EXECUTIVE SUMMARY

Nutrition represents an easily modifiable factor able to contrast inflammation and oxidative stress. This idea results particularly significant considering that in the last years, among population it is emerging the tendency to consume food that in addition to their nutritional content may provide some benefits to health not only preventing the risk of diseases but also facilitating the path to recovery and improving survival. **Growing evidence indicates the beneficial and preventive role of the MedDiet in the onset of diseases associated with increased level of inflammation**. However, studies considering in a comprehensive and integrated way the effect of a whole balanced MedDiet, followed for a consistent period of time, on inflammageing, the chronic and systemic inflammation typical of ageing, are still scantly in literature.

The **overall aim of the NU-AGE project is to improve health and quality of life in the EU ageing population by counteracting inflammageing through a whole MedDiet approach**. The project has the objective to fill in the current lack of knowledge on how the whole diet (and thus the integration of different nutrients) can impact on and counteract age-related decline.

NU-AGE proposes a comprehensive dietary strategy, involving not only scientists, but also a significant number of companies, including SMEs, from different EU countries who are fully committed to design advanced traditional food tailored for consumers over 65 years. The NU-AGE consortium is enriched by European Federations targeting all the stakeholders from consumers, to EU Food & Drink Industries, and policy makers.

The core activity of the project is the nutritional trial administered to a final number of 1149 volunteers in Italy, France, UK, The Netherlands and Poland, well balanced per gender (male percentage is 44%) and including a 20% of pre-frail subjects. All the subjects underwent an in depth characterization covering anthropometry, health and medical status, cognitive and physical functions and a series of biochemical and inflammatory measures. Moreover, in a selected subgroup of 120 volunteers advanced immunology, genetics, epigenetics, transcriptomics, metagenomics and metabolomics measures have been performed to test the efficacy and effectiveness of the NU-AGE diet on functional decline in older people through the evaluation of a series of cellular and molecular parameters as well as the general health status in all the over 65 subjects enrolled in NU-AGE.

The first outcomes obtained in the project indicate that that the NU-AGE dietary intervention was well accepted by European elderly population showing that old people are able to change dietary habits towards their nutritional needs taking advantages on specific health outcomes. Indeed, those volunteers who were most adherent to the NU-AGE diet decreased the plasma

levels of C-reactive protein, one of the main inflammatory marker that represented the primary outcome of the NU-AGE intervention and improve their lipid profile. Also the gut microbiota diversity is preserved in volunteers following the NU-AGE diet.

The NU-AGE study demonstrate that nationality and gender and, to a smaller extent, other factors, such as genetics, and a individual overall lifestyle could impact on the effectiveness of a specific dietary regime (in this case, MedDiet). Thus, this research has great potential to contribute to the improvement of the quality of life of European senior citizens.

Eight new advanced traditional food tailored made for over 65-years consumers and 6 elderly-tailored food prototypes have been designed within the framework of the NU-AGE project, thanks to the intense and continue collaboration between researchers and food industries.

Therefore, we can foresee that NU-AGE will have a large impact in Europe on all the stakeholders stemming from consumers to EU Food and Drink Industries and policy makers.

SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

List of Abbreviations

ADL/IADL: Activities of Daily Living/ Instrumental Activities of Daily Living
ASAT: Aspartate Transaminase
ALT: Alanine Transaminase BMD: Bone Mass Density
BMI: Body Mass Index
CES-D: Center for Epidemiologic Studies – Depression scale
CHD: Coronary Heart Disease
CTLs: Cytotoxic T Lymphocytes
CVD: Cardiovascular Diseases
DC: Dendritic Cells
DHEAS: Dehydroepiandrosterone
DXA: Dual-energy X-ray Absorptiometry or bone densitometry
ETP: European Technology Platform
GDS: Geriatric Depression Scale
HBa1c: Glycosylated Haemoglobin
HCMV: Cytomegalovirus
HITChip array: Human Intestinal Tract Chip array
hsCRP: High Sensitive C-Reactive Protein
IFN-ɣ: Interferon-ɣ
IL-6: Interleukin-6
IL-10: Interleukin-10
IL-17: Interleukin-17
MMSE: Mini Mental State Examintion
NF-κB: Nuclear Factor-κB
PBMC: Peripheral Blood Mononuclear Cells
PTH: Parathyroid hormone
PUFA: Polyunsaturated Fatty Acid
PWV/ FMD: Pulse Wave Velocity / Flow-mediated Vasodilation
RDA: Recommended Daily Allowance
RDP: Ribosomal Data Base Project
ROS: Reactive Oxygen Species
SPES: Spread European Safety
TDU: Training and Dissemination Units
TLR: Toll Like Receptor
TGF-β1: Transforming Growth Factor- β1
TNF-α: Tumor Necrosis Factor-α
TSM: Techno Scientific Mediators
WBC: White Blood Cells

Summary Description of project context and objectives

CONTEXT

Future changes in both population demographics and lifespan demand that European public health policies focus on "healthy ageing", which not only includes the prevention of diseases but also delaying the deterioration of health status.

Human ageing is currently defined as a complex dynamic process involving the continual adaptation of the body to life-long exposure to internal and external damaging, conceptualized in the ''remodelling theory of ageing''. Accordingly, the ageing phenotype in humans is very heterogeneous and can be described as a complex mosaic resulting from the interaction of a variety of variables. **One of the key mechanisms of the ageing process is the development of a chronic, low grade and sterile inflammatory status named "Inflammageing"** (Franceschi et al., 2014) and this condition has emerged as critical in the onset of the pathogenesis of major age-related chronic diseases such as sarcopenia, frailty, and disability, thus contributing to elderly mortality. Taking into account the diversity of factors associated with ageing and longevity (environmental, stochastic and genetic-epigenetic variables) the environment is the most easily modifiable. Among environmental factors, life-style and diet play a central role in determining the quality of ageing. All available literature data suggest that the crucial pathological phenomenon of **inflammageing is reversible and can be at least in part counteracted and slowed down through changes in diet**.

The overall aim of the NU-AGE project is to improve health and quality of life in the European ageing population by **counteracting inflammageing through a whole diet approach**.

A number of FP6- and FP7-funded European projects have focused on different aspects of the diet that affect ageing. In most cases, these projects examined how a single nutrient or micronutrient impacts on the functionality of specific organs or systems. In contrast, **NU-AGE proposes a comprehensive dietary strategy employing not only scientists, but also industries and consumers as full partners, to deliver healthy ageing to elderly people**. Through this approach, NU-AGE project has the objective to fill the current lack of knowledge on how the whole diet based on Mediterranean pattern (and thus the integration of different nutrients) can impact on and counteract age-related decline.

The rationale of the project is based on evidence that single nutrients can impact on inflammatory parameters, and goes beyond that introducing the idea that a whole Mediterranean Diet (MedDiet) approach, could target not only a higher number of vulnerable processes involved in inflammation and ageing but also to study the synergy of multiple subtle effects.

Thus, NU-AGE project has studied in a comprehensive and integrated way, the effect of a whole MedDiet newly designed according to the nutritional needs of people over 65 years of age (hereafter called "NU-AGE diet"). 1296 volunteers (65-79 years old) have been characterized before and after the dietary intervention by measuring a number of robust parameters capable of providing reliable data about different domains/subsystems (health and nutritional status, physical and cognitive functions, immunological, biochemical and metabolic parameters). A sub-

group of subjects has been further characterized by advanced techniques (genetics, epigenetics) and high-throughput "omics" (transcriptomics, metagenomics, pyrosequencing, HITChip array) in order to identify cellular and molecular targets and mechanisms responsible for the effects of the NU-AGE diet intervention.

This approach has allowed, for the first time, an evaluation of the whole-organism response by a systems biology approach, considering several tissues and organs/systems as a functional network instead of assessing the single tissue and organ responses separately, as in previous studies, which thereby lost the fundamental cross-talk between tissues and organs/systems.

The NU-AGE interdisciplinary scientific partnership includes leading research centers in Europe on nutrition and ageing, with expertise in biogerontology, nutrition (dietary intervention and biomarkers), immunology and inflammation, intestinal health, genetics and epigenetics, advanced statistics and systems biology implementing a unique combination of traditional and novel measures to explore the effect of NU-AGE diet on functional decline in older people.

This expertise is complemented by leading EU multinational food industries interested in functional and enriched foods specifically tailored for the elderly market and by a significant number of companies, including SMEs, from different EU countries who are fully committed to design advanced traditional food tailored for consumers over 65-years. The NU-AGE consortium is enriched by European Federations targeting all the stakeholders from the fork to farm, from consumers to EU Food & Drink Industries, and policy makers. By considering the interactions among economic constraints, behavioral controls, socio-cultural and health factors, also the market and policy perspectives on dietary interventions will be carefully analyzed by leading economic and consumer science experts in order to promote the delivery of foods targeted to ageing populations. NU-AGE project has developed new foods in accordance to the Regulation (EC) N° 1924/2006 taking into account what the average consumer understands by claims on food.

This interdisciplinary trans-sectorial project integrates complementary approaches and perspectives into a unique knowledge basis that has been transferred to industries to deliver products, tools and services that will support the elderly in respect of recommended diets, lifestyles and advice for healthy longevity.

PROJECT SCIENTIFIC AND TECHNOLOGICAL OBJECTIVES

Along the five years course of the NU-AGE project, the following strategic, measurable, technological and specific objectives have been reached by the project partners in order to fulfill the aims of the project and gain the expected results.

STRATEGIC OBJECTIVES:

1. to design a new food pyramid specific for over 65-years EU citizens ("NU-AGE diet");
2. to enhance a multidisciplinary approach in unravelling the role of diet for the EU's ageing population, elucidating molecular and cellular mechanisms of action of the diet on healthy ageing and longevity;

3. to contribute to dietary standards, recommendations and food based guidelines for the elderly EU citizens;

4. to design industrially driven fortified foods specifically targeted to postpone ageing decline.

MEASURABLE AND TECHNOLOGICAL OBJECTIVES:

1. to demonstrate that a nutritional intervention based on NU-AGE diet can counteract the decline which occurs with ageing at the level of different organs, systems and subsystems by affecting the systemic inflammageing status;
2. to identify cellular and molecular targets responsible for the beneficial effect of NU-AGE diet on age-related decline and inflammageing, using an integrated approach to analyze both the low (see WPs 3 and 4) and high dimensionality "omics" data and in depth analysis of immune system and metabolismdata (see WP5).
3. to produce a series of functional food prototypes driven by large food companies to meet the nutritional, rheological and sensory requirements for the over 65s-ageing-targeted consumers;
4. to design new improved traditional foods which could better satisfy the specific nutritional needs of the elderly;
5. to produce tools to translate NU-AGE's findings into dietary recommendations for the elderly;
6. to produce best practice guidelines for communicating to ageing populations in Europe about food with nutrition/ health claims.

The above mentioned objectives have been reached with the following SPECIFIC OBJECTIVES:

A. To establish and standardize methodological tools (SOPs) for recruitment and characterization of elderly subjects. This specific objective was linked to: (i) Strategic objectives n. 1 and 2 (and functional to n. 3); (ii) Measurable and technological objective n. 1.

B. To design and execute a dietary intervention. This specific objective was linked to: (i) Strategic objectives n. 1 and 2 (and functional to n. 3); (ii) Measurable and technological objective n. 1.

C. To coordinate and execute non "omics" analyses on biological samples. This specific objective was linked to: (i) Strategic objectives n. 1 and 2 (and functional to n. 3); (ii) Measurable and technological objective n. 2.

D. To evaluate cellular and molecular effects of whole diets. This specific objective was linked to: (i) Strategic objectives n. 1 and 2 (and functional to n. 3); (ii) Measurable and technological objective n. 2.

E. To perform data management and systems biology modelling. This specific objective was linked to: (i) Strategic objectives n. 1 and 2 (and functional to n. 3); (ii) Measurable and technological objective n. 2.

F. To analyze socio-economic determinants of food choices and preferences of the elderly. This specific objective was linked to: (i) Strategic objectives n. 3 and 4; (ii) Measurable and technological objective n. 3 to 6.

G. To identify the best strategy for ensuring market access and consumers' acceptability for elderly-tailored foods by large companies. This specific objective was linked to: (i) Strategic objectives n. 4; (ii) Measurable and technological objective n. 3 and 4.

H. To design new advanced traditional foods. This specific objective was linked to: (i) Strategic objectives n. 4; (ii) Measurable and technological objective n. 3 and 4.

I. To carry out the industrial design and production of elderly-tailored food products. This specific objective was linked to: (i) Strategic objectives n. 4; (ii) Measurable and technological objective n. 3.

J. To analyze the legislative, economical and knowledge transfer framework. This specific objective was linked to: (i) Strategic objectives n. 3; (ii) Measurable and technological objective n. 5 and 6.

K. To implement dissemination activities. This specific objective was linked to: (i) Strategic objectives n. 3; (ii) Measurable and technological objectives from 5 and 6.

