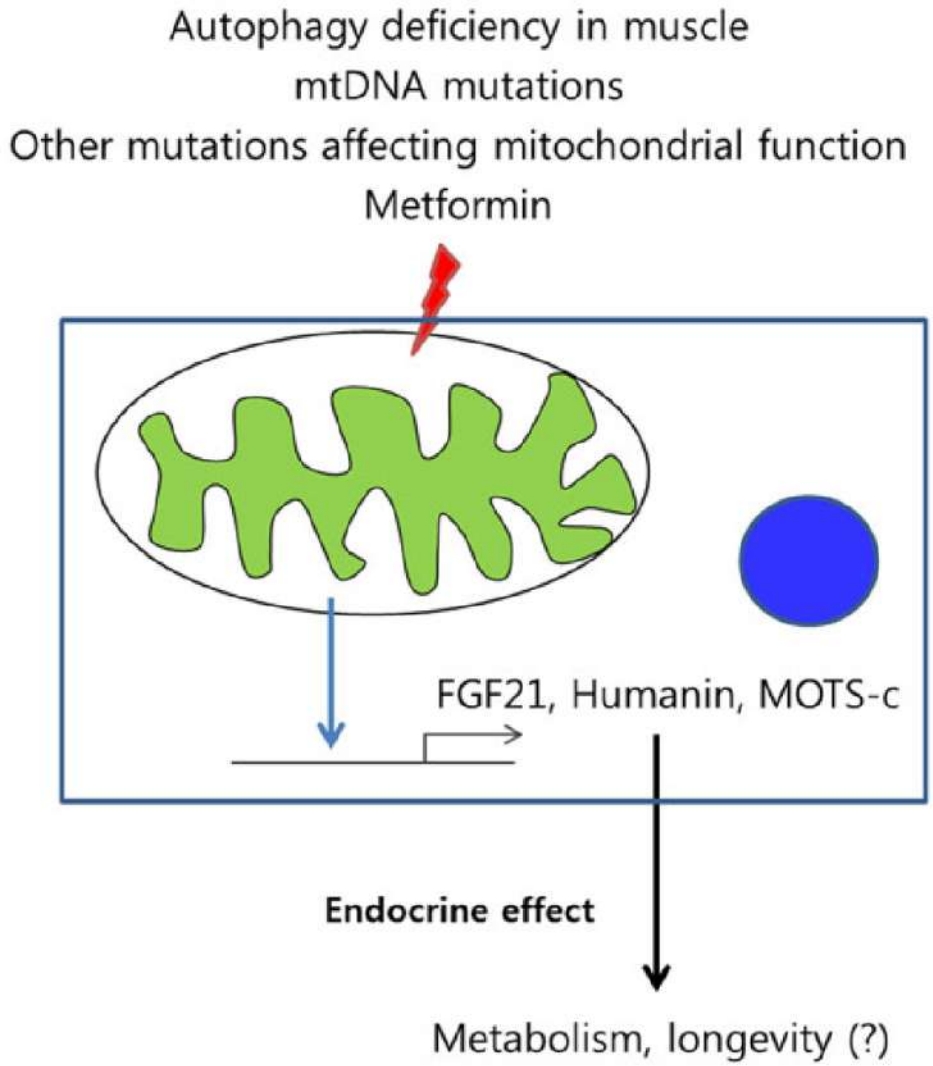


Figure 1. Non-cell-autonomous systemic effect of mitochondrial stress. Diverse mitochondrial stress can induce mitokines that can affect whole-body metabolism or longevity.

Lee 2015

- Mitokines are part of a stress-induced response that likely goes beyond mitochondria and entails the involvement of other organelles (ER, nucleus). It is debated whether they increase with age and how much they are expressed in long-lived people.
- Moreover, they are also found associated with a number of diseases, therefore it is not clear whether this response is always beneficial or can turn detrimental in certain conditions.

We then wondered whether centenarians were characterized by elevated levels of these mitokines with respect to elderly persons

THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2018, Vol. XX, No. XX, 1–8
doi:10.1093/gerona/gly153
Advance Access publication June 27, 2018

OXFORD

Original Article

Human Aging and Longevity Are Characterized by High Levels of Mitokines

Maria Conte, PhD,[1,2,*] Rita Ostan, PhD,[1,2,*] Cristina Fabbri, PhD,[1] Aurelia Santoro, PhD,[1,2] Giulia Guidarelli, MStat,[1] Giovanni Vitale, MD,[3,4] Daniela Mari, MD,[5] Federica Sevini, MSc,[1] Miriam Capri, PhD,[1,2] Marco Sandri, MD,[6,7] Daniela Monti, PhD,[8] Claudio Franceschi, MD,[9] and Stefano Salvioli, PhD[1,2]

