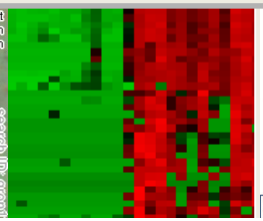
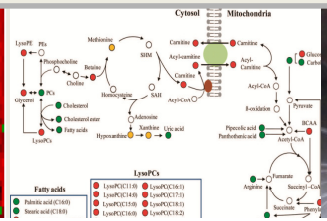
Health effects of plant bioactive compounds
Nutrigenomics approaches

Blandine COMTE

"Innovative Health-Promoting Food"
International Event on Functional Food
29th-30th September - Berlin - Germany



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JUR1019
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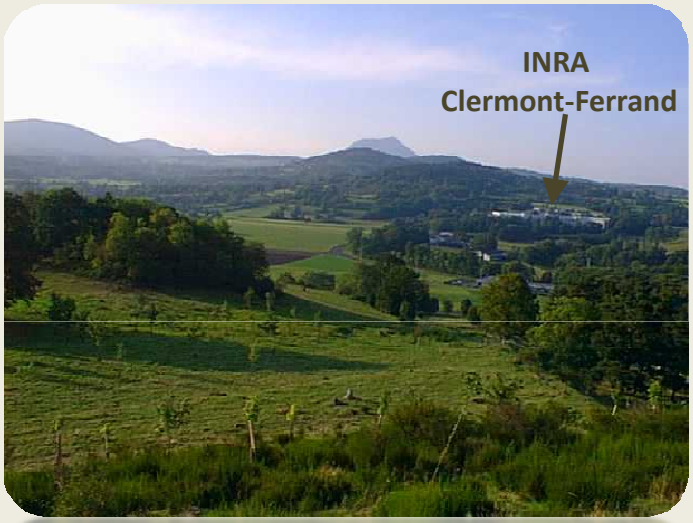
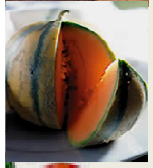
The Human Nutrition Unit - JRU1019

The Joint Research Unit gathers 150 persons in 10 teams (2 platforms: Metabolic Exploration Platform (MS) and an animal facility), including a permanent staff of over 50 scientists

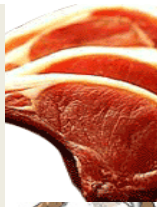
Elucidation of key mechanisms involved in the prevention of aged associated diseases by nutrients and foods



Impact of micronutrients on cardiovascular disease prevention and understanding the cellular and molecular mechanisms involved