L. To these, an overall objective should be added, related to the coordination and supervision of both the scientific-technical and administrative issues of the project.

MAIN SCIENTIFIC &TECHNOLOGICAL RESULTS/FOREGROUNDS

1 A STANDARDIZED MULTI-CENTRED RECRUITMENT OF HEALTHY ELDERLY EUROPEAN VOLUNTEERS

The NU-AGE project, involving a consortium of 30 partners from 17 European countries, was aimed to recruit 1250 volunteers aged 65-79 years and free from overt disease, in equal numbers of each gender and assessed as either non- or pre- frail (according to the definition of frailty by Fried et al., 2001) from 5 different European study centres located in Italy, France, Poland, Netherlands and the UK. These centres have been strategically selected to represent different geographical areas covering Northern, Eastern, Western and Southern Europe.

The first effort of the consortium was to establish a standardized procedure for recruitment, inclusion and randomization in the NU-AGE nutritional trial for the volunteers to avoid any recruitment bias among the study centres.

The procedure was set in three stages. The first stage involved the identification and targeting of potential volunteers through a number of different recruitment strategies (advertisements, promotional leaflets, mail drops, recruitment lists and databases and the use of primary care facilities as well as networks, clubs and residences specifically for elderly people). Each centre adopted a specific range of tactics and these will be reassessed periodically to ensure that recruitment stays on track. All volunteers received detailed written information by members of the study team (in accordance with local practices). Following the exchange of information and basic eligibility questions, the volunteer underwent the next stage of recruitment if they were a) still interested in participating, and b) deemed eligible.

The second stage of recruitment involved the consenting process. Due to differences in local legislation some centres asked the volunteer to complete a consent form at home and return it to the study centre, whereas others have been asked to attend the local centre to sign the form with a member of the study team as a witness. Once consent is complete the admissions questionnaires will be administered as the final step of recruitment.

The final stage of recruitment included two admissions questionnaires. Part 1 assessed the current health status of each volunteers ensuring their eligibility for the study. If the outcome of this questionnaire was favourable, Part 2 of the admissions questionnaire was administered assessing the frailty status. Volunteers with a score indicating they are frail were excluded from the study.

Once all three stages are complete, successful volunteers were randomised to one of the two study groups (control or diet), stratified by gender, age, BMI and frailty score (non frail or pre-frail) and were scheduled for an appointment to attend the study centre for their baseline measurements.

Table 1 summarises all the measures that has been performed on the volunteers to characterize their health status before and after the 12-month dietary intervention. The general and the interview questionnaires were set and standardized among centres to collect information about clinical data, health and cognitive status, medical treatments, physical functioning, dietary and nutritional assessment and blood analyses. The recruiting staff from the five recruiting centres was ad hoc trained to avoid test administration and measurements bias. A detailed description of the NU-AGE study design, the recruitment strategy and the complete characterization of the volunteers has been published in Santoro et al., 2014 (Mechanisms of Aging and Development).

From January 2012 to January 2014 a total of 2,665 apparently healthy, independently living European participants aged 65–79 years were recruited of which 1,512 were screened for inclusion. A total of 1296 volunteers were included in the dietary intervention trial, of which 645 were randomized into the diet group and 651 into the control group.

Table 1. Complete overview of NU-AGE study parameters and endpoints.

	Measures	**Description**
Primary parameters		
Inflammatory status	C-Reactive Protein (hsCRP), positivity for HCMV, IL-1β, IL-12, INF-γ, IL-6, sIL-6R, IL-1RA, TNF-α, IL-17, IL-8, IL-10, TGF-β1	Plasma levels
Secondary parameters		
Insulin sensitivity	glucose, insulin, Hba1C, HOMA	Serum and whole blood levels
Liver function status	ALAT, ASAT, GGT, alkaline phosphatase, creatinine	Serum levels
Hormonal function status	leptin and adiponectin	Plasma levels
Bone health	Total, femur and spine bone mineral density 25-OH vitamin D, parathyroid hormone	Dual-energy X-ray Absorptiometry (DXA) Serum levels
Cardiovascular health status	Lipid profile (triglycerides, total, HDL- and LDL-cholesterol) Blood pressure	Plasma levels Electronic blood pressure monitor
Cognitive status	General health and cognitive status Episodic memory Depressive symptoms Attention, working memory, executive functions Speed, attention, working memory, executive functions CERAD test battery	Subjects cognitive complaints Babcock story recall Geriatric depression scale (GDS) Pattern Comparison and number cancellation tests Trail making test Global measure of cognitive function
Mental health and quality of life	Depression and health related quality of life	SF-36v2
Physical functioning	Hand grip strength Physical performance Functional status and physical activity	Hand Dynamometer to the nearest 0.1 kg Sum score of 6 minute walking distance, Activities of Daily Living (ADL) scale, Instrumental Activities of Daily Living scale ADL and IADL, PASE questionnaire, Short Physical Performance Battery (SPPB), Triaxial accelerometer for a week (Actigraph or Armband)
Digestive health status	Bowel function	Evacuation frequency

Anthropometry	Height Weight Waist- and hip circumference Body composition, visceral fat	Person standing erect, wearing no shoes, to the nearest 0.1 cm. Person wearing light garments, no shoes and empty pockets, to the nearest 0.5 kg Waist: either at the narrowest circumference of the torso or at the midpoint between the lower ribs and the iliac crest. Hip: measured horizontally at the level of the largest lateral extension of the hips or over the buttocks Dual-energy X-ray Absorptiometry (DXA)
Nutritional status	Micronutrient status (vitamin B12, folate, 25-OH vitamin D)	serum levels, SNAQ
Genetics	Genome wide analysis	Illumina microarray (Illumina OmniExpress Bead chip) containing 713014 SNPs on DNA from whole blood
Biomarkers of ageing	Telomeres Length	Real time PCR on DNA from PBMC and whole blood
Tertiary parameters*		
Metabolic profile	Targeted and Untargeted NMR and MS metabolomics	serum and urine levels
Inflammatory and immunological status	Transcriptomics Proteasome and immunoproteasome composition and activity Inflammatory molecules and regulatory cytokines	Microarray on mRNA from PBMC Western blot analysis, fluorimetric and ELISA assay on protein and cell lysate from PBMC Plasma levels and proliferation assay
Intestinal health and composition	Metagenomics	16 S sequencing and phylogenetic microarray profiling on faeces.

***(subgroup of selected 120 subjects before and after diet)**

2 A NEW ELDERLY TAILORED DIET BASED ON A MEDITERRANEAN DIETARY PATTERN: THE NU-AGE DIET

2.1 METHOD

The dietary intervention was performed between April 2012 and February 2015. Participants in the diet group received dietary advice aimed at meeting the nutritional requirements of the ageing population, as based on national existing nutrient reference values and food based dietary guidelines. Special attention was paid to nutrients that may be inadequate or limiting in diets of elderly, such as vitamin D, vitamin B12, and calcium. The NU-AGE diet emphasizes greater intakes of whole grains, fruits, vegetables, low-fat dairy and cheese, fish, low-fat meat and poultry, nuts, and olive oil, the use of a vitamin D supplement (10 µg) and lower intakes of alcohol, sodium and sweets.

Participants in the intervention group received a number of commercially available foods meeting the criteria of the NU-AGE dietary guidelines, i.e. wholegrain pasta [UNIBO (Coop Adriatica), for all centers], frozen vegetables soup [UNIBO (Coop Adriatica), Italy only], extra virgin olive oil (LESIEUR, for all centers), vitamin D supplements [WU (i.e. 10 µg, MCO Health), for all centers], margarine enriched with omega-3 and -6 [WU (Unilever Health Institute), for UK, the Netherlands and Poland only], low-salt and low-fat Milner cheese [WU (FrieslandCampina), for all centers]. With monthly meetings, dieticians supported and motivated the participants to follow the NU-AGE diet. The control group received leaflets with the national healthy eating guidelines only. All volunteers were contacted after 4 months and after 8 months to complete a follow-up questionnaire and at month 12 for the final visit. The details of the NU-AGE diet have been published in Berendsen et al., 2014 (Mechanisms of Aging and Development).

2.2 RESULTS

The NU-AGE dietary intervention was successfully completed in all 5 countries. A total of 576 participants in the intervention group and 573 in the control group completed the intervention, so the total number of drop-outs was 79 in the intervention group and 83 in the control group. Mean age of the participants was 71 years, 44% were males and the mean BMI as 27 kg/m^2. Dietary intake was assessed by means of 7 day food records. A NU-AGE diet score was developed to assess the adherence to the NU-AGE diet. NU-AGE diet score includes all 16 components of the NU-AGE diet and ranges from 0 to 160 points.

Compared to participants in the control group, participants in the intervention group of all countries managed to increase their NU-AGE diet score after on year of follow-up, ranging from 10 points in Italy to even up to 39 points in France (details are showed in Figure 1). In most countries, participants significantly increased their whole grain, low-fat dairy, fish, nuts/seeds, fluids and vitamin D intake at T1 compared to the baseline (T0). Fruits, vegetables, legumes and extravirgin olive oil intake were significantly increased in some of the countries. Interestingly differences emerged considering these results by gender.

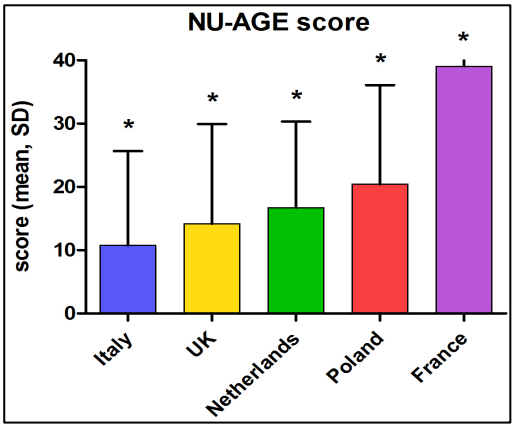


Figure 1. Change in the NU-AGE Diet score after the 1 year intervention by countries.

2.2.1 Acceptance and feasibility of the intervention

In all research centres the freely provided products - olive oil, margarines, pasta, cheese - were well accepted by the majority of participants. At the beginning of the intervention, there were some comments from the participants on the wholegrain pasta because this food is not commonly used by elderly population. For the same reason, some remarks were registered regarding extravirgin olive oil in the non-Mediterranean countries, cheese in the Southern countries and margarine in Poland. Issues were solved by handing out recipes and advices about how to use wholegrain pasta, olive oil, cheese and margarines and by providing information on the healthy aspects of these products. Despite these minor difficulties at the beginning of the study, all products were accepted by the majority of participants. In conclusion, NU-AGE dietary intervention was well accepted in an apparently healthy, independently living, ageing population.

2.2.2 Other Results

- Standardized and co-ordinated nutrients database and food groups database across all research centres.

- Availability of SOPs that were developed and used to standardize and align the intervention, the food supplementation, the monitoring of food intake and the compliance to NU-AGE diet in the 5 recruiting centres.

In conclusion, beneficial changes were observed regarding dietary intake after one year of dietary intervention providing dietary counselling, advices regarding the purchase, preparation and consumption of healthy foods, provision of foods and a vitamin D supplement. There were some differences per country though in which dietary goals they achieved. This study showed that elderly are able to change their dietary intake towards their nutritional needs. When effects of health outcomes are also confirmed, this research has great potential to contribute to improving the quality of life of European senior citizens.

3 THE IMPACT OF THE NU-AGE DIET ON THE INFLAMMATORY AND IMMUNOLOGICAL STATUS

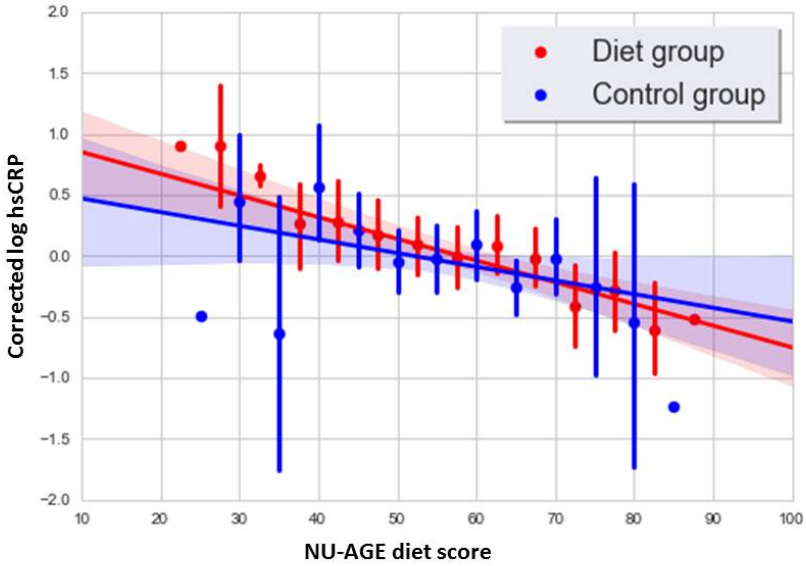
A variety of tissues, organs, systems (immune system) and ecosystems (gut microbiota) of the body can contribute to the onset and progression of inflammageing by increasing the production of a number of pro-inflammatory mediators, or lowering that of the anti-inflammatory ones, thus tilting the equilibrium toward inflammation.