[1]Department of Experimental, Diagnostic and Specialty Medicine (DIMES) and [2]Interdepartmental Centre "L. Galvani" (CIG), University of Bologna. [3]Department of Clinical Sciences and Community Health, University of Milan. [4]Laboratory of Geriatric and Oncologic Neuroendocrinology Research, Istituto Auxologico Italiano IRCCS, Cusano Milanino. [5]Geriatric Unit, Fondazione Ca' Granda, IRCCS Ospedale Maggiore Policlinico, Milan. [6]Venetian Institute of Molecular Medicine, Padova. [7]Department of Biomedical Science, University of Padova, Padova. [8]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence. [9]IRCCS, Institute of Neurological Sciences of Bologna, Bologna, Italy.

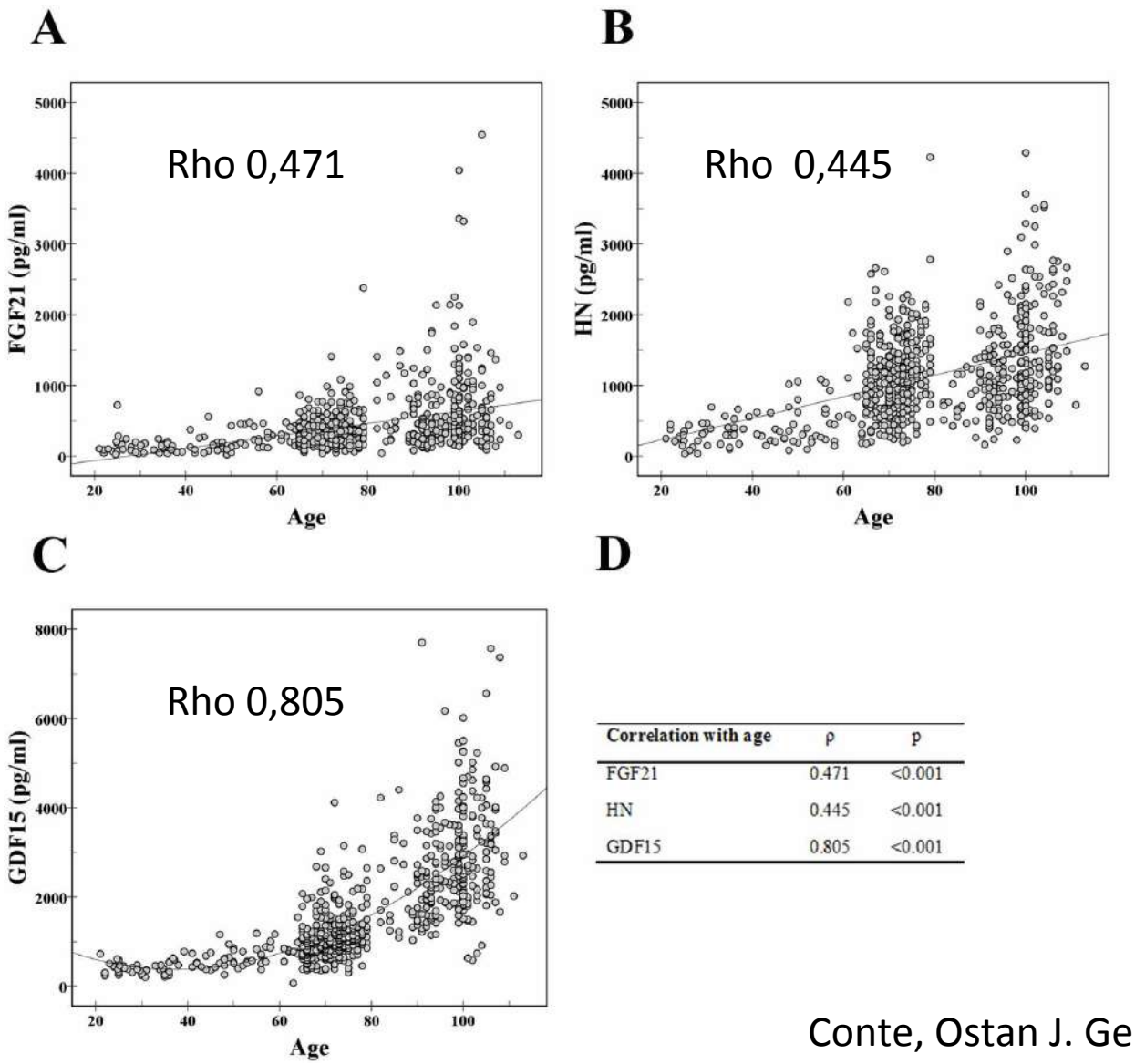
FGF21, Humanin and GDF15
Studied cohort

	YA	EL	OO	CENT
N	79	336	155	123
Men/Women, N	22/57	163/173	66/89	32/91
Age (yrs)	41.0 [20.0]	72.0 [6.0]	94.0 [7.0]	102.0 [5.0]
BMI (kg/m^2)	22.97 [4.53]	26.9 [4.6]	25.56 [4.79]	23.95 [4.31]
Handgrip (Kg)	33.25 [12.6]	31.0 [16.0]	18.0 [10.6]	11.5 [9.0]
HOMA-IR	1.37 [0.61]	2.11 [2.14]	0.99 [1.17]	0.9 [1.23]
HDL cholesterol (mg/dL)	53.0 [24.0]	54.76 [22.2]	55.0 [24.0]	45.0 [17.0]
Triglycerides (mg/dL)	73.75 [59.3]	98.4 [53.2]	103.0 [51.0]	108.0 [55.5]
CRP (mg/dL)	0.95 [1.8]	1.09 [1.9]	4.0 [1.0]	3.8 [10.2]
Albumin (g/dL)	4.45 [0.5]	4.33 [0.4]	4.5 [1.9]	3.7 [0.8]
Total protein (g/dL)	7.19 [0.5]	7.2 [0.84]	6.89 [0.88]	6.67 [0.9]
Uric acid (mg/L)	4.25 [1.76]	5.32 [1.81]	1.43 [3.74]	5.5 [2.33]
Creatinine (mg/L)	0.89 [0.14]	0.84 [0.26]	1.11 [0.46]	1.03 [0.55]

Median and [interquartile range]

Conte, Ostan J. Gerontol, 2018

FGF21, Humanin and GDF15 plasma levels show a positive correlation with age



Correlation with age	ρ	p
FGF21	0.471	<0.001
HN	0.445	<0.001
GDF15	0.805	<0.001

Conte, Ostan J. Gerontol, 2018

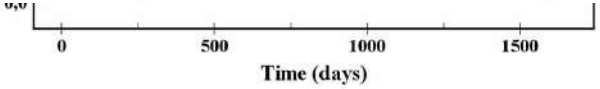
