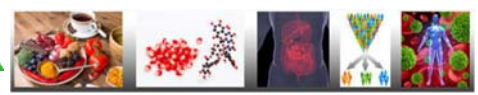




# POSITIVE NEWSLETTER



EUROPEAN COOPERATION IN THE FIELD OF SCIENTIFIC AND TECHNICAL RESEARCH



ISSUE V, JUNE 2017

## WELCOME

**S**ummer season is at hand with temperatures almost up to boiling point, yet POSITIVE has not slowed down its pace. We had no sooner met in Olsztyn for the 5<sup>th</sup> Working Group discussions than we started to get ready for the next gathering of POSITIVE partners this coming month of September in Thessaloniki (Greece). This time, the 6<sup>th</sup> WG meetings will be accompanied with the 2<sup>nd</sup> Scientific Workshop and a 2<sup>nd</sup> Training School. Meanwhile, we fulfilled the scheduled Action's work and budget plan for Grand Period 4 which was approved by the Management Committee. So, well done to all members of the POSITIVE family! Thank you for your hard work and commitment.

In this issue of the POSITIVE newsletter you will catch up with some of the recent activities undertaken within the project including the presentation of POSITIVE at VitaFoods Europe, a global nutraceutical event held in Geneva the past May. As usual, we invite you to have a first glance at some recent publications looking at interindividual variability published by POSITIVE members and don't miss the opportunity to read the Scientific Expert's opinion, on this occasion written by our POSITIVE Chair. Take a look at the 'Getting-to-know your POSITIVE partners' section to get familiarized with more senior and young researchers belonging to this COST Action. And, last but not least, save the dates for some of the most important POSITIVE events ahead.

The FG

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## NEWS FROM THE ACTION

**5<sup>th</sup> COST Action POSITIVE Meeting, 21-22 February, 2017**  
**Organized by the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences**  
**Olsztyn, POLAND**



## RESEARCH HIGHLIGHTS

The 5<sup>th</sup> WG meeting was started with a general review by the Chair Dr. Christine Morand who welcomed everyone and presented among other things the work & budget plan for GP4 and associated schedule events for 2017-2018, new application for STSM, articles and reports published, the rules for open access publication, etc. There was also a presentation of the 2<sup>nd</sup> training school on the 'Use of nutrigenetics and nutri(epi)genomics in nutrition'.



### Work Group 1

Leader: **Tom VAN DE WIELE**

Co-leaders: **Claudine MANACH & Rikard LANDBERG**

The WG1 was then initiated by the leaders Tom van De Wiele and Claudine Manach who gave an overview of the tasks and actions carried out. In relation with the Compounds Subgroup, there was a general discussion



about the problems associated with the drawing of the synthetic pathways of the compounds followed by several presentations by Dr- Torsten Bohn, Dr. AnaMarja Aura and Dr. Sofia Moco.

The work will continue by focusing on those most relevant metabolic and absorption steps to try to identify the main proteins involved using a specific template

and PathWhiz. The information will be passed on the microbiome and gene variants subgroups. Other points discussed were related to the comparison with pharmacogenomics studies of drugs metabolism and the use of predictive tools such as Biotransformer (under construction).

Next, Dr. Manach talked to the audience about the free access database Phytohub (<http://phytohub.eu>) to be used in metabolomics. Data curation and biological effects information can be added to this database and may be done by trained POSITIVE partners from WG1 and WG2. More information about PhytoHub and its use was provided in a webinar (11<sup>th</sup> May). On the same date, there was also an additional informative webinar on eBASIS (<http://ebasis.eurofir.org/Default.asp>) by Jenny Plumb from IFR (Norwich, UK). This not-open access database collects information on biological effects of food bioactive and could be linked to PhytoHub.



Within the Metabolomics subgroup, Dr. Maria Bronze presented an update of the metabolomics multi-test. Due to shipping problems there are not yet sufficient results and thus the effectiveness of the test will be seen next. In addition, the analytical subgroup is still working on a template to collect specific analytical features of the bioactive compounds. These data will be made available through PhytoHub.

Finally, there was a presentation of FoodComex, an on-line platform developed by INRA, with the aim of sharing non-commercially available compounds and standards. The tool is open to non-POSITIVE contributors. See a special report on FoodComex by Dr. Claudine Manach on this issue (page 15).

## RESEARCH HIGHLIGHTS

### Work Group 2

**Leader: Ana RODRIGUEZ-MATEOS**

**Co-leaders: Eileen GIBNEY & Dragan MILENKOVIC**

#### *Meta-analysis subgroup*



The 5<sup>th</sup> WG2 meeting (meta-analysis subgroup) was led by Dr. Ana Rodríguez Mateos who as usual presented an overview of the progress of the work carried out and the status of the ongoing meta-analyses. Next, the leaders of each of the different studies presented the progress done. Some of the meta-analyses (flavanols and blood

lipids, flavanols and glucose metabolism, ellagitannins & anthocyanins) are well advanced. In addition to very significant effects found for some of the investigated variables, a number of factors are also emerging as potential modulators and, at least, partially responsible for the inter-individual variability. A future revision of all the analyses will reinforce the relevance of these factors and will greatly contribute to understand the variability in the efficacy of the different bioactive compounds. Other meta-analyses are still at an early stage and need to be pushed forward. A potential collaboration with the COST Action EuroCarotenoid was proposed and will be pursued.

As a novelty, during the meeting in Olsztyn, a joint meeting between WG1 and WG2 was held for the discussion of several common issues such as: data fusion, co-authorship, and the planning and development of a special 'POSITIVE study' which would be carried out between various partners to generate novel data on inter-individual variability for a particular bioactive compound. This study will be given further thoughts *via* a questionnaire sent to all partners.

#### *Cell & Molecular Targets subgroup*

Dr. Dragan Milenkovic (cell and molecular targets subgroup) also summarized the activities done in this subgroup for the main three topics: human, animal and *in vitro* studies. The human studies have all been collected, a template prepared and data extraction initiated. Because the extraction is complex and needs an extra amount of work, a STSM for this task was proposed and a call will be launched. The activities will take place during early summer under the supervision of MGC. The animal and *in vitro* studies continue progressing according to the proposed data extraction procedures and data analyses.

Bioinformatic analyses and docking structure analyses are still pending on the results of these studies and the selection of candidate genes. The objectives are to find and select a number of genes as new targets of the bioactive compounds as well as understanding the biological pathways underneath the beneficial effects of these compounds. Also, a future task in collaboration with the Genetic Variant Group will involve the identification of genetic variants in these genes that may contribute to the inter-individual variability.



## RESEARCH HIGHLIGHTS

### Work Group 3

Leader: Baujke DE ROOS

Co-leaders: Marina HEINONEN

The 3<sup>rd</sup> WG3 meeting of POSITIVE was presented by the group leader Dr. Baujke de Roos *via* teleconference. She first introduced the results of a survey carried out across several European countries to try to identify the main deliverables that are relevant for different stakeholders and end-user groups. The questionnaire was responded by about 100 participants and revealed that most stakeholders and end-users understand the importance of the main objectives of POSITIVE, i.e. to improve the knowledge of the efficacy of plant bioactive compounds to produce better foods and food products so that these can be directed to specific group populations with improved benefits and recommendations. They also perceived the importance of clarifying the metabolism of these compounds and the completion and improvement of databases with information about all these compounds and their beneficial properties.