The overall aim of the NU-AGE project is to improve health and quality of life in the EU ageing population by counteracting inflammageing using a whole diet intervention and evaluating the whole-organism response by a systems biology approach that considers different tissues and organs/systems as a functional network estabilishing continuos cross-talk. To address this aim a series of omics and non omics analyses have been performed on the biological samples from all the NU-AGE volunteers before and after the NU-AGE intervention. Most of the omics analyses have been conducted on a subgroup of 120 volunteers (60 Italians and 60 Polish, half pre-frail and half non frail).

3.1 INFLAMMATORY PARAMETERS

On the whole, data from all the subjects show **an inverse relationship between the adherence to NU-AGE diet** (calculated by NU-AGE diet score) **and plasma levels of C-reactive protein (hsCRP) (Figure 2)**, one of the main inflammatory marker that represented the primary outcome of the NU-AGE intervention.

Figure 2. Correlation between the adherence to NU-AGE diet (calculated by NU-AGE diet score) and plasma levels of hsCRP in diet and control group.



Leptin and adiponectin were measured at T0 and T1 with the ProcartaPlex™ multiplex Immunoassay from Affymetrix eBioscience® with the Luminex system, on the whole sample set. Interestingly, even if the cumulative statistical analysis did not show significant diet-associated differences in the levels of leptin and adiponectin and in the adiponectin/leptin ratio, **country-specific patterns were observed**. Indeed, in the pre-frail volunteers from The Netherlands there is a significant decrease of the leptin levels after NU-AGE dietary intervention. Furthermore, looking

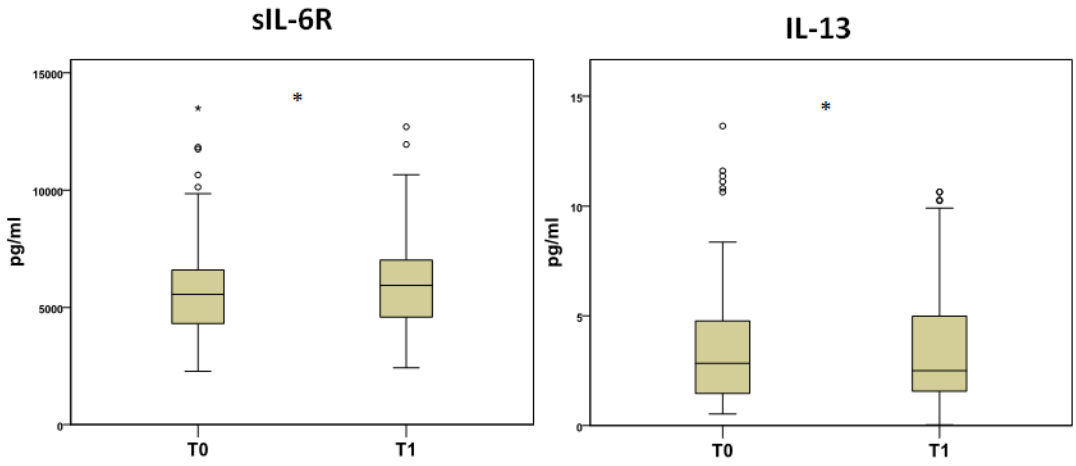
at the response of each country the results suggested that the UK, The Netherlands and Italy follow the same pattern while this appeared to be different for Poland and France. **Data would suggest that long-term local diet might affect the way individual population responded to the intervention**.

The analysis of plasma levels of inflammatory, anti-inflammatory cytokines and chemokines was conducted on the 120 subjects (at T0 and T1) of the subgroup for a total of 240 samples belonging to the intervention group.
The following molecules were analyzed by the Bio-Plex® Assays (Bio-Rad Laboratories, Inc, U.S.):

- Multiplex 17-plex Panel assay, for dosing IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17A, G-CSF, GM-CSF, INF-γ, MCP-1, MIP-1β, TNF-α. IL-1RA was added to this assay.
- Single assays for: IL-18, TGF-β1, sIL-6Rα.

As shown in figure 3 there was a significative increase in the level of the soluble receptor of IL-6 (p=0.026) and a significant decrease in the levels of IL-13 (p=0.030) after one year of dietary treatment.

Figure 3. Plasma levels of sIL-6R and IL-13 at T0 and T1.



Moreover in order to understand if the country (i.e. the life style and food habits) could impact on the inflammatory state, we compared the plasma levels in Italian and Polish samples (Mann-Withney comparison test) at baseline, before the diet intervention. As shown in table 2, the two countries are different, in particular Italian subjects have higher values of IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, MCP-1 and G-CSF with respect to the Polish ones, while the Polish subjects have significantly higher values of IL-18 and TGF-β.

Table 2. Comparison of plasma levels of cytokines and chemokines between Italian and Polish subjects at baseline (T0).

	n ITALY/POLAND	ITALY T0, median (min-max)	POLAND T0, median (min-max)	p value
IL-1β (pg/ml)	58/47	↑ 0.93 (0.03-7.54)	0.48 (0.01-5.7)	0.003
IL-4 (pg/ml)	58/44	↑ 1.78 (0.13-12.18)	1.07 (0.1-11.17)	0.032
IL-5 (pg/ml)	60/55	↑ 4.35 (0.28-24.91)	2.73 (0.0-17.82)	0.002
IL-6 (pg/ml)	60/54	↑ 7.50 (1.45-39.8)	4.74 (1.54-32.6)	0.002
IL-7 (pg/ml)	60/54	↑ 6.34 (1.8-26.95)	3.5 (1.04-21.35)	0.001
IL-8 (pg/ml)	60/54	↑ 11.93 (2.97-62.04)	8.62 (3.49-52.78)	0.011
IL-18 (pg/ml)	60/60	47.21 (6.46-176.19)	↑ 63.32 (23.22-146.37)	0.004
TGF-β (ng/ml)	60/60	26.43 (88.53-61.44)	↑ 38.33 (20.34-11.40)	<0.001
MCP-1 (pg/ml)	60/54	↑ 33.42 (15.83-73.02)	27.44 (9.7-102.6)	0.001
G-CSF (pg/ml)	59/54	↑ 23.79 (3.03-127.47)	11.56 (1.06-82.74)	0.005

Comparing the plasma levels of of cytokines and chemokines separately in Italian and Polish group before and after

the 1 year dietary intervention, no significant variation is observed in Italians, while in Polish there is a significant increase of sIL-6R ($p<0.0001$) and a significant decrease of TGF-β ($p=0.003$).

3.2 IN-DEPTH IMMUNOLOGY

A large variety of immunological parameters has been investigated in a cohort selected and recruited by the UEA (Norwich). The expression of co-stimulatory molecules (CD1, MHC Class II, CD40, CD80, CD86, CD152, CD154) has been studied after *in vitro* stimulation (with TLRS ligands) of PBMCs, then staining with fluorochromes and analyzed by Flow cytometry (Sony EC800 Analyser®). The results show that there is weak evidence ($p=0.0655$) that **the dietary intervention is associated with a reduction in MHC II % + cells upregulation.**

Total number of DC subpopulations, Myeloid DCs (MDCs) and plasmacytoid DCs (PDCs) were also determined by flow cytometry in whole blood using the "Blood Dendritic Cell Enumeration human kit" from Miltenyi Biotec®. No significant difference were found between control and intervention groups for the both MDCs and PDCs. However, there is a trend ($p>0.1574$) indicating that the dietary intervention is associated with a reduction in the ratio between MDCs and PDCs.

The number of NKT cells (TCR Vα24-Jα18$^+$ cells) were determined by flow cytometry in isolated PBMCs. No differences were found for the intervention group comparing to the control group.

The expression of NK cell and T cell markers (CD161, CD28, CD158d) was determined on isolated PBMCs. Flow cytometry analysis did not show any difference in the expression of these markers between control and intervention groups.

T cell (CD3$^+$CD8$^+$) proliferation was measured after *in vitro* stimulation (with Staphylococcal Enterotoxin B, SEB) of PBMCs, by flow cytometry using COULTER DNA PREP Reagents Kit®. We observed that dietary intervention did not have any effect on the T cell response to mitogen.

Also, oxidative burst activity of monocytes and granulocytes was measured in heparinized human whole blood with the Reagent kit Phagoburst (Phagoburst™ *Glycotope Biotechnology*), and analysed by Flow cytometry. No differences were found between the control and intervention groups.

Furthermore the CD8 T cell function was monitored. To this end, isolated PBMCs were stimulated with SEB as above and the expression of CD107 was assessed on CD3+CD8+ cells by flow cytometry. Dietary intervention had no effect ($p=0.13$) on the % of CD8 T cell degranulation.

Production of cytokines was analysed in the supernatant of the TLRs stimulated samples at before (T0) and after (T1) dietary intervention with the ProcartaPlex™ multiplex Immunoassay from Affymetrix eBioscience® (IFN-α, IFN-β, IFN-γ, IL-12p40, IL-12p70), using the Luminex® 100/200™ analyzer and with the ELISA kits from Wuxi Donglin Sci&Tech development CO., LTD (IL-12Rβ1, IL-12Rβ2, and SOCS3), using the microplate spectrophotometer (Benchmark Plus, Biorad®). No statistical differences were found for the production of cytokines after stimulation between the control and intervention groups. Minor diet-associated changes were observed. There is a trend towards increased production of IFN-β ($p=0.1056$) for the intervention group; in contrast a trend towards decreased production was seen for IL-12Rβ1 ($p=0.0929$) and Suppressor of Cytokine signalling (SOCS3; $p=0.1108$). A minor decrease of the IL-12p40/IL-12p70 ratio was observed ($p=0.1122$).

Analysis of the plasma levels of the cytokines (IL-2, IL-9, IL-22, IL-23) was performed at T0 and T1 by the ProcartaPlex™ multiplex Immunoassay from Affymetrix eBioscience®, using the Luminex® 100/200™ analyzer. IL-9 or IL-23 were not detected in the plasma samples of the volunteers and no differences were found in the plasma levels of IL-2 and IL-22 between control and intervention groups.

3.3 PROTEASOME AND IMMUNOPROTEASOME FUNCTION

Inflammageing is cause of the establishment of a chronic inflammatory environment in the cells and a source of oxidative stress which in turn can give origin to an accumulation of oxidized proteins, either due to increased protein damage or decreased removal of oxidized proteins. This process is directly associated with functional and structural modifications of a key cellular component, the proteasome.

Within the NU-AGE project, levels of oxidised proteins, along with proteasome composition and activity, on a selected subgroup of 120 volunteers from Italy and Poland were analysed before (T0) and after (T1) the NU-AGE dietary

intervention. In order to further elicit subjects' immunological status, a specialized type of proteasome complex (induced by inflammatory stimuli and constitutively active in immune cells), the so called the immunoproteasome, was also detected and quantified.

In general terms, statistical analysis confirmed a significant negative correlation between levels of oxidised/carbonylated proteins and proteasomal function both at T0 and T1.

Furthermore, regarding proteasome content and activity, subgroups of non-frail subjects and women seemed to receive a greater benefit from the NU-AGE dietary intervention.

Higher levels of proteasome content and activity were found in subjects with specific dietary habits (such as choosing foods that expect to keep them healthy, or high in proteins), and for whom in general the healthiness of food has significant impact on their choices, as well as in subjects that claim low levels of fatigue and sleep well, combined with generally positive attitude (considering themselves happy most of the time) and sensibly drink alcoholic beverages within the frames of a healthy diet. Lower levels of proteasome content were found in subjects with higher Body Mass Index (BMI).

Higher levels of immunoproteosome were observed in pre-frail subjects as well as in obese subjects (BMI > 30) at T0.

Regarding the pharmacological profile of the 120 volunteers, higher levels of oxidised proteins were found in subjects of both Italian and Polish subgroups under medication for high cholesterol at T0. Lower levels of oxidised proteins were detected after NU-AGE dietary intervention in subjects of the Polish subgroup who take aspirin as cardiovascular prevention. Lower levels of immunoproteasome at T0 were observed in subjects of the Italian subgroup that use prescribed medicine regularly.

3.4 METABOLIC MARKERS

An increased adherence to NU-AGE diet is associated to an improved lipid profile (in terms of total/HDL cholesterol ratio and triglycerides levels). NU-AGE dietary intervention had no significant effects on the metabolic markers (Albumin, Alkaline phosphatase, alanine Transaminase, aspartate aminotransferase, creatinine, gamma-glutamyl transpeptidase, glucose and uric acid).

4 THE USE OF ADVANCED OMICS TO ELUCIDATE MOLECULAR AND CELLULAR MECHANISMS MODIFIED BY THE NU-AGE DIET

4.1 METABOLOMICS

Mass spectrometry metabolomic untargeted analysis revealed subtle changes (~ 5% of significant differences between groups) between non-frail and pre-frail subjects at T0, with several markers of pre-frailty ($p<0.05$), both in urine (3 for males and 10 for females) and serum (between 20 and 30 for subjects who remained stable, improved or worsen their frailty status) samples.

