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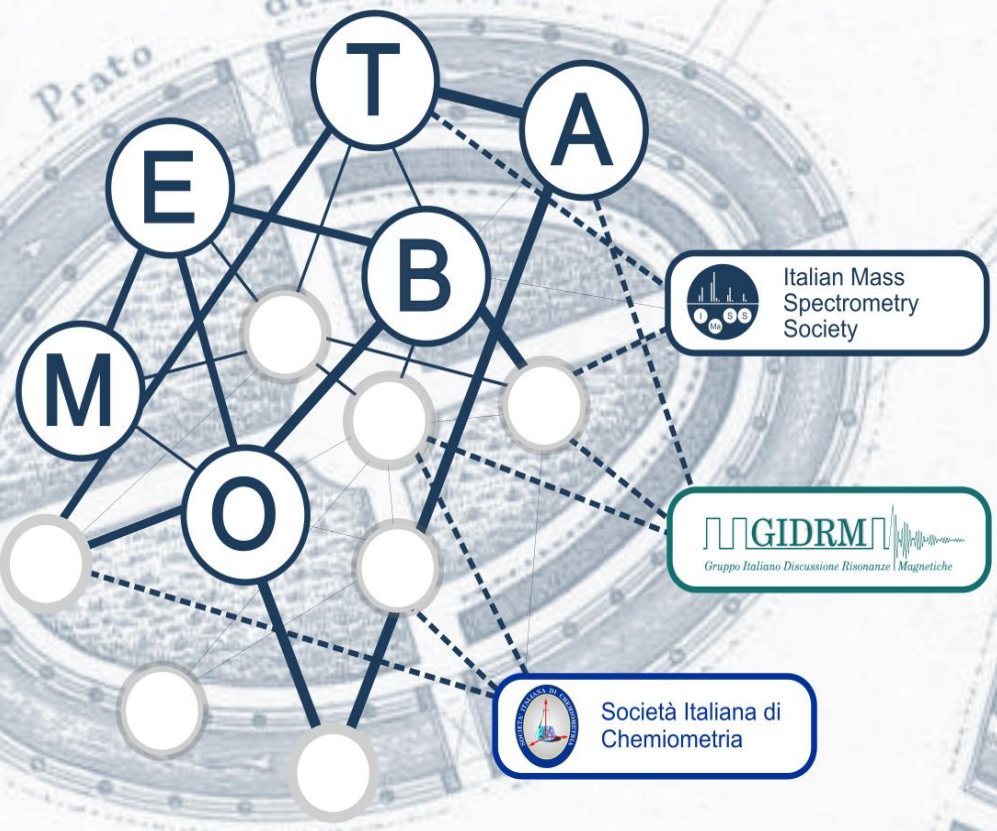

Advances in NMR and MS based Metabolomics

Padova, November 14 - 16, 2017

Palazzo del BO - Via 8 Febbraio, 2
Orto Botanico - Via Orto Botanico, 15

Plenary Speakers

Theodore Alexandrov - *EMBL, DE - UCSD, USA*
Benedicte Elena-Herrmann - *University of Lyon, FR*
Ana Gil - *University of Aveiro, PT*
Elaine Holmes - *Imperial College London, UK*
Serge Rezzi - *Nestlè Institute of Health Sciences, CH*
Johan Westerhuis - *University of Amsterdam, NL*

Network Italiano di Metabolomica

Italian Mass Spectrometry Society
Gruppo Italiano Discussione Risonanze Magnetiche
Società Italiana di Chemiometria

Keynote Speakers

Silvia Carraro - *University of Padua, IT*
Claudio Luchinat - *GiottoBiotech - University of Florence, IT*
Luisa Mannina - *University of Rome "La Sapienza", IT*
Francesco Savorani - *Politecnico di Torino, IT*

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Roberta Seraglia (CNR-ICMATE, Padova

Matteo Stocchero (Università di Padova)

Program

Tuesday, 14th November 2017

09:00-12:30 **Tutorials** *(Aula Nievo, Palazzo Bo*)

- 09:00-9:30 **Experimental Design in Metabolomics**
  (*Federico Marini*)

- 09:30-10:15 **NMR based Metabolomics: Experiments and Data Processing**
  (*Stefano Mammi*)

- 10:15-11:15 **MS based Metabolomics: Experiments and Data Processing**
  (*Giuseppe Giordano, Pietro Franceschi*)

- 11:15-12:15 **Data Analysis; Principles and Tools**
  (*Marina Cocchi, Matteo Stocchero*)

12:00-15:00 **Registration** (*Palazzo Bo*)

Chairman: Giuseppe Giordano, Stefano Mammi

14:00-15:00 **Welcome and Introduction to the Congress** (*Aula Magna, Palazzo Bo*)

15.00-15.30 **IMaSS, GIDRM and ASIC Presentation**

15:30-16:20 **Elaine Holmes**
(Tu1) What can Metabolic Profiling and the exposome tell us about chemical risk?

Metabolomics Society Grants:

16:20-16:40 **Monica Scognamiglio**
(Tu2) Metabolomics as a tool to study allelopathic interactions between Mediterranean plants.

16:40-17:00 **Veronica Ghini**
(Tu3) Cell metabolomics- an innovative tool to investigate cellular processes.

17:00-17:20 **Alessia Trimigno**
(Tu4) A multi-platform metabolomic approach for the identification of urine and serum dairy intake biomarkers.

17:20-18:10 **Ana Gil**
(Tu5) Cell Metabolomics: stories and challenges.

18:10-21:30 **Appetizer** (Cavour Cafe)

Wednesday, 15th November 2017

(Orto Botanico)

Impact of Metabolomics in Clinical Medicine

Chairs: Pietro Franceschi, Luigi Atzori

09:10-10:00 **Theodore Alexandrov**
(W1) Spatial Metabolomics in tissues and single cells.

10:00-10:30 **Silvia Carraro**
(W2) Metabolomic approach in asthma: a clinician's perspective.

10:30-10:50 **Andrea Armirotti**
(W3) Single neuron lipidomics made real.

10:50-11:10 **Pierluigi Caboni**
(W4) A lipidomics approach for the diagnosis of inflammatory bowel disease using QTOF-LC/MS and Ion Mobility-QTOF-LC/MS.

11:10-11:30 **Nicola Cavallini**
(W5) Discrimination of glioma brain tumor grades through Multivariate Data Analysis on ^{1}H-HR-MAS NMR *ex-vivo* spectra.

11:30-11:50 ***Coffee Break***

Metabolic Phenotyping

Chairs: Paola Turano, Andrea Armirotti

11:50-12:20 **Manfred Spraul**
(W6) NMR Based Tools in Clinical/Translational Metabolomics.

12:20-12:50 **David Heywood**
(W7) Platforms for the Precision Measurement of the Metabolome Powering biological insights: precisely profiling complex samples from large experimental cohorts.

12:50-13:10 **Rosaria Cozzolino**
(W8) Urinary volatile organic compounds (VOCs): application in clinics.

13:10-13:30 **Giuseppe Corona**
(W9) Pharmacometabolomics approach to identify new biomarkers of response to HER-2 targeted anticancer therapy.

13:30-14.30 ***Lunch and Poster Session***

Food / Nutrition

Chairs: Matteo Stocchero, Luisa Mannina

14:30-15:20 **Serge Rezzi**
(W10) Perspectives for Metabolomics in Human Nutrition .

15:20-15:50 **Claudio Luchinat**
(W11) Profiling visible and invisible metabolites in urine by NMR.

15:50-16:10 **Flaminia Cesare Marincola**
(W12) Impact of early postnatal nutrition on the NMR urinary metabolic profile of infant.

16:10-16:30 **Stefano Dall'Acqua**
(W13) The use of metabolomics in the study of nutraceuticals: cranberry and green coffee.

16:30-16:50 ***Coffee Break***

Food / Nutrition

Chairs: Marco Roverso, Graziano Guella

16:50-17:20 **Luisa Mannina**
(W14) NMR based Metabolomics in food Science: outcomes and critical points.

17:20-17:40 **Francesco Fanizzi**
(W15) Harvest year effect on Apulian EVOOs assessed by ^{1}H NMR based Metabolomics: a deeper study on altitude-based classification.

17:40-18:00 **Federica Angilè**
(W16) Metabolic profile comparison of different compartments of scyphomedusa *Rhizostoma pulmo*: preliminary results.

18:00-18:20 **Roberto Consonni**
*(W*17) Multi-spectroscopic investigations of *Crocus sativus* L. tissues.

18:20-19:10 **Bénédicte Elena-Hermann**
(W18) NMR Metabolomics in action: from real-time analyses to longitudinal snapshots in biological systems.

Thursday, 16th November 2017

(Orto Botanico)

Computational and Statistical Tools

Chairs: Federico Marini, Marina Cocchi

09:10-10.00 **Johan Westerhuis**
(Th1) Searching for metabolic changes in human nutritional intervention studies.

10:00-10:30 **Francesco Savorani**
(Th2) Untargeted NMR metabolomics: from raw data to valuable knowledge through chemometrics.

10:30-10:50 **Eleonora Amante**
(Th3) Optimization of a GC-MS method for endogenous anabolic androgenic steroids (EAAS) detection and application for new screening strategies of prostatic diseases.

10:50-11:10 **Vito Gallo**
(Th4) New metrics for performance assessment in NMR fingerprinting and simultaneous multicomponent qNMR analysis.

11:10-11:30 **Emanuela Locci**
(Th5) Metabolomics signature reveals distinctive patterns in an animal model of mechanical asphyxia and dysrhythmic cardiac arrest.

11:30-11:50 ***Coffee Break***

Metabolomics Applications

Chairs: Giuseppe Pieraccini, Claudio Luchinat

11:50-12:10 **Luigi Atzori**
(Th6) Pitfalls and Caveats in Clinical Metabolomics.

12:10-12:30 **Anatoly Sobolev**
(Th7) NMR and MS metabolomics of baobab (*Adansonia digitata* L.) products.

12:30-12:50 **Giuseppe Paglia**
(Th8) Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry based metabolomics workflow.

12:50-13:10 **Matteo Stocchero**
(Th9) The untargeted mass spectrometry analysis for the study of the "pediatric community acquired pneumonia".

13:10-13:30 **Zeeshan Shah**
(Th10) A detailed lipidomics and proteomics exploration of the FAAH knockout mice brain.

13:30-14:30 ***Lunch and Poster Session***

14:30-15:30 **Discussion Group**
Chairs: Federico Marini, Pietro Franceschi, Marco Geppi

15:30-15:45 **Best Poster Award**

15:45-16:00 ***Closing Remarks***

Abstracts

Tu1

What can metabolic profiling and the exposome tell us about chemical risks?

Elaine Holmes[1]

[1]*Division of Computational and Systems Medicine, Department of Surgery and Cancer. Imperial College London, Exhibition Road, London, SW7 2AZ.*

The complexity and metabolic regulation of the human ecosystem is partially controlled by environmental factors such as diet, lifestyle, toxic exposures and the gut microbiome, which interacts with the mammalian system at the level of genes, proteins and metabolism. Metabolic profiling of biofluids such as urine, plasma or fecal water encompassing high-resolution spectroscopic methods (NMR spectroscopy, LC-MS, GC-MS etc) in combination with multivariate statistical modeling tools, can provide a window for investigating the impact of toxins on human health since these metabolic profiles carry information relating both to genetic and environmental influences, including contributions from the microbiome, diet and xenobiotics [1]. Examples of urinary or faecal metabolites that are products of the metabolism of toxins or toxic/detoxification products of microbiota, or microbiota-host interactions include phenols, indoles, bile acids, short chain fatty acids and choline derivatives, all of which can be quantitatively profiled using spectroscopic technology.
The microbiome is highly metabolically active and has been shown to be capable of modulating toxins to either enhance or ameliorate the host response to toxicity. A range of examples taken from pre-clinical and clinical studies will be explored and the use of various models of microbial modulation discussed in the context of understanding drug metabolism and toxicity. Additionally the wider role of metabolic profiling in the context of biomonitoring applications is discussed.

References

[1] Nicholson JK, et al Xenobiotica. 1999; 29(11):1181-9. PubMed PMID: 10598751.

Tu2

Metabolomics as a tool to study allelopathic interactions between Mediterranean plants

Monica Scognamiglio, Bernd Schneider

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Allelopathy plays a very important role in ecosystems. It is defined as any direct or indirect, harmful or beneficial effect of one plant on another through the production of chemicals released into the environment.[1]
The understanding of this phenomenon has been partially constrained, among other things, by the methods available to study the secondary metabolites involved. A new method based on metabolomics has been recently developed,[2,3] and it is herewith applied to the study of allelochemicals from selected plant species of the Mediterranean region. Donor plant (*Arbutus unedo*, *Myrtus communis*, *Medicago minima* and *Daphne gnidium*) extracts were analysed by ^{1}H and 2D NMR in order to define their chemical composition. They were tested for their phytotoxicity on a receiving plant species (*Aegilops geniculata*) on which morphological and metabolomics analyses were performed. Tests were carried out also with partially purified fractions and with the pure putative allelochemicals. The extracts of the four plant species showed a strong inhibitory activity on the receiving plant. NMR paired with multivariate data analysis of the receiving plant let to hypothesize the main metabolic pathways affected. Studies with the pure compounds confirmed in some cases the putative allelochemicals, while in other cases it was possible to determine the occurrence of synergistic effects. Some of the compounds were taken up and, in some cases, modified by the receiving plant.

References

1. Rice E. L., Allelopathy, 1984
2. D'Abrosca B. et al., 2013. Phytochemistry 93, 27
3. Scognamiglio M. et al., 2014. Phytochemistry 106, 69

Tu3

Cell metabolomics- an innovative tool to investigate cellular processes

Veronica Ghini[1], Claudio Luchinat[2], Paola Turano[3].