There are also a number of short videos in progress in which researchers and industrial collaborators will present successful projects and will explain how these projects worked.



The development of an on-line tool for users to search for information about plant bioactives, health effects and interindividuals variability is still in progress with the collaboration of various other POSITIVE partners.

Dr. Baujke has also recently attended the VITAFOODS meeting in Geneve (May the 9<sup>th</sup>) where she presented the COST Action FA1403-POSITIVE aims by delivering a lecture entitled 'Addressing the Inter-Individual Variation in Response to Consumption of Plant Polyphenols: Insights from the COST Action FA1403-POSITIVE' (see on page 19).



### Focus Group

Leader: Mayte GARCÍA-CONESA

Co-leader: Iwona KIEDA

During the meeting, the FG is working on the preparation of a document that collects the responses of a preliminary questionnaire directed to the consumers. The document will summarize the impressions of the participants about metabolic diseases, the role of diet in health and the importance of understanding differences between individuals. Following this first exercise, an improved questionnaire will be prepared and distributed to other partners to investigate and compare the responses of the public in different countries.

Last but not least, the FG co-leader and some of her colleagues in Olsztyn have elaborated a very nice and informative short video featuring POSITIVE that can now be accessed at the POSITIVE website and *via* Facebook and Twitter.

The plenary Focus Group meeting was held the afternoon of February the 21<sup>st</sup>. The FG leader presented a summary of the activities in progress. In addition to the general tasks of keeping up with the POSITIVE website and preparation of the 5<sup>th</sup> edition of the newsletter, the FG has initiated the tasks of communicating the research carried out within POSITIVE to the Food Industry (in collaboration with WG3). Some of the contributions are the publication of a blog in the website of VITAFOODS (see page...) as well as the preparation of a short diffusion article in *The world of Foods Ingredients*.

Further, and following the previous activities where POSITIVE was presented to the general public at local scien-



## RESEARCH HIGHLIGHTS

**Think Thank Group**  
**Leaders: Rocío GARCÍA-VILLALBA & Pedro MENA**

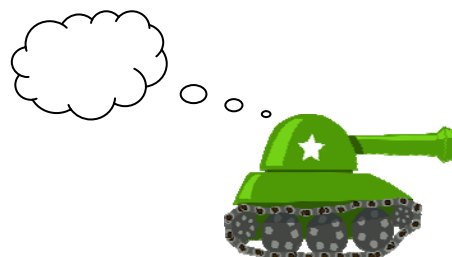
The 3<sup>rd</sup> TTG meeting in Olsztyn was led by Antonio González-Sarrías, as representative of the TTG. The meeting gathered about 15 participants. It started with a short introduction to the new ECI members about the goals reached in the last two year and the progresses that some ECI members have achieved in the TTG project ‘How to report interindividual variability in scientific publications’.



During the meeting, it was decided that the new representatives of the TTG for the next year and a half will be: Dr. Rocío García Villalba, from CEBAS-CSIC (Murcia, Spain) and Dr. Pedro Mena Parreño, from the University of Parma (Parma, Italy). Accordingly, they will be responsible for coordinating the activities developed by the TTG up until the end of this Action. Monthly webinars via Google Hangouts will continue to be carried out and will include: i) “Get-to-know sessions” to learn more about the ECI members and their research lines and, ii) further interesting discussions about the progress and next steps of the project “How to Report Inter-individual Variability”. At present, the ECIs involved in this project are concluding discussions about which parameters should be included, developing a template for the assessment of a ‘quality index’, and evaluating a number of manuscripts reporting inter-individual data. These activities promote exchanges between the ECIs and would be very useful in future works.

In addition to the above, a specific committee integrated by various ECIs are in charge of the 3<sup>rd</sup> Scientific Workshop of POSITIVE, that will be held in Thessaloniki (Greece) the 20<sup>th</sup> and 21<sup>st</sup> of September (see further information on page 9 of this issue and on the web <https://www6.inra.fr/cost-positive/Home/News/Upcoming-events-2017>). The title of this year workshop is: “Omics breakthroughs in the health effects of plant food bioactives” and will focus on how all

«Omics» sciences (including Nutrigenetics, Nutri(Epi)genomics, Metabolomics, and Microbiomics) have already contributed to provide new findings on the health effects of plant food bioactives. Several excellent researchers will present their scientific findings resulting from the use of these approaches. For this Workshop, both renowned and promising young researchers have also been invited. We hope you enjoy their talks and discussions.



## RESEARCH HIGHLIGHTS

### SOCIAL EVENTS

The day-long discussions held by all POSITIVE partners attending the meeting were followed by a dinner in a traditional Polish restaurant located in a small village outside Olsztyn. The participants had a chance to taste the typical delicacies of the Warmia & Mazury region cuisine served in interiors reflecting the old Polish land chambers.



Even though Olsztyn welcomed the partners with no snow whatsoever yet very “generous” rain, some of us also decided to take a stroll around the city’s Old Town, visiting the Castle of Warmian Bishops, once inhabited by the famous astronomer Nicolaus Copernicus. The guided tour was then followed by an evening in the Astronomical Observatory, where we enjoyed a “Journey to a Billion Suns”.



## FIRST ANNOUNCEMENT

**7<sup>th</sup> WG meeting in Dubrovnik,  
Croatia,**

**February-March, 2018**



## SAVE THE DATES



**Thessaloniki (GREECE) welcomes you to the**

**JOINT COST Action POSITIVE  
6<sup>th</sup> WG Meeting & 4<sup>th</sup> MC Meetings,  
3<sup>rd</sup> Scientific Workshop & 2nd Training School  
19<sup>th</sup> to 22<sup>nd</sup> of September 2017**

**VENUE:**

**Porto Palace Hotel, 65,  
26th Octovriou Str.,  
Thessaloniki, 546 28**

**ORGANIZERS:**

Dr. Christos Kontogiorgis [ckontogi@med.duth.gr](mailto:ckontogi@med.duth.gr),

Dr. Antonia Kaltsatou [akaltsat@gmail.com](mailto:akaltsat@gmail.com)

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**SAVE THE DATES**

## 3rd Workshop of the POSITIVE COST Action



Thessaloniki, Greece  
September, 20<sup>th</sup> - 21<sup>st</sup>

Dear all,

On behalf of the Scientific and Organizing Committee we would like to invite you to participate in the **3<sup>rd</sup> Scientific Workshop of the POSITIVE COST Action** to be held in **Thessaloniki (Greece)** from the **20<sup>th</sup> to 21<sup>st</sup> of September 2017**.