Results revealed gender differences, as no common marker was found between male and female subjects.

Serum metabolomic data also highlighted frailty status differences, with the identification of three sub-phenotype (stable, improvement, degradation) with specific markers. These markers are different for male and female subjects.

Regarding the effects of the Mediterranean diet analysed in stable subjects, serum metabolomics allowed identifying food intake markers; 50 significant metabolites were common between males and females. Urine metabolomics also revealed food intake markers but with gender differences.

Finally, regarding markers of frailty, the results of T0 *vs* T1 ANOVA analyses on serum samples of changers subjects revealed i) one biomarker of improvement (in females, diet specific), also marker of pre-frailty at T0, ii) one biomarker of pre-frailty status change, iii) one biomarker of deterioration (diet not specific), also marker of pre-frailty at T0. Among them, few markers of diet were identified as 8 metabolites were also found to be correlated with the nutrients (in stables subjects). Moreover, serum metabolomics enabled to phenotype frailty status changes, as three biomarkers were identified.

In conclusion, these results showed that untargeted metabolomics:

- enables to discriminate sub-phenotypes of pre-frailty with gender differences and pre-frailty status,

- allows identifying food intake markers,
- enables to phenotype frailty status changes.

Moreover, nutritional assessment data and vitamins, metals, and amino acid compositions of plasma samples from individuals at baseline, by NMR, were analyzed. It has not been possible to find solid differences between not frail and pre-frail individuals on the basis of their blood levels of vitamin, metals and amino acid. These results confirm the high degree of similitude between phenotypes observed previously.

4.2 TRANSCRIPTOMICS

The study of the transcriptome was performed on PBMCs (Peripheral Blood Mononuclear Cells) collected from blood of participants at T0 and T1. A selection of genes related to inflammation and immune function was analyzed using low density TaqMan arrays (Applied Biosystems) comprising 90 genes of interest and 6 housekeeping genes. Total RNAs were isolated (Qiagen RNeasy kit, 74106*)* and their quality was checked by capillary electrophoresis from dry pellets of PBMCs from Italy: 120 samples (60 samples at T0 and 60 samples at T1) from control subjects and 120 samples (60 T0 samples and 60 T1 samples) from subjects belonging to diet group. PCR-grade RNAs were obtained from 86 subjects from the initial 120. Those RNAs were reverse-transcribed in cDNAs and the cDNAs were then amplified by quantitative PCR (Polymerase Chain Reaction) using a 7900HT Fast Real-Time System *(Applied Biosystems)* to establish gene signatures among the 90 selected genes. To compare the gene expression levels between T0 and T1, a 2-tailed paired *student-t* test was performed from *ΔCt* values at T0 and T1. The table below shows the genes that are statistically differentially expressed after the dietary intervention as compared to before intervention.

Dietary intervention group			
Gene symbol	*Up-or down-regulation*	*Mean of the Fold changes*	*Statistical level*
CCR2	↗	*1.41*	**
CD68	↗	*1.16*	*
CD8A	↗	*1.14*	*
CXCR3	↗	*1.27*	*
IFNG	↗	*1.37*	*
SELP	↗	*1.30*	**
SKI	↗	*1.13*	**
STAT3	↗	*1.15*	***
TGFB1	↗	*1.09*	**
TNF	↗	*1.25*	*

Within the control group), 11 genes were differentially expressed (2-tailed paired *student-t* test) at T1 as compared to T0. They are showed in the table below:

Control group			
Gene symbol	*Up-or down-regulation*	*Mean of the fold changes*	*Statistical level*
BAX	↗	*1.11*	***
CCR7	↘	*0.95*	*
CD3E	↘	*0.94*	*
GNLY	↗	*1.11*	*
IL12A	↗	*1.15*	*
IL15	↗	*1.23*	***
IL4	↘	*0.85*	***
PRF1	↗	*1.22*	**
SELP	↗	*1.23*	*
SKI	↗	*1.07*	*
TGFB1	↗	*1.07*	*

Despite the fact that all these genes are statistically differentially expressed, we can notice that the changes in expression levels are of very weak amplitude (fold change means closed to 1). To confirm the observations and identify novel blood biomarkers, it could be interesting to analyze the levels of expression of the corresponding proteins.

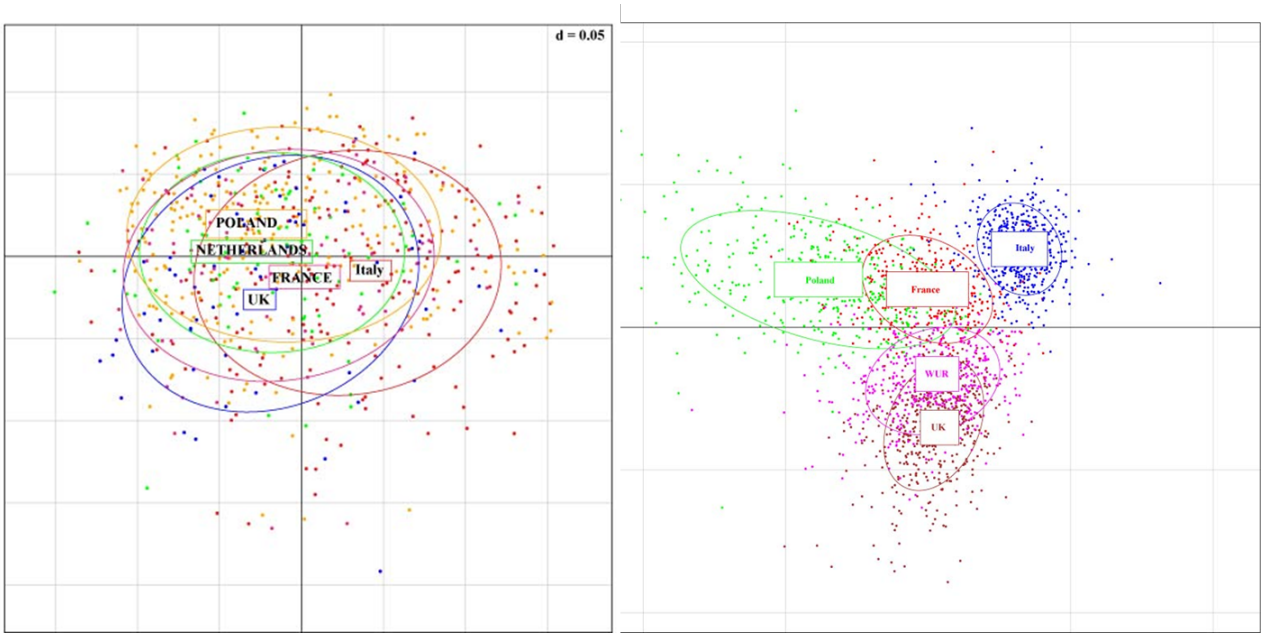
4.3 METAGENOMICS

The 16S Ribosomal RNA Gene sequencing on Illumina MiSeq System has been analysed in the NU-AGE volunteers from the five NU-AGE countries also the analyses of the microbiota composition of the fecal samples from the Dutch cohort (250 samples at T0 and 250 samples at T1) was conducted by using the HiTCHIP array.

In summary, the effect of dietary intervention was investigated using quantile regression analysis on the full dataset. A number of significant taxa were identified. These results are sensitive to adjustment for confounding variables and so more work is needed to identify significant taxa after adjustment. The HitChiP results reported an overall decrease in diversity across the whole dataset between T0 and T1. Analysis of the full dataset of subjects belonging to diet and control group confirmed this finding. The HitCHIP results also reported that this reduction in diversity was more pronounced in the control group. Diet group maintained a trend to lower diversity (p=0.058), whilst the control group showed a significant decrease in diversity (p=0.037). The dataset was further divided according NU-AGE diet score. Subjects were dividing in i) NU-AGE diet score under the median (53) at T0, ii) NU-AGE diet score over the median at T0, iii) NU-AGE diet score under the median at T1 and iv) NU-AGE diet score over the median at T1 for diet and control group; results showed that control **subjects with a poor diet at the end of the study were most affected by the loss of diversity**.

Moreover country differences emerged between the 5 recruiting centres (results summarized in Figure 4).

Figure 4. Left panel; PCoA ordination of samples from the five centres as plotted using the microbiome OTU dataset with the Spearman distance. Right Panel; PCoA ordination of the samples using the Food group information and Euclidean distance.



4.4 GENETICS

The genetic characterization of the cohort recruited in NUAGE was performed at genome wide levels by an Illumina microarray (Illumina OmniExpress Bead chip) containing 713014 SNPs. 1200 samples were genotyped and quality controls (QC) in attempt to remove false positive associations were performed. After quality controls 711631 SNPs and 1178 samples were retained. Since the high level of genetic diversity between populations and the impact of gene-environment interaction a discriminant analysis of principal component, with the aim of identifying and describing genetic clusters, has been performed. Starting from 711631 SNPs, we removed SNPs having an R2 value of greater than 0.1 with any other SNP within a 50-SNP sliding window (advanced by 10 SNPs each time), after filtering 81530 SNPs were retained for this analysis. In Figure 5 the results of the discriminant analysis of principal components (DAPC) are reported. All these analyses were performed using PLINK, Perl and R packages.

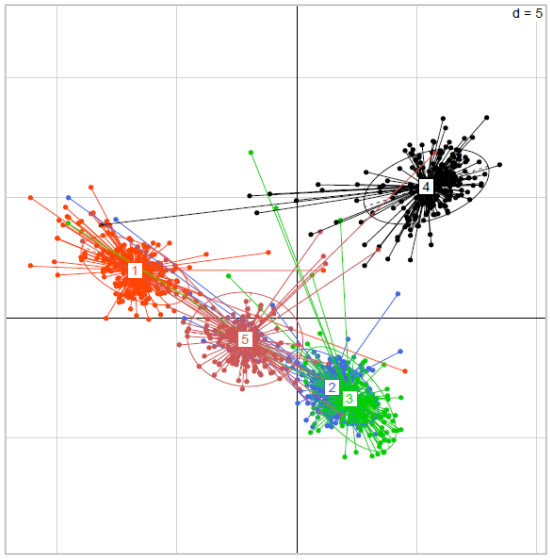


Figure 5. Discriminant analysis of principal components (1: Italy - red, 2: UK - blue, 3: The Netherlands - green, 4: Poland - black, 5: France - dark red).

4.5 EPIGENETICS

Epigenetics is influenced by environment and particularly life-style and nutrition could strongly impact on the methylation of the DNA. To this purpose we studied the epigenetic signature of a subgroup of NU-AGE subjects belonging to diet group at T0 and T1 to unravel the possible changes. The analysis of genome-wide blood DNA methylation profiles was conducted on the 120 subjects from the subgroup (60 Italians and 60 Polish at T0 and T1) using the Illumina Infinium technology. After quality checks to remove low quality samples and probes, different comparisons were run in order to identify the main sources of epigenetic variation among the samples. First, population-specific DNA methylation patterns were identified by comparing Italian and Polish subjects. This analysis showed a clear separation of the two populations (Figure 6, panel A). While the methylation status of some CpG probes was affected by the presence of SNPs in the target site (Figure 6, panel B), the differences observed for other CpG sites could be ascribed to proper epigenetic basis (Figure 6, panel C), thus suggesting the existence of factors that differently shape the epigenome of Italian and Polish populations.

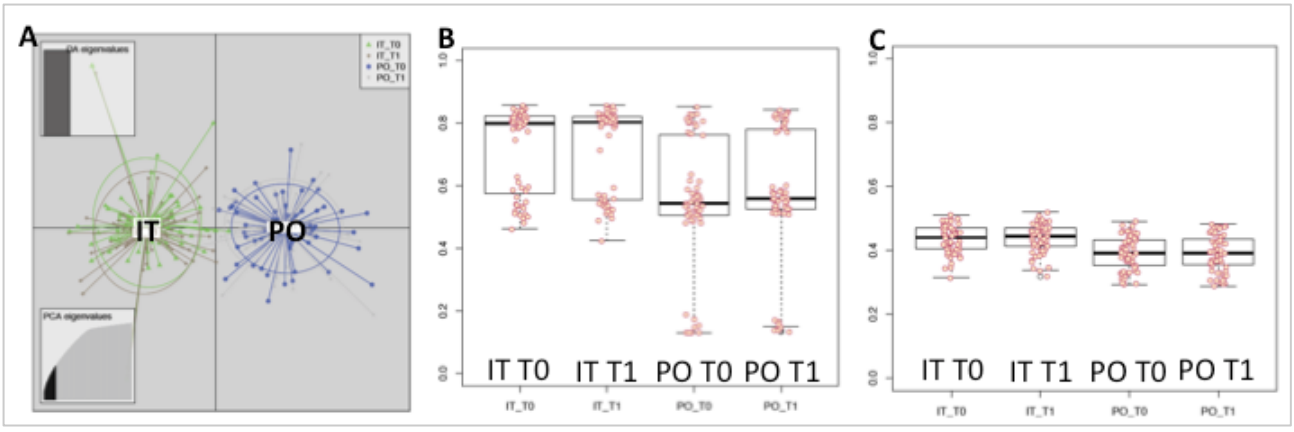


Figure 6. (A) Discriminant analysis of principal components on DNA methylation profiles. (B) Boxplots of methylation values for the probes cg13800652 (B) and cg16705929 (C).