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Cell metabolomics has emerged as a promising tool with wide applications in many research areas [1]. Metabolite concentrations represent sensitive markers of responses of cells to different stimuli; thus, non-targeted metabolomics represents a very good approach to explore the cell phenotype and to have an overall portrait of the biological process under investigation. In this framework, high-field ^{1}H NMR-spectroscopy (900 MHz) is an efficient and highly reproducible platform for the analysis of cell cultures both in terms of their endo- and exo-metabolome. The type and abundance of metabolites detected in cell lysates and their respective growing media can be viewed as a global fingerprint that unambiguously describes the current metabolic status of the system [2].
There are many possible applications for cell metabolomics, including studies aimed at characterizing cellular responses to toxic and environmental factors, as well as to treatment with bioactive molecules or drugs [3]. In particular, metabolomic analysis of cancer cells can be extremely useful for understanding the mechanisms of tumor insurgence and progression, identifying key players in these events and studying the response and/or resistance to therapeutics.
In this context, human cell metabolomic analysis has been used to study the role of enzyme sphingosine kinase 1 (SK1) in oncogenesis in ovarian cancer, using an original multidisciplinary approach combining NMR with biochemical analyses [4]. Moreover, we are presently applying the same approach to study the metabolic effects introduced by a promising new drug in brain tumor cell lines.

References

[1] Zhang, A. Sun, H. Xu, H. Qiu, S. Wang, X. *Omics J Integr Biol*. **17**,10 2013.
[2] Nicholson, JK. Lindon, JC. *Nature*. 455, (2008).
[3] Ghini, V. Di Nunzio, M. Tenori, L. Valli, V. Danesi, F. Capozzi, F. Luchinat, C. Bordoni, A. *Int J Mol Sci.* **18**, 2 (2017)
[4] Bernacchioni, C. Ghini, V. Cencetti, F. Japtok, L. Donati, C. Bruni, P. Turano, P. *Mol Oncol.* **11**, 5 (2017).

Tu4

A multi-platform metabolomic approach for the identification of urine and serum dairy intake biomarkers

Alessia Trimigno[1], Gianfranco Picone[1], Linda H Münger[2], Carola Freiburghaus[2], Grégory Pimentel[2], Kathryn J Burton[3], Nathalie Vionnet[3], François Pralong[3], René Badertscher[2], Guy Vergères[2], Francesco Capozzi[1]

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[2] Federal Department of Economic Affairs, Education and Research EAER, Agroscope, Berne, Switzerland
[3] Service of Endocrinology, Diabetes and Metabolism, Lausanne University Hospital, 1011 Lausanne, Switzerland

Food intake biomarkers (FIBs) found in human biofluids can be used as objective tools for the assessment of the intake of specific foods and the investigation on dietary habits. An acute cross-over randomized intervention study was carried out in order to determine candidate FIBs for milk and cheese consumption. Urine and serum were collected from eleven young healthy participants at baseline and different time-points and analysed employing a multi-platform metabolomic approach, through GC-MS and 1H-NMR. Some of the candidate FIBs were found in both urine and serum, such as lactose, galactose and galactonate for milk, 3-phenyllactic acid for cheese and pinitol for the control soy drink. The investigated kinetics of the candidate biomarkers also gave interesting results, showing how glucose had a decreasing blood concentration right after the intake of soy drink. These FIBs have to be validated in observational studies, though they could be very helpful for the assessment of dairy intake in nutritional and dietary investigations.

Tu5

Cell metabolomics: stories and challenges

Ana M. Gil[1], Iola F. Duarte[1]

[1]Department of Chemistry, CICECO- Aveiro Institute of Materials (CICECO/UA), University of Aveiro, 3810-193 Aveiro, Portugal; agil@ua.pt

Cell metabolomic strategies have been increasingly exploited in studies spanning from drug testing and development, to assessment of materials performance and biotoxicity. In this presentation, examples are given of the use of Nuclear Magnetic Resonance (NMR)-based metabolomics to analyse cells and/or cell extracts in the contexts of 1) anti-cancer drug development and 2) nanoparticle (NP) function and biotoxicity assessment. In vitro NMR studies of newly synthesized metal-based drugs (with Pt or Pd centers) are shown to provide insight into the response of cancer and healthy cells to treatment, both in single-drug and combination protocols, unveiling interesting drug synergetic effects. In the context of materials, silver NPs action and biotoxicity are addressed for several cell types. Here, in vitro metabolomics helps understand the roles of particle size/composition in triggering cell response and biotoxicity. The advantages and shortcomings of in vitro metabolomic strategies are discussed and an outlook on the translation to in vivo systems is presented.

References

[1] Lamego, I., Marques, M.P.M., Duarte, I.F., Martins, A.S., Oliveira, H., Gil, A.M., Journal of Proteome Research 16, 1773 (2017).
[2] Carrola, J., Bastos, V., Jarak, I., Oliveira-Silva, R., Malheiro, E., Daniel da Silva, A.L., Oliveira, H., Santos, C., Gil, A.M., Duarte, I.F., Nanotoxicology 10, 1105 (2016).

W1

Spatial metabolomics in tissues and single cells

Theodore Alexandrov[1]

[1] *EMBL, DE - UCSD, USA*

Metabolite imaging mass spectrometry promises to localize small molecules, metabolites, and lipids in tissues, microbial and cell cultures, and to interpret them in the context of cellular heterogeneity. Just until recently the molecular interpretation of the big data generated by this technique was hampered by the lack of bioinformatics for metabolite identification. We developed a bioinformatics approach that allowed us to reveal images of localization of hundreds of metabolites in a variety of biological systems. First, I will present how our community big data analytics of data from hundreds of datasets paves an avenue to creating comprehensive metabolite atlases on the levels of tissues and organs. Second, I'll present our recently developed approach to spatial single-cell metabolomics of adherent cell cultures that enabled us to associate fluorescent phenotype with intracellular metabolites and to discover metabolically-determined cell subpopulations.

W2

Metabolomic approach in asthma: a clinician's perspective

Silvia Carraro[1]

[1] *Unit of Allergy and Respiratory Medicine, Wmone's and chidlren's Health Department, University of Padova*

Asthma is a particularly interesting disorder for the application of metabolomics, given the interplay between genetics and environment in its etiology, and the somewhat limited understanding of its pathophysiology.
Several studies have been based on the application of a metabolomic approach to different biofluids for the investigation of asthma. Dealing with the pediatric population the studies conduced on urine and exhaled breath samples are of particolar interest inasmuch as they do not require invasive procedure to obtain such samples.
As for the application of metabolomics in asthma research, 4 areas seem presently the most promising.
The first area is represented by the characterization of asthma phenotypes. In particular a number of studies provided evidence that different metabolomic arrangements underlie severe and non-severe asthma, with the potential for the identification of biomarkers specifically related to the most severe forms.
In second place, a promising field of research is represented by the study of the relationship between human metabolome and anti-asthma. Metabolomics provides a tool both for studying the effects of antiasthma drugs on human metabolism and for identifying biomarkers predictive of the response to such drugs .
In third place the metabolomic approach may have a role in the early identification of children with asthma within the large group of children with recurrent wheeze during preschool age.
Last, the longitudinal monitoring of the metabolic arrangement may have a role in the characterization of specific profiles predictive of the loss of control in asthma

W3

Single neuron lipidomics made real

Andrea Armirotti[1], Collin B. Merrill[2], Abdul Basit[1], Yousheng Jia[2], Christine M. Gall[2,3], Gary Lynch[2] & Daniele Piomelli[1,2]

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[3] *Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA, 92697, USA.*
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Our understanding of the physiological and pathological functions of brain lipids is limited by the inability to analyze these molecules at cellular resolution. Here, we here present a method that enables the detection of lipids in identified single neurons from live mammalian brains. Neuronal cell bodies are captured from perfused mouse brain slices by patch clamping, and lipids are analyzed using an optimized nanoflow liquid chromatography/high-resolution mass spectrometry protocol. In a first application of the method, we identified more than 40 lipid species from dentate gyrus granule cells and CA1 pyramidal neurons of the hippocampus. This survey revealed substantial lipid profile differences between neurons and whole brain tissue, as well as between resting and physiologically stimulated neurons. The results suggest that patch clamp-assisted single neuron lipidomics could be broadly applied to investigate neuronal lipid homeostasis in healthy and diseased brains.

References

Merrill CB., Basit A., Armirotti A., Jia Y., Gall CM., Lynch G., Piomelli D. Patch clamp-assisted single neuron lipidomics. Sci Rep. 2017 Jul 13;7(1):5318.

W4

A lipidomics approach for the diagnosis of inflammatory bowel disease using QTOF-LC/MS and Ion Mobility-QTOF-LC/MS

Pierluigi Caboni[1], Antonio Murgia[1,2,] Christine Hinz[2], Sonia Liggi[2], Zoe Hall[2], Luigi Atzori[3], Julian L. Griffin[2]

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[3] Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria di Monserrato S.S.554

Inflammatory Bowel Disease (IBD) is a group of pathologies defined as chronic inflammation of the intestine with an unclear aetiology. Crohn's disease and ulcerative colitis are the most common manifestations. Currently, the most common diagnostic practices are very expensive and invasive. Serological biomarkers have been recently proposed for the diagnosis, but they remain unused in clinical applications. In this work, the lipidomic profiles of plasma and faecal samples of IBD patients have been compared with healthy individuals using a QTOF-LC/MS and an IM-QTOF-LC/MS. Through the use of QTOF-LC/MS and IM-QTOF-LC/MS, variations in the lipid profile between IBD and healthy individuals were highlighted. In particular, dyacilglycerols, phosphatidylcholines, lyso-phosphatidylcholines and fatty acids were found significantly changed among the groups of samples. Moreover, a solid phase extraction was achieved for a restricted group of lipid plasma extracts. Three different lipid phases were obtained: glycerophospholipids, neutral lipid and free fatty acid fractions. Comparing UC vs CD the statistical analysis reported significant differences in the glycerophospholipid and neutral lipid fractions. In conclusion, given the improvement of resolution and identification power of lipid species, the use of lipidomics and mass-spectrometry can be considered a valuable tool for the IBD diagnosis investigation.

W5

Discrimination of glioma brain tumor grades through Multivariate Data Analysis on ^{1}H-HR-MAS NMR *ex-vivo* spectra

Nicola Cavallini[1,2], Valeria Righi[3], Adele Mucci[1], Antonella Valentini[4], Marina Cocchi[1]

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Gliomas account for about 40% of total primitive brain tumors, and discrimination between high and low glioma grades is a vital diagnostic decision, determining the most effective treatment and having an important impact on patient management and outcome.
In vivo MRS can support the diagnosis of cancer based on MRI, but it can only be used when the molecular markers are well established. Their identification can be derived from the spectroscopic analysis of *ex vivo* biopsy samples using HR-MAS NMR technique.
45 specimens of brain tissue were obtained from 35 patients diagnosed either with glioma or lymphoma, and analyzed using HR-MAS NMR. With the aim of retrieving as much information as possible, three different pulse sequences were used, giving rise to three spectral datasets.
Multivariate data analysis was performed to identify informative metabolites and their interactions. The key goals were: 1) explore the data; 2) integrate the spectra to enhance information retrieval and interpretability; 3) distinguish among glioma grades III and IV.
After proper alignment [1], the datasets were explored by Principal Component Analysis (PCA). The spectra were integrated using Multivariate Curve Resolution (MCR, [2]) on selected intervals. MCR is a method able to resolve overlapping peaks and separate them, by extracting their "pure" peak shapes and their relative concentration in each sample. The concentration information was used to produce the interval-resolved data, which are in principle a more compact and cleaner version of the original data [3].
Class-modeling (SIMCA) and Discriminant Analysis (PLS-DA) were finally applied to the interval-resolved data considering each sequence dataset both separately and datafused. The differences in performance were assessed.

References

[1] F. Savorani, G. Tomasi, and S. B. Engelsen, "icoshift: A versatile tool for the rapid alignment of 1D NMR spectra," *J. Magn. Reson.*, vol. 202, no. 2, pp. 190–202, 2010.
[2] A. de Juan and R. Tauler, "Multivariate Curve Resolution (MCR) from 2000: Progress in Concepts and Applications," *Crit. Rev. Anal. Chem.*, vol. 36, no. 3–4, pp. 163–176, 2006.
[3] F. Savorani, M. A. Rasmussen, Å. Rinnan, and S. B. Engelsen, "Interval-Based Chemometric Methods in NMR Foodomics," 2013, pp. 449–486.

W6

NMR Based Tools in Clinical/Translational Metabolomics

Manfred Spraul[1] , Hartmut Schäfer[2] , Claire Cannet[2], Fang Fang[2]

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NMR for long has been considered a tool for structure elucidation. In the last years, mixture analysis has come into the focus of NMR driven by Metabolomics needs. High throughput screening applications have been established in food analysis and in the analysis of body fluids, tissues and cell extracts. Despite the lower sensitivity, NMR has other important advantages for Metabolomics. Standardization and automation in Metabolomics NMR range from preparation to final data analysis. Taken e.g. the complexity of human urine and its daily variability, highest effort is needed deliver meaningful quantification results and statistical models with high specificity and sensitivity. With the introduction of 2D-NMR a new level of quantification is possible, reducing the signal overlap in one-dimensional spectra. It can be shown, that wet spiking is not sufficient to guarantee correct limits of detection and quantification. The efficiency of numerical spiking procedures is explained, solving the problem. Besides quantification of small molecules also lipoproteins and their subclasses in plasma/serum can be analyzed. Application examples are shown. Ring- and proficiency tests are discussed as well as biobanking use.

W7

Platforms for the Precision Measurement of the Metabolome
Powering biological insights: precisely profiling complex samples from large experimental cohorts.