The POSITIVE COST Action is focused on inter-individual variation in response to consumption of plant food bioactives and determinants involved. The focus of this year workshop, titled "**Omics breakthroughs in the health effects of plant food bioactives**", is on how all «Omics» (including Nutrigenetic, Nutri(Epi)genomics, Metabolomics and Microbiomics) have helped to provide new findings on the health effects of plant food bioactives. Several excellent researchers will show their scientific findings resulting from the use of these approaches. You may see the detailed scientific programme of this workshop and other practical information about abstract submission and registration on the **website: to be announced**.

Please, do not hesitate to contact to us for further details:

[positive.ws2017@gmail.com](mailto:positive.ws2017@gmail.com)

We hope that you will join us for this interesting workshop!

*Thank you for your consideration*

SPECIAL THANKS TO OUR SPONSORS





## SAVE THE DATES

This Training School will combine webinar sessions open to all partners (scheduled between June and September) and a practical session for a limited number of partners.



21<sup>st</sup> to 22<sup>nd</sup> of September 2017

**COST Action POSITIVE 2<sup>nd</sup> Training School**

**Use of Nutrigenetics and Nutri(epi)genomics**

**in Nutrition Research**

**Organized by**

**Dragan MILENKOVIC,**

**Anne-Marie MINIHANNE,**

**Baujke DE ROOS,**

**Wim VAN DEN BERGHE**

**Thessaloniki, Greece**

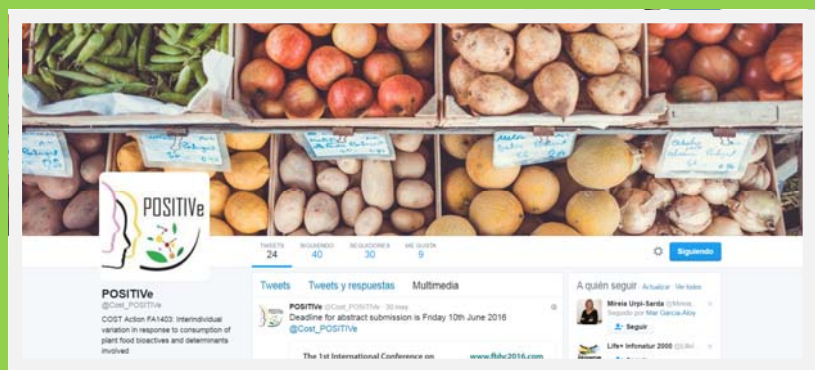


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## SHORT TERM SCIENTIFIC MISSIONS

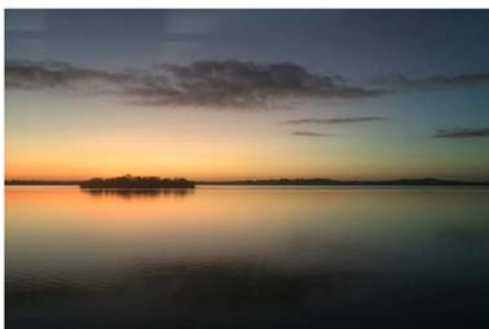
### IRINA UZHOVA from Spain went to UCD, Dublin, IRELAND

I chose to perform my short scientific stay at the UCD Institute of Food and Health which belongs to the University College Dublin and is located in Dublin, Ireland. The project I was working on is the "Fusion" pilot study involving the analysis of individual datasets. The idea was to create a large dataset of raw data from intervention studies conducted in different centres/institutions within COST network, which could be used to specifically understand the factors related to interindividual variation in response to food bioactives. This STSM allowed me to liaise with COST partners in order to identify the available studies within COST, work on the study documentation and plan further analysis, and thus I can affirm that my short stay was very successful in forwarding the project.

The Institute of Food and Health is such a fun place to work. Once you are there you are 100% involved in some of the activities they conduct at the centre, and I loved it. During that period, there was a UCD Healthy Eating Week, which consisted of all kinds of fun food and sport included challenges. Thus, we took a part in the orange eating competition trying to beat the world record by eating 3 x 300g oranges in under 1 minute and 8 seconds. The prize wasn't ours but we had such a great time.

As for the country itself, I could definitely say that Ireland is breathtaking! It is absolutely beautiful! An Emerald island with its rolling hills and vales of green, infinite beaches and mysterious foggy forests I felt in love with it from the first sight. Irish weather is unpredictable and you can't trust the forecast. And, yes, it is raining almost every day. Now I know "there is no bad weather, there are bad clothes".

### STSM Topic: Interindividual variation in response to consumption of plant food bioactives and determinants involved – Analysis of individual datasets



## SHORT TERM SCIENTIFIC MISSIONS

This past April I had the valuable opportunity to visit the Protein chemistry, Proteomics and Epigenetic-signalling (PEPS) lab of the University of Antwerp in Belgium within my Short Term Scientific mission. This mission, focused on the extraction of data from published papers on cellular and molecular targets of plant food bioactives, allowed me to directly contribute to the work of COST Action POSITIVE and achievement of its scientific objectives. Also, it gave me a unique chance to follow the experiments of the investigation about kinase activity using PamGene station in which the host team of Prof. Wim Van den Berghe is an expert. I was able to gain knowledge in a new, state-of-the-art technology and also establish new connections, which will hopefully result in fruitful collaborations and improve my future research. This mission also gave me a chance to meet some great people. The host team was on top of its game, being friendly in and out of the office, showing me the wonderful Antwerp, its social life and guiding me through the tour of famous Belgian chocolates and beers. This STSM was all together a really great experience and I strongly encourage young researchers from the COST POSITIVE network to take the opportunity and apply for their STSM.



**IRENA KRGA from France went to the Protein Chemistry, Proteomics and Epigenetic-signalling (PEPS) lab of the University of Antwerp, BELGIUM**

**STSM Topic: Data extraction from published papers on cellular and molecular targets of plant food bioactives**



## OF INTEREST TO THE POSITIVE COMMUNITY

<http://icph2017-quebec.org/en>

Congress Information ▾

Scientific Program ▾

Speakers

Partnership & Exhibition

Our Partners

Registration ▾



**ICPH 2017**

**8th INTERNATIONAL CONFERENCE ON POLYPHENOLS AND HEALTH**

Québec City Convention Centre

OCTOBER 3 - 6, 2017  
QUÉBEC - CANADA

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## SHORT TERM SCIENTIFIC MISSIONS

### ANA JELENKOVIC from Serbia went to the School of Medicine, University of Glasgow, SCOTLAND

My STSM lasted from the 3<sup>rd</sup> until the 21<sup>st</sup> of April, and I was hosted by Dr. Emilie Combet Aspray, at the School of Medicine, University of Glasgow. I saw this visit as a unique opportunity to learn a statistical tool I have been eager to for a long time, explore an unknown culture and work with professionals from this field of research.

This STSM was an extension on previous work carried out by WG2 team, and the main objectives were to gain practical knowledge in conducting meta-analysis using the Comprehensive Meta-Analysis software, and apply it in order to explore inter-individual variability in platelets response to the intake of certain polyphenols (flavonols, anthocyanins, ellagitannins). Prior to working with the software itself, there was a need for performing a thorough data review, extraction and categorization. Due to the great variability between studies in terms of platelet outcomes, methods, agonist concentrations – this was a quite challenging work to do. But after hours of concentrated and dedicated effort, and with Dr. Emilie's support, I managed to complete both.