Then, the effect of NU-AGE diet on the epigenetic age of the subjects, estimated using to the Horvath's epigenetic clock (Horvath, 2013), was evaluated. This analysis showed that the subjects experienced an epigenetic rejuvenation

(and, possibly, a biological rejuvenation) after the NU-AGE dietary intervention (Figure 7), which was sharper for Poland female subjects (paired t-test, p=0.007). Preliminary analysis performed taking into account the frailty status and its changes after the intervention suggest a trend to rejuvenation in the Polish subjects that changed their status from pre-frail to non-frail during the course of the study.

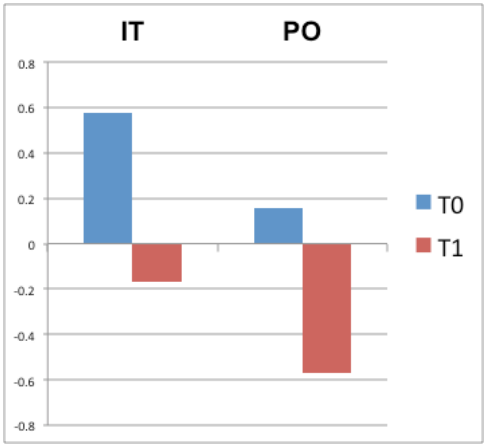


Figure 7. Intrinsic (unconfounded by differences in blood cell types) epigenetic age acceleration for Italian and Polish subjects at T0 and T1.

4.6 TELOMERE LENGTH

Telomeres are repetitive sequences that prevent end-to-end fusion and maintains chromosomal stability. Telomere length is becoming an important biomarker for cellular and biological stain put on tissues and can be affected by disease as well as lifestyle factors such as psychological stress. It is currently suggested that telomere length can serve as a biomarker of an individual's cumulative exposure to oxidative stress, thus, an indicator of health status. NU-AGE allowed to improve our understanding of the determinants of telomere length in European elderly individuals and to evaluate the effects of dietary changes on telomere length. Quantitative PCR-based analysis of telomere length was used to address these issues. This method is based on the measurement of the factor by which the sample differed from a reference DNA sample in its ratio of telomere repeat copy number to single copy gene copy number. We obtained a relative Telomere to Single copy gene (T/S) ratio, which is proportional to the average telomere length. Measurements were performed on on whole blood samples, which were frozen after collection in EDTA vacutainers and stored at -80^{o}C . Blood DNA kit (Macherey-Nagel, Germany) was used to extract genomic DNA according to manufacturers' instructions. PCR-based telomere length assessment method was improved by using a commercially available assay developed by Qiagen (Rotor-Gene SYBR Green Kit, Qiagen, USA). A standard deviation of less than 0.3 was required to accept the sample as valid. An intra-assay CV of less than 0,6% and inter-assay CV of less than 2,1% was always observed.

Females show longer telomeres than males indicating significant differences between gender in telomere length. Moreover, participants from Italy, Netherlands and France showed longer telomere than Poland and United Kingdom ones indicating significant differences in telomere length between countries, where (Figure 8).

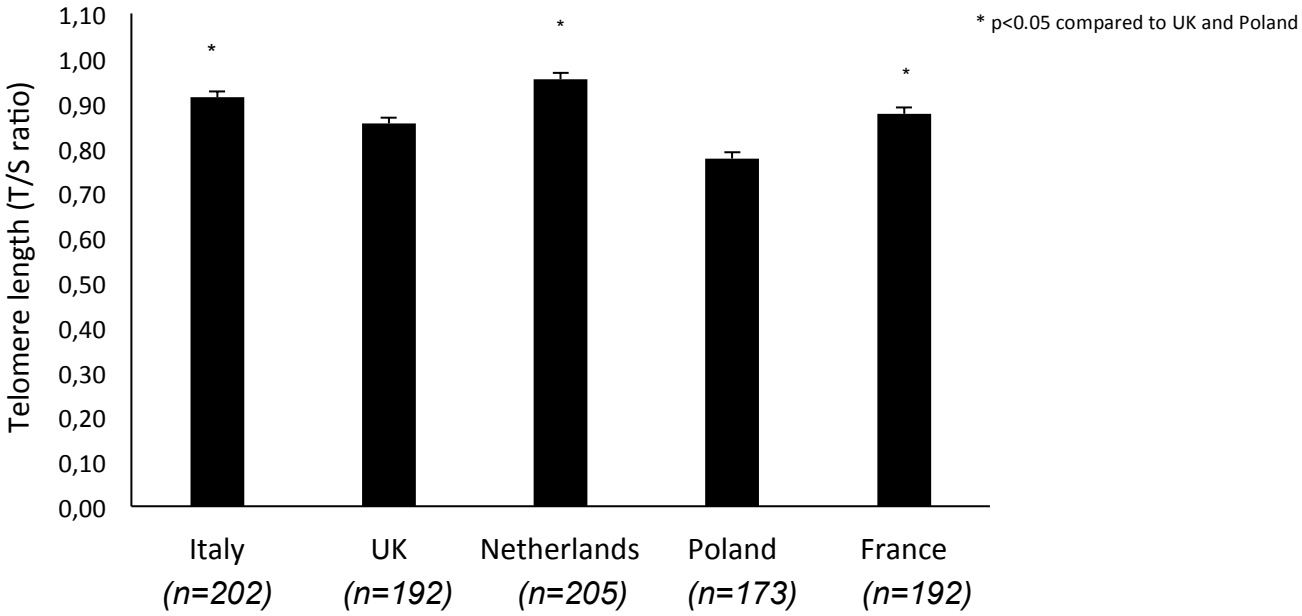


Figure 8. Telomere length in subjects from Italy, UK, Netherlands, Poland and France.

Elderly over 75 years had shorter telomere length than elderly aged between 65-75 yr. Following the dietary intervention, telomere length in the elderly with the shorter telomere length was significantly improved.

5 NU-AGE DATABASE

The NU-AGE project developed the templates necessary for long-term storage of multi-centre data in the Phenotype and Omics database. In addition, the design and meta-data of the study were uploaded to the database.

The NUAGE study is composed of several sub-datasets, where several varieties of measurements have been performed on different subgroups. This has been done to allow to obtain as many information as possible on the patients; to optimize the information gathered we had to reduce the number of subjects for the most expensive tests, so the sub-populations were carefully chosen. To assess the population effect of the diet the two most different populations has been chosen, Italian and Polish. To investigate the effects on the frailty development, a subset of subjects whose frail status changed with time was selected. The resulting database contains both wide range information about subjects in all of Europe, and extremely detailed information about hundreds of subjects undergoing the diet. This database will become public in four years, and has been designed to be maintainable for long time and for ease of access even for researcher that are not familiar with it. It will help understand the questions posed by the project, such as the relationship between inflammation and frailty, the effect of the diet on morbidity and inflammation, how different form of exercise influences the health outcome; It will also allow the partners to explore more specialized questions, such as the present habits of nutrition and physical activity of the European population, the relationship between socio-economical factors and diet habits, characterizing the various population from the body soft tissue composition in a more quantitative way and be able to relate all these variables with health outcome after one year. The great number of different dataset collected, such as cytokines, metabolomics, metagenomics, methylation status and so on, will also be precious to develop a higher understand of the system biology of nutrition and inflammation. It will also be precious to asses a new standard for the definition of frailty, being able to characterize this value in different population across Europe, making it possible to find good, independent marker that are easy to measure in the clinical practice and can be used as an indirect way of measuring the underlying patterns of ageing and morbidity.

Moreover, NU-AGE proposed the most promising mechanistic modeling approaches and analysis methods that may help in interpreting the co-regulation of inflammatory and metabolic processes that are involved in the physiological decline observed with aging, at multiple biological organization levels and time scales (Calcada et al., 2014, MADhttp://www.ncbi.nlm.nih.gov/pubmed/24462698). Moreover to better interpret the inflammatory parameters produced within the NU-AGE consortium, it has been produced a mechanistic mathematical model of interleukin-6 (IL-6) signaling, calibrated with biochemical and biophysical parameters found in the scientific literature (i.e. chemical association rates and cellular receptor kinetics) and which allows for simulations of the cellular response to a stimulus of IL-6 in different inflammatory conditions.

6 ANALYSES ON SOCIOECONOMIC DETERMINANTS OF FOOD CHOICES

6.1 IMPACT OF ELDERLY FOOD CHOICES

In addition to knowledge about how nutritional factors affect the ageing process, the promotion of healthy eating for heathy ageing requires a clear understanding of how the elderly choose their foods, and what factors may be used in order to steer those choices to improve their diets. The WP aimed at contributing to this improved understanding by first analysing the determinants of diet quality and nutritional health status within the elderly populations of several EU countries. A literature review summarised the scientific knowledge on the subject from a broad disciplinary perspective covering, in particular, the fields of public health nutrition, economics, psychology and consumer research. That preliminary work put to light the complexity of food choice behaviours, the literature emphasizing a great number of potential drivers of diet quality without being able to establish their relative importance. Those factors include the individual's economic environment (e.g., financial situation, affordability of healthy foods), health status (e.g., masticatory efficiency), socio-demographic factors (e.g., household structure, education, social networks) as well as other characteristics of the living environment (e.g., accessibility of grocery stores). Few attempts have been made to apply behavioural theories to analyse the elderly's food choices. The literature review also revealed the need to consider the heterogeneity of the elderly population, in particular in its age dimension (the old-old versus the young-old), as well the diversity of nutritional health problems faced by this population group (anorexia of aging versus obesity).

The WP proceeded to analyse the determinants of diet quality among the elderly of four EU countries (Italy, UK, Sweden, Finland), using secondary data sets on individual intakes and/or household consumption. An index of dietary quality was calculated and regressed on a set of potential determinants derived from the literature review, taken into account the data available across the four countries. The model's structure (i.e., regressors) was defined ex-ante, and the statistical analysis was then performed separately by each partner, hence limiting the risks of excessive data mining. The work produced several new insights: i) diet quality among the elderly in the four countries was both low on average and extremely variable, leaving much room for improvement through the promotion of healthy eating among the elderly, ii) the socio-economic variable included in the model explained a small but significant share of the variability in diet quality in all four countries, iii) the influence of many variables was found to be similar in terms of sign and significance across the four countries. The comparison of results across countries represents, for this WP, the main European value added of the project, iv) as expected from the literature review, education, not living alone and female gender were characteristics positively associated with diet quality across the four countries, v) more surprisingly, resource availability was associated with diet quality either negatively (Finland and UK) or in a non-linear or non-statistically significant manner (Italy and Sweden), vi) socio-professional status, retirement, age and seasonality explained very little of the differences in diet quality, vii) regional differences among countries persisted even after controlling for the other sociodemographic variables.

We conclude from that analysis that poor diet quality among the elderly is not primarily an economic issue, which implies that specific public health policies, as opposed to broad macroeconomic policies, could be successful in promoting healthy eating among the elderly. Those policies should target populations currently chosing diets of particularly poor quality, such as men living alone with a low level of education. However, the finding that age and retirement status have little impact in terms of diet quality may indicate that food habits are largely set in the latter part of life, thus making behavioural change difficult for the elderly.

The WP extended the previous analysis, which focused on broad associations between diet quality and socio-economic characteristics, by developing a structural model of diet choice and health determination. The model was grounded in the human capital model of the demand for health and estimated using the two richest data sets for Italy and the UK. The results establish that diet quality and other lifestyle choices affect health even in old age. While expected, this finding differs from what found in epidemiological studies showing that health is here self-assessed by the individual herself as opposed to being objectively measured on the basic of medical information. Thus, the results support the view that adopting healthier lifestyles, including better diets, generates relatively quick rewards in terms of health as evaluated by the subject himself – or, in other words, that it is never too late to benefit from eating more healthily.

We also found that the causal relationship between diet quality and health was bi-directional: diet quality influences health but health also influences diet quality. While intuitive, this bi-directional relationship has been strangely absent from the behavioural models of diet choice and health determination developed to date. It follows that complex feedback or offsetting mechanisms in the relationship between diet quality and health should be considered carefully when analysing the benefits from promoting healthy eating to the elderly. For instance, the results suggest that although an exogenous improvement in diet quality would enhance health among the elderly, the benefits would be partially offset as improved health would ultimately lead to people being less concerned about their diets and, as a result, adopting relatively less healthy diets.