David Heywood

Sr Manager Omics Business Development, Waters

In order to be successful, the researcher is faced with the challenge of balancing metabolomic coverage with accurate, reproducible and robust LC-MS measurements. In this presentation we will describe how hyphenated high resolution mass spectrometry techniques, including Ion Mobility and SONAR, a fast and selective data independent acquisition mode, are combined with UPLC and informatics workflows to address these challenges. We will review how these solutions have been successfully applied to a multi-omics study of metabolic disorder.

W8

Urinary volatile organic compounds (VOCs): application in clinics

Rosaria Cozzolino, Antonella Martignetti, Beatrice De Giulio

Institute of Food Science, ISA CNR Via Roma, 64 83100 Avellino; rcozzolino@isa.cnr.it

Volatile organic compounds (VOCs) are emitted from the human body reflecting the metabolic condition of an individual and creating a unique chemical signature that pathological or mental diseases and genetic disorders can alter by a qualitative and/or quantitative change in the odours. Accumulating evidence has shown that this "smellprint" can be crucial in diagnosing, monitoring and developing novel therapies of human disease [1].

Among the various biological fluids, urine shows specific features that make it an option of choice for volatile metabolomic profiling. Many studies show that urinary VOCs could be perturbed in some physiological and pathological states, including several diseases and different dietary exposures [2-3].

VOCs can be analysed as disease specific 'fingerprints' using gas chromatography (GC) coupled with mass spectrometry (MS). In this communication, we describe two studies based on solid-phase microextraction (SPME) coupled GC-MS.

In the first study, the volatile urinary profile of 24 autistic and 21 healthy children was investigated. Urine samples were analyzed both under acid and alkaline pH, to profile a range of urinary components with different physicochemical properties. Bioanalytical data, submitted to data analysis, allowed us to discriminate autistic and control groups, and to identify 15 possible VOCs biomarkers [4].

In the second study, the investigation of urinary VOCs profile allowed the discrimination of 21 overweight/obese (OW/Ob) and 28 normal-weight (NW) children. Samples were analyzed both under acidic and alkaline conditions. Univariate and multivariate statistics permitted to distinguish two clusters of cases and discover 14 VOCs able to characterize the two groups [5].

References

[1] Buljubasic, F. and Buchbauer, G. *Flavour Fragr. J.* **30**, 5 (2015).
[2] Silva, C., Cavaco, C., Perestrelo, R., Pereira, J. & Câmara, J. S. *Metabolites* **4,** 71 (2014).
[3] Alkhouri, N. *et al. Pediatr. Obes.* **10,** 23-29 (2015).
[4] Cozzolino, R., De Magistris, L., Saggese, P., Stocchero, M., Martignetti, A. *et al. Anal Bioanal Chem.* **406,** 4649 (2014).
[5] Cozzolino, R., De Giulio, B., Marena, P. Martignetti, A., Günther, K. *SCREP* ***submitted*** (2017).

W9

Pharmacometabolomics approach to identify new biomarkers of response to HER-2 targeted anticancer therapy.

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The knowledge of the individual variation in drug response represent a key step for the effective development of the precision medicine. Pharmacometabolomics represent a complementary omics approach to identify the individual characteristics of response to pharmacological interventions based on the analysis of the individuals pre-dose metabolomics profile. In this study we have explored the potential of this metabolomics approach to predict the pharmacological phenotype of HER-2 breast cancer treatments which are characterized by a great inter-patients variability. The pre-dose serum targeted metabolomics profiles of 34 HER2 patients treated with neoajuvant trastuzumab-paclitaxel, have been investigated by FIA and LC-tandem mass spectroscopy. The patients were stratified according to their pathological response and the metabolomics profiles differences investigated by PLS-DA. The most relevant metabolites that contribute to differentiate the responders from non responders were: spermidine, tryptophan, propylcarnitine and the two phosphatidylcholine diacyl phospholipids: PCaaC26:0 and PCaaC30:2. Further metabolite selection revealed that responders showed higher levels of spermidine and lower amounts of tryptophan compared to poor responders ($p<0.001$, $q<0.05$). Based on the level of these two metabolites was possible to identify patients who achieved pathological complete response to trastuzumab-paclitaxel treatment with a sensitivity of 90% [0.79-1.00] and specificity 0.87% [0.67-1.00]. These encouraging preliminary results strong support the role played by patients' metabolism in determining the response and may be useful in selecting patients that are more likely to benefit from trastuzumab-paclitaxel treatment.

W10

Perspectives for Metabolomics in Human Nutrition

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Nutrition is key in determining health status and predisposition to develop disease throughout lifespan. By looking at the concentration dynamics of metabolites, e.g. the endpoints of physiological regulatory processes, Metabolomics is well suited to investigate the molecular interplay between genetic and environmental factors including nutrition. As such, Metabolomics owns great premises to help the definition of individual-specific nutritional requirements and thus to enable personalized nutrition for health maintenance, disease prevention and therapeutic management in future.

Nowadays, recommendations for daily intakes of nutrients and micronutrients are mainly based on data generated from epidemiological research and sometimes depletion/repletion studies. These reference systems, not always harmonized between countries, are meant for population nutritional management. Although the current recommendation systems includes some degree of stratification of the needs according to age, gender and physiological status, moving towards personalized nutrition will require additional granularity on the individual-specific nutritional requirements and their variation over time. Achieving a comprehensive nutritional status analysis through the quantitation of circulating micronutrient concentrations and functional markers can help determining someone's nutritional needs. However, there are still knowledge gaps and lack of scientific consensus on the best biomarkers to be used for several micronutrients. Furthermore, available methodologies for nutritional status are often specific to single biomarker and the actual ability to cover a comprehensive set of nutrient and micronutrient biomarkers is hampered by the lack of fast, robust and cost effective profiling methodologies. So far, Metabolomics, either through targeted or untargeted approaches, has mainly been deployed to assess metabolic modifications of dietary patterns or nutrient intakes, without paying much attention to developing novel biomarkers of nutrient/micronutrient status. Yet several works highlight the potential of metabolic profiling approaches to deliver such biomarkers. Recently new analytical methodologies were developed and validated for the quantitative profiling of elements (including trace elements) and liposoluble vitamins in human biofluids by tandem mass spectrometry. Such methodologies and their extension to other classes of biologically relevant nutrients and micronutrients are expected to provide a holistic analysis of nutritional status at the molecular levels. A key advantage of such profiling approaches, beyond analytical performance, relies in the ability to easily compute several combinations of nutrients and micronutrients to be tested as potential novel nutrition-related biomarkers.

This lecture will briefly review deployment of Metabolomics in the field of nutrition and will report recent analytical chromatography and mass spectrometry developments for the quantitative profiling of multiple nutrients, micronutrients and their metabolites in biological fluids. It will report on the development of a novel nutritional phenotyping analytical platform that is currently used to assess nutritional requirements in both patient groups and general population. Future technological perspectives will also be discussed as regards with nutritional status biomarker discovery, instrument sensitivity, and miniaturization of sample collection and analytics.

W11
Profiling visible and invisible metabolites in urine by NMR

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Metabolic fingerprinting of body fluids by NMR can be obtained in minutes, has unsurpassed reproducibility, and low costs. Examples of successful serum metabolomics fingerprinting from our laboratory are diagnosis of potential celiac disease, prediction of relapse for breast cancer, prediction of survival of metastatic CRC, and early diagnosis of heart failure. We and others have shown that individual metabolic fingerprints exist also in urine, that they are stable over periods of many years, insensitive to alterations of lifestyles or mild disease states, but sensitive to the onset of major diseases from a very early stage.
Passing from fingerprinting to profiling provides also information on complex biological processes and interactions that occur at the systemic level. Profiling is straightforward in serum, but much less so in urine, due to the variability of metabolite chemical shifts, in turn arising from weak inter-metabolite interactions that change with the variable chemical composition of urine. This variability has until now hampered automated assignment and quantitation of urine metabolites. We have realized a significant step forward by modelling inter-metabolite interactions in > 4000 artificial urine samples [1]. We established clear relationships between urine composition and metabolite chemical shifts. As a consequence, chemical shifts of many metabolites in a given spectrum can be predicted form those of not more than five “navigator” signals, and the concentrations of several metabolites can be back predicted from the spectra. Remarkably, we are also able to predict concentrations of “invisible” metabolites like inorganic ions, making NMR profiling of urine a powerful, fast and inexpensive analytical tool.

Reference

[1] P. Takis, H. Schaefer, M. Spraul and C. Luchinat, *Nature Communications, in press*.

W12

Impact of early postnatal nutrition on the NMR urinary metabolic profile of infant

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Metabolomics appears to be a promising technique in neonatology and pediadrics. Research in these fields is evolving continuously, providing contributions to the improvement of pediatric and neonatological practices in hospitals and clinics as well as to a deeper understanding of infant nutrition and growth.[1]
In this study we used NMR-based metabolomics to investigate the metabolic urinary profiles of exclusively breast-fed term infants (n = 11) and compare with those of a double-blinded controlled trial with formula-fed term newborns randomized to receive either an infant formula enriched by functional ingredients (n = 24) or a standard formula (n = 25). Anthropometric measurements and urine samples were taken at three time points: at the enrollment (within the first month of life), at around 60 days of life, and at the end of study period (average age of 130 days). The urinary metabolome was examined in relation to time and diet strategy. A common age-dependent modification of the urine metabolome was observed for the three types of nutrition, mainly characterized by similar temporal trends of choline, betaine, myoinositol, taurine, and citrate. Contrarily, metabolic profiles differed according to the type of diet (human *vs* formula milk), while no significant difference was observed between the two formulas. These modifications were ascribed mainly to compositional differences of milks. The findings of this study pointed out the potential of the metabolomics approach for neonatal nutritional science, in particular to provide important contributions to the improvement of the nutritional properties of infant formulas.

References
[1] Dessì, A.; Cesare Marincola, F.; Masili, A.; Gazzolo, D.; Fanos, V. *BioMed Res. Int.* **2014,** 981219 (2014)

W13

The use of metabolomics in the study of nutraceuticals: cranberry and green coffee

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Metabolomics is a useful tool to study and obtain new information about the target of action of different natural products used as nutraceuticals or food supplement [1,2,3]. In this paper we describe the application of MS-based metabolomic approach to evaluate the mode of action of cranberry in both rats and human volunteers, and the effects of supplementation of green coffee consumption in humans. Changes of urine composition were studied by targeted and untargeted approach. For cranberry experiments, antiadhesive activity of urine after oral administration of standardized extract to rats (n= 12) and to a small number of healthy human volounteers (n=6) was also evaluated. Antiadhesive activity of cranberry was not related to the presence of procyanidins in urines, but to colon metabolites of procyanidins and phenolics, suggesting a crucial role of microbiota in the bioactivity of cranberry extract. Green-coffee bean extract (GCBE) was administered to a small group of healthy volounteers (400 mg) and analysis revealed changes of urinary composition that could be related to the catabolism of GCBE constituents and to induced fatty acid metabolism, mainly related to carnitine derivatives. This latter result could be considered, at least in part, as a further proof of the mode of action of GCBE.

References

[1] Peron, et al., *J Pharm Biomed Anal. 140:62-70.* (2017).
[2] Peron, et al., *J Agric Food Chem.* 65:5657-5667 (2017).
[3] Peron, et al., *Fitoterapia.* In Press, Accepted Manuscript (2017).

W14

NMR-based metabolomics in food science: outcomes and critical pointsNMR

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The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics [1,2] . ^{1}H high field NMR spectroscopy has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolic profiling of food stuff such as olive oils, sea bass, truffles, blueberries, etc. The analysis of the metabolic profiling together with the application of a suitable chemometric approach has allowed food characterization in terms of geographical origin, genetic origin and farming. The potential of NMR spectroscopy to detect food adulterations has been also demonstrated. Here, the NMR methodology used to study foodstuffs is discussed reporting some significant examples together with some critical points that it is important to face to make this approach fully useful for solving problems of great interest also for consumers.

References

[1] Mannina L., Sobolev A.P. , Viel S. *Progress in Nuclear Magnetic Resonance Spectroscopy*, **66**, 1 (2012)
[2] Sobolev A.P., Circi S., Capitani D., Ingallina C., Mannina L *Current Opinion in Food Science* **16**, 59 (2017)

W15

Harvest year effect on Apulian EVOOs assessed by ^{1}H NMR based Metabolomics: a deeper study on altitude-based classification

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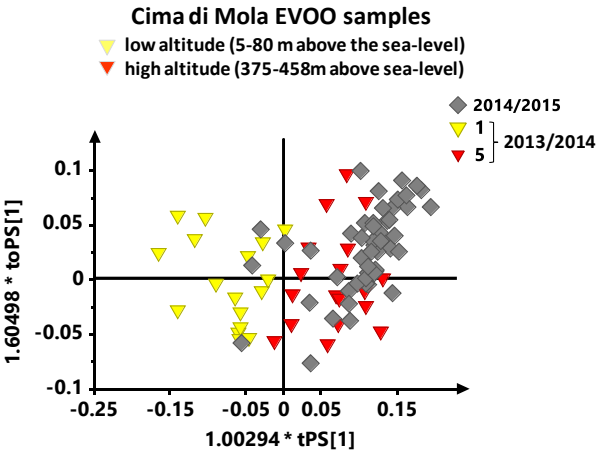
In a recent work [1], we quantitatively assessed the differences among four different cultivars and in two subsequent harvesting years (2013/14, 2014/15) by collecting ^{1}H NMR data of EVOO samples. Following genetic identification of single olive trees, a detailed Apulian EVOO NMR database was built using 900 oils samples obtained from 450 cultivar certified single trees (Coratina, Peranzana, Ogliarola and Cima di Mola). All the analyses made on the ^{1}H NMR based metabolomics confirmed that the overall variations for Coratina EVOOs are the smallest. Therefore Coratina based blends could be useful to maintain EVOO characteristics, even in the case of seasons with very different climatic conditions. A further comparison between the two harvest years has been performed by classifying oil samples according to the altitude of the olive groves. Five levels of altitude (above sea-level) have been identified and the potential effects on EVOOs metabolomic profiles have been evaluated. Interestingly a particularly wet year (2014/15) shifts the EVOOs characteristics toward the highest altitude sample class. This has been observed for Cima di Mola, by predicting 2014/15 (wet year) oils in the 2013/14 (ordinary year) model, built with the lowest (5-80 m above sea-level) and the highest altitude (375-458m above sea-level) class samples.