Nonagenarians and centenarians with low level of mitokines (LL) have a slight but significant survival advantage

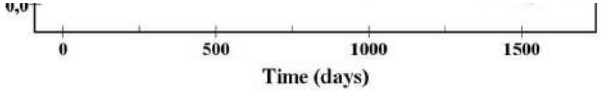
Nonagenarians

Centenarians

Hazard ratio			
	B coefficient	Exp(B) (95% C.I.)	p
FGF21	0.25	1.29 (1.04-1.60)	0.019
HN	0.32	1.37 (1.01-1.86)	0.042
GDF15	**0.61**	**1.84 (1.19-2.85)**	**0.006**

Supplementary Table 4. Association of mitokines with mortality in nonagenarians and centenarians estimated by a Cox regression model with log-transformed variables adjusted for age and gender.





Estimated survival:
LL 1,142 days
HL 691 days

Estimated survival:
LL 530 days
HL 386 days

Hazard ratio			
	B coefficient	Exp(B) (95% C.I.)	p
Low mitokines	-	-	-
High mitokines	0.789	2.2 (1.4-3.4)	**0.000**

Hazard ratio			
	B coefficient	Exp(B) (95% C.I.)	p
Low mitokines	-	-	-
High mitokines	0.554	1.7 (1.1-2.7)	**0.018**

Elderly subjects with low levels (LL) of FGF21 and GDF15 have better biochemical parameters than those with high levels (HL)

Median [interquartile range]

Group (N)	LL (273)	HL (63)	p
Men/Women (N)	127/146	36/27	0.130
Age, mean (SD)	71.0 (4.0)	72.7 (3.9)	0.011
FGF21	284.1	431.5	0.000
HN (pg/mL)	1086.2	1047.1	0.876
GDF15 (pg/mL)	952.0	1835.2	0.000
BMI (kg/m^2)	26.6 [4.6]	28.6 [5.0]	0.007
Glycemia (mg/dL)	98.2 [14.7]	103.2 [21.0]	0.012
Insulin (μU/mL)	8.6 [7.9]	10.0 [8.5]	0.007
HOMA-IR index	2.0 [2.0]	2.6 [2.6]	0.002
Total cholesterol	200.0 [44.6]	186.5 [49.5]	0.036
HDL cholesterol	55.9 [22.0]	49.8 [25.7]	0.015
Triglycerides	96.3 [49.7]	107.6 [78.7]	0.037
C-Reactive Protein	1.0[1.7]	1.3 [2.5]	0.012
Creatinine (mg/dL)	0.8 [0.2]	0.9 [0.3]	0.003

Conte, Ostan J. Gerontol, 2018

Conclusions /1

- Centenarians are characterized by elevated levels of mitokines, suggesting that they have a working mechanism of response to (mitochondrial) stress.