6.2 NUTRITION AND HEALTH CLAIMS IN RELATION TO THE ELDERLY POPULATION

A second task collected primary data to investigate nutrition and health claims in relation to the elderly population. All participants in the NU-AGE interventions were asked to fill out an exit questionnaire focusing on their attitudes towards nutrition and health information/claim and sources of information on healthy eating. Subsequently, interviews were carried out with four GPs in each of the five recruiting centres. This was rounded up by an additional interview with a member of the Standing Committee of European Doctors (CPME). Overall, about half of the elderly stated that they used labels as a source of nutritional information when purchasing foods, while one third stated never to use them, usually because those labels were too difficult to read or contained too much information. Those two issues – information overload and poor readability of labels – were also emphasized by GPs, who therefore recommended a simplification and tailoring of labels to the elderly population. CPME was aware of this type of problems, and, consequently, its policy stresses the need for clear labelling understandable to older people.

To the elderly, the most important information on food products packaging is the best-before date, followed by sugar content, with nutrition claims in only fifth position. Participants stated that the top five influences on their purchase decision were: best-before date, price, taste, ingredients, and habit. Across the countries, participants in The Netherlands and the United Kingdom scored significantly higher on understanding nutrition claims than Poles and Italians, with the French in-between. Nutritional claims only seem to be helpful to those who already read labels. GPs confirmed those findings and shared an overall impression that people did not know what information to trust. This points to the need for education, raising awareness of the nutritional-health connection, and encouraging healthier eating among elderly people in order to improve quality of life in that population group. In fact, all interviewed GPs as well as the CPME agreed on the importance of improved education for the general public and health practitioners about nutritional claims.

Among sources of information on nutrition, food labels were mentioned most often by the elderly, followed by books and health magazines, with dieticians and doctors only coming in fourth place. When seeking advice on specific diet-related questions, the majority of the elderly turned to their doctors, dieticians, family and friends. GPs were aware that the main source of nutrition information was the opinions of acquaintances, friends, family or magazines. The interviews finally revealed that GPs' nutrition knowledge should be improved, and referral to dietary counselling should be made easier.

7 RECOMMENDATION TO POLICY MAKERS

The active participation of FoodDrinkEurope in the NU-AGE project started with a first desk analysis concerning the EU legislation on food consumption, diet and health with particular reference to the nutritional and health claims regulation. The overall goal of this analysis was to identify features of the EU legislation, particularly on health claims, and to define training and technology transfer tools that would help transmit NU-AGE relevant results to the food sector stakeholders and the EU policy authorities. The 'Report on EU legislation' (D.11.1) provided an overview of the

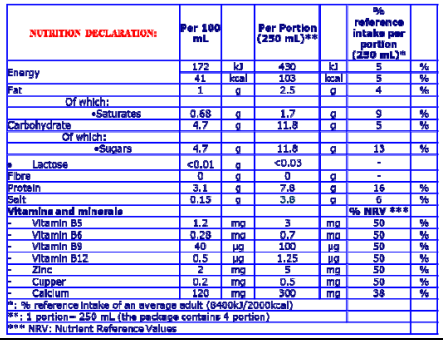
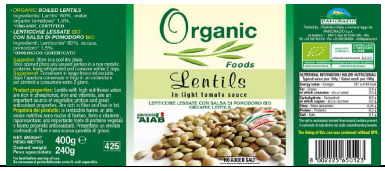
legislative provisions of relevance for the NU-AGE project, either because they are addressed to specific consumers groups - such as the elderly - or because they indirectly have an impact on this category of consumers. The specific conditions and requirements in the EU for the use of nutrition and health claims, for the addition of vitamins and minerals to foods and for the composition and marketing of food supplements and dietary foods create the conditions for encouraging the production of foods that, due to their nutritional or health properties, can better suit the specific needs of the elderly. Therefore, by providing an in-deep analysis of the most relevant provisions, the Report showed the crucial role that the legislation can play for meeting the elderly's exigencies, and laid the foundations for achieving the long-term objective of the WP 11: identifying the possible scenarios based on the scientific NU-AGE outcomes and drafting specific recommendation for EU Policy makers and relevant stakeholders.

FoodDrinkEurope has also put forward a commitment on the NU-AGE Project to the EU Platform for Action on Diet, Physical Activity and Health (Action no. 1318), increasing awareness with other relevant stakeholders.

During the last months of the project, the research data generated by WPs 2-9 was examined by FoodDrinkEurope to provide remarks, comments and suggestions on the impact that these new evidences of research will have on the health claims regulation, and collect them on a 'Recommendation to Policy Makers' (D.11.2). Thus, the research conducted within the project show that diet can play a significant role not only in counteracting or slowing down the processes associated with ageing, but also in improving the overall health status of elderly people. This effect on ageing varies considerably based on nationality and gender and – to a smaller extent – on other factors, such as genetics, and a person's overall lifestyle. Based on this fact, FoodDrinkEurope experts highlighted the importance of supporting initiatives that positively contribute to healthy ageing, both at EU and at national level, and that address specifically the various factors (nationality, gender, genetics, overall lifestyle, etc.) that influence the impact of diet on ageing. Importantly, the outcomes of NU-AGE underline the need for a coordinated and multidisciplinary approach between various policy domains.

8 THE NU-AGE ELDERLY TAILORED FOOD PRODUCTS

The reformulation and design of a series of food products were carried out on a laboratory scale according to existing procedure and machineries associate also to a detailed evaluation of the current regulatory framework with regard to food products which have been "reformulated" based on RTD inputs. The full list of products developed by the eight SMEs of the NU-AGE consortium is presented in table 3.

COMPANY (Country)	FOOD PRODUCT (name and picture)	FOOD PRODUCT (brief description)
YORUK SUT (Turkey)	FRESH KASKHAVAL CHEESE 	FRESH KASKHAVAL CHEESE, low fat (max 25% fat in dry material). Testing production has done without adding salt but external analysis found 0.28 gr salt in 100gr of products because the rennet enzyme used as ingredient has salt in its structure.
EC6 (France)	MOLECULAR CUISINE 	WHOLE DAILY DIET for elderly people
MIRELITE (Hungary)	WILD SAUCE 	QUICK FROZEN WILD SAUCE made of chopped roots vegetables with sour cream, and spicy, cooked together and get a perfect flavour to meat products. The sauce visually get golden colour, good density for serving it. The chopped vegetables are visible in the sauce, so the fibre is raise up the products side of healthy tone
VIDRERES (Spain)	UHT MILK with GINGSENG 	UHT DAIRY PRODUCT, composed by lactose-free and low-fat (1%) milk, enriched with ginseng and vitamins and minerals
PANCRAZIO (Italy)	ORGANIC LENTILS IN TOMATO SAUCE 	ORGANIC BOILED LENTILS IN BRINE, with organic tomatoes and no salt added
ZEELANDIA (Czech Republic)	BARLEY BREAD 	BREAD MADE WITH USAGE OF BARLEY FERMENT AND BARLEY PEARLS with the aim to increase Beta-glucans content
WIESBAUER (Austria)	MEAGER BEEF OF “BEIRIED”	MEAGER BEEF OF “BEIRIED”, 100% pure beef

NUTRITION DECLARATION:	Per 100 mL		Per Portion (250 mL)**		% reference intake per portion (250 mL)*	
Energy	172	kJ	430	kJ	5	%
	41	kcal	103	kcal	5	%
Fat	1	g	2.5	g	4	%
Of which:						
•Saturates	0.68	g	1.7	g	9	%
Carbohydrate	4.7	g	11.8	g	5	%
Of which:						
•Sugars	4.7	g	11.8	g	13	%
• Lactose	<0.01	g	<0.03		-	
Fibre	0	g	0	g	-	
Protein	3.1	g	7.8	g	16	%
Salt	0.15	g	3.8	g	6	%
Vitamins and minerals					% NRV ***	
- Vitamin B5	1.2	mg	3	mg	50	%
- Vitamin B6	0.28	mg	0.7	mg	50	%
- Vitamin B9	40	µg	100	µg	50	%
- Vitamin B12	0.5	µg	1.25	µg	50	%
- Zinc	2	mg	5	mg	50	%
- Cupper	0.2	mg	0.5	mg	50	%
- Calcium	120	mg	300	mg	38	%
*: % reference intake of an average adult (8400kJ/2000kcal)						
**: 1 portion= 250 mL (the package contains 4 portion)						
*** NRV: Nutrient Reference Values						

	 	
MEVGAL (Greece)	OLIVE DROPS YOGURT  	GREEK YOGURT WITH OLIVE OIL 2% fat from olive oil, high in unsaturated fats and vitamin E. Also high protein content and with lactic acid bacteria that aid digestion

Table 3. Full list of elderly tailored products obtained within the NU-AGE projects

9 ELDERLY TAILORED FOOD PROTOTYPES

The three large food industries of the NU-AGE partnership developed the following prototypes:

9.1 LESIEUR

LESIEUR presents the developments of 2 products namely a seed oil high in omega-3 and fortified in vitamin D, and an olive oil high in polyphenols.

The seed oil will be a blend of:

- 70% of rapeseed oil, having omega-3,
- 30% of high oleic sunflower oil, a very stable oil, also high in vitamin E,
- 7.5 µg/100g of vitamin D. We checked the acceptability of the product, which was good, as well as the stability of the nutrients of the product during all the shelf life and during its use. We chose to use a light PET bottle of 50cL, in order to have a practical andnot heavy for the elderly. Finally, we performed a Life Cycle Analysis of the product, and shown that the developed product has a better environmental impact that the average oil on the French market.

The olive oil will be a blend of:

- 70% of an olive oil specifically high in polyphenols, from an Italian supplier and coming from an Italian production,
- 30% of standard extra virgin olive oil.

This blend will contain at least 41mg/100g of hydroxytyrosol and its derivative, which is above the minimum required by the European regulation. The olive oil has an acceptable taste, especially in bitterness and piquant, and an acceptable price, it was in fact less expensive than the specific olive oil. We checked the stability of the nutrients during all the shelf life of the product, and during its use. We chose to use a light PET bottle of 50cL, in order to have a practical, not too heavy product for the elderly.

LESIEUR presents the developments of another product defined after WP 8, namely a mayonnaise high in omega-3, in fibers and low in fat. A specific recipe was developed to meet the criteria defined in WP8: water, rapeseed oil, free range egg yolk, Dijon mustard, fibers, vinegars, modified maize starch, sugar, salt, flavor, colors, concentrated lemon juice, preservative (potassium sorbate), thickener (xanthan gum).

A sensory test and a study to measure the necessary strength to open the mayonnaise jars were performed. Accordingly, for this product, it has been decided to employ a small lid, so that the elderly can open the product easily, and a small packaging (235g) because many elderly live alone and it is easier to carry.

9.2 VILLANI

VILLANI performed a low salt salami trial prototype using the chicken charcass reduced 4 times as a broth.
The sodium cloride is an important ingredient for the dry salami process, because of the antimicrobic activity on the raw material and the role to extract the salt soluble proteins from the meat in order to glue the slice in the final product ready to eat. It is not possible remove the salt during meat processing, therefore VILLANI tested a different method to reduce the salt, covering the surface of the minced meat with a solution based on meat soluble proteins at the beginning of the mixing processing step, with promising results.
Liquid meat: produce the same ionic strength on the surface of the minced meat inspite of the reduction of NaCl added. Reduced chicken broth is a source of Umami taste and will balance the reduced salty taste by the reduction of the NaCl added. The reduction of the amount of NaCl in the meat mass forced the R&D staff to process a low temperature seasonig period. This is the reason why the pork meat mass was processed in the Villani plant in Castelnuovo Rangone (MO, Italy) where is located the salami processing facility, while the first step of the salami seasoning was processed in the Villani plant in Pastorello (PR, Langhirano area, Italy) where is located the Parma ham processing plant and where is available the low temperature seasoning processing plant. Several trials were performed and the trial n°27 was well accepted and considered as a low sodium salami acceptable by the market. The cost is around 1€/kg more than the standard salami, because of the longer time request to dry the salami and to season it at low temperature. The other meat prototypes produced by VILLANI was a Mortadella based on turkey meat enahnced with chicken reduced broth.

9.3 GRANAROLO

In the framework of NU-AGE project, GRANAROLO completed several laboratory tests to develop a new functional milk product addressed to the elderly designed to be good, safe and nutritious while affordable and low in calories to be proposed in case of some disorder such as dysbiosis, osteoporosis and lactose intolerance.

GRANAROLO (plant of Soliera, MO, Italy) developed a synbiotic functional UHT milk packaged in 500 ml and 1000 ml octagonal shaped carton packages. This NU-AGE functional milk is lactose-free, hyperproteic (5%), low-fat (1%) and enriched with fructooligosaccharides (FOS), longchain Omega-3 PUFAs, vitamins and mineral salts. The product also includes a separated probiotics supplement (concentrated freeze-dried colture of infant type bifidobacteria, maltodextrin) packaged in sachet made with coupled material PET / AL / PE; it is managed separately in such a way that the consumer can add by himself the probiotic to the milk.

