Nestlē Institute of Health Sciences

NuGO

Nutrition 2.0 – Molecular Phenotyping in Humans

Martin Kussmann[1,2,3] Head of Molecular Biomarkers

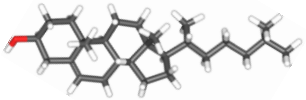
[1] *Nestlé Institute of Health Sciences*

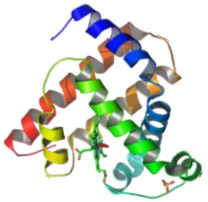
[2] *Ecole Polytechnique Fédérale Lausanne (EPFL)*

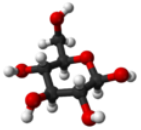
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

[3] *Aarhus University (AU) Denmark*

AARHUS UNIVERSITY







World Healthcare in 2020…

… 1 out of 5 will be over 65

- 70% of developed countries with more 50+ than 50-
- Over 200M people aged 65+ in China

➢ ***Requires healthcare systems better adapted to needs of the elderly***

… 3 out of 5 will die from a chronic disease

- 50M Alzheimer's patients
- 7% of the world's adult population will live with Diabetes

➢ ***More emphasis on prevention and treatment of chronic diseases***

… 1 out of 5 will be overweight or obese

- 120M people in the US
- 20% of people under 18 year in China

➢ ***Need to treat increasing co-morbidities such as cardiovascular diseases and diabetes***

… Malnutrition causes 35% of disease burden in children <5 years

- Maternal and child malnutrition is the underlying cause of 3-5 million deaths
- Associated to increased susceptibility to chronic disease later in life

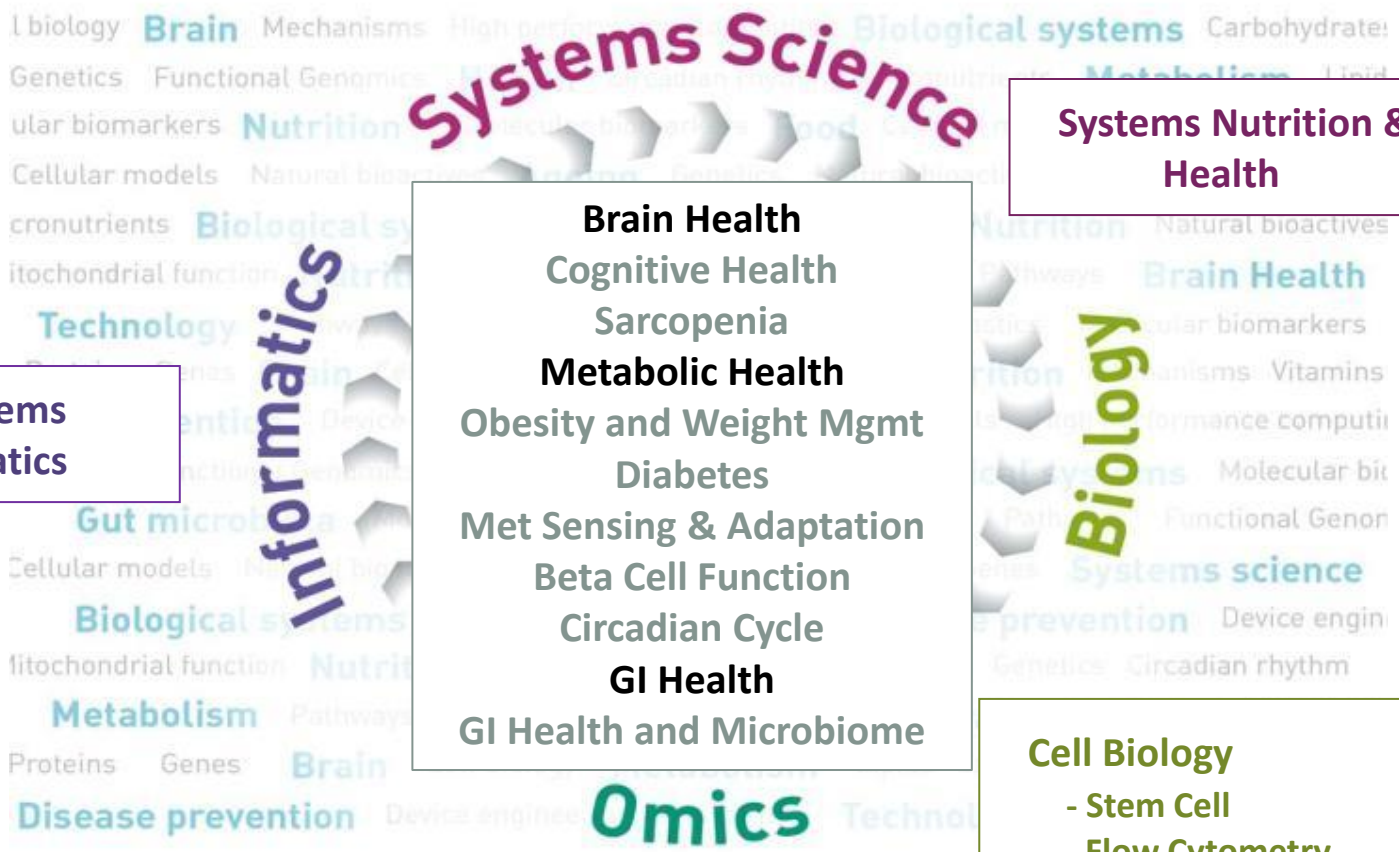
… US$ 5 to 10 Trillion will be spent on healthcare