References

[1] Girelli, C.R., Del Coco, L., De Pascali, S.A., Papadia, P., Fanizzi, F.P., Harvest year effects on Apulian EVOOs evaluated by ^{1}H NMR based metabolomics *PeerJ.* 2016; 4: e2740.

W16

Metabolic profile comparison of different compartments of scyphomedusa *Rhizostoma pulmo*: preliminary results

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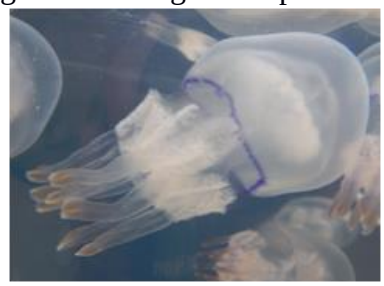
The Mediterranean Sea is among the heavily affected regions by jellyfish blooms due mainly to scyphozoans such as *Rhizostoma pulmo*. The jellyfish have few natural predators, and their bodies represent an organic-rich substrate as well as a source of nutrients that could support rapid bacterial growth with great impact on the structure of marine food webs. In Asiatic countries, jellyfish are



widely studied for its antioxidant, anti-hypertensive and anti-hyperlipidemic activity, but their nutritional and nutraceutical values still remain poorly characterized [1,2]. In this study the differences in the metabolic profiles of the compartments of *R. pulmo* such as the umbrella, oral arms, and female gonads were evaluated. For each compartment, both the aqueous and lipid extracts were studied by multivariate analysis (Principal Component Analysis, PCA) of their ^{1}H NMR metabolic profiles. From the PCA analysis of the aqueous extracts, a higher content of molecules with important metabolic actions in marine invertebrates [3] acting as osmolytes, such as homarine, betaine, taurine, alanine and glycine was observed in female gonads. From the PCA analysis of the lipid extracts a higher content of n-3 Polyunsaturated Fatty Acids (n-3 PUFA) was observed in gonads, while a higher content of Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA) and Diunsaturated Fatty Acids (DUFA) characterize oral arms and umbrella.

References

[1] Leone, A., Lecci, R. M., Durante, M., Meli, F., Piraino, S. *Marine drugs*, **13**(8) (2015).
[2] Khong, N. M., Yusoff, F. M., Jamilah, B., Basri, M., Maznah, I., Chan, K. W., Nishikawa, J. *Food chemistry*, **196** (2016).
[3] Zotti M., De Pascali SA., Del Coco L., Migoni D., Carrozzo L., Mancinelli G., Fanizzi F.P. *Food chemistry*, **196** (2016).

W17

Multi-spectroscopic investigations of *Crocus sativus* L. tissues

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The increasing interest on saffron, the most expensive spice worldwide obtained from dried red stigma of *Crocus sativus* L., over the last decades, is mainly due to its potential employment as a source of biologically active molecules and not only because of its use as a food additive. Other tissues of *Crocus sativus* L. could contain potentially bioactive molecules as well, like tepals, generally considered as byproducts and high resolution NMR spectroscopy allowed to investigate their content. The use of ^{1}H NMR data combined with chemometrics allowed moreover to asses quality characteristics of saffron, like geographical origin, the storage period during which saffron can be considered as fresh and the detection of possible chemical- and bio-adulterants[1-4]. In addition, the volatile fraction of stigma was characterized by GC-MS while the metabolic content of *C. sativus* L. tissues (stigma, stamens and tepals) was characterized with different spectroscopic techniques like HPLC-DAD, FT-IR, and Raman comparing the results with the NMR findings. The outcomes, highlighted the limitations of the current ISO procedures [5,6], thus suggesting a possible integration of ISO with advanced analytical techniques.

References

[1] Cagliani, L.R. Culeddu, N. Chessa, M. Consonni, R. *Food Control* **50**, (2015) 342-348.
[2] Ordoudi, S.A. Cagliani L.R. Lalou, S. Naziri, E. Tsimidou, M.Z. Consonni, R. *Food Res. Int.* **70**, (2015) 1-6.
[3] Petrakis, E.A. Cagliani, L.R. Polissiou, M.G. Consonni, R. *Food Chem.* 173, (2015) 890-896.
[4] Petrakis, E.A. Cagliani, L.R. Tarantilis, P.A. Polissiou, M.G. Consonni, R. *Food Chem.* **217**, (2017) 418-424.
[5] International Standard ISO 3632-2: Saffron (*Crocus sativus* L.) Test Methods. Geneva: The International Organization for Standardization (2010).
[6] International Standard ISO 3632-1 : Saffron (*Crocus sativus* L.) Specification. Geneva: The International Organization for Standardization (2011).

W18

NMR metabolomics in action: from real-time analyses to longitudinal snapshots in biological systems

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Nuclear Magnetic Resonance (NMR) is a versatile technique that can contribute to biological investigations across time scales as a powerful metabolic phenotyping platform.
We first illustrate how real-time NMR investigation of metabolites kinetics can deliver a detailed picture of the energetic metabolism for hybrid cell-free protein synthesis (CFPS) systems composed of rabbit reticulocyte lysates (RRL) ribosome-free supernatant complemented with ribosomes from different mammalian cell-types. A counterintuitive strategy, based on reducing the ribosomal fraction in RRL, is rationalized using a real-time NMR metabolomics investigation. We show that persistent ribosome-associated metabolic activity consuming ATP is a major obstacle for maximal protein yield, and reveal the potential of real-time NMR for optimization of CFPS systems.(1)
Meanwhile, NMR metabolomics contributes, among a range of phenotyping studies, to *de novo* characterization of model biological systems. We report a ^{1}H NMR metabolomic study that evaluates the impact of different mutations in the thyroid hormone receptor alpha (TRα1), encoded by the *THRA* gene, which are involved in Resistance to Thyroid Hormone (RTHα), a recently discovered genetic disease. Germline mutations in the mouse *Thra* gene were introduced using CRISPR/Cas9 genome editing, and evaluated from urine and plasma metabolomics in 3 and 6 months-old adult mice. We provide a proof-of-principle that NMR metabolomics could be used to diagnose RTHα.

References

[1] Panthu B., Ohlmann T., Perrier J., Schlattner U., Jalinot P., Elena-Herrmann B., and Rautureau G. J. P., *ACS Synth. Biol.* **Article ASAP,** doi: 10.1021/acssynbio.7b00280 (2017).

Th1

Searching for metabolic changes in human nutritional intervention studies

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Metabolomics data from human nutritional intervention studies are often characterised by large variations between the subjects. This is different from most animal studies where metabolic variation between the test animals is usually less abundant. The large variation between human subjects can give rise to two problems in the analysis. The first is that small and subtle treatment effects (e.g. dietary responses) can easily be overlooked, especially when the effect is smaller than the intrinsic variation between the subjects. The second problem is that the response and the impact of the treatment effect may differ between the subjects. This implies that an average treatment effect may not be the most relevant in studies where subsets of subjects respond differently upon a dietary intervention. To deal with the large variation between individuals, specific experimental designs are used in which the same individuals are subjected to multiple or to all treatments. Therefore, each subject in the study population can act as his or her own control and the data is often called paired. Multivariate extensions of the paired t-test can be used to analyse such data. In this presentation examples the metabolic effects as a result of drinking tea, coffee and also of drinking a large amount of alcohol are presented. It is shown that exploiting the paired data structure underlying the studies improves the power and the interpretability of the estimated metabolic effect.

Th2

Untargeted NMR metabolomics: form raw data to valuable knowledge through chemometrics

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In the last decades NMR spectroscopy, though being a much less sensitive analytical technique than Liquid- and Gas-Chromatography hyphenated to mass spectrometry, has increasingly proven very effective on providing researchers in the metabolomics filed with plentiful amount of information-rich data. Still, the relevant information carried by the NMR spectra is often buried under an overwhelming number of dominant features which mask the weak whispers of crucial biomarkers. Furthermore, it is becoming more and more clear that a univariate approach, based on the quest of one or a few direct biomarkers, is very likely doomed to produce partial and sometimes misleading results [1]. In other words, raw metabolomic NMR data are rough diamonds which most of the times need several tools and several steps to fully shine. Chemometrics is the toolbox.
Starting from the experimental design to end with complex multivariate data analytical approaches, passing through data pre-processing and pre-treatment along with the boost of data-fusion, chemometrics is an ever-growing set of tools made to extract unbiased and valuable knowledge from the collected chemical data.
In this presentation, examples of metabolomic studies taken from the food, the human nutrition and the human health fields will be given to set the light on the teamwork made by NMR spectroscopy and chemometrics towards the solutions to previously non-responded scientific hypotheses.

References

[1] R. Bro, M.H. Kamstrup-Nielsen, S.B. Engelsen, F. Savorani, M.A. Rasmussen, *Metabolomics* **11,** 5 (2015), 1376-1380

Th3

Optimization of a GC-MS method for endogenous anabolic androgenic steroids (EAAS) detection and application for new screening strategies of prostatic diseases

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Actually, the prostatic specific antigene (PSA) is the unique biomarker used to detect prostatic malignancy[1]. Unfortunately, it has problems of specificity, with increasing levels in presence of several urological diseases. So, it is necessary to find new biomarkers that improve the performances of the diagnosis of first level. The occurrence of prostatic diseases, like benign prostatic hypertrophy (BPH) and prostatic carcinoma (PCa)[2], are related to an alteration of steroidal biomarkers. For this reason, the evaluation of the urinary steroidal profile (USP) could be used as a support during the screening analysis in men. A GC-MS analytical methodology for the detection of 17 EAAS plus the formestane, a natural anti-estrogen, was optimized by means of DoE[3] and validated. In collaboration with the San Luigi Hospital of Orbassano (Turin), a cohort of 212 healthy individuals, 89 subjects affected by BPH and 70 patients suffering from PCa was enrolled, in order to: (i) make an explorative analysis over the age as bias factor and (ii) build a cascade of PLS-DA classification models. Then, two classification steps were developed: the first one for the discrimination between healthy and pathologic subjects (both BPH and PCa), while the second one distinguishes between PCa and BPH patients. The performances of the second model, in terms of sensibility and specificity, were compared with those obtained with the evaluation of the PSA, showing an improvement for both the parameters.

References

[1] Slate, E. H. & Turnbull, B. W. Statistical models for longitudinal biomarkers of disease onset. *Stat. Med.* **19,** 617–637 (2000).
[2] Carson, C. & Rittmaster, R. The role of dihydrotestosterone in benign prostatic hyperplasia. *Urology* **61,** 2–7 (2003).
[3] Leardi, R. Experimental design in chemistry : A tutorial. *Anal. Chim. Acta* **652,** 161–172 (2009).

Th4

New metrics for performance assessment in NMR fingerprinting and simultaneous multicomponent qNMR analysis

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In the framework of the validation of fingerprinting and simultaneous multicomponent qNMR methods, the statistical equivalence of suitably processed NMR spectra must be demonstrated. Once ascertained the statistical equivalence of the spectra, new and appropriate metrics for performance assessment in NMR analysis can be considered.
This presentation is based on the results collected in three different NMR interlaboratory comparisons (ILCs)[1,2,3,4] and shows how can experimental conditions allowing for statistical equivalence be fulfilled.
Performance assessment is discussed in terms of both single component quantification, by the popular and traditional z-score, and multi-component analyses by means of a new performance index (named Q_p-score). Q_p-score is related to the difference between the experimental and the consensus values of the slope of the calibration lines. By an analogous reasoning followed for z-score, performance assessment by Q_p-score is considered satisfactory when $|Q_p| \leq 2.0$, questionable when $2.0 < |Q_p| < 3.0$ and unsatisfactory when $|Q_p| \geq 3.0$.
Qp-score is a parameter suitable for harmonization of fingerprinting protocols and simultaneous quantitative multi component analysis. Such parameter, that was designed considering consolidated internationally agreed statistics, represents an unbiased evaluation tools for NMR method validations.