Whereas the composition of the product is confidential, in Table 3 the nutritional information of 100 ml of functional milk are reported in table 3.

Table 3. Nutrition information per 100 ml of NU-AGE functional milk.

Energy	kcal	55	
	kJ	231	**% LARN**
Protein	g	5,00	8%
Carbohydrates	g	5,00	8%
of which sugars		5,00	7%
of which lactose		< 0,01	0%
Fat	g	1,00	5%
of which saturated		0,65	1%
of which LC PUFA (omega3)	*mg*	*100,00*	*40%*
Dietary Fiber (FOS)	g	3,00	10%
Sodium	g	0,05	4%
Calcium	g	0,24	20%
Vitamin B6	mg	0,60	40%
Vitamin B12	mcg	1	40%
Vitamin E	mg	5	40%
Vitamin C	mg	34	40%
Vitamin D3	mcg	6	40%
Folic acid	mcg	80	20%
Bifidobatteri infant type	cfu	1,0E+09	

10 THE NU-AGE WEBSITE AND SOCIAL MEDIA

The NU-AGE website was launched in November 2011 (http://www.nu-age.eu/).

Links to newsworthy deliverables and to external mentions which have come out of the project were included under the 'News' section of the website. This includes a podcast, a Food Today article and a press release. The number of publically available communications deliverables was expanded and external mentioned in the media. There are five external media mentions which can be viewed on the 'News' section of the NU-AGE website. During the whole period analysed, the section 'consortium' of the NU-AGE website was updated with the mentions of the NU-AGE project on consortium partner websites. This was carried out as part of the mutual linking task in order to encourage collaboration between the NU-AGE project, consortium partners and relevant external organisations.

Social media have been also important tools to reach people. NU-AGE Facebook counts currently 117 likes while Twitter reached 56 followers. The interaction with the NU-AGE Twitter account has especially increased during the final conference on the 5th April 2016.

In regards to website statistics, Google Analytics has been used to monitor a number of parameters. These parameters include the number of visits, the countries which visitors are from and the length of time they spent on the website. The highest peak in unique visits was during the period November 2014 and April 2015, while the highest peak in total page views was between May 2014 and October 2014, when figures reached 3,264 and 8,096 respectively. In regards to countries, during the entire period analysed, the majority of visitors have come from Italy, the UK and France. United States and Belgium appeared among the top three countries only during the semesters May 2015-October 2015 and November 2015-April 2016 respectively.

On the whole, figures showed an increase in unique visits, visitors and total page views until April 2015, while a slight decrease occurred during the semester May 2015-October 2015. However, the comparison between the period analysed in this report (May 2014 – April 2016) and the report on the first 36 months of the project shows a general positive trends in almost all parameters considered.

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NU-AGE POTENTIAL IMPACT

RESEARCH IMPACT

The overall aim of the NU-AGE project is to improve health and quality of life in the EU aging population by counteracting inflammaging through a whole Mediterranean Diet (MedDiet) approach. We are proud to remark that the NU-AGE project and its study design has been mentioned, in a special issue of Nature journal devoted to aging studies, as "NU-AGE is exactly the kind of large, longitudinal study that scientists the world over are clamoring for" (Hughes V., 2012).

NU-AGE has accepted the ambitious challenge to identify the molecular mechanisms underpinning the effect of a whole diet based on Mediterranean pattern on the prevention and delay of the decline occurring with age, as a result of the derangement of a variety of organs and systems. To properly evaluate the complex deterioration of physiological functions responsible for the age-related increased risk of morbidity and mortality, NU-AGE has adopted a systems biology approach, which is highly innovative and timely in the field of human elderly nutrition.
Through this approach, the project has the objective to fill the gap in knowledge on how a whole MedDiet can impact on and counteract the age-related decline.

In line with the EC expected impact, NU-AGE main outcomes shall contribute to a better understanding of the nutritional needs for a better quality of life in the over 65 years-EU population. . This will support European strategies on nutritional recommendations, by contributing, on one hand, to the substantiation of nutritional and health claims, and, on the other, to increase competitiveness of the European food industry through the development of both over 65-consumers-targeted functional food prototypes and innovative SME-driven design of advanced traditional food products.

The core activity of the project is the nutritional trial that has been administered to a final number of 1149 volunteers in Italy, France, UK, The Netherlands and Poland, well balanced per gender (male percentage is 44%) and including a 20% of pre-frail subjects (according to the frailty criteria by Fried et al., 2001). All the subjects underwent an in depth characterization covering anthropometry , health and medical status, cognitive and physical functions and a series of biochemical and inflammatory measures. Moreover, in a selected subgroup of 120 volunteers an advanced immunology, genetics, epigenetics, transcriptomics, metagenomics and metabolomics measures have been performed to test the efficacy and effectiveness of the NU-AGE diet through the evaluation of a series of cellular and molecular parameters as well as the general health status in all the over 65 subjects enrolled in NU-AGE. During the last year of the project all data has been collected and entered in the database and researchers analyzed the main results.

The results of the NU-AGE project will pave the way to the definition of the key mechanisms of action and their interactions as well as of the relative impact of a whole diet approach. This findings will allow the EU to act on several levels to obtain:

• a feasible dietary solution to reduce health related problems of an increasing ageing population in EU, reducing the

burden and the costs related to age- and nutrition-related diseases,

• the possibility to develop new products or to implement traditional foods with healthier traits that can integrate and hence improve elderly diets, triggering the industrial interest into this new market sector. Optimization of the use of traditional ingredients into different food types, together with the production of novel foods, will lead to the development of new industry business, increasing the competitiveness of the European food industry;

• understanding the integrated biological changes that occur by changing the whole diet to improve health ageing at a cellular level. Moreover, by considering the relations among economic limitations, behavioral preferences, socio-cultural and health determinants, the impact of NU-AGE will affect the production of elderly-tailored foods and over 65 consumers habits.

The first outcomes obtained in the project indicate that the NU-AGE dietary intervention was well accepted by European elderly population showing that old people are able to change dietary habits towards their nutritional needs taking advantages on specific health outcomes. Indeed, those volunteers who were most adherent to the NU-AGE diet decreased the plasma levels of C-reactive protein, one of the main inflammatory marker that represented the primary outcome of the NU-AGE intervention and improve their lipid profile (in terms of total/HDL cholesterol ratio and triglycerides levels).

The metagenomic analyses showed that subjects with a poorer diet at the end of the suffer a significant loss of diversity in gut microbiota composition.

The NU-AGE study was the first dietary intervention investigating the effect of a healthful MedDiet providing targeted nutritional recommendations for optimal health and quality of life in a healthy European elderly population. When effects of health outcomes are also confirmed, this research has great potential to contribute to improving the quality of life of European senior citizens.

Genetics analyses well distinguished the five recruiting centers and also the epigenetic analyses showed a clear separation of the two populations studied. Metabolomics enables to discriminate sub-phenotypes of pre-frailty with gender and frailty status differences, identifying markers of food intake and frailty status changes. Results showed that the pre-frailty could be better characterized using new markers, that new biomarkers could be predictive of health evolution towards pre-frailty and that elderly men and women remain different regarding metabolic status with only few or no common markers of pre-frailty.

Identification of sub-phenotypes of frailty (stable, improvement, degradation) with specific markers can contribute to better identification of at-risk subject and therefore to more accurate prevention with nutritional recommendations.

Intriguingly, what is strongly emerging is that there are the several important differences among subjects coming from various countries and between men and women both regarding the baseline status and the effect of NU-AGE intervention. These observations possibly indicate that a MedDiet pattern as the NU-AGE diet might not have the

same outcomes on all the populations and underline the importance of considering gender and geographical origin in further studies evaluating the effects of dietary intervention on diseases, aging and longevity.

SOCIO ECONOMIC IMPACT

In addition to knowledge about how nutritional factors affect the aging process, the promotion of healthy eating for heathy aging requires a clear understanding of how the elderly choose their foods, and what factors may be used in order to steer those choices to improve their diets.

The socioeconomic research carried out within NU-AGE project will have an impact by informing policy-makers interested in promoting healthy eating for healthy aging. For example, a message emerging with consistency from the different sub-components of the WP is that poor dietary choices among the elderly are driven primarily by a lack of knowledge about healthy eating rather economic circumstances, at least on average (i.e., this could be different when considering the most deprived segment of that population). Thus, the research supports the promotion of healthy eating through traditional public health measures, such as informational campaigns or nutritional education. Moreover, although it also points to the need to improve the clarity, trustworthiness, and readability of labels on food products consumed by the elderly. Food manufacturers seeking to target the elderly population will also benefit from the detailed description of how the elderly obtain information on nutritional health issues, as well as how they interpret and respond to nutritional/health claims.

Importantly, the NU-AGE study demonstrates that the impact of a certain dietary regime on ageing varies considerably on the basis of nationality and gender and – to a smaller extent – on other factors, such as genetics and personal overall lifestyle.

Based on these outcomes, specific national dietary recommendations may be established for over 65-year citiziens in order to help improving the dietary habits of elderly people. In order to be effective, such policy initiatives should take into account all the factors (i.e. nationality, gender, genetics, overall lifestyle) able to influence the impact of diet on ageing. Dietary recommendations may also need to be adapted to the various dietary cultures and traditions and should consider the different impact that the same dietary regime (in this case, the MedDiet) has shown to have on diverse European populations.

INDUSTRIAL IMPACT

A series of advanced traditional food tailored for over 65-year consumers and elderly-tailored food prototypes have been designed within the framework of the NU-AGE project, thanks to the intense and continue collaboration between researchers, food industries and food federations.

NU-AGE has given the potential to increase the European food industry's competitiveness with new food formulations specifically tailor-made for elderly populations. Over the last decade, EU's share of the global food market has declined from 24% to 19.8% suggesting that EU can not longer compete with the emerging economies of China, India and Brazil on raw material and/or labor costs. Therefore, the only strategy that European food industries can employ

is to compete by adding value, efficiently transferring and exploiting EU's outstanding basic science and technology. This project will drive the development of new market sectors that are currently under-evaluated and under-exploited. SMEs have indeed benefited from the project outcomes by producing foods which do not require technological conversion of standard machinery and by supplying the local population at low cost.

Main dissemination activities and exploitation of results

Along the 5 years project duration, all the partners largely contribute to disseminate the project, from its design and innovative aspects to its results. To this purpose, a large set of tools have been used such as websites, video clips, social networks, posters, presentations, press releases, articles, interviews.

Due to the large inter-disciplinarity characterizing the consortium, the project was presented to scientific congress mainly related to aging, nutrition and inflammaging as well as other very specific on epigenetics, metabolomics, metagenomics.

Also the Food Federations the industrial counterpart presented the advancement of the project in specific food fair and consumers meetings.

Workpackage 12 of the project was aimed to promote the use of results from the project among the target groups: opinion leaders, policy makers, regulators, media, the food and beverage industry, SMEs, RTD performers, communication agencies and other information multipliers, professional associations, health professionals, educators, consumer associations and associations for ageing in Europe (e.g. AGE and European Older People's Platform).

NU-AGE project dedicated special issue in MAD

An entire Special issue has been dedicated to NU-AGE in "Mechanisms of Ageing and Development", vol.136-137(2014); The complete list of papers is shown in Table I. Aurelia Santoro, Stathis Gonos, Wilhelm Bohr, Patrizia Brigidi and Claudio Franceschi acted as Guest Editors (Figure 1). http://www.nu- age.eu/science_publications

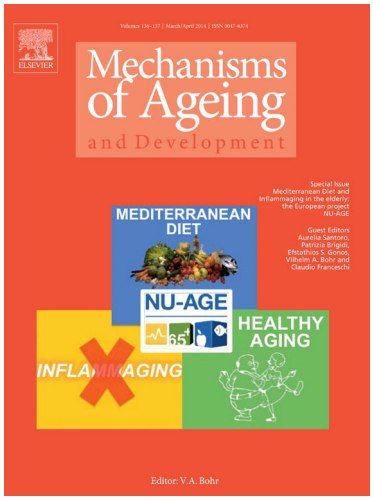

Figure 1: Cover page of the NU- AGE special issue on MAD

Tabella 1 List of 15 papers published in the special issue dedicated to NU-AGE on Mechanisms of Aging and Developments

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

Collaboration with other EU projects and UE initiatives:

- Since 2014 it was establishe the collaboration with Prof. Oberdan Parodi, coordinator of the **FP7 project DOREMI**. UNIBO sent details on the NU-AGE trial and Diet to Prof. Parodi in order to set up the nutritional trial in DOREMI and invited Prof. Parodi to give a talk during the NU-AGE final conference.
- NU-AGE also collaborated with Prof. Catherine Renard, coordinator of the **FP7 OPTIFEL** project since 2014. NU-AGE was presented at EXPO 2015-Dietary Strategies for a healthy Ageing via EU-funded R&D Work shop" on May 18th 2015, invited by Prof. Renard.
- UNIBO, as representative of the NU-AGE consortium within the **EIP-AHA (A3-nutrition Group),** contributed to the preparation of the poster to be presented during the eHealth week in Athens on 12-14 May 2014 and attende some meeting in Brussels. The image of the poster is reported below (Fig2).