- The preponderant and most upregulated mitokine appears to be GDF15, which is also emerging as the most altered serum protein during aging.

- However, high levels of mitokines are correlated with diseases and mortality (in particular GDF15) indicating a compromised health condition that is not rescued by the positive effect of these mitokines.

- GDF15 is more sensitive to mitochondrial stress and health status than FGF21 and HN.

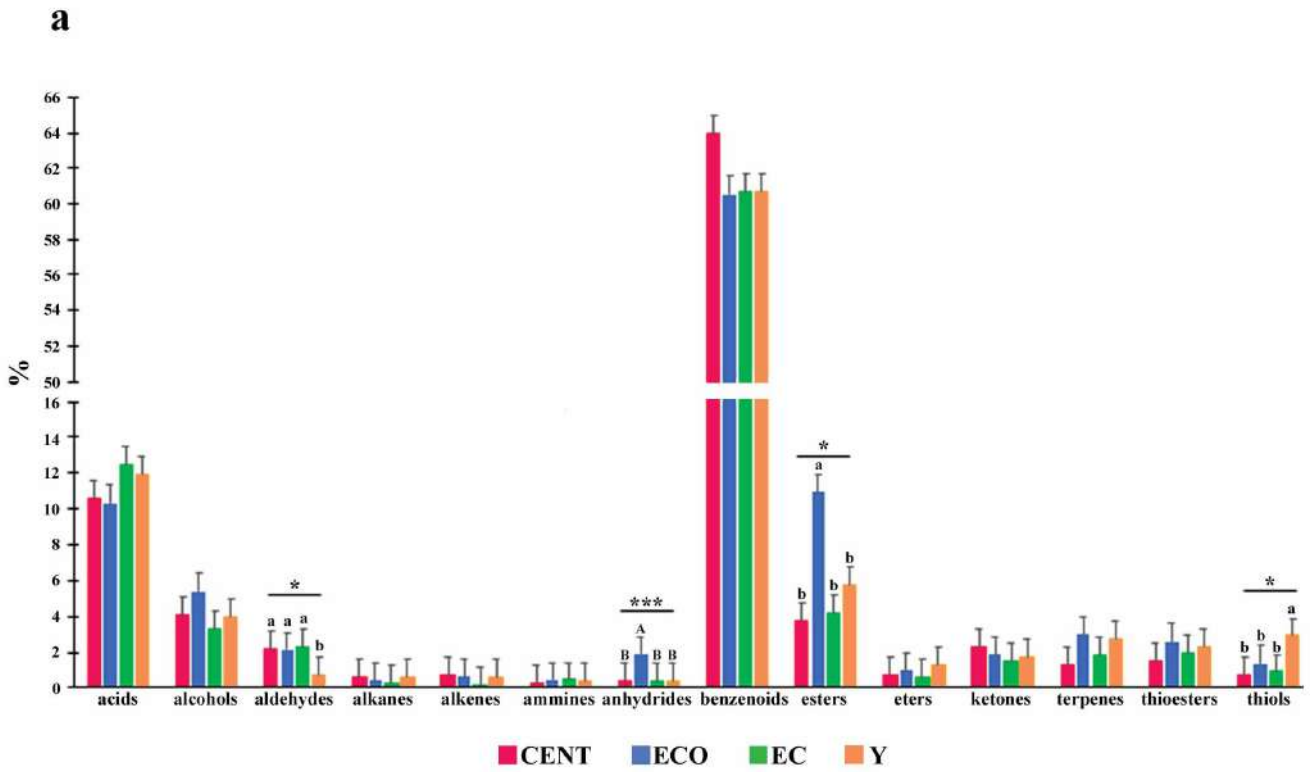
Looking for metabolic markers that could discriminate age groups, we studied Volatile Organic Compounds (VOCs) in feces and urine of 73 volunteers from 24 to 106 years of age, including CO and their spouses…

Group (N°)	Age range	Sex (N°)	Average number of diseases	Type of diseases (N°)
Y **(15)**	24-36	4M, 11F	0	---
EC **(20)**	61-85	12M, 8F	1.8	arthrosis (7), chronic obstructive pulmonary disease (3), chronic renal failure (1), diabetes (2), hypercholesterolemia (5), hypertension (10), hypothyroidism (2), osteoporosis (4)
ECO **(16)**	67-76	6M, 10F	0.6	arthrosis (2), hypercholesterolemia (2), hypothyroidism (2), osteoporosis (4)
CENT **(22)**	100-106	4M, 18F	3.1	angina pectoris (2), autoimmune diseases (1), cancer (in remission) (1), chronic obstructive pulmonary disease (4), chronic renal failure (3), dementia (2), depression/anxiety (3), diabetes (3), heart failure (5), hepatitis (1), hypertension (14), hyperuricemia (2), hypothyroidism (2), irregular heart rhythm (6), osteoporosis (6)

- A total number of 110 VOCs were detected in fecal samples and 33 in urine samples by means of microextraction-gas chromatography-mass spectrometry technique (SPME-GC/MS) .