References

[1] V. Gallo, N. Intini, P. Mastrorilli, M. Latronico, P. Scapicchio, A. Rizzuti, *Analytical Chemistry*, **87**, 6709–6717 (2015).
[2] "Validation of a 1D 1H-NOESY experiment for fingerprinting of wheat and flour". V. Gallo, N. Intini, P. Mastrorilli, M. Latronico, P. Scapicchio, A. Rizzuti et al. "NMR Inter-Laboratory Comparisons" Series, Vol. 1 (Sept 2015). ISBN: 97888999259112 –Ed. NeP Edizioni, Rome
[3] "Validation of NMR fingerprinting methods: effects of processing on measure reproducibility and laboratory performance assessment". V. Gallo, N. Intini, P. Mastrorilli, M. Latronico, P. Scapicchio, A. Rizzuti, S. Todisco et al. "NMR Inter-Laboratory Comparisons" Series, Vol. 2 (Sept 2016). ISBN: 9788899259709 – Ed. NeP Edizioni, Rome
[4] "Validation of a 1D 1H-NOESY experiment for fingerprinting of grape juices" V. Gallo, N. Intini, P. Mastrorilli, M. Latronico, A. Rizzuti, S. Todisco, R. Ragone, P. Scapicchio, P. Dambruoso, et al. "NMR Inter-Laboratory Comparisons" Series, Vol. 3 (Sept 2017). ISBN: 9788885494060 – Ed. NeP Edizioni, Rome

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Th5

Metabolomics signature reveals distinctive patterns in an animal model of mechanical asphyxia and dysrhythmic cardiac arrest

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Cardiac arrest (CA) is a poorly understood phenomenon. Different metabolic pathways may be activated in arrest and post-resuscitation (1). The purpose of this study is to apply metabolomics to identify the metabolic profiles characteristic of two different experimental models of CA, namely asphyxial and ventricular fibrillation CA (ACA and VFCA). To this aim, we have used 20 landrace/large-white female pigs in which the ACA and VFCA were induced and plasma samples collected at different time points: at CA, during cardiopulmonary resuscitation and in the post-resuscitation period. All samples were analyzed by ^{1}H NMR spectroscopy and LC-MS/MS spectrometry coupled with univariate and multivariate statistical analysis. The metabolomics profiles characterizing the two pathological entities during the different phases of the experiment were identified and discussed. ACA and VFCA differed significantly with regard to the metabolic disturbances during the peri-arrest period. Major alterations in plasma concentrations of metabolites involved key energy production pathways. A potential prognostic role for succinate was emphasized.

References

[1] E. Locci, G.Varvarousi, P.Scano, A. Noto, G.Ferino, F. Rosa, G. Finco, N. Iacovidou, E. d'Aloja, T. Xanthos, **96**, 8 (2015)

Th6

Pitfalls and Caveats in Clinical Metabolomics

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Clinical metabolomics has the potential to deliver diagnostic biomarkers for the detection and prognosis of diseases, and the prediction of the efficacy and safety of pharmaceutical interventions, providing insights into the biochemical mechanisms of diseases and the modulation by drugs and move into the clinic. Unfortunately, many of the metabolomics studies are hampered by studies not well designed, findings not validated in independent replica cohorts, no proper clinical phenotyping available. Metabolomics represent a paradigm shift in biomarkers research, away from approaches that focus on a limited number of reactions or single pathways, to approaches that attempt to capture the complexity of metabolic networks to better understand physiopathology and improve diagnosis. It is reasonable to expect that the metabolomics approach, together with functional genetics, will have substantial impact in clinical (personalized medicine) studies. If metabolomics aims to became the new clinical chemistry and convince more clinicians about its relevance, the basic rules of clinical chemistry need to be applied, e.g. control of the preanalytical phase, quantitation, validation, interlab validation, etc. At present, there are still significant challenges in answering biological questions by using a metabolomics approach. We would like to discuss theses pitfalls and caveats.

Th7

NMR and MS metabolomics of baobab (*Adansonia digitata* L.) products

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In African tradition, the baobab (*Adansonia digitata* L.) is considered as the "tree of life", due to its water-holding capacity and numerous ethnomedicinal and nutritional uses [1]. Various parts of plant (fruits, leaves, seeds etc) are used for food and in ethnomedicine. The powdered leaves served as a tonic possess antioxidant [2], anti-inflammatory, and antiviral activities [3]. Since 2008 the European Commission authorized the import of the baobab fruit pulp as a novel food. Besides its rich nutritional profile the pulp of baobab fruit shows also anti-inflammatory, analgesic, gastroprotective, and hypoglycemic properties, both *in vitro* and *in vivo* studies [4]. The present study has been focused on the application of metabolomics to characterization of the fruit pulp and powdered leaves of baobab. A multi-methodological approach, which included the use of RP-HPLC-PDA-ESI-MSn and NMR, was adopted. Primary and secondary metabolites of different classes both in water-soluble and liposoluble extracts were identified and quantified.

References

[1] Jackson, S., *HerbalGram - The Journal of the American Botanical Council*.**108,** 42 (2015).
[2] Vertuani, S., Braccioli, E., Buzzoni, V., Manfredini, S., *Acta Phytotherapeutica* **86**, 2 (2002).
[3] Vimalanathan, S., Hudson., J. B. *J. Med. Plants Res.* **3**, 576 (2009).
[4] Garvery, R., Clegg, M., Coe, S. *Nutr. Health*, (2017), doi: 10.1177/0260106017704361.

Th8

Pre-analytic evaluation of volumetric absorptive microsampling and integration in a mass spectrometry based metabolomics workflow

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Volumetric absorptive microsampling (VAMS) is a novel approach that allows single drop blood collection. The aim of this study was to develop a suitable protocol for integrating VAMS technology with mass spectrometry-based metabolomics workflow by evaluating some key pre-analytical strategies that are pivotal in metabolomics studies [1].
We first evaluated the best extraction procedure by comparing six different protocols finding that the highest number and amount of metabolites was extracted using acetonitrile:water.
We then tested the stability of the blood metabolome sampled by VAMS. While the storage at room temperature causes significant changes in the composition of the metabolome (75% of the metabolites changed significantly over time), VAMS samples are stable when stored at -80°C up to 6 months. Our results also indicate that samples can be stored for 1 day in sealed bags with desiccants at room temperature or at 4°C.
Based on the these results, we suggest to keep VAMS samples at room temperature for a maximum of 1 day and store them at -80°C for untargeted metabolomics.
We finally employed the developed VAMS metabolomics workflow for investigating the alteration of iron homeostasis in mice fed diet rich in iron vs controls. Several metabolic pathways were affected by high intake of iron, such as urea cycle and beta-oxidation. Moreover, in the high iron diet, we detected increased blood level of pyruvate and glucose suggesting that high iron intake affects systemic glucose homeostasis.
In this study, we integrated for the first time VAMS with MS-based metabolomics and we have shown that VAMS sampling can be successfully incorporated in the metabolomics workflow if standardized procedures are used.

References

[1] Volani, C. Caprioli, G. Calderisi, G. Sigurdsson, B.B. Rainer, J. Gentilini, I. Hicks, A.A. Pramstaller, P.P. Weiss, G. Smarason, S.V. Paglia, G. *Anal. Bioanal. Chem.* **409**, 26 (2017).

Th9

The untargeted mass spectrometry analysis for the study of the "pediatric community acquired pneumonia"

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Introduction: Community Acquired Pneumonia (CAP), a frequent cause of hospitalization in pediatrics, could be bacterial or .viral. Available diagnostics often fail to distinguish viral from bacterial cases of pediatric CAP owing to lack of specificity. Metabolomics, an analytical approach aimed at characterizing disease states based on unique metabolic signatures, can expand the pathophysiological understanding of many diseases. Our hypothesis was that metabolomics can positively identify pediatric CAP etiology based on specific metabolic profiles.
Methods: we used liquid chromatography and mass spectrometry to perform an untargeted metabolomic analysis of metabolites extracts from urine samples. The urine samples were collected from children hospitalized for CAP of pneumococcal or viral etiology, in three different pediatric hospitals
Results: we enrolled 59 children over sixteen months (12 viral, 15 pneumococcal and 32 mixed/undetermined). Statistical modeling robustly segregated pneumococcal and viral samples based on 93 metabolite species with significantly different abundances. 20 metabolites were annotated and considered as putative biomarkers. Among the pathways involved in the discrimination between the cohorts, the most prominent is adrenal steroids' synthesis and degradation.
Conclusion: this study shows that viral and pneumococcal pneumonia have different impacts on the systemic metabolome, which may lead to the discovery of novel diagnostic biomarkers or therapeutic targets.

Th10

A detailed lipidomics and proteomics exploration of the FAAH knockout mice brain

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Fatty acid amide hydrolase (FAAH) is a serine protease, which hydrolyzes bioactive endocannabinoids (ECs). ECs are lipid mediators that bind to and activate cannabinoid receptors CB1 and CB2. Upon binding ECs initiate a number of signaling pathways which control diverse set of biological functions including memory, learning, social behavior, appetite and inflammation. Modulating the metabolism of ECs by inhibiting FAAH holds therapeutic promise in a wide range of neurological diseases. Since the discovery of FAAH, it has been a very good target for pharmacological studies, many natural and synthetic analogues have been designed to target this enzyme. Given its large number of biological functions, a deeper understanding of its lipidome and proteome could generate useful information for future pharmacological strategies to combat neurological disorders. The aim of our work is to investigate the difference in lipidome of FAAH knockout mice brain compared to its wild type using Ion mobility based mass spectrometry, difference in proteome using TMT labelled quantitative proteomics and a deeper targeted lipidomics of all the biosynthetic intermediates of anandamide pathway which is the most important signaling lipid in the brain.

By using ion-mobility assisted, untargeted mass spectrometry, we discovered the upregulation of pro-apoptotic ceramides, besides previously known fatty acid ethanolamides and acyl taurines. Further we extended our study to TMT labelled quantitative proteomics to explore the effect of upregulated bioactive lipids on the proteome of FAAH knockout brain. The experiment generated relative expression data on various mouse proteins, out of which the majority of significantly upregulated proteins are related to cell growth, apoptosis, transport processes, cell to cell adhesion and RNA splicing. Our targeted lipidomic analysis of anandamide pathway showed a downregulation of its metabolic precursors, which could be a possible feedback inhibition to fine tune the levels of anandamide. Overall our study gives a deeper understanding of the lipidome and proteome of FAAH knockout mice brain.

References:

1. WA devane et al., (1992) *Science* **258-5090** (1946-1949)
2. Bluett RJ et al., (2014) *Transl. Psychiatry* **4** 1-5
3. Fabio et al (2016) *Progress in lipid research* **62** 107-128
4. Esbroeck et al (2017) *Science* 356-6342 (1084-1087)
5. Pal Pacher et al., (2006) *Pharmacol Rev*. **58 3** 389–462.
6. Saghatelian, A., et al.,Life Sci, 2005. **77**(14): p. 1759-66.
7. Saghatelian, A., et al., *Biochemistry.* 2004 43(45):14332-

Poster

P1

Metabolic profile of extremophile plants and propolis from Argentina: from the suspect screening to a metabolomic approach

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Some plants from Argentina grow at high altitudes and resist to extreme environmental conditions, which support the production of interesting bioactive secondary metabolites sometimes described also in propolis. A liquid chromatography coupled to a diode array-quadrupole-Time of Flight (LC-DAD-QTOF) detection system was proposed for characterizing the phenolic profile of some argentinean plants and propolis.
Zuccagnia punctata and four different propolis were firstly characterized following a suspect screening approach in tandem MS mode, identifying some new and biologically relevant biomarkers [ ]. Only two propolis showed large amounts of molecules present in Z. punctata. Fifty suspect compounds evidenced by LC-MS analysis of four different plants and 21 propolis samples were then subjected to PCA analysis. The first two components described about 45 % of the variance with a clear indication of the propolis biological sources.
A metabolomic approach, based on non-target LC-MS acquisition was thus attempted. The free software XCMS [ ] was used for data analysis, showing more than 360 significant MS signals. Its automatic PCA processing showed the first two components describing about 60% of the dataset variance, proving that this approach can empower the achievable information.

References

[1] Solorzano, E. Bortolini, C. Bogialli, S. Di Gangi, I.M. Favaro, G. Maldonado, L. Pastore, P. J. Funct. Food., 33, 425 (2017).
[2] https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage

P2

NMR metabolomics of blood samples from heart transplanted patients related to different organ conservation systems

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Despite the progress in mechanical circulatory support and stem cell therapy, heart transplantation is still the best treatment of advanced heart failure [1]. Although universally adopted, cold ischaemic preservation is not optimal because low levels of anaerobic metabolism deplete ATP storage and increase acidosis and myocardial oedema [2,3]. An alternative to cold ischaemia is a new method of normo-thermic donor heart perfusion: the Organ Care System (OCS) developed by TransMedics Inc [4]. Although a better preservation of the cardiac muscle using ex vivo perfusion has been demonstrated to improve follow up of survival length in animal models, the same evidence is still missing for human patients.
Here we present our encouraging preliminary results about nuclear magnetic resonance (NMR) systematic analysis of plasma and serum samples obtained from cardiac and peripheral vessels of donors and acceptors of heart transplantation. Typically, samples obtained from transplanted patients with hearts that underwent different preservation protocols show that the lactate/glucose ratio proves reproducibly larger when the heart is preserved by classic cold ischaemia with respect to the condition where the OCS protocol is employed. This indicates that the metabolic state of the organs that are treated by OCS protocol corresponds to an appreciably lower fatigue condition.

References

[1] Kirklin JK, Naftel DC, Kormos RL, et al. *J Heart Lung Transplant* **32**, 141 (2013).
[2] Banner NR, Thomas HL, Curnow E, et al. *Transplantation* **86**, 542 (2008).
[3] Russo MJ, Iribarne A, Hong KN, et al. *Transplantation* **90**, 444 (2010).
[4] Ozeki T, Kwon MH, Gu J, et al. *Circ J* **71**, 153 (2007).

P3

^{1}H NMR-based Metabolomics approach to characterize urine samples of female obese patients

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According to the World Health Organization, obesity is defined as a complex multifactorial disease characterized by abnormal or excessive fat accumulation that may impair health. Mostly it is caused by unhealthy lifestyles: an excessive calories consumption accompanied by sedentary habits.
The present study has been focused on the application of metabolomics in order to characterize urine samples of female obese patients. The study of metabolic profiles can be regarded as the response of biological systems to environmental changes in relation to pathology.
A ^{1}H NMR-based approach, combined with chemometric analyses, was adopted. We analyzed 11 urine samples of female obese patients (Body Mass Index, BMI >25) and 11 urine samples from control subjects with BMI between 18 and 25. It should be noted that urinary metabolic profile of male obese subjects was previously studied by NMR [1]. ^{1}H NMR spectra data were submitted to the Principal Component Analysis (PCA) to detect possible analogies and differences between the samples analysed. The preliminary analysis displayed a higher content of three organic acids (lactic acid, β-hydroxyisovaleric acid, 3-hydroxybutyric acid) in urine of obese patients whereas in urine of control subjects a higher content of hippuric acid and trigonelline was observed.
Comparing our results with those obtained for male obese patients [1], it is evident that low levels of trigonelline and hippuric acid are potential markers of obesity independently on gender.