ENERGETIC DENSITY

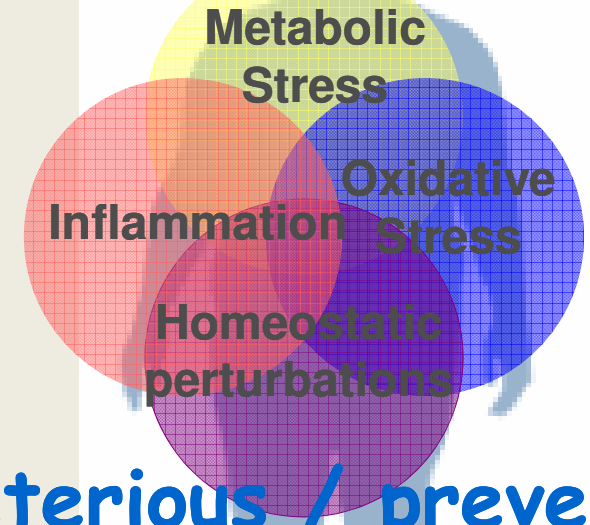







Metabolic dysfunctions

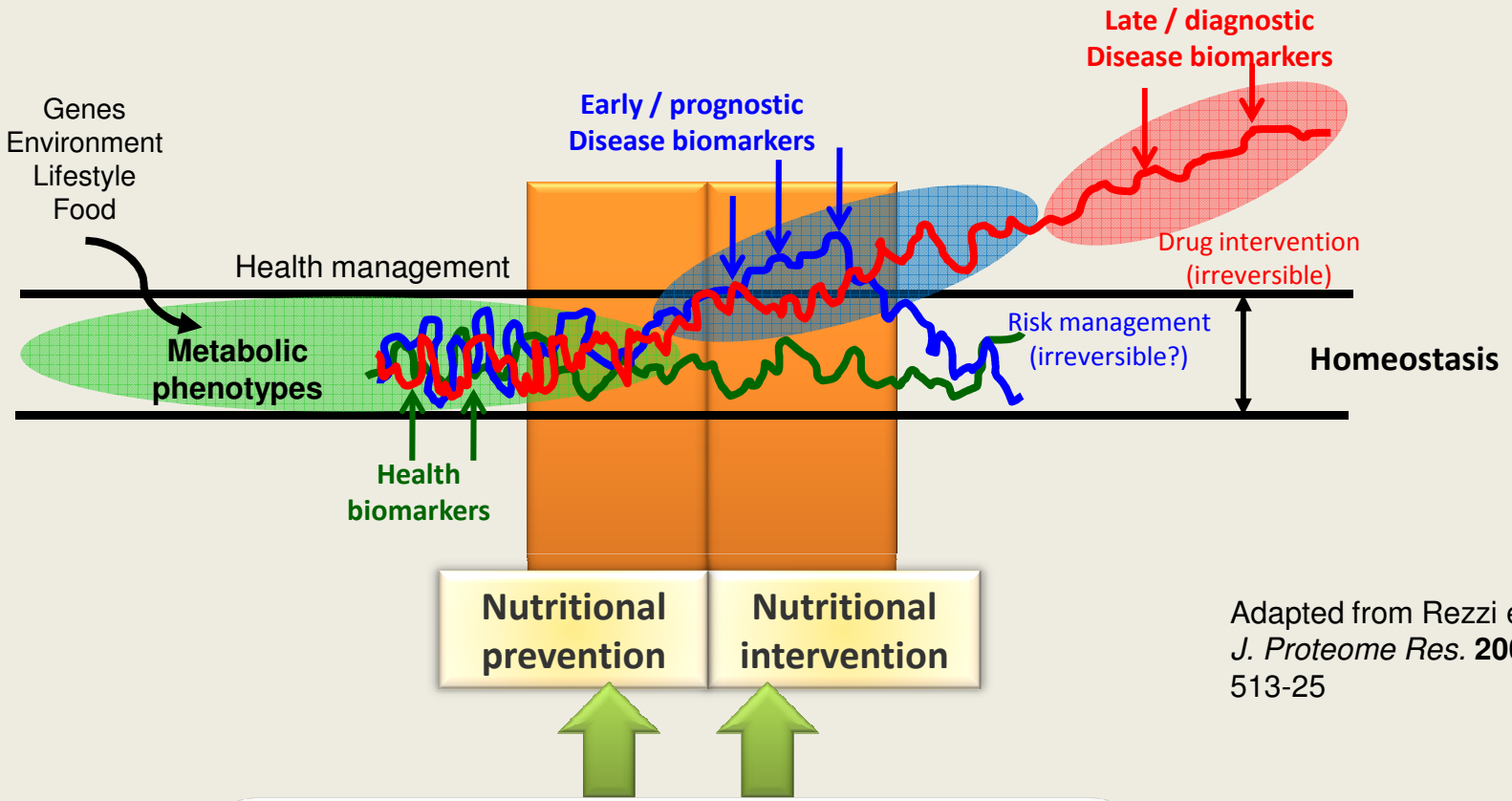
Cardiovascular diseases



Deleterious / preventive effects of nutrients

NUTRITIONAL DENSITY

Dynamics of metabolic phenotypes



Adapted from Rezzi et al; *J. Proteome Res.* **2007**, 6: 513-25

Complex food / Dietary patterns

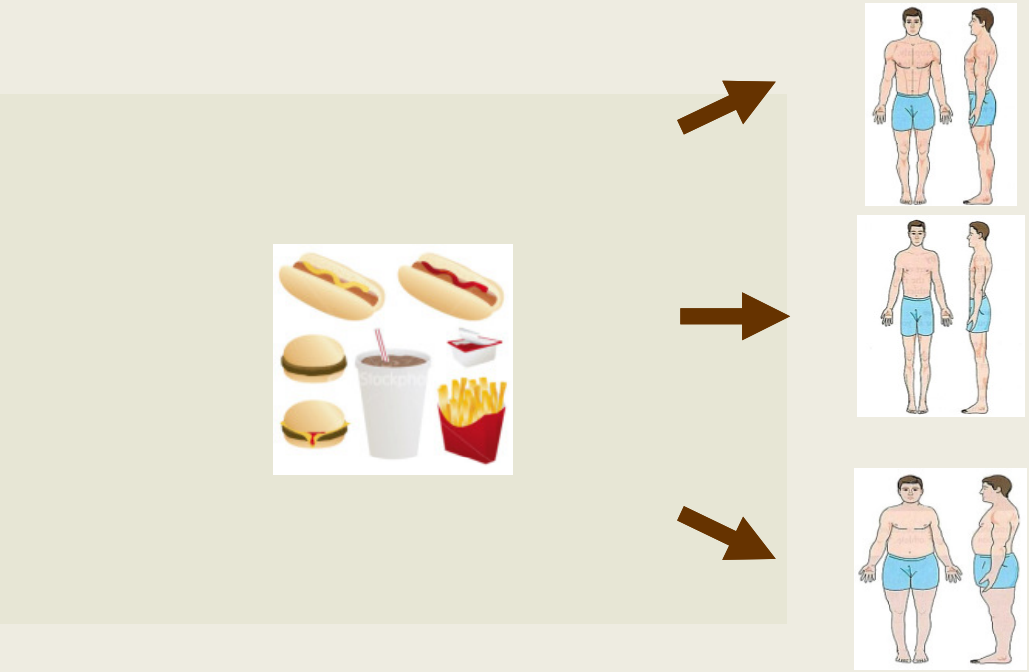
- Macronutrient balance
- Diversity bioactives
- Interactions between nutrients
- Matrix effects

Health effects of bioactive compounds

- UTILIZATION OF THE METABOLOMIC APPROACH TO CHARACTERIZE THE EXPOSURE TO NUTRIENTS AND BIOACTIVES AND IDENTIFY DIET COMPONENTS/INTERACTIONS RESPONSIBLE FOR THE EFFECTS

- IDENTIFY CARDIOVASCULAR PROTECTIVE EFFECT OF POLYPHENOLS AT NUTRITIONAL DOSES AND DECIPHER POTENTIAL MOLECULAR MECHANISMS

Diets and metabolic phenotypes

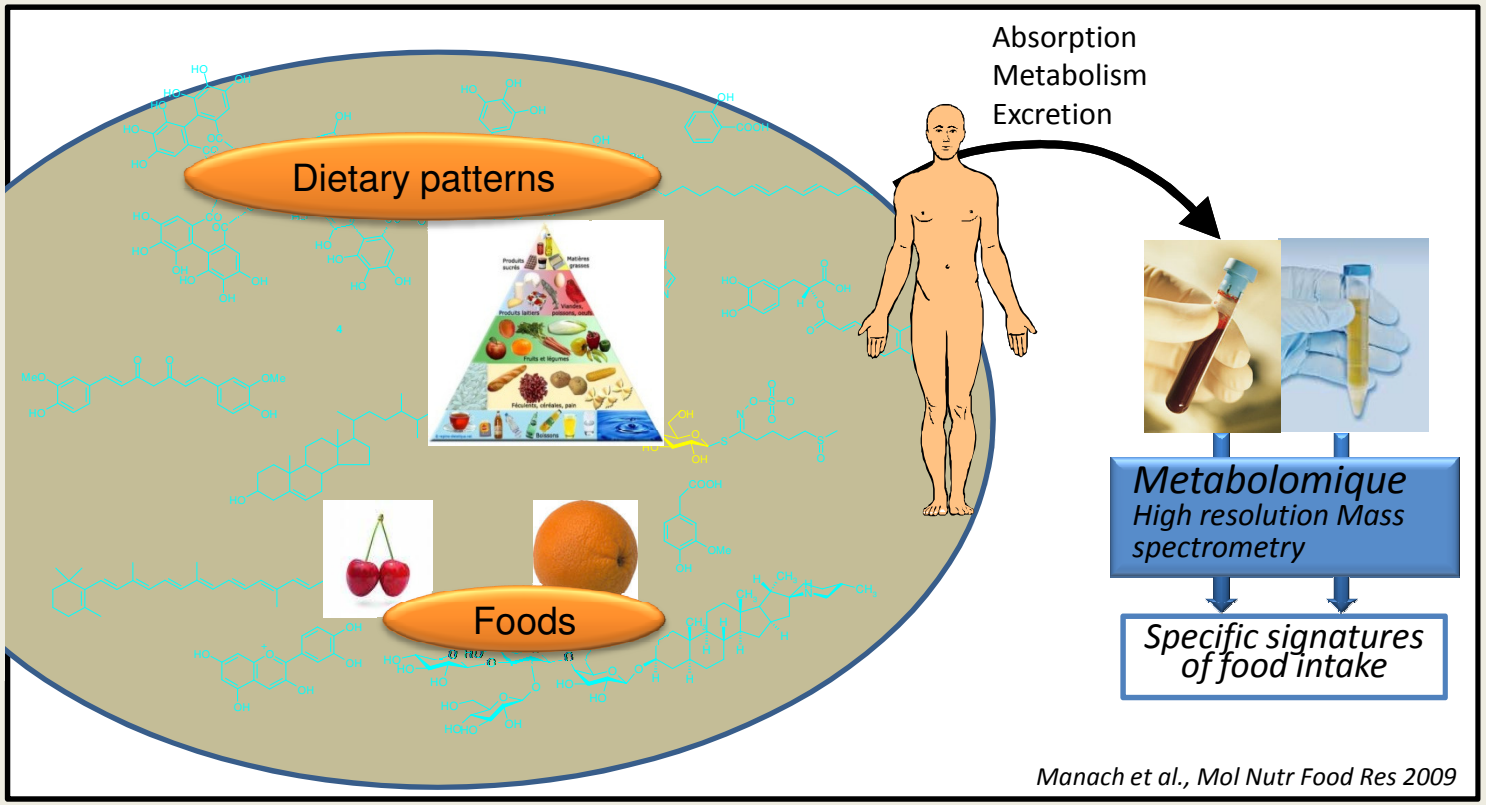


- Identify key markers of nutritionally responsive/non responsive phenotypes
- Contribution of inter-individual variability in nutrient and bioactive exposure

Customize diets for a healthier metabolic phenotype

Food metabolome

Food metabolome: all metabolites directly coming the digestion and metabolism of food components