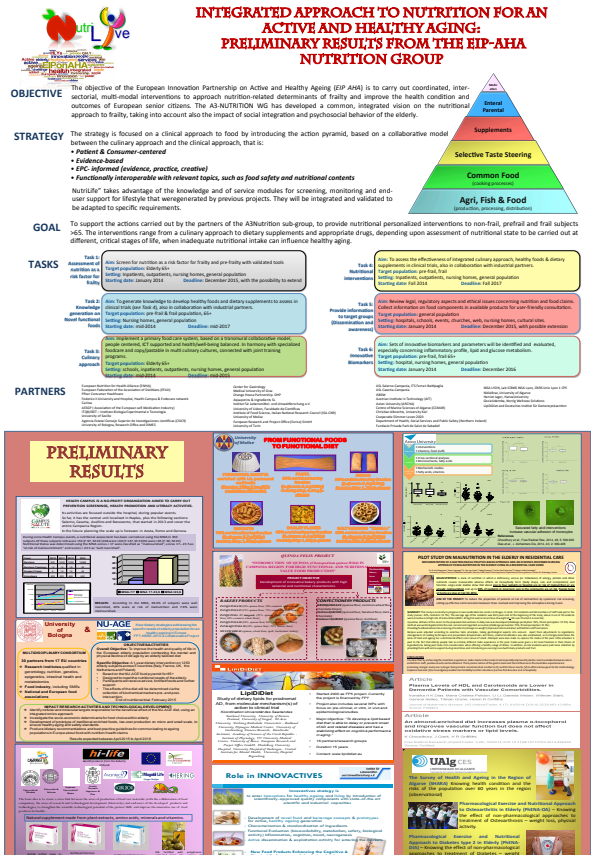

Figure 2: Poster presented by the A3 EIP-AHA group on nutrition at the eHealth week in Athens.

List of the main dissemination activities:

- Dissemination Workshop: Parallel session on NU-AGE at the IUNS 20th International Congress of Nutrition, Granada, entitled "The NU-AGE project: New dietary strategies about healthy nutrition for the elderly" Spain, 20 September 2013 (Presentations made by UNIBO, UEA, WU, UCC)
- Dissemination Workshop: Parallel session on NU-AGE at the SFRR-Europe 2013 Conference, Athens, entitled "The NU-AGE project: New dietary addressing the specific needs of the elderly population" Greece, 23 September 2013 (Presentations made by UNIBO, NHFR, UCC)
- Final Conference: Mediterranean Diet to combact ageingin European Countries, Brussels, Belgium 5 April

2016 (Presentation for researchers and industry made by most of the project partners, webinars available at: http://www.nu-age.eu/final_conf)

- April 2016: Judith Zwartz address, Wageningen, the Netherlands. Presentation for the general public (>230 attendants)
- January 2016: Workshop "Translational Aging Research: Challenges and opportunities", Leiden, the Netherlands. Presentation and poster
- June 2015: Nutrition Society, Irish Section Meeting, Cork, Ireland. Presentation
- November 2013: Symposium Healthy diet, Healthy ageing – 25 years of nutrition research in elderly at Wageningen, Wageningen, the Netherlands. Poster and presentation
- *June 2011: 4th International Symposium on "Nutrition, Oxygen Biology and Medicine", Paris, France, presentation*
- *August 2011: 43rd IUPAC World Chemistry Congress, Puerto Rico, USA, presentation*
- *September 2011: SFRR-Europe meeting, Istanbul, Turkey, presentation*
- *October 2011: 13th IUBMB Conference on "Cell Signaling Networks", Merida, Mexico, presentation*
- *December 2013: 6th Workshop on Geriatric Oncology, Athens, Greece, Oral presentation*
- *December 2013: 'Searching for the elixir of youth', Athens, Greece, talk*
- *February 2014: 17th Seminar of the Greek Society of Clinical, Chemistry and Biochemistry, Athens, Greece, Oral presentation*
- *April 2014: 1st World Congress on "Geriatrics and Neurodegenerative Diseases", Corfu, Greece, Oral presentation*
- *September 2014: SFRR EUROPE conference Paris, France, presentation*
- *September 2014:SFRR-e conference, Spetses, Greece, presentation*
- *2014 Open Health Alliance workshop, Athens, Greece, presentation (Civil society)*
- *2014 How many years will our children live? Athens Science Festival (Civil society), Athens, Greece, presentation*
- *November 2015: Advanced School on Molecular and Cell Biology to Unravel the Physiology/Pathology of Diverse Biological Paradigms, Uruguay, presentation.*
- IVth International symposium nutrition, oxygen biology, and medicine: free radicals, nutrition, and aging
- from fundamental aspects to clinical applications. Invited Presentation, 15-17/06/2011, Paris (France)
- International symposium on "health benefits of foods - from emerging science to innovative products". Invited Presentation, 05-07/10/2011, Prague (CzechRepublic)
- EU Platform for Action on Diet, Physical Activity and Health: "The NU-AGE project" Invited Presentation 09/02/2012 Bruxelles (Belgium)
- 7th Nutrition taste and health. Invited Presentation, 20-21/03/2012, Dijon (France)
- International Conference of Nutrition (ICN) 2013: Parallel Symposium 'The NU-AGE project: New Dietary Strategies about Healthy Nutrition for the elderly''. NU-AGE session Chair, 20/09/2013, Granada (Spain)

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- 7th Probiotics, prebiotics and new foods meeting: "Metagenomics in Aging". Oral Presentation, 10/09/2013, Rome (Italy)
- International Symposium" the challenge of biological Research on Aging in the 21st century: from cells to clinics. Lecture, 2-4/11/2014, Sherbroook (Canada)
- "Diet, gut microbiota, inflammation and epigenetic changes with age". Lecture, 7-8/7/2014, Los Angeles (USA)
- Moscow Institute of Technology and Physics (MITP); Talk: "Biomarkers of Aging". Oral Presentation, 11/04/2014, Moscow (Russia)
- Congress on "Law and food safety" La Dieta Mediterranea ed il Progetto Europeo NU-AGE" Presentation, Santoro A. & Franceschi C.03/12/2015 Ravenna, Italy
- The 2015 Gordon Research Conference on Biology of Aging Discussion Leader Franceschi C 19-24/07/2015 Newry, ME, USA

- Biology of Aging ANIS- - conference:“Inflammation, garbaging and nutritrion”. Oral Presentation, 22-24/10/2015, Singapore
- NU-AGE Final Conference:” NU-AGE: MedDiet for the health of the elderly across Europe. Oral Presentation, 5/04/2016, Bruxelles (Belgium)
- NIA-Theories of Aging and translational perspectives workshop “Inflammation and biomarkers”. Oral Presentation, 27/01/2016, Baltimore (USA)

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NU-AGE at EXPO 2015

- EXPO 2015-Dietary Strategies for a healthy Ageing via EU-funded R&D Work shop”: “Nutrition is critical for healthy aging: the EU project NU-AGE”EXPO. Oral presentation Santoro A. & Franceschi C., 18/05/2015, Milan (Italy)
- EXPO 2015 Workshop-Dieta e strategie nutrizionali per la popolazione “senior” europea: il progetto NU-AGE e le altre iniziative sul tema. Oral presentation Franceschi C., 29/05/2015, Milan (Italy)
- Milano Bicocca for EXPO 2015- Dietary Needs of healthy and frail older people: “Inflammation and Diet” . Oral Presentaton, 03/08/2015, Milan (Italy)
- EXPO: Un approccio integrato alla vita attiva e in buona salute: le possibili sinergie tra gli interventi nutrizionali. Round table Santoro A., 20/10/2015, Milan (Italy)

The results of socioeconomic team of the project have already been communicated to a broad range of stakeholders. The following three conference presentations reached the scientific community in the fields of nutrition, public health and food economics:

1. Mazzocchi, M., Irz, X., L. Modugno, and W B. Traill (2015). Too late to get healthy? A behavioural analysis of the diet-health relationship in the older Italian population. IAAE conference, Milan, August 2015, available at http://ageconsearch.umn.edu/handle/229070.
2. Mazzocchi, M., Irz, X., Modugno, L., Traill, W. B. (2014). A behavioural analysis of the diet-health relationship in the older Italian population, Annual Conference of the Agricultural Economic Society, Paris, 09-11 April.
3. Irz, X., Fratiglioni, L., Kuosmanen, N., Mazzocchi, M., Modugno, L., Nocella, G., Shakersain, B., Traill, W. B., Xu, W. & Zanello G.(2013). Socio-economic determinants of healthy diet and healthy aging among the EU elderly. Paper presented at the 20th International Congress of Nutrition, Granada, Spain, 15-20 September. Abstract published in Annals of Nutrition and Metabolism 63(supp 1): 42-43.

The main results of the analysis of the socio-economic determinants of diet quality in Italy, UK, Sweden and Finland were also summarised in the following peer-reviewed paper: Irz, X., Fratiglioni, L., Kuosmanen, N., Mazzocchi, M., Modugno, L., Nocella, G., Shakersain, B., Traill, W. B., Xu, W. & Zanello G. (2014). Socio-demographic determinants of diet quality of the EU elderly: A comparative analysis in four countries. Public Health Nutrition 17(5): 1177-89. A related article targeting the general public was then published in Finland (in Finnish): Varakkaat eivät sijoita terveelliseen ruokaan, MTT ELO PR Magazine, 2013. Both articles were picked up by the general and specialised online media – see for instance the article by Food&Drink Business Europe at http://www.fdbusiness.com/health-may-not-be-dependent-on-wealth-in-old-age-suggests-study/.

The WP results were presented to all NU-AGE stakehodlers, including SMEs and policy makers, at the project’s annual meetings and final conference. Further, the presentation of the research about nutrition and health claims at the final conference was recorded and diffused via social media as well as an invited webinar at the French association for nutrition in the elderly (ALIM 50+).

As regards dissemination activities, FoodDrinkEurope has provided regular updates on the NU-AGE project developments, outcomes and achievements to FoodDrinkEurope membership, composed of 25 National federations, 25 European Sector Associations and 19 Companies. This update has been done through both oral briefings in internal meetings and special sections dedicated to the NU-AGE project in FoodDrinkEurope documents.

In addition, FoodDrinkEurope has periodically presented NU-AGE project activities at the EU Platform for Action on Diet, Physical Activity and Health meetings and at other external events related with the scope of NU-AGE.

More information on the NU-AGE dissemination activities:

http://www.nu-age.eu/home

A detailed list of all dissemination activities is shown in Template A2: list of all dissemination activities.

Public Website:

www.nu-age.eu

NU-AGE PROJECT LOGO:

LIST of BENEFICIARIES:

ALMA MATER STUDIORUM- UNIVERSITA' DI BOLOGNA - University of Bologna - UNIBO, Italy
UNIVERSITY OF EAST ANGLIA - UEA, United Kingdom
WAGENINGEN UNIVERSITEIT - Wageningen University -WU, Netherland
INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE - French National Institute for Agricultural Research - INRA, France
SPREAD EUROPEAN SAFETY GEIE – SPES, Italy
UNIVERSITY COLLEGE CORK, NATIONAL UNIVERSITY OF IRELAND - UCC, Ireland
INSTITUTE OF FOOD RESEARCH – IFR, United Kingdom
SZKOLA GLOWNA GOSPODARSTWA WIEJSKIEGO – Warsaw University of Life Sciences - WULS-SGGW, Poland
FoodDrinkEurope, Belgium
EUROPEAN FOOD INFORMATION COUNCIL - EUFIC, Belgium
LUONNONVARAKESKUS – Natural Resources Institute Finland – LUKE, Finland
ETHNIKO IDRYMA EREVNON, National Hellenic Research Foundation – EIE NHRF, Greece
STRATICELL SCREENING TECHNOLOGIES - STRATICELL, Belgium
UNIVERSITY OF READING – UREAD, United Kingdom
KAROLINSKA INSTITUTET – Karolinska Institute – KI, Sweden
ÖREBRO UNIVERSITET - Örebro University - ORU, Sweden
LESIEUR SAS, France
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NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK – Dutch Research Organisation –TNO, Netherland
CENTRE DE RECHERCHE EN NUTRITION HUMAINE D'AUVERGNE - Nutrition Research Center d'Auvergne - CRNH, France
NESTLE INSTITUTE OF HEALTH SCIENCES SA -NIHS, Switzerland
EC6 SARL, France
MIRELITE MIRSA Co. Ltd, Hungary
GRANAROLO SPA, Italy

Contact Details:

Aurelia Santoro, PhD
NU-AGE project Scientific Manager
Alma Mater Studiorum-University of Bologna
Dept. of Experimental, Diagnostic and Specialty Medicine
Section of Immunology, II floor
Via S. Giacomo 12, 40126 Bologna, Italy
Tel: +39 051 209 4758;
Fax: +39 051 209 4747
email: aurelia.santoro@unibo.it