References

[1] Calvani, R., Miccheli, A., Capuani, G., Tommassini Miccheli, A., Puccetti, C., Delfini, M., Iaconelli, A., Nanni, G., Mingrone, G., *International Journal of Obesity* **34,** (2010).

P4

^{1}H-NMR metabolomic profiling approach on cancer patient sera

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At National Cancer Institute in Naples there is a 600-MHz NMR spectrometer with cryoprobe, equipped with an automation system able to acquire spectra in automation on twenty-four samples.
Our group is performing studies on the evaluation of metabolomic profiling on liquid biopsy samples collected in melanoma and colorectal cancer (CRC) patients at different stages of the disease and/or at different time points before and during treatments. In detail, we evaluated the serum metabolome on metastatic colorectal patients subjected to first line bevacizumab plus chemotherapy and on metastatic melanoma patients subjected to different immunotherapy treatments.
Through this approach, we identified the metabolites that present statistically significant different levels in the patients groups compared to healthy donors, and those that increase or decrease during the melanoma and CRC progression. Moreover, we were able to group in separate clusters the patients with different outcome in terms of overall survival and to identify a set of metabolites that, either before or during treatments, can discriminate patients with favorable than those with worst outcome.
Overall, the obtained results suggest that metabolomic profiling by NMR is a potent and affordable method to select the patients, to predict outcome for cancer treatment, and, hence, to improve the early detection and prognosis definition of cancer disease.

P5

^{1}H NMR-based metabolomic analysis on caulerpin accumulation effects from the invasive *Caulerpa cylindracea* in muscle of *Diplodus sargus*

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The introduced green alga *Caulerpa cylindracea* has widely invaded the Mediterranean coasts, becoming an important dietary component of a native edible fish, the white sea bream *Diplodus sargus*. Remarkably, one of the algal secondary metabolites, the bisindole alkaloid red pigment caulerpin (CAU), enters the food chain and accumulates in the fish tissues. Therefore, CAU has been used as an indicator of the trophic exposure of the fish to the exotic seaweed [1,2]. To evaluate the effects of *C. cylindracea*-based diet on metabolomic profiles (both metabolites and fatty acids composition), a total of 88 specimens, experimentally fed with different levels of caulerpin (high, P, natural, N and null, C, dose), were analyzed by ^{1}H NMR spectroscopy and multivariate analysis (MVA). A marked difference was observed in the OPLS-DA statistical model of aqueous extracts between treatments, in particular between fish fed with caulerpin at the natural level in the alga (N), which are clearly distinct from those fed with high (P) and null (C) levels of the toxin. In particular, a higher relative content of lactate and inosine and inosine 5'-phosphate was found in N samples, while a high relative content of creatine/phosphocreatine, taurine, alanine and choline was found in P and C samples. Moreover, ^{1}H NMR-based metabolomic analysis of lipid extracts showed that, fish fed with high level of caulerpin had an altered muscle fatty acid composition, with a reduction of essential polyunsaturated fatty acids content, as already reported by Felline et al. 2014 [3]. The metabolomic approach could provide new insights for further research in order to assess the possible transfer of such molecules to humans through seafood consumption.

References

[1] Felline, S., Mollo, E., Cutignano, A., Grauso, L. Andaloro, F. , Castriota, L. , Consoli, P., Falautano, M., Sinopoli, M., Terlizzi, A., *Aquat Biol* ***26***, 27-31 (2017)
[2] De Pascali, S.A., Del Coco, l., Felline, S., Mollo, E., Terlizzi, A., Fanizzi, F.P., *Marine Drugs*, ***13***, 3550-3566 (2015)
[3] Felline, S., Mollo, E., Ferramosca, A., Zara, V., Regoli, F., Gorbi, S., Terlizzi, A., *Mar. Biol.*, ***161***, 1275–1283 (2014)

P6

Drug effects on metabolic profiles of *Schistosoma mansoni* adult male parasites by ^{1}H-NMR spectroscopy

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Schistosomiasis, caused by trematodes of the *Schistosoma genus*, is one of the most devastating and neglected tropical parasitic diseases. Eggs laid within the definitive host by mature pairs are responsible for both pathology and life cycle maintenance [1]. Schistosomiasis treatment relies on praziquantel which is a safe and very effective drug against mature worms but poorly active on larval and juvenile parasite stages. Therefore, the search for new pharmacological treatments appears mandatory [2].
The aim of this study is to evaluate the metabolic profile in adult male parasites treated with perhexiline maleate (PHX), previously shown to be very effective on *S. mansoni* schistosomula larval stage, juvenile and adult worms [2]. To this purpose, a ^{1}H-NMR spectroscopy study was performed on adult male worm extracts to evaluate the metabolic perturbations due to PHX, gambogic acid (GA), and DMSO. The effects of PHX and GA compound treatments were compared in order to highlight different metabolic profiles and specificity of the PHX action. Finally, the effect of DMSO, the vehicle of PHX and GA compounds was assessed to ascertain the outcomes of this study. For the first time the most abundant soluble metabolites of adult male *S. mansoni* were characterized. Our results also present significant different metabolic profiles in PHX and GA treated samples showing that PHX-treatment associates with some specific variation of metabolites in schistosomes. Further investigations are necessary to relate the significant metabolite differences to biochemical pathways.

References

[1] You, R.J. Stephenson, *Front. Genet.* **5,** 176 (2014)
[2] Guidi, C. Lalli, *PLoS Negl Trop Dis.***10,** 8 (2016)

P7

The physiopathological role of mitochondrial calcium uptake in skeletal muscle homeostasis

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Muscle activity leads to major swings in mitochondrial [Ca^{2+}] which control aerobic metabolism, survival pathways and cell death. Recently, we showed that mit Ca^{2+} uptake positively modulates skeletal muscle trophism by impinging on two major pathways, PGC-1α4 and IGF1-AKT/PKB. In addition, metabolomics analyses of MCU silenced muscles revealed a clear shift towards β-oxidation. Here, we aimed to discern the metabolic route regulated by mitochondrial Ca^{2+} uptake that is responsible for muscle trophism, for this purpose, we generated a skeletal muscle specific Mcu KO mouse (skMCU KO). Our preliminary data confirm that PGC-1α4 and IGF1-AKT/PKB signaling pathways are negatively regulated in these animals. We also observed a slight decrease of fibre size. Most importantly, when these mice were exercised on a treadmill, an impaired running capacity became evident, indicating that mit Ca^{2+} uptake is required to guarantee skeletal muscle performance. Finally, a clear metabolic alteration is present in skMCU KO animals. Specifically, skMCU KO mice show decreased glucose, increased lactate, free fatty acids and ketone bodies, suggesting an impaired crosstalk between skeletal muscle and liver. These data indicate that mit Ca^{2+} uptake plays a pivotal role in the control of muscle trophism. Further investigations of MCU-dependent effects on skeletal muscle homeostasis will represent an important task for the future. Indeed, this research will provide new possible targets for clinical intervention in all diseases characterized by muscle loss.

P8

NMR-based metabolomic profiles of different sweet melon (*Cucumis melo* L.*)* Salento varieties: analysis and comparison

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From the 18th century in the Salento area (Apulia region) the cultivation of sweet melon (*Cucumis melo* L.) varieties has always been intense, affecting the provinces of Brindisi and Lecce [1]. Over the years, the production of this fruit has involved a large number of selected and preserved varieties in the different local districts. Unfortunately, most of the characteristics of locally grown vegetable varieties do not match the food industry requirements. Moreover, the agricultural land abandon leads these varieties to quickly disappear, thus affecting the intraspecific biodiversity [2].
In order to characterize the inter-variety diversity of *Cucumis melo* and the potential differences in the nutritional quality of fruits, a preliminary investigation on the juice of six sweet melon varieties (locally known as "Allungato", "Scurzune", "Minna de la Monaca", "Pinto", "Egiziano", "Gialletto Tondo") cultivated in the Salento area, was performed by ^{1}H NMR spectroscopy and Multivariate Analysis (MVA). Interestingly, the analysis grouped the samples into clusters according to the different variety. In particular, a different sugars (mono and disaccharides) content was observed among the grouped varieties. The results strongly support the capability of NMR and chemometrics to be used in quality assessment [3] and traceability of several products such as food with specific geographical origin which require to be assessed by analytical methods.

References

[1] Mannarini, A., *Orticoltura salentina : monografia: Ambiente, Importanza economica, Pratiche colturali, Studio botanico-agrario delle razze ortive locali* , tip. Editrice Leccese (1914)
[2] Accogli, R., Nicolì, F., De Bellis L., *Thalassia Sal*. **36,** 43-52 (2014)
[3] Girelli, C.R., De Pascali, S.A., Del Coco, L., Fanizzi, F.P., *Food Research International,* **90**, 281-287 (2015)

P9

NMR Metabolomic Analysis of the kidneys from a novel mouse model of Renal Cell Carcinoma (RCC) might reveal a progressive metabolic reprogramming

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Renal Cell Carcinomas (RCCs) are commonly found in syndromic forms of cancer such as Von Hippel Lindau (VHL) disease [1] and Tuberous Sclerosis Complex (TSC) [2]. A novel mouse model was generated in OSR (Drusian et al, submitted for publication), in which the *Tsc1* gene is inactivated in a specific segment of the renal tubule. This resulted as expected in upregulation of the mTORC1 cascade. Of interest, the mouse developed progressive renal carcinogenesis at different time points with cysts observed at P20, cystadenomas at P50 and carcinomas at P80. Because the phenotype affects the entire kidney and not only a few lesions, this allowed to perform global metabolomics on these tissues to understand the progressive metabolic changes. We applied ^{1}H-NMR to study the endometabolome of WT and cystadenomas (P50) and carcinomas (P80). Importantly, the mouse model only develops cancerous lesions in the renal cortex, similar to humans and this allowed for dissection and analysis of the cortice as an enrichment strategy. Supervised multivariate analysis highlighted 48 and 42 bins/metabolites (VIP score >1) that were able to discriminate between WT and KO at P50 and at P80, respectively. Moreover, enrichment pathway analysis revealed that 20 pathways were altered at P50 and 16 at P80, with 15 being conserved between the two conditions. Among the pathways that appeared differentially enriched were glutaminolysis, glycolysis and purine biosynthesis, all pathways implicated in the mTORC1 cascade. Among all metabolites one that appeared to change the most between WT and KO kidneys both at P50 and at P80 was fumarate, an oncometabolite [3] found accumulated in FH-deficient renal cancers classified as papillary type II.
This analysis, combined to MS targeted analysis and biochemical assays, revealed that the inactivation of *Tsc1* gene leads to a global metabolic rewiring. In particular, *Tsc1* inactivation causes progressive accumulation of different types of metabolites and one pathway which is only enriched at P80, possibly representing an interesting metabolic change associated with progression. Finally, fumarate is one of the metabolites that accumulates in these RCC and that might be involved in the progression of the phenotype.

References

[1] Grubb III R.L et al. 2005. Management of von Hippel-Lindau-associated kidney cancer. Nature clinical practice. 2: 248-55.
[2] Henske EP. 2005. Tuberous Sclerosis and the kidney: from mesenchyme to epithelium and beyond. Pedriatioc Nephrol. 20:854-7.
[3] Sciacovelli M. and Frezza C. (2016) Oncometabolites: Unconventional triggers of oncogenic signalling casade, Free Radical Biology and Medicine 100: 175 -181Surname, B. Surname, *Forensic Science Int.* **28,** 5 (1985).

P10

Metabolomic profiling of green extracts of *Corylus avellana* leaves by ^{1}H NMR spectroscopy and multivariate statistical analysis

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With the aim to exploit the leaves of Corylus avellana, cultivar "Tonda di Giffoni", as source of bioactives with beneficial health properties, a metabolomic study was carried out. Our previous investigations of the leaves of C. avellana led to the isolation of cyclized diarylheptanoids and diaryletherheptanoids, some of which highly hydroxylated, named giffonins A-P, along with flavonoid derivatives, which displayed the capacity to prevent oxidative damages of human plasma lipids [1,2].
Therefore, the polyphenolic content and the antioxidative activity of different extracts obtained through the use of "green" solvents, i.e. macerations with ethanol:water mixtures, infusion extractions and SLDE-Naviglio extraction, were determined.
Nuclear magnetic resonance (NMR) spectroscopy is one of the most commonly used analytical tools for plant metabolomics applications [3].
1H NMR experiments of C. avellana extracts were performed. Based on the NMR data, a total of 31 metabolites were identified. The metabolite variation among the extracts obtained by macerations, infusions and SLDE-Naviglio extractions was further evaluated using principle component analysis (PCA) and partial least squares (PLS-DA) analysis. PLS-DA gave a preliminary classification of samples based on the extraction technologies, consolidated by PLS correlation of data matrix with phenolic content. This approach based on NMR analysis combined with chemometric tools confirmed its high investigative potential in plant metabolomics.

References

[1] Masullo, M. Cerulli, A. Olas, B. Pizza, C. Piacente S., *J. Nat. Prod.* **78**, 17 (2015).
[2] Masullo, M. Cantone, V. Cerulli, A. Lauro, G. Messano, F. Russo, GL. Pizza, C. Bifulco, G. Piacente S., *J. Nat. Prod.***78**, 2975 (2015).
[3] Choi, H. Choi, Y.H. Verberne, M. Lefeber, A.W.M. Erkelens, C. Verpoorte, R., *Phytochemistry* **65**, 857 (2004).