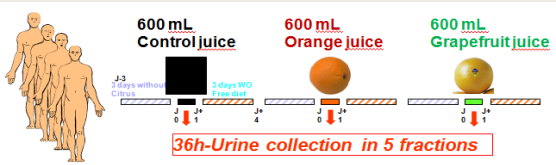


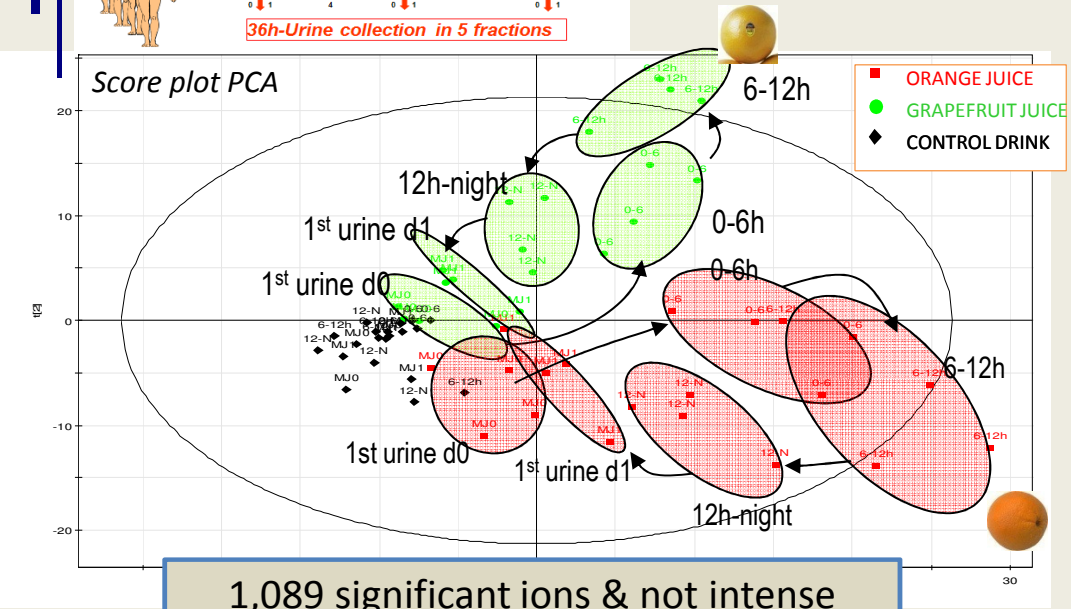
- **Biomarkers of intake for specific foods & dietary patterns**
- **Extensive phenotyping of dietary exposures, including phytochemicals**
- **Identification of new bioactives**

Biomarkers of citrus intake

PNRA-AGRUVASC

Controled intervention study, one single dose

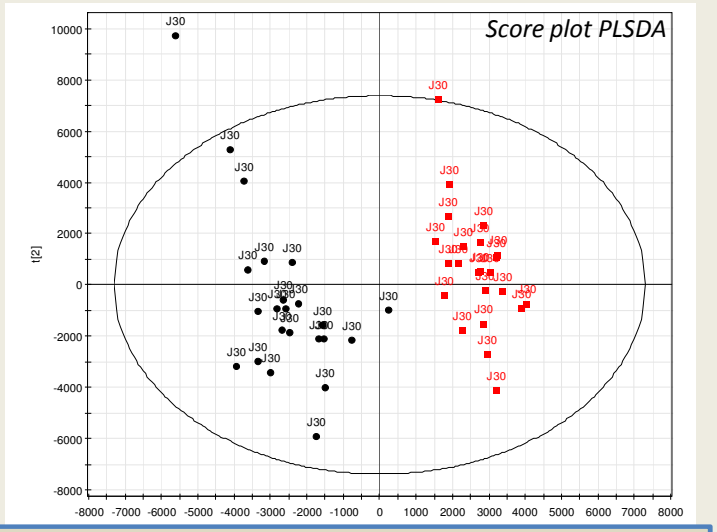




1,089 significant ions & not intense after juice consumptions of citrus juice

One month controled intervention study

24 volunteers
600 ml/d Orange juice / Control drink
Free feeding, cross-over study, morning urine spot D30



57 significant ions & more intense after orange juice consumption

Good discrimination of citrus consumption by urinary metabolites

The approach in the SU.VI.MAX2 cohort

Coll. S. Hercberg, P. Galan,
UREN, Inserm/INRA/CNAM/Paris 13

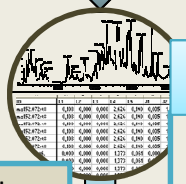
The SU.VI.MAX2 cohort *(6,850 M & F; 55-70 y.o.)*

FFQ between 2007 & 09
+ 6 24h recalls between
1994-2002 (SU.VI.MAX1)

40 **LOW** citrus consumers
<1.5 g/day; median=0.9 g/d

40 **HIGH** citrus consumers
>25 g/day; median=45.7 g/d

Biobank
(Urine spot 2007-2008)



LC-QToF metabolomics analysis

1/2 dilution; UPLC- ESI positive mode - (QToF)MS
Scan mode *m/z* 70-1000, 26 min gradient

Markerlynx

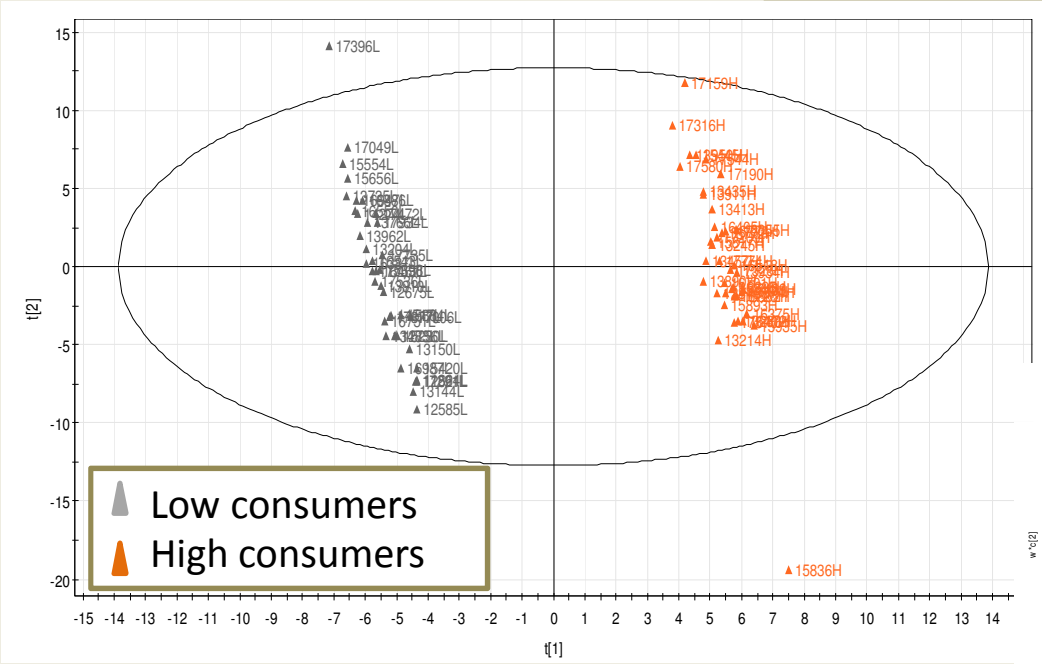
868 detected ions → Multivariance statistical analyses

One way ANOVA:
167 significant ions/citrus consumption *(P-value<0.05)*

Discrimination of citrus consumption in the SU.VI.MAX2 cohort

OSC-PLS (Log Pareto)