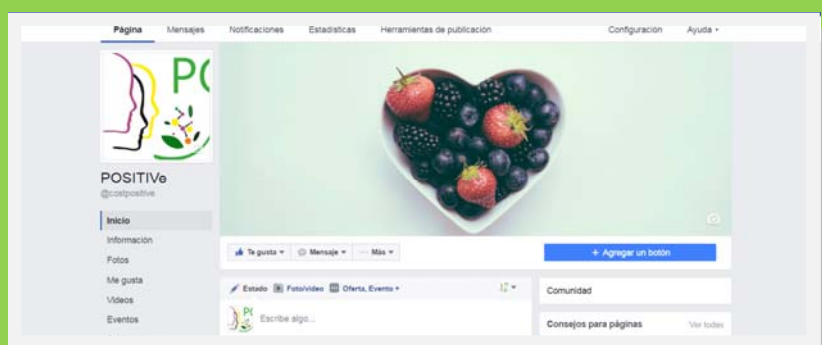
After having the hard work done, I used to stroll around the beautiful city of Glasgow, and seek for Mackintosh's and Alexander Greek Thomson's architectural signatures, or wander through the fairy-tail like Edinburgh, while the stunning Scottish nature left me breathless. I could feel irritated and amused at the same time when all four seasons started changing in one single day, but I always felt enormously happy when I felt the sun warming my face. Scotland is one extraordinary country and Glasgow is one extraordinary city, with vivid music culture and fabulous people. All of this, together with the valuable gained research experience made me have a really unique experience, and I would wholeheartedly recommend to POSITIVE young researchers to apply for STSM grant!

### STSM Topic:

**Meta-analysis training and its application in evaluating polyphenols' effects on cardio-metabolic health**

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## SCIENTIFIC EXPERT'S OPINION

### Dr. Christine MORAND COST POSITIVE Chair

The main physiological functions of the body are known to be developed during the first years of life, reach a peak in early adulthood and then decline. The rate of the decline highly depends on the lifestyle and dietary behaviour. A general consensus raising from a number of population-based and intervention studies is that the most protective diets for human health are those rich in plant foods (Katz & Meller, 2014). A specific feature of these foods is to be the exclusive and abundant dietary sources of a wide range of phytochemicals. This large group of compounds might not be essential throughout life or cause clinically manifested deficiencies, however they are considered "essential" for health and well-being in adulthood and in the elderly population (Holst & Williamson, 2007). Especially, the wide spectrum of their biological activities and associated mechanisms are of interest for the prevention of a diversity of chronic diseases, including cardiometabolic diseases, age-related cognitive decline and some cancers. Thanks to the recent development of databases on phytochemical contents in plant foods, the inverse association between their intake and the incidence of pathologies has been significantly strengthened.

However, to date much of the pleiotropic effects ascribed to dietary phytochemicals are derived from cell studies and animal models and only some of them are backed-up by



## POSITIVE initiatives towards the understanding of interindividual variability and CVD prevention by plant bioactive compounds

human intervention studies. Therefore, the available knowledge is still too limited to establish recommendations for the general population or for populations at risk of specific diseases. In addition, phytochemicals are considered by the body as xenobiotics, underlying a potential risk of toxicity if consumed excessively. For these reasons, research efforts must continue to convince the whole scientific community of nutritionists and physicians about the actual contribution of these non-essential compounds in promoting the health benefits associated to the consumption of plant foods. This is all the more important given that there is no doubt that the real prospects for innovation in the field of nutrition, food and health will come from these plant food bioactive compounds rather than from other macro or micro-nutrients.

We know from clinical research that depending on their physical/genetic make-up individuals respond differently to nutritional challenges, and thereby may experience more or less benefit/risk associated with particular dietary constituents. Even if still poorly explored, this inter-individual variation in responsiveness is of particular relevance for dietary plant bioactives (Manach et al, 2016). Indeed, most of these compounds are absorbed and metabolized through the same polymorphic carriers and enzymatic systems than drugs and other xenobiotics, meaning that the efficiency of their bioavailability is likely to depend on individual genetic background.

In addition, gut microbiota is known to be extensively involved in the metabolism of a number of plant bioactives, especially that of polyphenols. The gut microbiota converts polyphenols into active and bioavailable metabolites; hence variations in its composition and functionality can affect polyphenol bio-efficacy (Tomas-Barberan et al, 2016). Together with genetic background, gut microbiome composition, others factors like age, gender, lifestyle and physio-pathological state could also be responsible for the heterogeneity in responsiveness to plant food bioactives consumption that has often led to inconclusive results in clinical trials aiming to demonstrate the health effects of specific dietary bioactive compounds (Milenkovic et al, 2017).

A clear understanding of why some bioactive plant compounds work effectively in some individuals but not or less in others is crucial for a full consideration of these compounds in future strategies of personalized nutrition to prevent chronic diseases, as well as to underpin the development of new functional and customized foods. Reaching this aim implies first to identify the main determinants of variability and how these different factors interact to influence subject-specific response to the consumption of the main families of plant food bioactives, regarding both their bioavailability and bio-efficacy. This work has been initiated by the European scientific experts involved in the COST Action POSITIVE.

## SCIENTIFIC EXPERT'S OPINION

From the findings of the POSITIVE network and of other complementary initiatives, the relative contribution and possible interactions of the main determinants of inter-individual variability identified should be further validated through dedicated randomized controlled trials and large-scale prospective studies for the different families of plant food bioactives. To show the relative contribution of various factors controlling the bioavailability of the compounds and the individual biological responsiveness to their consumption, as far as possible, these studies will integrate omics approaches for an in-depth characterisation of individuals, including genotyping, gut microbiota analysis, food metabolome profiling, transcriptomic response profile. This information rich datasets will be very useful to establish correlations with health outcomes in both intervention and prospective studies. The power of these studies will also have to be calculated to allow for different 'responsive' subgroups analyses and/or wise targeted recruitments must be performed based on the factors likely to affect the individual response. A major challenge will also be to develop methods and tools to phenotype and stratify individuals based on their ability to respond to the intake of plant food bioactives. Several approaches can be foreseen to develop methods for subjects' stratification, including modelling and challenge tests (Manach et al, 2016). Models may be built from the acquired knowledge to predict the internal exposure of individuals and more complex ones should also be developed to predict biological responsiveness to plant food bioactives intake. To assess the individual's capacity to respond to plant food bioactives intake, the challenge test approach could include the use of standardized supplement containing various bioactives, together with that of standardized postprandial nutritionally challenging conditions defined to induce disturbances impacting health outcomes. This set of approaches will be helpful both to strengthen the scientific knowledge of the determinants of inter-individual variability and to estimate the personal health benefits that an individual can gain from different bioactive compounds.