- Some of these VOCs were present only in specific age groups, suggesting that they can be used as markers of age.

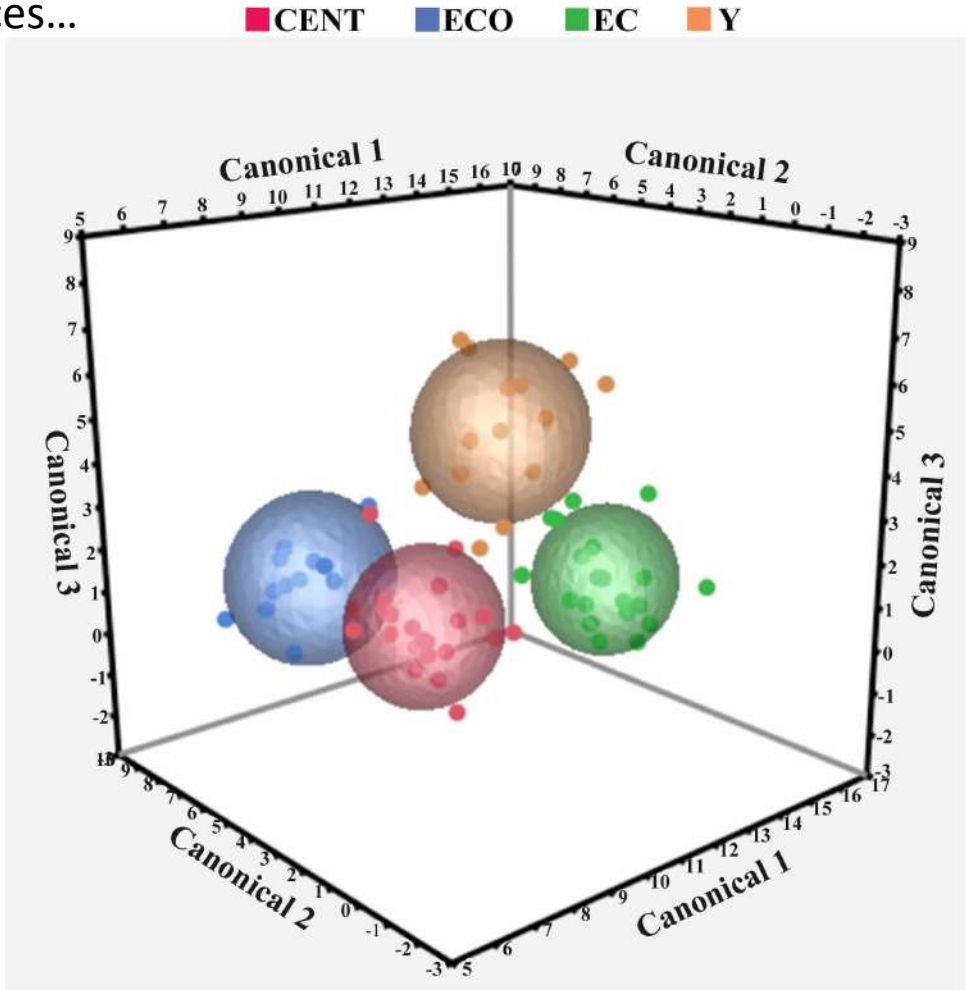
- Relative abundance is also modified according to age for specific classes of VOCs, in particular in feces.