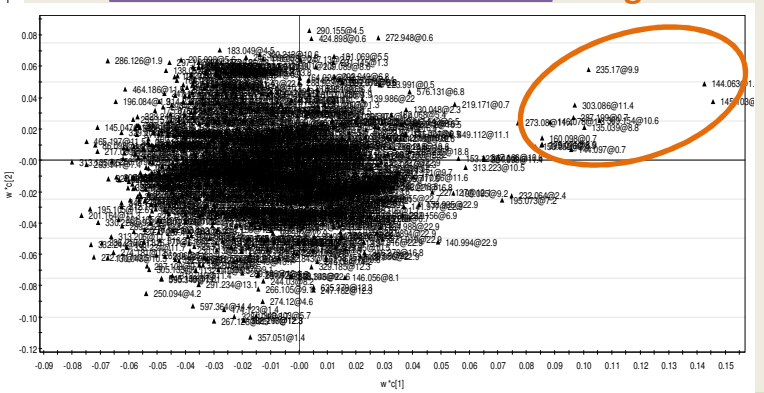
$Q^2Cum = 0.84$



Very good discrimination of the high *vs* low citrus consumers

OSC-PLS *Loading plot*

Ions with a high VIP



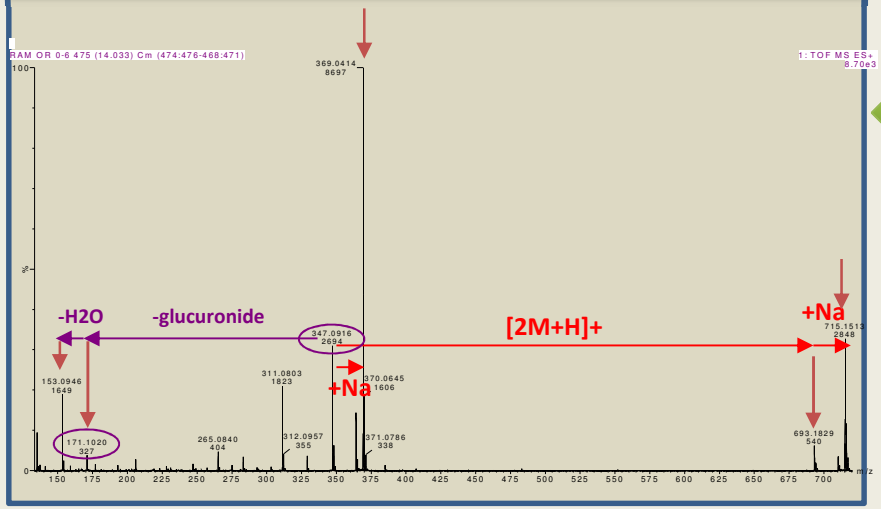
Selection of the ions to identify biomarkers of citrus consumption

VIP PLS >1.5 or P-value AVOVA <0.001 & more intense if citrus consumption

23 ions

Identification strategy

Analysis of the mass spectra

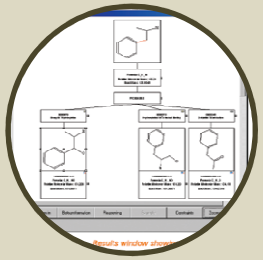


DB of citrus phytomicronutrients metabolites

224 known phytomicronutriments

+

Metabolites Known + predicted by meteor (Lhasa Ltd)



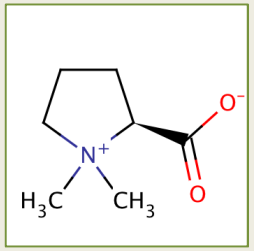
➢1 700 entries with monoisotopic masses

Hypothesis – Plausibility Analysis (/literature)

- ✓ Standard analysis if available
- ✓ LTQ-Orbitrap™: Monoisotopic mass + MS/MS spectra + UV-Vis spectrum

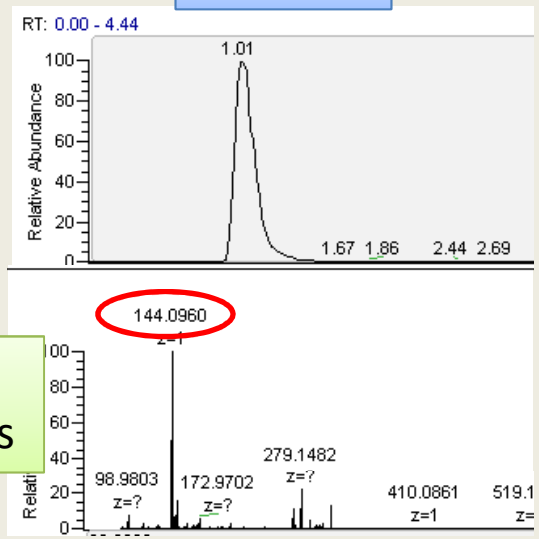
Validation - identification of the Ion 144.097: Proline Betaine

LTQ-Orbitrap

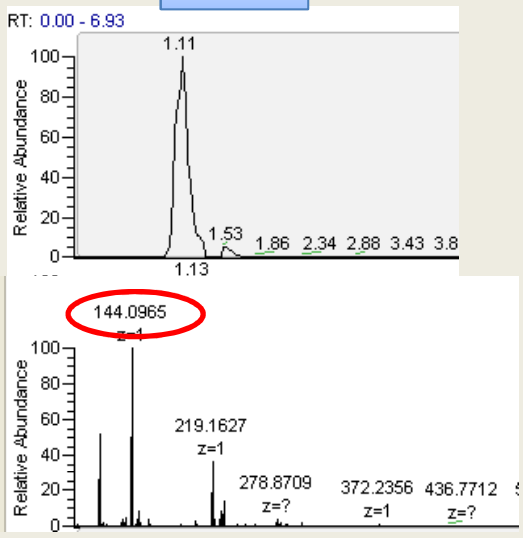


Retention time
MS/MS Monoisotopic mass

Standard



Urine



Already identified as a biomarker of citrus consumption

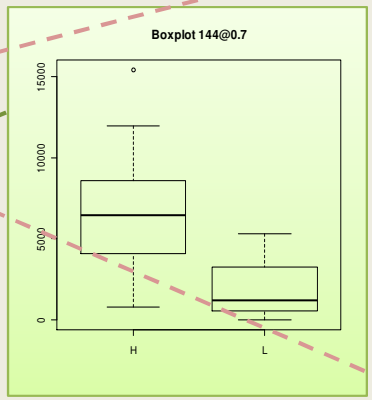
Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption[1–3]

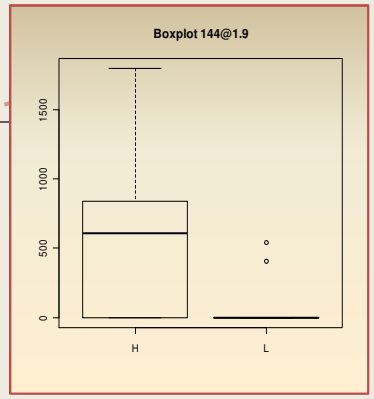
Silke S Heinzmann, Ian J Brown, Queenie Chan, Magda Bictash, Marc-Emmanuel Dumas, Sunil Kochhar, Jeremiah Stamler, Elaine Holmes, Paul Elliott, and Jeremy K Nicholson

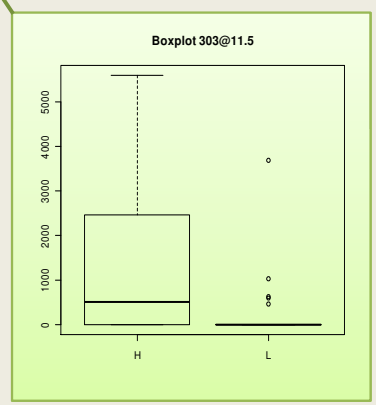
AJCN 2010, 92:436-43

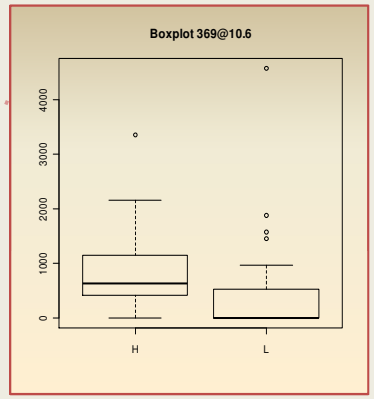
Biomarker identifications

IONS	Identification
144.063@1.9	Unknown $C_6H_{10}O_3N$
144.097@0.7	Proline betaine
369.154@10.6	Adduct Na 347
235.17@9.9	Several Hypotheses
303.085@11.5	Hesp 3'gluc (fragment)
146.078@1.3	Several hypotheses
160.098@0.7	Hydroxyproline betaine (tentative)
153.052@7.1	Unknown $C_8H_9O_3$
232.064@2.4	Several Hypotheses
273.08@11.4	Naringenin 4'-gluc (fragment)
347.164@10.6	Several hypotheses
449.112@11.1	Naringenin 7-gluc