This research development will undoubtedly lead to innovative applications for these plant food bioactives in the area of personalized nutrition to maintain best health conditions through a balanced and properly administered daily nu-

trition and will offer exciting opportunities for the food and nutraceutical industry to produce healthier and customized foods.

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## CONTACT US



EUROPEAN COOPERATION IN THE FIELD OF  
SCIENTIFIC AND TECHNICAL RESEARCH

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### Webpage:

[www6.inra.fr/cost-positive](http://www6.inra.fr/cost-positive)

**RESEARCH DISSEMINATION**
**FoodComEx – a chemical library for food compounds and food**

The Food Compound Exchange – FoodComEx (<http://foodcomex.org/>) is a virtual chemical library developed as part of the Food Biomarkers Alliance – FoodBALL project (<http://foodmetabolome.org/>) under the coordination of Claudine Manach (INRA, France), Christoph Weinert (Max Rubner Institute – Germany) and David Wishart (University of Alberta - Canada). The aim of FoodComEx is to maintain an online catalog of food compounds and derived metabolites that are not usually commercially available, but could be shared by academic laboratories.

The lack of standards is a major limitation in nutrition studies, especially when it comes to plant food compounds. Authentic standards are useful for validating identifications of compounds in metabolomics profiles of foods, extracts, and samples obtained in human or animal studies. They can also serve to develop quantification methods. Pure standards are crucial for the investigation of the health effects of bioactive metabolites. Too many *in vitro* studies have been performed so far with non-physiologically relevant compounds, due to the unavailability of standards for the identified circulating metabolites of bioactives such as polyphenols. On the other hand, collections of precious molecules have been isolated or synthesized in academic laboratories all over the world.

The idea behind the collaborative platform FoodComEx is to make these molecules available for new studies and for a wider community. Chemists, food scientists or others that are interested in sharing compounds synthesized, isolated or stored in their laboratories can directly upload their compounds in the FoodComEx platform through a user-friendly interface and guided by a video tutorial. Anyone visiting the website will be able to browse the library and for every compound, obtain a description including the chemical structure, method of production, purity, storage conditions, spectral data and the contact details of the provider. Those interested in obtaining a given compound will be able to contact the group that is offering it and establish a cooperation. Providers can also describe their skills and research interests in the providers' directory, where they can be easily found by others reaching out for collaborations. FoodComEx also features a virtual board where users can add the names/structures of wanted molecules, to stimulate their sharing or synthesis by others. The use of FoodComEx is free of charge and the platform will be sustained over the years thanks to the solid bioinformatics infrastructure of University of Alberta, which already maintains major databases such as HMDB, FooDB, DrugBank and PhenolExplorer.

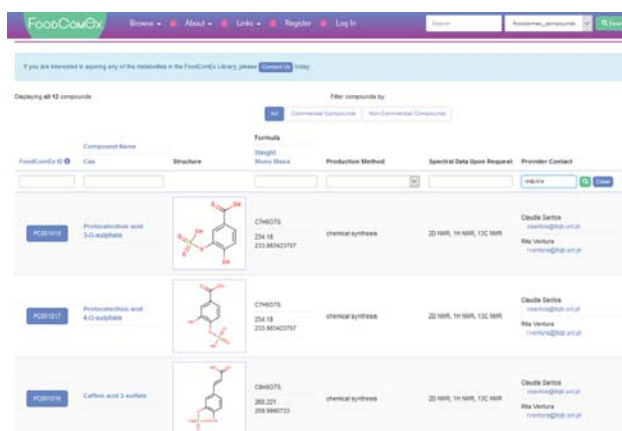
FoodComEx is on its initial development and its success depends on the participation of scientists willing to share their compounds and establish cooperations with other groups. Some of our enthusiastic POSITIVE partners (Claudia

Santos, Pedro Mena, Daniele Del Rio) have already uploaded polyphenol metabolites. Everyone is invited to participate by registering their compounds in the database and forwarding the information on FoodComEx to colleagues likely to be interested in this initiative.

If you have any question, please contact Jarlei Fiamoncini ([jarlei.fiamoncini@inra.fr](mailto:jarlei.fiamoncini@inra.fr)) who is currently leading FoodComEx development



or Claudine Manach ([claudine.manach@inra.fr](mailto:claudine.manach@inra.fr)).

FoodComEx ID	Compound Name	Structure	Molecular Weight	Molecular Mass	Production Method	Spectral Data (ppm Request)	Provider Contact
234 18	Protocatechuic acid 3-O-sulfate	<chem>O=C1C=CC(=C(C=C1)OS(=O)(=O)O</chem>	234.18	232.04622197	chemical synthesis	22 1695, 16 1695, 132 1695	Claudia Santos <a href="mailto:menap@inra.fr">menap@inra.fr</a> Ray Ventura <a href="mailto:menap@inra.fr">menap@inra.fr</a>
234 18	Protocatechuic acid 4-O-sulfate	<chem>O=C1C=CC(=C(C=C1)OS(=O)(=O)O</chem>	234.18	232.04622197	chemical synthesis	22 1695, 16 1695, 132 1695	Claudia Santos <a href="mailto:menap@inra.fr">menap@inra.fr</a> Ray Ventura <a href="mailto:menap@inra.fr">menap@inra.fr</a>
268 221	Caffeoyl acid 3-sulfate	<chem>O=C1C=CC(=C(C=C1)OS(=O)(=O)O</chem>	268.221	266.066713	chemical synthesis	22 1695, 16 1695, 132 1695	Claudia Santos <a href="mailto:menap@inra.fr">menap@inra.fr</a> Ray Ventura <a href="mailto:menap@inra.fr">menap@inra.fr</a>

**RESEARCH DISSEMINATION**
**PUBLICATIONS– WG1**

To date, very few human dose-response studies have been conducted looking at (poly)phenol rich foods and health outcomes; such studies are essential to assess cause-effect relationships and to develop dietary recommendations. Health effects of (poly)phenols are considered to be driven by circulating metabolites rather than the native compounds and, up to now, even less work has looked at plasma and urine (poly)phenol metabolite profiles in relation to dose-response intake of (poly)phenol-rich foods.

juices. A total of 14 of all analyzed plasma metabolites (60 compounds) positively correlated with the amount of (poly)phenols ingested in the drink. In contrast, no linear dose-response was found for total urinary excretion over 24 h, however 12 of the 60 (poly)phenol metabolites displayed a positive linear dose-response.

The inter-individual variability expressed as coefficient of variation of area under the curve (AUC) and maximum concentration in plasma (C<sub>max</sub>) was 53% and 51% for total plasma (poly)phenols, respectively. Of the 60 metabolites quantified the lowest variability in C<sub>max</sub> was found for dihydroferulic acid 4-O-sulfate (CV 43%) and highest variability was detected for vanillic acid (CV 216%). The CV for AUC varied be-

**Inter-individual variability in absorption and metabolism of cranberry (poly)phenols**

Feliciano et al. (Nutrients 2017) evaluated whether the plasma and urinary levels of phenolic metabolites follow a dose response after consumption of cranberry juice; they also assessed the extent of inter-individual variability of circulating metabolite concentrations. A randomized controlled crossover trial was performed in 10 healthy men, in which blood and urine samples were collected over 24 h after consumption of cranberry juices containing 409, 787, 1238, 1534 and 1910 mg total (poly)phenols.