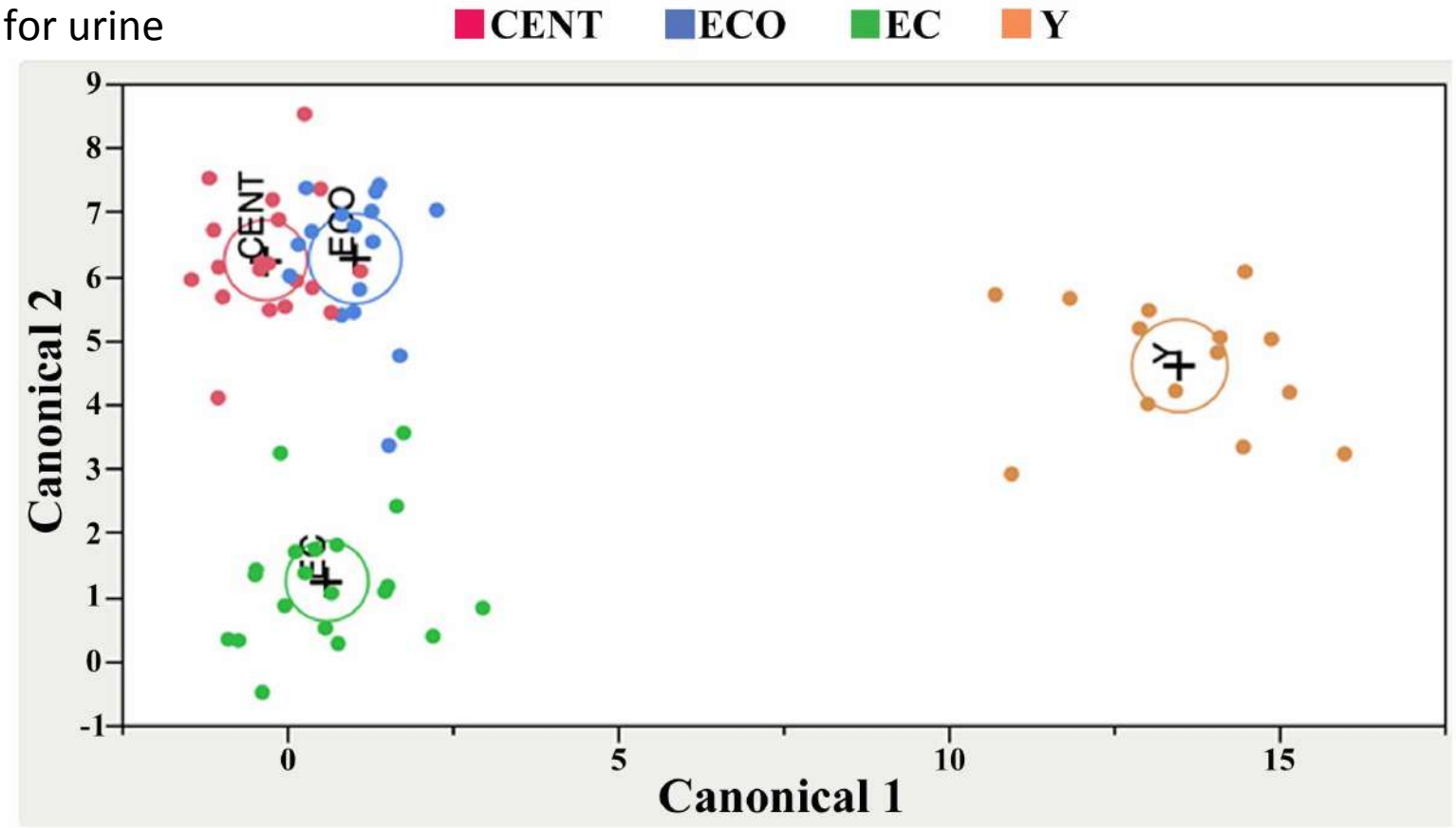
Conte M et al.,
Geroscience 2019, in press

❖ Accordingly, a discriminant analysis is able to clearly separate the 4 groups (young, elderly, centenarians and centenarians' offspring).