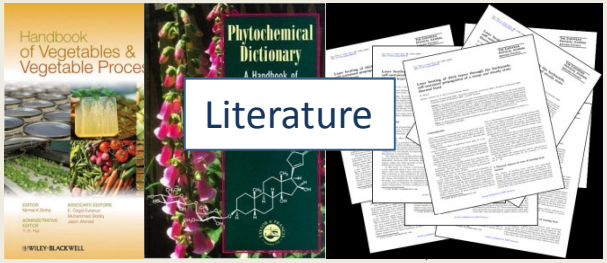
Coherent hypotheses with the Orbitrap mass measurement
But difficult validation (no available standards and low biological concentrations)

All the most discriminating ions were also present in the short term controlled study Agruvasc
But not in the lowest p-values

PhytoMetaBank

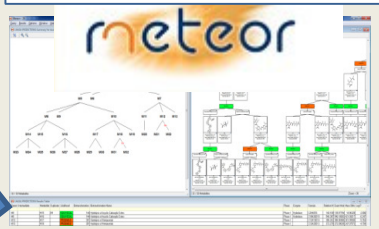
Internet database on food phytomicronutriments and their metabolites in human

USDA Phenol-Explorer
Database on polyphenol content in foods
Dictionary of Food Compounds
FooDB The Food Component Database
KNApSAcK
Composition tables



Literature

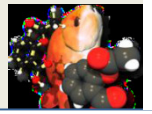
In silico prediction of metabolism



2,000 food phytomicronutriments
Classification
Known metabolites
In silico predicted metabolites
Food sources

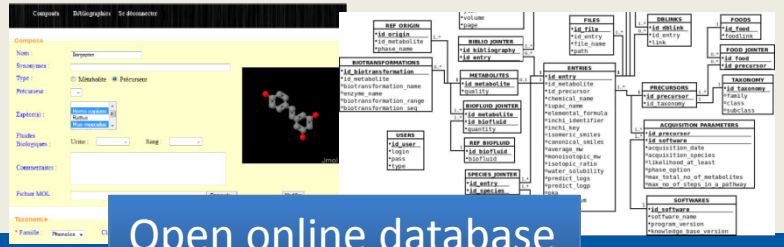
Physical & chemical data (logP, …)
Spectral data (monoisotopic mass…)
Links to other databases

Experimental data
MS/MS, UV…


ChemAxon

Collect / prediction of physical & chemical data

ChEBI
KEGG Kyoto Encyclopedia of Genes and Genomes
hmp human metabolome project
PubChem


Open online database

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Health effects of bioactive compounds

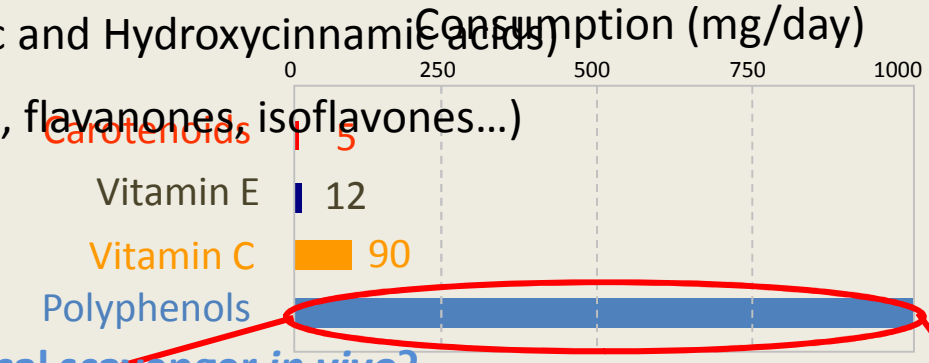
- UTILIZATION OF THE METABOLOMIC APPROACH TO CHARACTERIZE THE EXPOSURE TO NUTRIENTS AND BIOACTIVES AND IDENTIFY DIET COMPONENTS/INTERACTIONS RESPONSIBLE FOR THE EFFECTS

- IDENTIFY CARDIOVASCULAR PROTECTIVE EFFECT OF POLYPHENOLS AT NUTRITIONAL DOSES AND DECIPHER POTENTIAL MOLECULAR MECHANISMS

Polyphenols and health

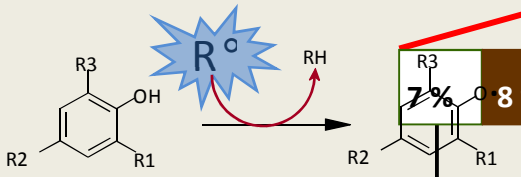
➢ Phytomicronutrients, most abundant dietary antioxidants

- **Phenolic acids** (Hydroxybenzoic and Hydroxycinnamic acids)
- **Flavonoids** (Flavanols, flavones, flavanones, isoflavones…)
- **Lignans**
- **Stilbenes**



Free radical scavenger *in vivo*?

(Guilland *et al.*, 1986; Kühnau, 1976)



* In plasma: polyphenols ≈ 1µM

Cardiovascular protective properties are not related to direct anti-oxidant effects

Polyphenols and cardiovascular diseases

Epidemiological studies

- Beneficial effect of flavonoids consumption on CVDs (coronary artery disease risk and stroke risk) (Arts and Hollman, 2005)

- Inverse association between flavanons intake and CHD mortality (Mink et al., 2007)

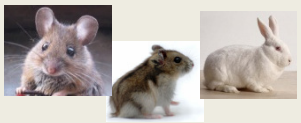
Clinical trials

Meta-analysis: flavonoid- rich food & beverages: tea, cocoa, soya

Improvement of:
- Systolic and diastolic blood pressure
- Endothelial function

(Hooper *et al.*, 2008)

Animal studies



- Reduction of atherosclerotic lesions upon consumption of tea extracts, wine, pomegranate juice, grapes in apoE$^{-/-}$ mice model (Furhman et al., 2005) (Hayek et al., 1997)

Dietary polyphenols and cardiovascular function

Protective effects and molecular mechanisms

Human Intervention Studies

Flavonoid rich foods / isolated compounds

Ex: Orange juice / Hesperidin

- Assessment of vascular function

(FMD by echodoppler, microvascular function by PAT index, microvascular reactivity by LD imaging coupled to iontophoresis, arterial compliance)

- Systemic markers of cardiovascular risk
- Gene expression analyses *(transcriptome study)*

Animal studies

apoE $^{-/-}$ mice

Polyphenol supplementation at nutritional doses

- *catechin*
- *curcumin*
- *anthocyanidin*
- *naringenin*

- Histomorphometry of aorta
- Biochemical parameters
- Gene expression analyses (transcriptome study)
- miRNA expression profiling
- Immunohistochemistry

In vitro studies

HUVECs

Polyphenol metabolites at nutritional doses

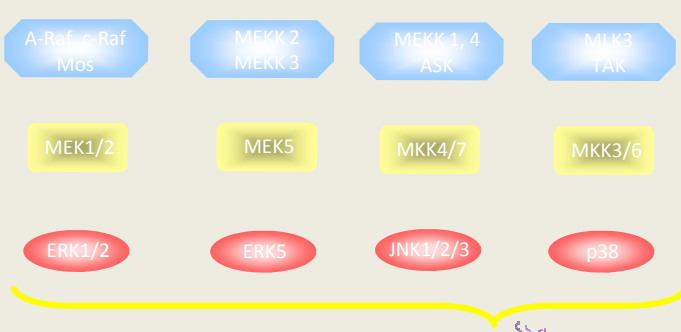
Ex: Flavanones metabolites

- Adhesion of monocytes
- Proliferation of HUVEC
- Migration of HUVEC (wound healing test)
- Transendothelial migration of monocytes
- Target gene expression analyses (LDTArrays)