A linear dose-response increase in total plasma (poly)phenol metabolites was found after intake of the cranberry

between 48% for 4-hydroxybenzaldehyde and 163% for 1-methylpyrogallol-O-sulfate. Despite the homogeneous test population (healthy, young, males) many of the metabolites assessed exhibited a very large inter-individual variability, probably dependent on an individual's ability to generate specific metabolites. The authors identify a number of factors that may influence the metabolism and absorption of (poly)phenols including differences in the gut microbiome, genetic polymorphisms in transporters or metabolizing enzymes and environmental impact.


**nutrients**


Article

**Absorption, Metabolism and Excretion of Cranberry (Poly)phenols in Humans: A Dose Response Study and Assessment of Inter-Individual Variability**


Dr. Rodrigo Feliciano



Dr. Charlotte Vills



Geoffrey Istas



Dr. Christian Heiss



Dr. Ana Rodriguez-Mateos



## RESEARCH DISSEMINATION

## PUBLICATIONS-WG2



### An insight into human tissue gene expression interindividual variation in response to supplementation with a pomegranate extract rich in the bioactive compounds ellagitannins

Numerous pre-clinical studies have shown that the beneficial effects attributed to plant bioactive compounds may be mediated, at least partially, by the orchestrated activation in the cells of a range of genes in response to the presence of these compounds. However, how gene expression regulation by plant bioactive compounds may happen *in vivo* remains an unresolved question. An accepted hypothesis is that the compounds that might trigger the gene response in the organism are the metabolites formed in the body and detected in the cells and tissues.

The aim of this human intervention randomized study was to explore some gene expression changes occurring in colon tissues that could be associated with the intake of the bioactive compounds ellagitannins (ETs) or with presence in these tissues of the urolithins, the main gut microbial metabolites derived from ETs. Colon cancer patients consumed a pomegranate extract rich in ETs for several days. We attained colon tissue samples before (baseline biopsies) and after the intervention (post supplementation surgical pieces), extracted the RNA and measured gene expression in comparison with a control group. We also analysed the presence of urolithins in these tissues.

Inter-individual variability in the gene expression response to the intake of bioactive compounds has been scarcely investigated. Gene expression variation is inherent to genes and is influenced by many factors, i.e. disease status, tissue heterogeneity, sampling protocols, etc. Our study corroborated: i) a large inter-individual variability and ii) a considerable impact of the experimental protocol on gene expression. One important issue was that these two aspects were gene- and tissue-specific. Like this, some genes exhibited a lower inter-individual variation (CV=30%) whereas others displayed a

larger one (CV=70%). Equally, some genes were more affected than others by the experimental protocol and some genes resulted induced whereas others were found to be downregulated in the colon samples from the control volunteers. These circumstances, greatly enhanced the difficulty in discerning gene expression changes attributable to the specific supplementation with ETs (expected to be moderate or small changes). Despite all this, we were able to detect some significant and specific gene expression changes in the colon samples from the individuals that consumed the pomegranate extract. These changes suggested a counteracting effect of the consumption of this product to the changes occurring in the control participants. However, these changes could not be associated with the quantity or profile of urolithins in the tissues. Importantly, we were not able to find similarities between the human *in vivo* changes and those previously reported *in vitro* for these metabolites.

Overall, our study shows the feasibility of detecting gene expression changes in human tissues in response to supplementation with bioactive compounds but also points out to various key issues that have yet to be addressed and improved before we can definitively demonstrate these molecular effects *in vivo*: 1) establish an appropriate sample size for these studies, 2) improve designs to reduce protocols impact and enhance samples quality, 3) understand the intrinsic variability in gene expression in human tissues, e.g. factors affecting this variability: epi(genotype), chronobiology, regulation by microRNAs, etc. Gene expression regulation by bioactive compounds is a complex and multifactorial process that will require the implementation of many more complementary *in vitro* and *in vivo* studies before we may be able to reveal the molecular mechanisms underlying the effects of these compounds.

## RESEARCH DISSEMINATION

### INTRODUCING POSITIVE AT:



### Dr. Victoria MORENO ARRIBAS presenting POSITIVE at NUTRACEUTICALS EUROPE (Madrid, SPAIN)

The 1st Edition of the NUTRACEUTICALS Europe Summit & Expo gathered more than 2000 professionals in the sector of the functional and novel ingredients from all over Europe during the past 22<sup>nd</sup> and 23<sup>rd</sup> of February at the Madrid Fair (Madrid, Spain).

With the presentation of more than 50 lectures by national and international leading speakers, Nutraceuticals Europe exposed the latest scientific research, as well as the latest advances in R+D+I of the most relevant companies in the functional and novel ingredients sector.

On this occasion, Dr. Moreno-Arribas from CIAL-CSIC was one of the invited speakers and talked to the audience about: **Wine and Polyphenols, understanding their health benefits through the intestinal microbiome**. After her conference, Dr. Moreno-Arribas also introduced POSITIVE to the attendants using the set of POSITIVE slides prepared by the FG and available to all partners on request.



DETROP, the International Exhibition of Food & Beverage Industry took place at the Exhibition Centre HELEXPO in Thessaloniki (GREECE) during the past 3<sup>rd</sup> to 6<sup>th</sup> of March. DETROP is the a major exhibition in Greece within the food & beverage sector and every year gathers a very high number of exhibitors and visitors. This year, DETROP has been characterized by an extensive international participation from the European Union, Asia and Middle-East, Russia, USA and other places.

Also, a large number of parallel events were organized, including a meeting of the International Observatory of Oxidative Stress, designated: "The anti-oxidant power of the food on our plates". Dr. Christos Kontogiorgis was invited to give a lecture entitled: "**Natural Antioxidants: Truth and myths regarding their role in Public Health**". As part of his presentation, Dr. Kontogiorgis dedicated some time to introduce the aims of the Cost Action POSITIVE using the slides prepared by the FG.



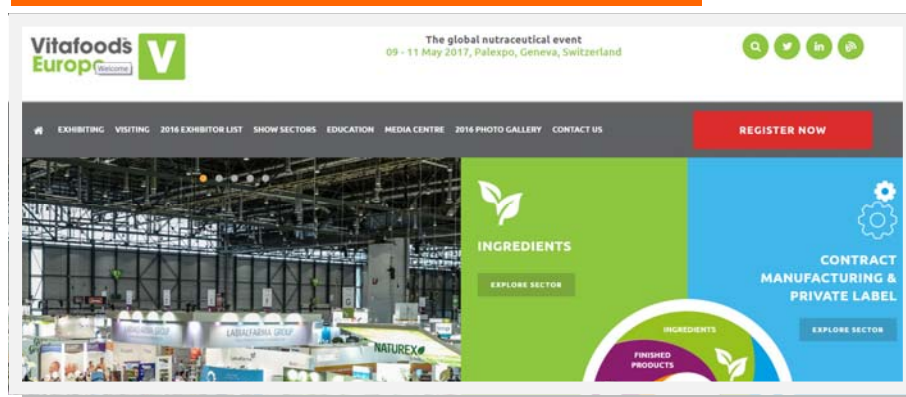
### Dr. Christos KONTOGIORGIS presenting POSITIVE at DETROP (Thessaloniki, GREECE)

All the attendants recognized the important mission of the Action and the innovative role that plays to impulse the research on the benefits of plant foods for health.