P11

Metabolic stratification of breast cancer

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Remodeling of cancer cell metabolism is essential for tumour formation and development. Consequently, it is also cancer's major vulnerability, since tumour survival and growth, as well as the sensitivity of specific cancers to chemotherapy rely on a limited number of specific metabolic pathways. However, a major hurdle to exploit these vulnerabilities is to determine the metabolic phenotype of individual, in situ tumours with existing diagnostic methods. We developed a novel approach to overcome this barrier, based on defining mitochondrial cancer subtypes.
We developed a novel method for massively correlated biclustering (MCBiclust www.bioconductor.org) in expression data of ~1K mitochondrial genes to study the fundamental biology of how mitochondrial gene expression determines cellular metabolism. Applying this method to large breast cancer gene expression datasets revealed mitochondrial breast cancer subtypes, based on correlated expression of specific gene sets (nuclear encoded mitochondrial gene expression patterns – nMGEPs). Crucially, by using mitochondrial functional imaging and biochemical approaches together with metabolomics and flux analysis in cellular models, we have shown that nMGEP subtypes identify major cancer cell metabolic phenotypes of ER-positive breast cancers (Luminal A and B pathological subtypes). The mitochondrial subtypes showed unique redox properties, mitochondrial activity and distinctive dependence on either glucose derived pyruvate or glutamine utilizing anaplerotic pathways.
Finally, by using flux balance analysis and kinetic models we have generated predictions for the minimal gene set required to determine the metabolic phenotype of the mitochondrial BRCA subtypes, which have been verified experimentally.

References

[1] Bentham RB, Bryson K, Szabadkai G. MCbiclust: a novel algorithm to discover large-scale functionally related gene sets from massive transcriptomics data collections. Nucleic Acids Res. 2017 Sep 6;4:8712-8730.
[2] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012 Oct 4;490:61-70.

P12

ICP-MS & HR-NMR: a marriage towards the innovation in Authenticity and analytical Traceability

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This study shows as a fusion of different analytical techniques such as ICP-MS/ICP-OES and ^{1}H HR-NMR can improve the performance of classification and prediction models applied in the field of authenticity[1] and analytical traceability of food matrices.
ICP-MS/ICP-OES targeted analysis focuses on the quantitation of Ca, Na, K, Mg, Zn, Fe, Cu, Mn, Se in 121 samples of a "traditional" ovine cheese coming from five different dairies and in 90 additional Italian ovine cheeses.
Untargeted food fingerprinting approach by HR-NMR was also applied for all samples.
ICP-MS/ICP-OES and HR-NMR data were evaluated with a multivariate statistical analysis considering both data sets separately and with a data fusion approach. Results were compared in order to determine the best model to discriminate between traditional and all other ovine cheeses and at the same time to distinguish the dairies for the traditional one. The best model were obtained with variables coming from ICP-MS and HR-NMR techniques.

References

[2] Downey, G. Advances in food authenticity testing, 1st Edition. Elsevier, Boston, MA (2016) (ISBN: 978-0-08-100220-9)

P13

Metabolomics approach to evaluate polyphenols in Berry Leaves, analyzed by LC-ESI-Orbitrap-MS

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Berry fruits are known as source of phenolic compounds, while their leaves are less studied but are one of the largest waste of berry fruit production, and their enhanced utilization level would be beneficial in improving the sustainability of agricultural practices [1].
Knowledge of the precise phenolic profile of berry leaves could take the underutilized berry leaves to good use as cheap raw materials for extract rich in polyphenol production. The results obtained with the present work suggest that berry leaves are a potential source of extract rich in phenolics with pro-healthy properties to contribute to human health [2].
In the present work, secondary metabolites of *Morus nigrum* [3], *Ribes nigrum* [4] and *Fragaria vesca* [1] leaves were investigated, following an approach based on untargeted and targeted metabolomics by using the combination of LC-ESI-FT-MS and LC-ESI-FT-MS/MS analyses coupled to chemometrics data analysis and specifically PCA (Principal Component Analysis).
PCA helped to differentiate metabolites in leaves from the different species, underlying marker compounds for different berry leaves samples under investigation.
Extraction of leaves were obtained with methanol and with greener solvent (ethanol : water mixtures) with and without the enhancement by ultrasound wave. The multivariate approach was successfully applied too to distinguish samples extracted in different way and the metabolites characterizing each extraction technique applied.

References

[3] A. Kårlund, K. Hanhineva, M. Lehtonen, G. J. McDougall, D. Stewartc, and R. O Karjalainena, *J. Sci. Food Agric*. **97**: 2182–2190 (2017)
[4] J. Oszmiański, A. Wojdyło, J. Gorzelany, I. Kapusta, *J Agric Food Chem.* **59** (24):12830-5 (2011)
[5] F. Sun, L. Shen, Z. Ma, *Food Chemistry* (2011), 126(3), 1337-1343
[6] M. Vagiri, A.Ekholm, S.C. Andersson, E. Johansson, K. Rumpunen. *J Agric. Food Chem.* **60**:10501−10510 (2012)

P14

Seasonal variations in fruit metabolism revealed by untargeted LC-MS-based metabolomics

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Fruits are plant organs able to accumulate a wide variety of compounds, especially secondary metabolites, which undergo major changes throughout the development and ripening processes. Metabolomics is, in this sense, a powerful tool that allows the characterization and monitoring of the phytochemicals produced within the fruit [1]. The garden strawberry (*Fragaria x ananassa* Duch.) is considered a model species in the molecular study of fleshy fruits, even though from a botanical perspective it is actually a false fruit. Despite strawberry development and ripening have already been examined in depth from a molecular point of view [2,3], no information is available so far regarding the possible impact of different season-dependent conditions on the metabolic rearrangements that characterize these processes.
In this work, a strawberry reblooming cultivar was grown in field tunnels from September through June, facing a first cycle of production in autumn and a second one during the next spring. Eight stages from pre-flowering till fruit over-ripening were sampled by relying on *ad-hoc* experimental design and studied by the mean of untargeted metabolomics. HPLC-ESI-MS revealed clear differences in strawberry secondary metabolites between the two productive seasons. These data, together with those obtained for primary metabolism explored by NMR, and the exploitation of transcriptomics techniques (microarray analysis) will shed more light on the molecular mechanisms involved in strawberry fruit formation, development and ripening.

References

[4] Hanhineva K. & Aharoni A. *Molecular Techniques in Crop Improvement*, Springer Netherlands (2010)
[5] Fait A., Hanhineva K., Beleggia R., Dai N., Rogachev I., Nikiforova V. J. & Aharoni A. *Plant physiology*. **148**,2 (2008)
[6] Zhang J., Wang X., Yu O., Tang J., Gu X., Wan X. & Fang C. *Journal of experimental botany*. **62**,3 (2010)

P15

Application of metabolomics in nutraceuticals research: insights into the metabolic and anti-aging effects of *Polygonum cuspidatum* in healthy rats

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The application of metabolomics in the *in vivo* bioactivity study of natural products and nutraceuticals is an attractive novel approach. The effects of a nutritional intervention could be studied by monitoring the changes of urine composition during treatment, being urine more prone to metabolic variations compared to other bio-fluids [1].
Polygonum cuspidatum is frequently used as a component of nutraceuticals due to its high content of resveratrol. This stilbenoid has received widespread attention because of its supposed beneficial health effects; some authors reported a lifespan extension related to treatment with resveratrol in several organisms [1,2,3] and, because of its antioxidant properties and its caloric restriction mimetic role [3], a preventive activity in many age-related and metabolic diseases has been attributed to this phytochemical [2,4]. In this paper we report the application of an integrated MS- and NMR-based untargeted approach to evaluate the effects of supplementation of healthy rats with a standardized *P. cuspidatum* dry extract (100 mg/kg containing 20% of resveratrol) for 49 days. Markers of *P. cuspidatum* supplementation effects were mainly related to its antioxidant activity and to effects on energy metabolism. Changes of urine composition were related also to rat aging. Targeted MS analysis of oxidative stress and aging markers in urine were also obtained using LC/MS/MS approaches. The overall results allowed to observe significant differences between treated animals and controls, suggesting the use of the integrated metabolomics approach as a powerful tool to evaluate the possible different mode of actions related to the "anti-aging" effects of *P. cuspidatum*.

References

[1] Peron et al., *J Pharm Biomed Anal. 140:62-70.* (2017).
[2] Baur et al., *Nature*. 444:337-342. (2006).
[3] Pearson et al., *Cell Metab.*, 8:157-168. (2008).
[4] Howitz et al., *Nature*. 425:191-196. (2003).

P16

Target metabolomic analysis of Red Blood Cells transfusion bags: role of hypoxanthine in neutrophil activation

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Red Blood Cells (RBCs) stored in blood bank conditions, undergo biological/biochemical changes collectively referred as "*storage lesions*". The RBCs bags metabolic profile was evaluated during storage (up to 42 days) by ^{1}H-NMR spectroscopy [1]. More than 30 metabolites were identified: we observed a significant increase of hypoxanthine (HX) concentration in aged RBCs units [2]. This intermediate of purine pathway is a pro-oxidant molecule: it's oxidation by xanthine oxidase (XO) produces reactive oxygen species (ROS, $O_2^{\bullet-}$ and H_2O_2) as by-products. ROS play a central role in inflammation, acting as direct mediators and/or being involved in neutrophil activation and downstream inflammatory response mediated by cytokines and chemokines. *In vitro* studies on neutrophil priming/activation, induced by RBCs storage medium, attributed this effect to the presence of bioactive substances, such as lipids [3]. The search of other mediators is still an open question. Since the role of HX was not previously investigated, our aim is to discern its role on neutrophil activation. The effect of 42 days old RBCs supernatants and HX (± XO in both conditions) on isolated neutrophils in culture, was assessed by flow cytometry and ELISA assays.
An increase of the intracellular cytokine TNFα and a release of chemokine IL-8 in the extra-cellular space, were observed. Both effects were absent in presence of allopurinol, XO inhibitor, thus demonstrating the role played by HX on neutrophil activation.

References

[1] Pertinhez, T.A., Berni, P., Casali, E., Lindner, L., Spisni, A., Baricchi, R. *Blood Transfus.* **12**, 548 (2014)
[2] Casali, E., Berni, P., Spisni, A., Baricchi, R., Pertinhez, T.A. *Blood Transfus.* **14**, 555 (2016)
[3] Silliman, C.C., Moore, E.E., Kelher, M.R., Khan, S.Y., Gellar, L., Elzi, D. J. *Transf.* **51**, 2549 (2011)

P17

A Metabolomic Study of Urinary Biomarkers in Bladder Cancer based on NMR Spectroscopy

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Bladder cancer (BC) is the most common urological malignancy. Early diagnosis of BC is crucial to improve patient outcomes [1]. Currently, a non-invasive diagnostic tool as biomarkers identification that is as sensitive and specific as cystoscopy, is lacking [2]. Indeed, metabolomics could be a complementary approach to differentiate bladder cancer from non-cancer controls and to monitor tumor recurrence and progression.
The aim of this study was to evaluate the metabolic perturbations occurring in non-muscle invasive (NMIBC) and muscle invasive (MIBC) urinary bladder cancer compared to a control group, focusing to the metabolic differences between the two stages of cancer. In this metabolomics work, we studied the metabolic profiles of 52 samples of urine (19 NMIBC, 21 MIBC, 12 healthy volunteers) using $_1$H-NMR spectroscopy.
Here we present preliminary results obtained combining two different metabolomics approaches (targeted and untargeted analysis). Statistical analysis was performed to confirm and discover potential biomarkers of bladder cancer in order to distinguish between control, NMIBC and MIBC cohort.
Significant altered concentrations of metabolites related to different pathways (lipid metabolism, purine and pyrimidine metabolism, glycolysis) were found in urine with cancer.

References

[1] Cheng, X. Yang, *Int. J Clin Exp Med* **8,** 7 (2015)
[2] Chan, KK Pasikanti, *J Proteome Res* **14,** 2 (2015)

P18

A new biomarker for COPD: Histone 3.3

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Chronic Obstructive Pulmonary Disease (COPD) causes tissue destruction in lungs of patients and breathing problems, leading finally patients to death for insufficient respiratory ability. Patients with moderate to severe COPD are very susceptible to recurrent Acute Exacerbations of COPD (AECOPD). AECOPD have been associated with accelerated decline in lung function, decreased the quality of life, increased morbidity and mortality. AECOPD is also the major cost component of the disease. H3.3 is elevated in the extracellular milieu in the lung and in the bronchoalveolar fluid. Also, neutrophil extracellular traps (NETs) release H3.3 during AECOPD.
Study: Collectively these data suggest that H3.3 is likely to be elevated in the plasma of AECOPD and probably correlates with the number of exacerbations and the progression of the disease. We compared STELLIRK peptide of H3.3 in non-smoker and different GOLD stage patients with different number of exacerbation per year, to determine a correlation with the state of the pathology. This study was performed measuring the level of the peptide in plasma patients using Multiple Reaction Monitoring-Mass Spectrometry (MRM-MS). With this technology is possible to quantify the proteins even in a very complex matrix and we found that the histones level in plasma samples is closely related to the severity and the progression of the disease.
Conclusion: This study shows that H3.3 is a promising biomarker for COPD as a mirror of lungs destruction.

P19

GC-MS-based metabolomics and NMR investigation of Formula Milk

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Breast milk (BM) is the gold standard in neonatal nutrition. When BM is not available, it can be substituted or integrated with commercial infant formulas, sold under different brands and formulations. The low-molecular-weight hydrophilic compounds in various formula milk (FM) brands and BM samples were analyzed by GC-MS and multivariate statistical data analysis. Results highlighted that FM formulations, besides being strongly different from BM, are diverse especially in regard to the addition of oligosaccharides and other nutrients. Moreover, the ^{1}H NMR spectra of the aqueous and of the lipid fractions of FM samples exhibited differences due to the diverse formulations. Strengths and weaknesses of the two analytical approaches will be discussed also in the light of the nutritional reports.