Identification of potential molecular targets

MAPK signaling pathway

MAPKKK → MAPKK → MAPK → Transcription factor → Gene expression

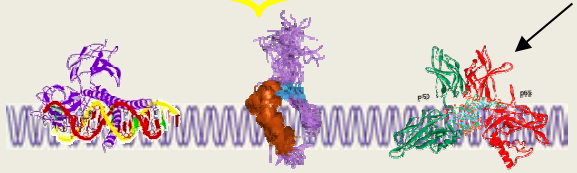


NF-κB signaling pathway

Phosphorylation of IκB by IKK

Degradation of IκB by proteosome

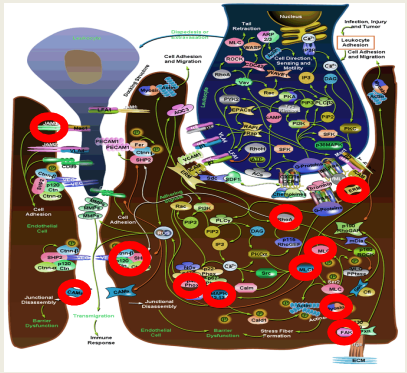
Liberation of NFκB complex



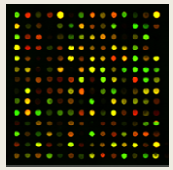
Transcription factor

miRNA

Bioinformatics analysis



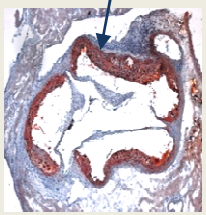
Modification of gene expression in aorta



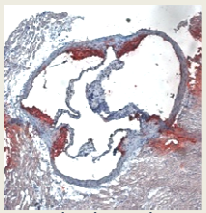
Cell adhesion / transendothelial migration

Decrease in atherosclerotic lesion development *in vivo* using nutritional doses

Atherosclerotic lesions



Control



Polyphenol

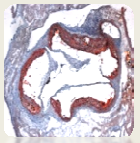
Experimental approach

*Models :
- Apo E$^{-/-}$
- C57Bl/6J on high fat high cholesterol diet
*6 week-old
*N=20 / group

16 weeks

- Control diet
- **Polyphenol (0.02%)**
  i) catechin
  ii) naringin
  iii) anthocyanidins
  iv) curcumin

Lipid deposit in aortic root by histomorphometry

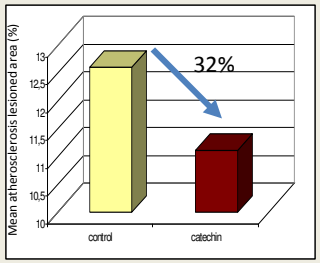


Lipid, inflammation and antioxidant parameters in plasma

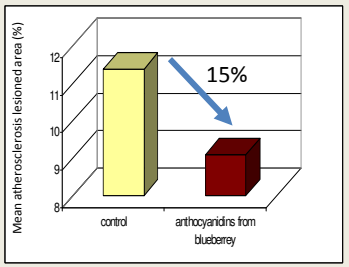
Lipid parameters in liver

Gene expression analysis in aorta

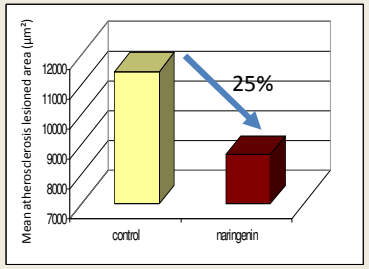
Catechin



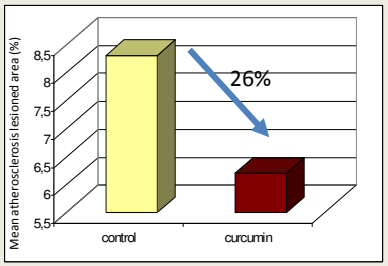
Anthocyanins



Naringin



Curcumin



Consumption at nutritional doses resulted in a significant reduction of lipid deposits in aorta, independently to changes in plasma lipid levels or antioxidant capacity

Auclair S., et al. 2009. *Atherosclerosis*. 204(2):e21-7

Mauray A, et al. 2009. *J Agric Food Chem*. 57:11106-11.

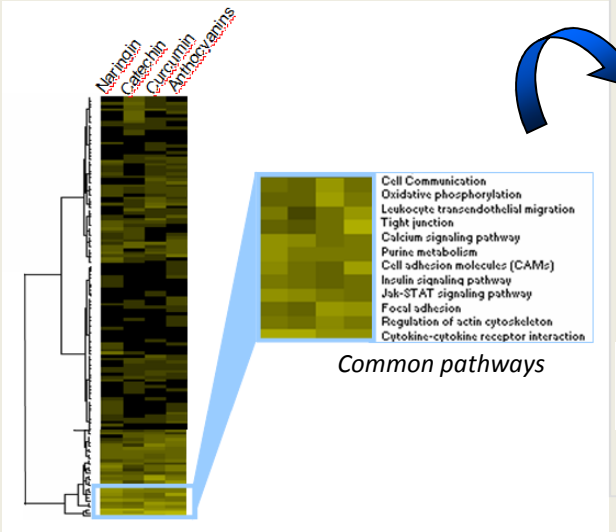
Chanet C, et al. 2011. *J Nutr Biochem* Epub 2011 Jun 16.

Coban et al. Under revision in *Mol Nut Food Res*

Molecular mechanisms of action of bioactives

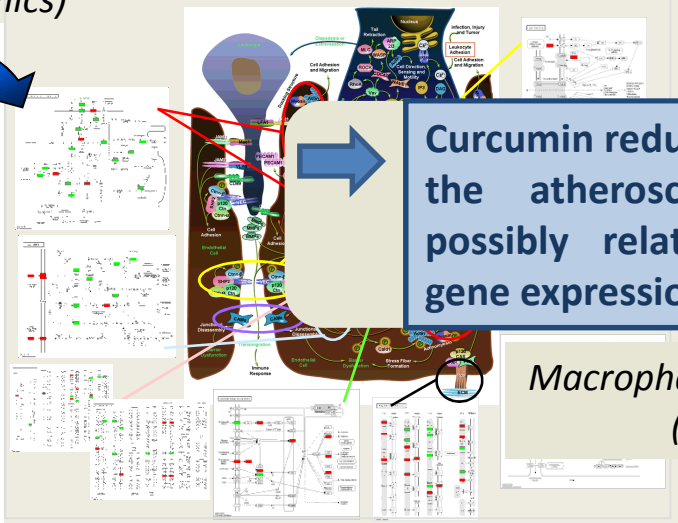
Nutrigenomic analysis: global (transcriptomics) or targeted approaches

Aorta gene expression (transcriptomics)



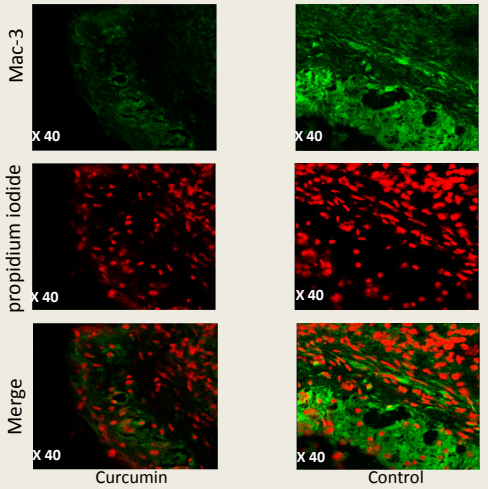
Common pathways

Hierarchical clustering of pathway profiles



Curcumin reduces macrophage number in the atherosclerotic lesion by 30%, possibly related with modification in gene expression

Macrophage quantification in aortic roots (immunohistochemistry)



- **Polyphenols modulate gene expression in aorta**
- **Functional analysis revealed a cluster of common pathways related to cell-cell adhesion, cell junctions, focal adhesion, and cell cytoskeleton that are related to transendothelial migration**

Auclair S., et al. 2009. *Atherosclerosis*. 204(2):e21-7

Mauray A, et al. 2009. *J Agric Food Chem*. 57:11106-11.