## RESEARCH DISSEMINATION

INTRODUCING POSITIVE AT:  
VITAFOODS

Professor Baukje de Roos  
 Deputy Director, Rowet Institute,  
 University of Aberdeen  
 United Kingdom



between consumption and effects of specific plant bioactives in population subgroups? This would certainly be of interest to the main stakeholder group attending the Vitafoods meeting, which were those involved in producing ingredients for other

On the 9<sup>th</sup> and 10<sup>th</sup> of May (2017) I visited the Vitafoods meeting in Geneva on behalf of the COST Action POSITIVE. This meeting was attended by over 18 thousand stakeholders from all over the world and therefore an ideal platform to present the first results of the POSITIVE consortium. During the Educational Sessions on 'Polyphenols' I had the opportunity to discuss how we can address the concept of inter-individual variation in response to consumption of plant polyphenols within the COST Action. The efficacy by which dietary interventions influence health is currently mainly determined by taking population-based approaches that can favourably shift disease risk factors in the entire population, but many of the large RTCs have effectively demonstrated that only 40% of a cohort responds to dietary interventions. Could we, somehow, overcome and indeed benefit from individual variability in responses to interventions? For example, could we work towards future health claims which would be based on a clear relationship

food businesses.

My presentation outlined examples of inter-individual variation in bioavailability, including absorption, distribution, metabolism and excretion of plant bioactives. It also discussed personal requirements - who needs what? This was illustrated by examples of increased efficacy of specific bioactives in specific subgroups such as the presence of specific polymorphisms, increased efficacy in specific age groups or enhanced efficacy in those that produce beneficial metabolites based on their microbiota. Finally, I presented the outcomes of our stakeholder questionnaire, indicating that stakeholders are specifically interested in producing optimised foods (where processing methods may lead to a higher bioavailability of bioactives); as well as in the availability of databases which provide knowledge on the metabolism of bioactives in the human body. The Phytohub activity within the POSITIVE network is an excellent example of this.

<http://www.vitafoodsinsights.com/blogs/speakers-corner/2017/04/developing-plantbased-foods-that-address-individual-needs.aspx>


 The banner features a vibrant image of fresh vegetables like tomatoes, green beans, and cucumbers in wooden crates. Overlaid on the image is the text "Developing Plant-based Foods that Address Individual Needs" and "by Prof Baukje De Roos - © April 16, 2017". At the bottom, there is the Vitafoods Europe logo and a call to action: "REGISTER NOW SAVE €145".

## GET TO KNOW YOUR POSITIVE PARTNER

adipose tissue. It was an exciting time of intense learning, as well as an academic and cultural exchange.

### SENIOR RESEARCHERS



**TATJANA RUSKOVSKA**  
 Faculty of Medical Sciences  
 Goce Delcev University  
 REPUBLIC OF MACEDONIA

#### What is the focus of your research?

My research has mostly been related to atherosclerosis risk factors beyond the conventional lipid status measurements in clinical settings. Biomarkers of lipid peroxidation and antioxidant defense, followed by autoantibodies against oxidized LDLs, protein carbonyls and hsCRP were among the key topics of my research.

Recently, my research has been focused on dysfunctional adipose tissue, since its inflammation and oxidative stress in obesity largely contribute to the whole body insulin resistance, which is an important determinant of metabolic syndrome. In this context, the interest of POSITIVE in cardiometabolic outcomes resulting from consumption of plant bioactives is highly complementary to my work, and helped me set my overall research in a new perspective.

#### In what countries/organizations have you studied or worked?

After obtaining the bachelor's degree in life sciences (biochemistry and physiology) at the University "Ss. Cyril and Methodius" in Skopje, Republic of Macedonia, I started working at the Military Hospital in Skopje as a biochemist in the Central Clinical Laboratory. In parallel to my work at the hospital, I completed my master and PhD studies at the same University. After obtaining the PhD degree, I was appointed Head of the Central Clinical Laboratory. In 2009 I was invited to join the Faculty of Medical Sciences at the University "Goce Delcev" in Stip, Republic of Macedonia, as a professor of clinical chemistry and biochemistry. From 2011 till 2014 I served as a Vice-Dean of the Faculty of Medical Sciences.

#### What has been the greatest achievement in your career?

So far, it has definitely been the Fulbright scholarship, awarded by the United States Department of State. This is a highly competitive grant, and as such it means a lot to me. As a Fulbright scholar, I spent the academic 2014/15 at the University of Minnesota, Twin Cities, in the laboratory of Dr. David Bernlohr, where I was working on the pathophysiology of obese white

#### Which is your favorite paper you have written/co-authored and why?

I will point out two papers which are my favorites at the moment. The first one is my first paper listed on the PubMed (from my perspective, it looked like an elusive goal for many years). This is the paper entitled "Comparative Analysis of Serum (Anti)oxidative Status Parameters in Healthy Persons", co-authored with Dr. Eugene Jansen from the National Institute for Public Health and the Environment, the Netherlands, with whom I have had an excellent cooperation for many years. The second paper which is also very important to me is the paper "Oxidative stress and protein carbonylation in adipose tissue - implications for insulin resistance and diabetes mellitus", co-authored with Dr. David Bernlohr, and written during the preparation of my Fulbright application.

#### Who is/was your most influential mentor/colleague and why?

There are several colleagues/professors who have had a remarkable influence on my career, which I highly appreciate. I already mentioned the cooperation with Dr. Jansen and Dr. Bernlohr. I will also highlight the role of my mentor Prof. Jordanka Dimovska from the University "Ss. Cyril and Methodius" in Skopje, whose extraordinary guidance was an essential determinant of my early career, as well as the influence of Prof. Helen Griffiths from the United Kingdom, an excellent scientist with a remarkable personality, who was practically my first contact with the European science and higher education.

#### What is your advice for young scientists?

Set your goals, and do your best to accomplish them. Science is exciting, definitely not easy, sometimes even frustrating, but if you like it, it's worth spending your life working on it.

#### Where is your favorite place in the world and why?

I have not decided yet, I still explore the world. It must be a place with a breathtaking nature and kind people. And there must be a lab.

#### What is your favorite music/book?

I listen to modern pop music and every few months I make my own selection of several favorite songs. Some of them attract me with the rhythm, sometimes it is the interpretation, and sometimes the lyrics... it is different for every song. I also like jazz and bossa nova. Regarding the books, it is completely different. For many years now, Coelho's "Alchemist" has been my favorite book. It seems it's all about (bio)chemistry after all.

