**Nutrigenomics and phytochemicals**

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**Phytochemicals and surrogates markers**

Several years!

Cardiovascular diseases: Arterial pressure, cholesterol...
Cancers: DNA damage, apoptosis...
Osteoporosis: Bone density, pyridinoline
Various diseases: Oxidative stress

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**Phytochemicals and health – Sources of evidence**

- Epidemiology
- Animal experiments
- Clinical trials
- Mechanisms of action

Nutritional recommendations
Food quality improvement

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**Surrogate markers**

Antioxidants and health

- Biomarkers
  - Oxidative stress
  - Lipemia
  - Inflammation
  - Endothelial function
  - Thrombosis
  - Arterial pressure
- Effects often contradictory
  - Markers sensitive enough?
  - Not the right markers?
- Links with pathologies often poorly understood
- Combination of biomarkers?

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**Phytochemicals and health**

Bone density, pyridinoline
Osteoporosis
DNA damage, apoptosis
Cancers
Arterial pressure, cholesterol
Mechanisms of action
Polyphenols and atherosclerosis

- Weak effects => examined at high doses
- Relevant in nutrition ???

Active polyphenol concentrations (µM)

Nutritional polyphenol concentrations

- Endothelial cells
- Aortic smooth muscle cells
- Hepatocytes
- Immune cells
- Lung epithelial cells
- Fibroblasts
- β-cells

KS483 cells from mice Genistein, 18 days

1 µM Differentiation into osteoblasts
10-50 µM Differentiation into adipocytes

Different effects at nutritional and pharmacological doses

Antioxidants – The chemical definition

Chemical reducing agents
Cofactors of antioxidant enzymes
Any compound stimulating antioxidant defense

Any compound present in low concentration able to delay or inhibit the oxidation of a substrate

Antioxidants « in vivo » - more than antioxidant effects

Red wine polyphenolic compounds inhibit atherosclerosis in apolipoprotein E-deficient mice independently of effects on lipid peroxidation

JACN, 2004, volume 79
Antioxidants and health claims

- Claim = “naturally contains antioxidants”

Metabolic effects of phytochemicals are complex

Nutrigenomics to characterize complex metabolic effects

Number of variables measured

- Patients: Urine, HPLC-UV, PCA
- 15 nucleosides
- 113 peaks (largely unknown)
- Liver cancer
- Hepatocirrhosis
- Hepatitis
- Yang J. et al., 2004, J Chromatogr
Nutrigenomics and phytochemicals

Phytochemical

Early metabolic changes associated to diseases

Omics

New hypotheses on mechanisms

New biomarkers

Different “omics” levels

Transcriptomics 25,000 genes
Proteomics 100,000 proteins
Metabolomics 20,000 metabolites

Tissues/cells
Plasma
Urine...

Data collection

Transcriptomics 25,000 genes
Proteomics 500 proteins
Metabolomics 5000 metabolites

CLOSED SYSTEM (chip)
- Finite number of transcripts
- Largely annotated

Mature
Technology development

OPEN SYSTEMS
- Variables not known a priori
- No universal technique for all proteins or metabolites

Quickly evolving

Data analysis

Samples
- Variables annotated or not
- Which structure within the matrix?

Variables
Intensities
**Data analysis**

**Visualization tools**
- Principal component analysis
- Hierarchical clustering analysis

- Distance between samples and groups
- Identification of variables affected in each group
- Distance between variables

**Data interpretation**
- Map experimental data on favorite metabolic and cellular charts
  - KEGG, GeneMAPP, Genomatix, Gene-GO, MetaCyc...
- Metabolic pathways
- Cellular and biological functions
- Gene regulation
- Integration

**Transcriptomics**

**Phytochemicals and biological functions**

- Catechin
- Apo E deficient mice
- Aorta
- 22 k DNA array

- 54 genes differentially expressed
  - (1.2 < Fold change < 2.4)

**Gene Ontology**
- Biological process level 3

- Nodes suggest implication of core transcription factors

**Transcriptomics**

**Phytochemicals and gene regulation**

- Catechin
- Apo E deficient mice
- Aorta
- 22 k DNA array

- Filtering for cardiovascular diseases

- Nodes suggest implication of core transcription factors
Combining transcriptomics and molecular biology

- Implication of a key transcription factor characterized
- Key pathways under the control of this transcription factor determined

Lee J-M. et al. (2003) JBC

Transcriptomics in nutritional conditions

- Identification of most probable mechanisms in nutritional conditions

Takahashi et al., 2004, Mol. Carcinogen.

Metabolomics

- Phytochemicals and biological functions

Fat +/- Catechin
Urinary LC-ESI-QToF
36 rats
554 variables

133 variables

- Nicotinic acid
- Dihydroxyquinoline
- Pimelic acid

From high- to low-level modelling

- New hypotheses with mechanisms

Phase A
Generate broad perturbations in the biological system
Statistical data mining
Identify modules affected by the perturbation
Generate an ensemble of model hypotheses

**Phytochemicals in epidemiology**

- Biomarkers of exposure needed
- Urine metabolome = Σ phytochemicals
  - Food metabolome
  - Exposome
  - Xenometabolome…

- Markers for phytochemical exposure

- **Food metabolome**

  - Controlled diets
  - Urine samples
  - LC-ESI-QToF
  - Exact mass = identification

- **Phytochemical and health**

  - From metabolic fingerprinting to metabolic profiling
  - Discovery of new associations between phytochemical exposure and health or diseases