Chanet C, et al. 2011. *J Nutr Biochem* Epub 2011 Jun 16.

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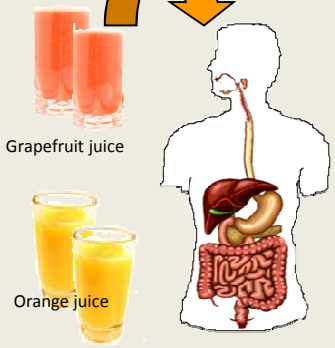
Polyphenol metabolites on cellular activity

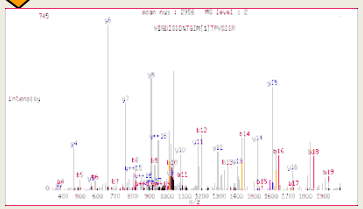
Phytomicronutrients of citrus fruits

1) Identification and synthesis of polyphenol metabolites:



Blood sample

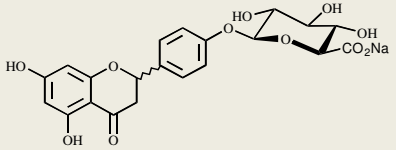
LC MS/MS analysis:



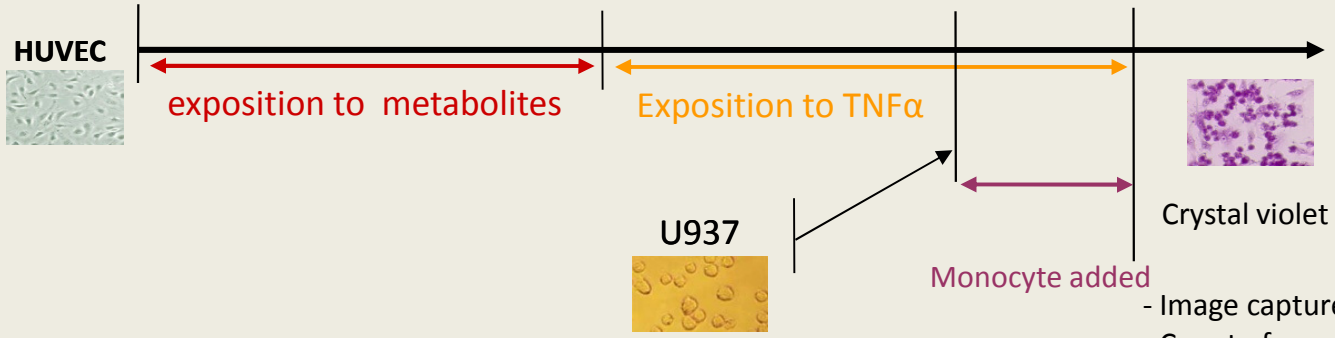
Identification of circulating metabolites

Bioactive-glucuronides
Bioactive-sulfates
. . . . . .

Chemical synthesis of circulating metabolites



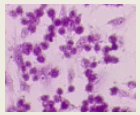
2) Monocyte to endothelial adhesion assay:

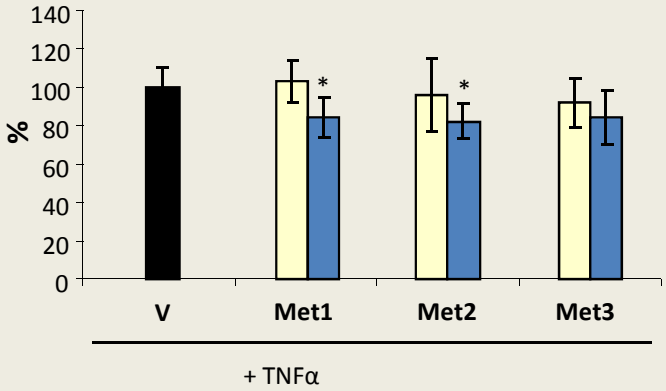


- Image capture
- Count of monocytes adhering to HUVEC

Effects on HUVEC activity *in vitro*

Monocyte adhesion

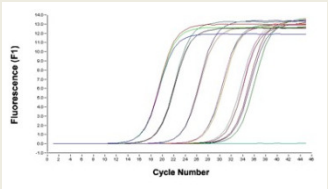




Pretreatment with metabolites reduce adhesion of monocytes to TNFα activated endothelial cells

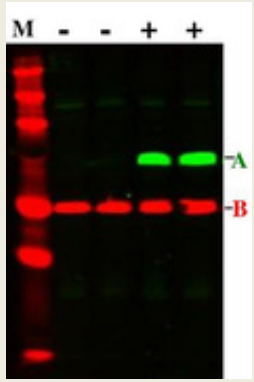
Expression analysis of targeted genes

- RT-PCR and microarray analyses of both mRNA and miRNA -



Metabolites modulate expression of genes

Cellular pathways and protein expression analysis



Polyphenols modulate protein expression

Physiological concentration of metabolites modulate initiation steps of atherosclerosis development by modulating expression of genes and proteins *via* modulation of signaling pathways activity

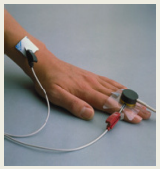
Role of hesperidin in the effects of orange juice?

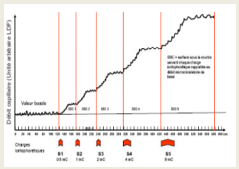
Inclusion → A, B or C (4 weeks) → Wash Out → A, B or C (4 weeks) → Wash Out → A, B or C (4 weeks)

A: 500 ml/d of orange juice
B: 500 ml/d of an isocaloric control drink + 1 placebo capsule
C: 500 ml/d of an isocaloric control drink + 1 capsule filled with 292 mg Hesperidin

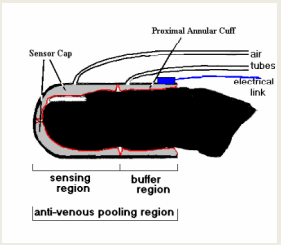
Functional & Systemic measurements

Microvascular reactivity measurement using laser Doppler coupled to Iontophoresis

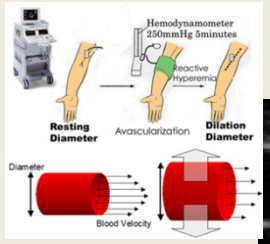


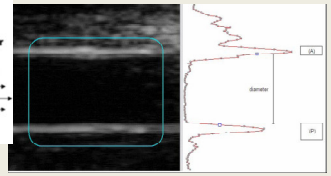


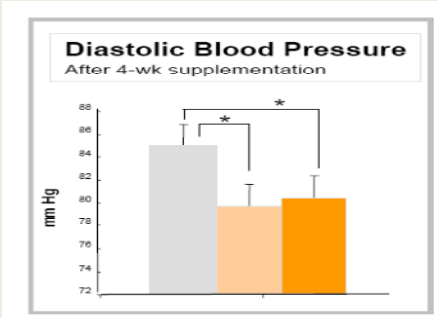
Peripheral Arteriel Tonometry

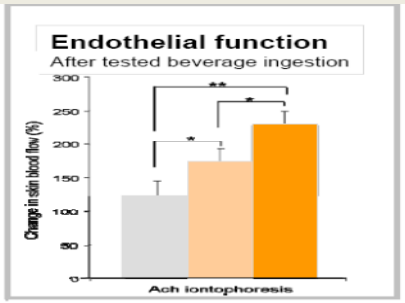


Flow mediated dilatation (FMD)