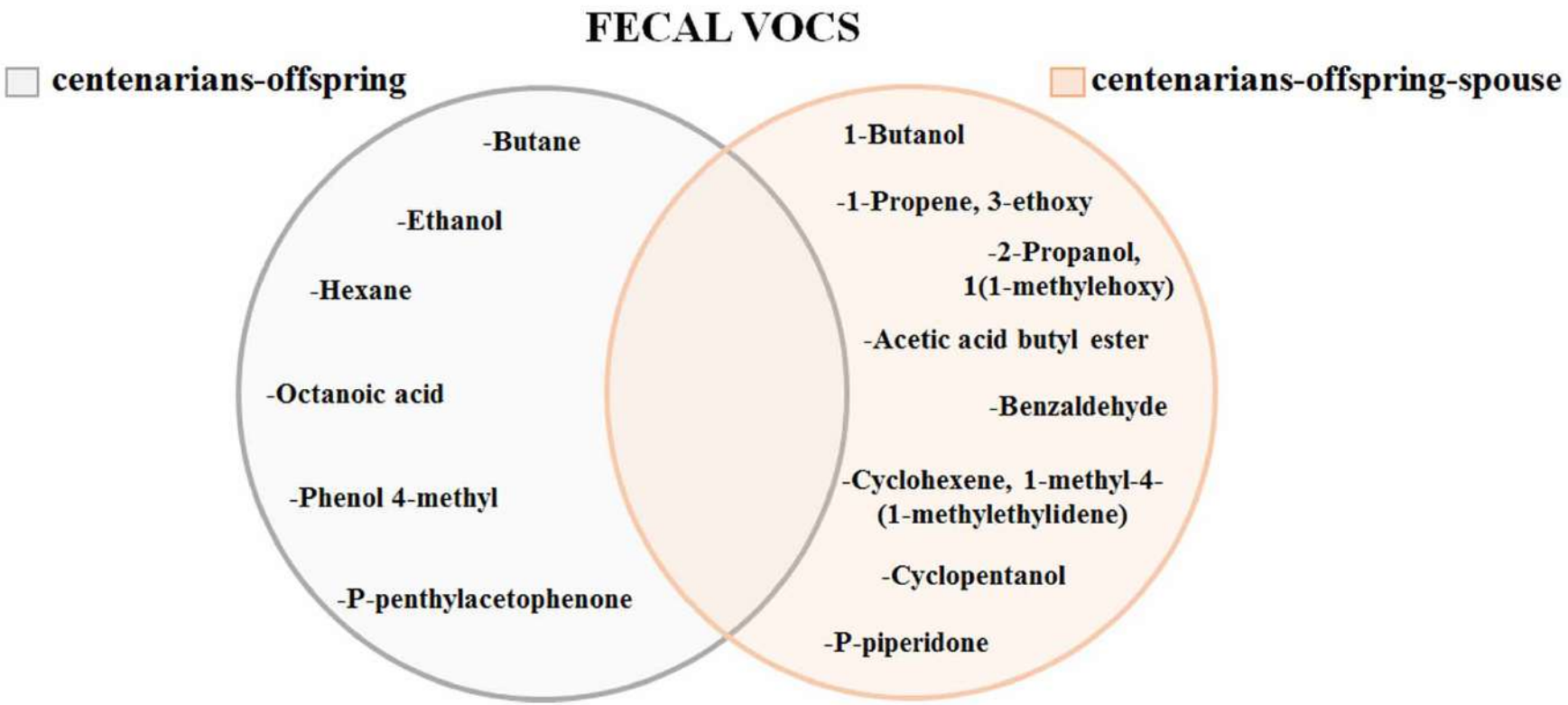
For feces…

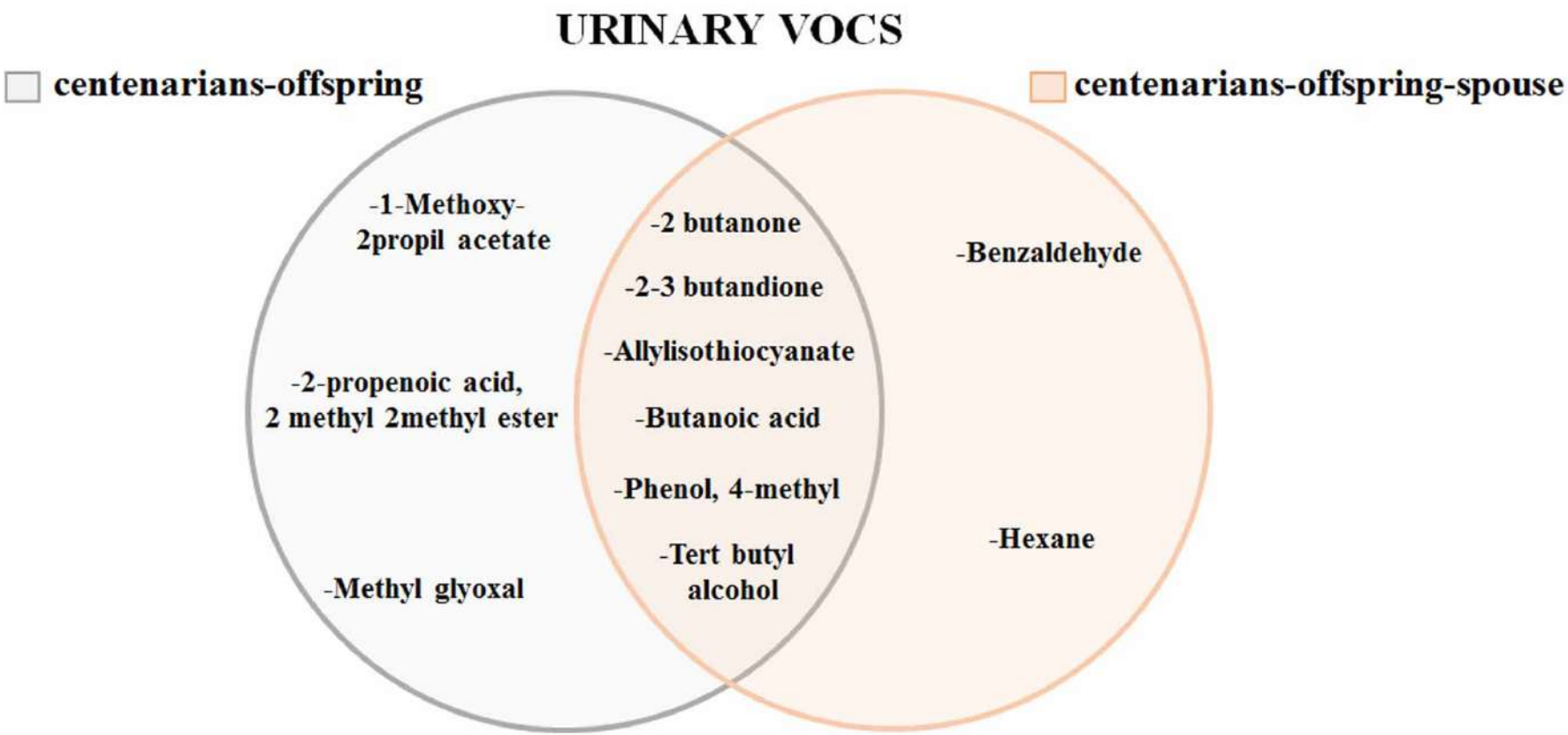


… and for urine



❖ Taking advantage of the presence of trios centenarian-offspring-spouse, we were able to identify VOCs that are shared by couples (family trait) or trios (environmental influence).




URINARY VOCS
centenarians-offspring
centenarians-offspring-spouse
-1-Methoxy-
2propil acetate
-2-propenoic acid,
2 methyl 2methyl ester
-Methyl glyoxal
-2 butanone
-2-3 butandione
-Allylisothiocyanate
-Butanoic acid
-Phenol, 4-methyl
-Tert butyl
alcohol
-Benzaldehyde
-Hexane

❖ *As a whole, the most discriminative VOCs associated with the different aging phenotypes appear to be the following:*

FECES		Discrimination
VOC	**ASSOCIATION WITH**	
formic acid, butyl ester	Healthy aging and longevity	EC from ECO and CENT
4-carene	Healthy aging and longevity	
1H-indole, 5 methyl	Healthy aging and longevity	
butanoic acid methyl ester	Age (within familiar longevity)	CENT from ECO
ethanol	Age (within familiar longevity)	
butanoic acid ethyl ester	Age (within familiar longevity)	
butanoic acid, 3-methyl-, 2-methyl propyl ester	Age (within familiar longevity)	
dimethyl trisulfide	Aging	Y from CENT, ECO, EC
1H-indole, 3 methyl	Aging	
URINE		
VOC	**ASSOCIATION WITH**	
2-butanone	Aging	Y from CENT, ECO, EC
isopropyl alcohol	Aging	
2-pentanone	Aging	
3-penten-1-ol	Aging	
pyrrole	Aging	