P20

High resolution MS analysis for resolving untargeted metabolic profiles

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The determination of fruit and vegetable metabolic profile represents an increasingly important topic for the characterization and quality ensurance of PDO (protected designation of origin) species.
In the present study the high sensitivity, accuracy and specificity of high resolution mass spectrometry (HR-MS) have been exploited for untargeted analysis, so allowing a broad phytochemical characterization of Bligh-Dyer extracts of two botanical species in the context of the e-ALIERB project, an open lab, with the aim of enhancing and defining foodstuff and botanicals of Lazio region (Italy). A combined approach based on FT-ICR (Fourier transform ion cyclotron resonance) MS coupled with electrospray ionization (ESI) and CID (collision induced dissociation) experiments has been applied to characterize both red sweet pepper "Cornetto di Pontecorvo" (*C. annuum*)[1] and Sperlonga white celery IGP (*A. graveolens L.*)[2]. The acquisition of elemental composition and structural information, besides further checks with reference compounds and specialized databank, have allowed us to identify a number of metabolites, including metallated mono- and disaccharides, choline, amino acids, organic acids, and metabolic intermediates such as flavones, glycosylated compounds and GABA.

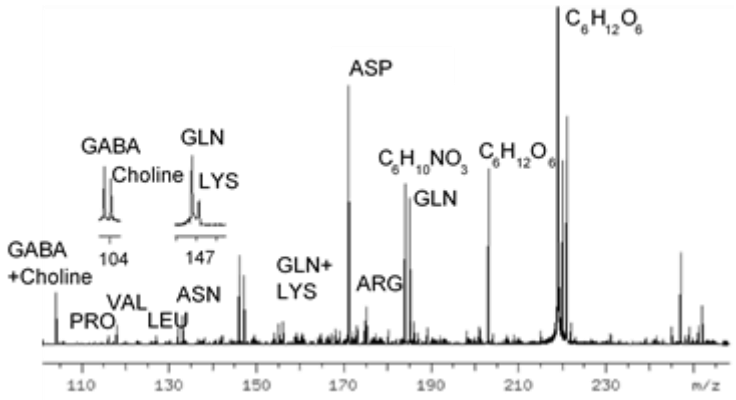

Figure 1: Excerpt of HR mass spectrum of the pulp hydroalcoholic Bligh-Dyer extract from open field "Cornetto di Pontecorvo" pepper.

References

[1] Lin, L. Shengmin, Lu. Harnly, J,M. *J. Agric. Food Chem.* **55** (2007).
[2] Marìn, A; Ferreres, F; Tomàs-Barberan, F,A; Gil, M,I; *J. Agric. Food Chem*. **52** (2004).

P21

Predicting NMR visible and invisible components of urine by a powerful analysis tool

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Urine is extremely rich in metabolic information, containing a total of more than 2000 endogenous metabolites, approximately 250 of which potentially detectable by NMR in minutes[1-2]. Accordingly, the NMR chemical shifts of a substance in a complex mixture strongly depend on the composition of the mixture itself, as many weak interactions occur that are hardly predictable. Chemical shift variability is the major obstacle to automatically assigning, and subsequently quantitating, metabolite signals in body fluids, particularly urine[3]. Rather than accepting this variability as a fact of life, we took the view that the variability of chemical shifts of the signals of a given metabolite must be a function, no matter how complicated, of the chemical composition of the mixture as a whole, i.e. of the variable concentrations of all the metabolites in the mixture. If this relationship were unraveled, chemical shifts would be predictable. This was achieved by constructing >4000 artificial mixtures, where the concentrations of the most abundant urine metabolites – including inorganic ions – are varied, to sparsely but efficiently populate an N-dimensional concentration matrix. Then, the assignment of >90 signals from > 60 metabolites in each mixture led to the corresponding matrix of chemical shifts. A strong relationship was established between the concentration and the chemical shift matrix, so that chemical shifts of metabolites can be accurately predicted in real urine samples[4]. Surprisingly, the concentrations of the invisible inorganic ions are also predicted, along with those of albumin and of several other abundant urine components within clinical accuracy[4]. Urine shift predictor is available for testing at: http://150.217.146.252:8080/.

References

[1] Bouatra, S. *et al*. *PLoS One* **8,** e73076 (2013).
[2] Nicholson, J. K. & Lindon, J. C. *Nature* **455,** 1054–1056 (2008).
[3] Puchades-Carrasco, L., Palomino-Schätzlein, M., Pérez-Rambla, C. & Pineda-Lucena, A. *Brief. Bioinform.* **17,** 541–552 (2016).
[4] Takis, P. G., Schäfer, H., Spraul, M. & Luchinat, C. *Nature Commun.,* In press, (2017).

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Untargeted analysis of intracellular samples of three winemaking yeast reveal new indole-based metabolites

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Untargeted metabolomics has emerged as an important tool in many disciplines between them food sciences. This comprehensive technique focuses on the detection of as many groups of metabolites as possible to obtain patterns or fingerprints without necessarily quantifying a specific compound(s). Particularly in food science, untargeted metabolomics has recently risen as a tool for gap mining and filling the lack of information regarding metabolic mechanisms of interest.
This study aimed to compare intracellular profiles of different yeast. The synthetic must was fermented by two *Saccharomyces* (Red Fruit and QA23) and non-*Saccharomyces* (*Torulaspora delbrueckii)*. Six folds performed and sampling was accomplished taken a volume equivalent to 10^9 cells after 2, 5, 7 and 15 days. The metabolites in a cells or produced by they represent integrative information about cellular function and therefore, link phenotype of a cell in response of environmental changes [1].
An untargeted LC-MS method was applied employing LTQ Orbitrap XL in positive and negative ionization. MS/MS assays operated in data dependent scan mode acquiring information at high resolution for Full Scan and MS2 and MS3 levels
Principal component analysis PCA revealed that time of fermentation was stronger differentiating factor than type of yeast. Indeed samples from fermentation days 2 and 15 were grouped in two clearly visible clusters. Among three tested yeast, clear visible were effects of *Saccharomyces* and non-*Saccharomyces* strains. *T. delbrueckii* exhibited more differences comparing to the rest mainly due to the increased number of features unique to this yeast. Statistical analysis and annotation of features revealed a series of new indole-based metabolites.

References

[1] Smart, K.F., Aggio, R.B.M., Van Houtte, J.R., Villas-Bôas, S.G., Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. *Nat. Protoc.* 2010, 5, 1709–1729

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Is dressing neutral in the digestion of foods? A study on balsamic vinegar

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Traditional balsamic vinegar is produced in the area of Modena and Reggio Emilia (Italy) and it is largely used for food preparation all around the world. In the last years, it has been considered as a source of antioxidant compounds and its biological activity has been largely studied [1]. Vinegar and balsamic vinegar have also demonstrated an anti glycemic effect in the insulin-resistant subjects [2]. However, the mechanism through which such effect is produced is still debated and hypothesis on possible interactions with the digestion processes have been formulated. Based on these premises, the present research work aims to evaluate the effect of Traditional Balsamic Vinegar on food digestion by using a validated *in vitro* systems. Three different foods with different matrices characteristics have been taken into account: Bresaola meat, Parmigiano Reggiano cheese, and boiled potatoes. Samples have been digested *in vitro* in triplicate according to the **INFOGEST** protocol [3]. For each food, 6 time points have been sampled. The resulting digestates were analyzed both by the application of high-resolution spectroscopy (HR-NMR) technique [4-5], which provides a more comprehensive profile for smaller proteins and metabolites released in the digestive juices, and by the Bradford protein assay, which provides a quantitative information limited to proteins larger than 3-5 kDa.
The obtained preliminary results show how, according to the type of food, traditional balsamic vinegar contributes to increasing the release of small molecules making them much more bioaccessible.

References

[1] Verzelloni E. et al.. From balsamic to healthy: Traditional balsamic vinegar melanoidins inhibit lipid peroxidation during simulated gastric digestion of meat (**2010**). Food and Chemical Toxicology, 48(8–9), 2097-2102. **[2]** Johnston C. S. et al.. Vinegar: Medicinal Uses and Antiglycemic Effect. C. A. (**2006**). Medscape General Medicine, 8(2), 61. **[3]** Minekus M, et al.. A standardised static in vitro digestion method suitable for food - An international consensus (**2014**) Food & Function, 5(6), 1113-1124. **[4]** Bordoni A. et al.. The foodomics approach for the evaluation of protein bioaccessibility in processed meat upon in vitro digestion (**2014**). Electrophoresis, 35, 1607−1614. **[5]** Bordoni A, et al.. NMR comparison of in vitro digestion of Parmigiano Reggiano cheese aged 15 and 30 months (**2011**). Magnetic Resonance Chemistry, 49, S61–S70.

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Skin damage exploration through NMR metabolomics

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Ultraviolet (UV) radiation is one of the major factor among hazardous environmental agents that induces acute and chronic reactions and melanoma formation in the human skin. UV exposure is responsible of inflammation, DNA damage, endogenous formation of free radicals and reactive oxygen species (ROS), tumor promotion and cutaneous autoimmune diseases[1]. Metabolomics is a powerful approach for the identification of biomarkers and metabolic pathways following perturbations of cellular homeostasis. However, its application to the recognition and quantification of specific dermal metabolites[2] is still lacking. In the present study, we developed an NMR approach to analyze skin and plasma metabolites' alteration after UV-B irradiation. We used the UV-B burn mouse model to set up the analytical method[3]. Skin burn have been generated with a dose of 500 mJ/cm^2 using a UV-B narrow-band lamp. Skin and plasma samples were collected from control and injured animals 48 hours after UV-B irradiation. Differences in the metabolic profiles between the two experimental conditions have been highlighted, both in plasma and in tissue extracts.

References

[1] Hruza L.L., Pentland .A.P. *J Invest Dermatol* **100**, 35S (1993).
[2] Li J., Xu W., Liang Y., Wang H. *Pharmacological Reports* **69,** 252 (2017)
[3] Hajiali H., Summa S., Russo R., Armirotti A., Brunetti V., Bertorelli R., Athanassiou A. and Mele.E. J*ournal of Materials Chemistry B.* **4**, 1686 (2016)

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Combining ^{19}F NMR functional screening and metabonomic analysis

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Over the last 15 years, the Fluorine NMR functional screening, *n*-FABS (*n*-Fluorine Atoms for Biochemical Screening)[1-2], has become a powerful, reliable and efficient screening technique for the identification of hits and/or leads in the drug discovery process both in academia and industry[3-6]. Its easy set-up results in fast and reliable detection of both potent and weak inhibitors when used in the search for modulators of enzymes' activity. The versatility of this technique allows its application to the screening of compound libraries directly in living mammalian cells[7]. With this approach, both functional screening and metabolic profiling can be performed simultaneously on the same sample. This represents a significant advantage over other screening techniques. The knowledge gathered with this approach could be useful in the prioritization and follow up of different hits by discarding those compounds that have toxic side effects thus reducing the time and costs of the early stages of the drug discovery process.

References

[7] Dalvit C., Ardini E., Flocco M., Fogliatto G.P., Mongelli N., Veronesi M. *J.A.C.S.* **125,** 14620 (2003).
[8] Dalvit, C., Ardini, E., Fogliatto, G.P., Mongelli, N., Veronesi, M. *Drug Disc.Today* **9,** 595 (2004).
[9] Fattorusso, R., Jung, D., Crowell, K.J., Forino, M., Pellecchia, M. *J Med Chem* **48,** 1649 (2005)
[10] Tarrago, T., Frutos, S., Rodriguez-Mias, R.A., Giralt, E. *ChemBioChem* **7,** 827 (2006).
[11] Keita, M., Kaffy, J., Troufflard, C., Morvan, E., Crousse, B., Ongeri, S. *Org Biomol Chem* **12,** 4576 (2014)
[12] Rydzik, A. M., Leung, I. K., Thalhammer, A., Kochan, G. T., Claridge, T. D., Schofield, C. J., Chem Commun, **50,** 1175 (2014).
[13] Veronesi M., Giacomina F., Romeo E., Castellani B., Ottonello G., Lambruschini C., Garau G., Scarpelli R., Bandiera T., Piomelli D., Dalvit C. Analytical Biochemistry **495**, 52 (2016).

P26

Metabolomic approach in the study of pre-diabetes pathologies by HPLC-MS and GC-MS lipid class characterization

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Type 2 diabetes mellitus is a complex disease [1], which is characterized by abnormal hepatic glucose output, insulin resistance and impaired insulin production [2][3]. The global increase of the disease necessitates early detection, even before the occurrence of hyperglycaemia. Metabolomics is the rapidly evolving field of the comprehensive measurement of ideally all endogenous metabolites in a biological fluid [4]. Changes in metabolic profiles are a potential source of such biomarkers: amino acids or fatty acids derivatives can be identified as biomarkers in preliminar stages of diabetes disease. The purpose of this work is to build up an HPLC-MS and a GC-MS method in order to identify as many as possible biomarkers in serum samples by comparing healthy populations and pathological ones.

References

[1] Leahy JL. Pathogenesis of type 2 diabetes mellitus. Arch Med Res, 2005; 36: 197–209
[2] Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E. Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. Diabetes Metab Rev, 1998; 4: 727–747.
[3] Xu F, Zou L, Ong CN. Multiorigination of chromatographic peaks in derivatized GC/MS metabolomics: a confounder that influences metabolic pathway interpretation. J Proteome Res, 2009; 8:5657–5665.
[4] Fiehn O. Metabolomics–the link between genotypes and phenotypes. Plant Mol Biol, 2002; 48: 155-171

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