A one month consumption of orange juice, or pure Hesperidin, induced a significant **decrease in diastolic blood pressure.**

Orange juice and Hesperidin ingestion significantly **improved postprandial microvascular endothelial reactivity.**

Morand C, et al. 2011. *Am J Clin Nutr*. Jan;93(1):73-80

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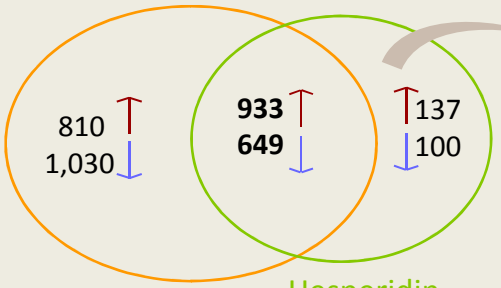
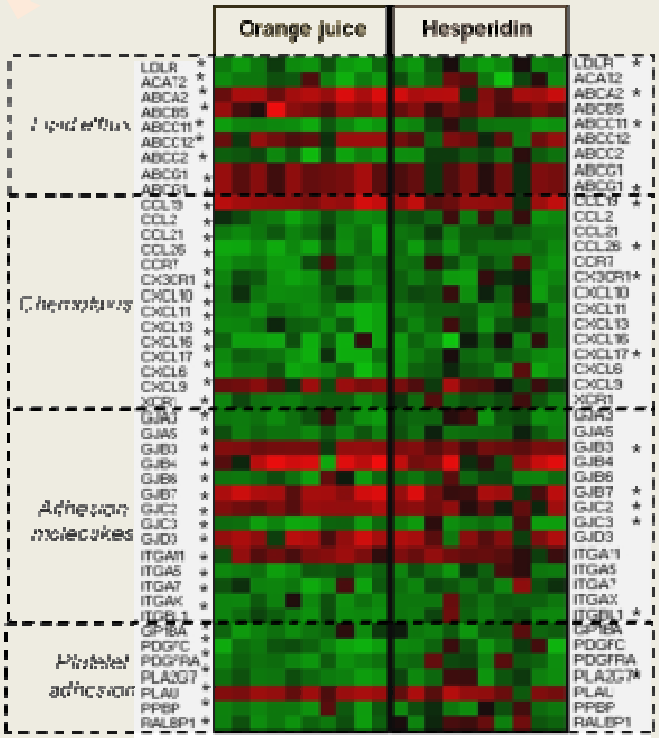
Role of hesperidin in the effects of orange juice?

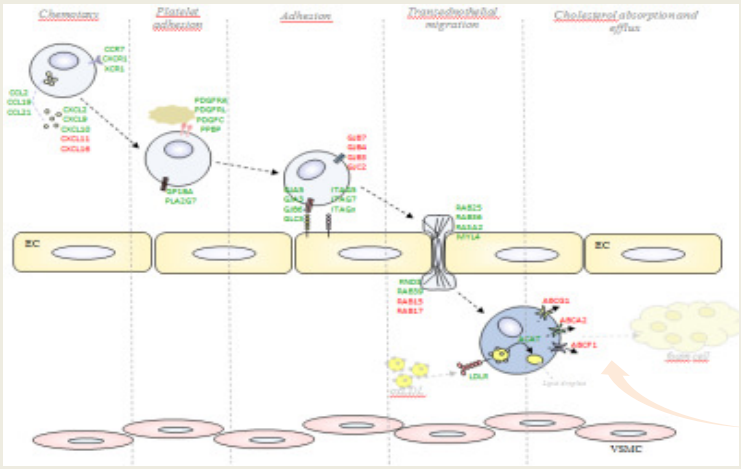
Number of differentially expressed genes in leukocytes



Microarray analysis:
1,582 genes are differentially expressed by orange juice and hesperidin consumption

Heat-map of atherosclerosis-related gene expression





Many of these genes are implicated in chemotaxis, adhesion, infiltration and lipid transport: suggesting **lower recruitment** and **infiltration of circulating cells to vascular wall** and **lower lipid accumulation**.

Milenkovic *et al* in press, PloS ONE

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CONCLUSION

- ✓ The metabolomic approach allows to identify biomarkers of food consumption directly in cohort studies.
- ✓ The best found biomarkers are in part different from those found in controlled intervention studies.

Food consumption biomarkers (cohorts)	*vs* ?	Exposition to metabolites of phytomicronutriments (interventions)

- ✓ The identification of the discriminating molecules is the bottleneck of the metabolomic approach. Regarding the Food metabolome it is related to the low concentrations in biofluids, of the lack of knowledge in databases and the absence of standards.

- ✓ Polyphenols present cardiovascular preventive properties, their bioactive effects are mediated by their capacity to modulate expression of genes.
- ✓ Potential cellular and molecular targets have been identified.

Acknowledgments

UMR1019-Unit of Human Nutrition

Claudine MANACH
Dragan MILENKOVIC
André MAZUR
Christine MORAND
Sergio POLAKOF
Mercedes QUINTANA
Augustin SCALBERT

Bruno CHABANAS
Audrey CHANEY
Sylvain CLAUDE
Dilek COBAN
Jane HUBERT

Dominique BAYLE
Catherine BESSON
Marie-Anne VERNY

METABOLISM EXPLORATION PLATFORM
From genes to metabolites

Estelle PUJOS-GUILLOT
Jean-François MARTIN
Bernard LYAN
Frank GIACOMONI
Jean-Louis SEBEDIO
Marie-Laure BAYLE
Charlotte JOLY
Daniel CESAIRE

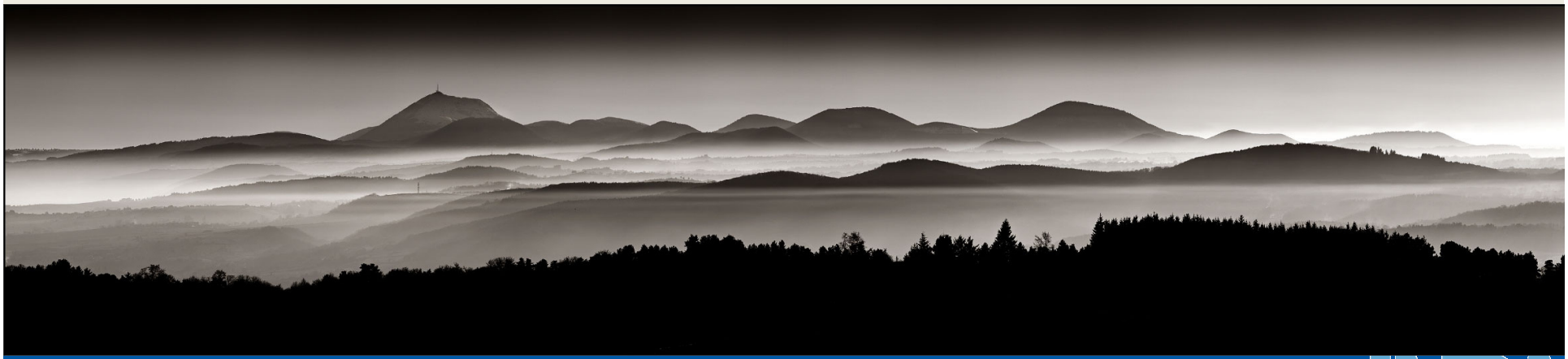
UREN, Inserm/INRA/ CNAM/Paris 13

Serge HERCBERG
Pilar GALAN

Financial supports: ANR Agruvasc,
Programme ANSSD2010 AlimH INRA
Citrus Dpt, Florida State, USA

ANR Agruvasc

Cathy BENNETAU-PELISSERO, ENITA Bordeaux
Annie BERARD, CHU Bordeaux



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Thank you for your attention