carbon dioxide	Healthy aging and longevity	CENT and ECO from EC
benzaldehyde 2,3,6-thrichloro	Healthy aging and longevity	
acetone	Healthy aging and longevity	
trichloromethane	Healthy aging and longevity	
benzaldehyde	Healthy aging and longevity	
butanoic acid	Healthy aging and longevity	

Conclusions /2

- VOCs can be a mirror of the organismal metabolism and proved to be able to discriminate age groups.

- Interestingly, VOCs appear able to discriminate also health status (EC vs ECO) and are affected by familiar components (genetics?).

- All together, mitokines and VOCs could add up to the list of biomarkers of age useful to dissect chronological and biological age, and to identify subjects at higher risk for age-related diseases. Further studies are needed to formally prove their predictive capacity as biomarkers.

Thanks to:
Maria Conte

Paolo Garagnani
Miriam Capri
Aurelia Santoro

Maria Giulia Bacalini
Salvatore Collura
Noémie Gensous
Cristina Giuliani
Giulia Guidarelli
Elena Marasco
Morena Martucci
Cristina Morsiani
Chiara Pirazzini
Francesco Ravaioli

Prof. Giuseppe Conte, Andrea Serra and Marcello Mele – Università di Pisa
Prof. Fabiola Olivieri, Emanuela Mensà, Jacopo Sabbatinelli – Università Politecnica delle Marche

European Commission

H2020 Project 634821

PROPAG AGEING

JPND research

ADAGE
Alzheimer's Disease pathology within the ageing physiology