#### What is your favorite sport(s)?

I am not a fan of sports, and I simply do not keep track of the sports events. For recreation, I like walking/hiking, and from time to time I practice a variation of yoga, for flexibility and relaxation.

## GET TO KNOW YOUR POSITIVE PARTNER

### SENIOR RESEARCHERS

#### What is the focus of your research?

I work in the Nutrition Section of the Food Engineering Department at Ege University, Turkey. It is one of the most important state universities of Turkey, situated in the heart of the Aegean. In this department, I have the pleasure to teach undergraduate and graduate courses. Further, I am supervisor for 3 MsD graduate and 2 PhD students. In addition, a branch laboratory with quite good facilities in scientific research is present. My main scientific approaches are on structuring foods with health benefits, release of bioactive components in the gut and demonstrating health benefits in food systems currently studied, dairy and fruit/vegetable. I took my degree at the department of food engineering. Later on, I made my speciality on food science and nutrition. After my undergraduate and graduate programs, being well informed on the subject of food and knowing the food processes well has been a great advantage for me in nutrition studies. I am currently studying topics such as *in vitro* static digestion of food and *in vitro* amylase and lipase inhibition in fat and carbohydrate digestions.

#### In what countries/organisations have you studied or worked?

I have not worked in any other countries, but I have partner projects with many countries. The newest is the European Union project named Pathway27 which is still going on.

#### What has been the greatest achievement in your career?

For me, my greatest achievement was my transition to academia after a professional career. Since then every day comes and feels as an achievement. For me greatest attainment is waking up every day and still having that zeal and enthusiasm to go to work like it's your first day. Therefore I would like to count my enthusiasm and love of 35 years in academia as my greatest achievement.

#### Which is your favourite paper you have written/co-authored and why?

My favourite one would have to be "El S. N., Simsek S. Food Technological Applications for Optimal Nutrition: An Overview of Opportunities for the Food Industry. 2012. Comprehensive Reviews in Food Science and Food Safety. Vol 11, 2-12."

That year, (2012) I had the chance to give inspiration to many young researchers on the subject of food and nutrition including my graduates in choosing the subject of their thesis. The years that followed, this paper and it's subject conceived many publications. I would like to think I had a good foresight.

#### Who is/was your most influential mentor/colleague and why?

That would be my colleague and friend Dr. Sibel Kara Kaya.

Our friendship started on our youthful first days at the university, and still goes on in the academic platform. We have been working on the same discipline for 30 years. This always gave us a good synergy and together we did fruitful studies and brought up students.



**SEDEF NEHIR EL**  
**EGE UNIVERSITY,**  
**Food Engineering Dept.,**  
**Nutrition Section,**  
**Izmir, TURKEY**

#### What is your advice for young scientists?

To young scientist, I would suggest them to always have a deadline for whatever their heart desires to study or research and to keep their deadlines. Yes, over time they may be more anxious, alert or even sometimes impatient when racing time. This is not something to worry, for these are all beneficial for the passion when one follows the path of science.

#### Where is your favourite place in the world and why?:

My favorite place in the world is my home. It embraces all the places I've had the chance to visit, where I walked, what I ate, how I lived. All these experiences come together in beautiful harmony that energies and reflects, reminds me.

#### What is your favourite music/book?:

There is one song that always holds the special place in my heart ever since my earlier years; it is called 'Living Next Door to Alice' by Smokie. I am attaching the youtube link for you to listen; <https://www.youtube.com/watch?v=Z6qnRS36EgEU>

Among the sea of books, it is very hard to pick one. But I can encourage everyone to read Momo by Michael Ende.

#### What is your favourite sport(s)?:

I am ashamed to say that I am not the athletic type therefore I do not have any favorite sports. But I have always taken pleasure from watching artistic skate championships and never miss to do so.

## GET TO KNOW YOUR POSITIVE PARTNER

### EARLY STAGE RESEARCHERS

#### What is the focus of your research?

In the Laboratory of Vascular Biology and Nutrigenomics of the CNR Institute of Clinical Physiology (CNR-IFC)-Lecce (Italy) we studied the effect and related mechanisms of food and dietary ingredients, mainly of the Mediterranean dietary pattern, on the pathophysiology and clinic of cardiometabolic diseases. To this aim, we use relevant *in vitro* model systems including human endothelial cells, monocytes/macrophages, and adipocytes to perform cellular and molecular studies. Furthermore, we perform human studies to evaluate changes in health indices or discover new biomarkers of exposure/effect in response to diet or dietary constituents. We are particularly interested in the health effect of polyphenols (from olive oil, red wine, etc.) and fatty acids (mono- and poly-unsaturated fatty acids).

#### In what countries/organisations have you studied or worked in?

I completed all my academic studies in Italy. I graduated in Biology at the University of Salento, Lecce, and received my PhD in Innovative Strategies in Biomedical Research at the Sant'Anna School of Advanced Studies, Pisa. During my PhD studies and then as a Postdoctoral Researcher I conducted my research activities at the CNR-IFC, Lecce, Laboratory of Vascular Biology and Nutrigenomics. Now I work at the CNR-IFC Lab as a permanent researcher.

#### What has been the greatest achievement in your career?

The greatest achievement in my career is the joint collaboration and networking with other researchers to exchange scientific ideas, doubts, research projects and results. This has helped to open my mind to the critical thinking, to adopt a multidisciplinary approach to the research and to be prone to discussion with my peers as a step for my personal and scientific growth.

#### Which is your favourite paper you have written/co-authored and why?

My favourite paper relates to the important effect of two extra virgin olive oil compounds, the monounsaturated fatty acid oleic acid and the polyphenol hydroxytyrosol, on the regulation of adiponectin, an adipokine with beneficial properties against cardiometabolic diseases (Additive regulation of adiponectin expression by the mediterranean diet olive oil components oleic Acid and hydroxytyrosol in human adipocytes. *PLoS One*. 2015 Jun 1;10(6):e0128218. doi: 10.1371/journal.pone.0128218. eCollection 2015). After a years-long and hard research activity, this publication represents an important personal milestone and also would contribute to the advancement in the understanding of the Mediterranean diet health benefits.



**EGERIA SCODITTI**

**CNR - Institute of Clinical Physiology  
Lecce, ITALY**

#### Who is/was your most influential mentor/colleague and why?

Professor Raffaele De Caterina is the most important mentor during my career. Besides being my PhD thesis supervisor, he introduced me to the research field inspiring ideas and projects, and importantly encouraging me to be self-confident and independent.

#### Where is your favourite place in the world and why?

My favourite place in the world is Sicily in Italy, because of its history, art and archeology all around the island, sunny places, breath-taking scenery, and unforgettable Mediterranean food.

#### What is your favourite music/book

I love reading Isabel Allende, but also crime novels. Regarding music, I like listening to pop music.

#### What is your favourite sport(s)?

I like walking in the countryside with my 14-years old husky Jack.