- More than 16% of GDP spent on healthcare
- National health expenditures in USA per capita will reach US$ ~14'000

➢ ***Requires radical ways to contain costs and / or increase available funding***

Source: UN, WEF, IBM, PWC, Litsearch, Bain analysis / estimate

Scientific Platforms…



Brain Health
Cognitive Health
Sarcopenia
Metabolic Health
Obesity and Weight Mgmt
Diabetes
Met Sensing & Adaptation
Beta Cell Function
Circadian Cycle
GI Health
GI Health and Microbiome

Systems Nutrition & Health

Biosystems Informatics

Functional Genomics
Molecular Biomarkers

Cell Biology
- Stem Cell
- Flow Cytometry
- Device Engineering

Mitochondrial Function
Natural Bioactives & Screening

…to understand the interplay between…

Kussmann, Hager, Morine, Sonderegger, Kaput. Frontiers 2013
Kussmann, Kaput. Appl. Transl. Genomics 2014

NIHS 2015

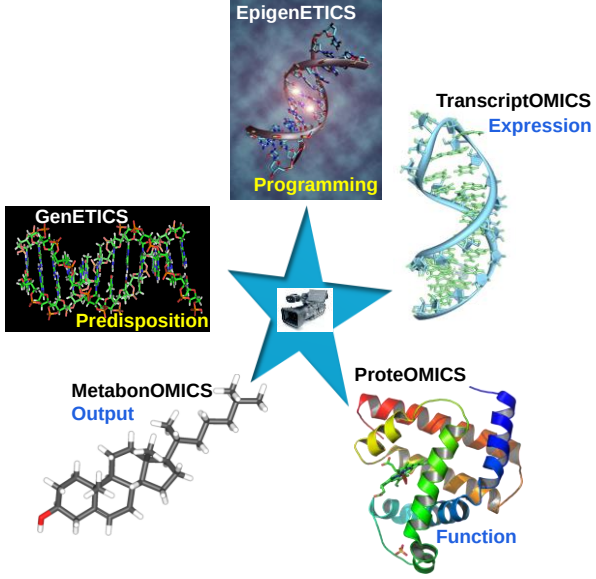
- **Employees: 150**
  - → **61% Ph.D., 19% Master**
  - → **28 nationalities**
  - → **Average age : 39**

- **Scientific Development and Interaction:**
  - **9 Adjunct Lecturers/Professors PhD & Master program with 20 mandates**
  - **> 100 publications**
  - **4 grants**

- **Numerous collaborations with national and international partners**

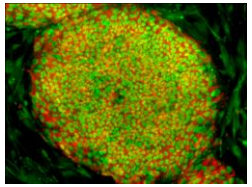
What makes us special…

1. **Integrated omics and systems biology**
   Genomics, Genetics, Epigenetics, Transcriptomics, Proteomics, Lipidomics, Metabonomics, Micronutrients
   Meta- and omics data. Translation into Diagnostics
   Data acquistion ← → processing ← → interpretation

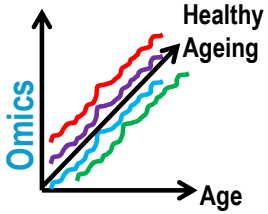


2. **Natural human cell models**
   ViaCyte partnership for human iPSC models
   Encapsulation and *in vivo* differentiation

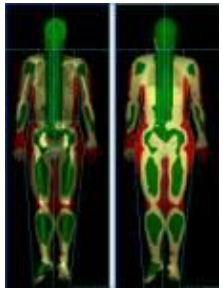
VIACYTE



3. **Longitudinal human studies with safe challenges**
   Systems view of recovery from challenge and restoration of homeostasis rather than analysis of system « at rest » (fasting)
   Metabolic (e.g. high fat, high glucose), cognitive and physical (endurance / resistance exercise) challenges



4. Classical **« top-down » phenotyping meets molecular « bottom-up » phenotyping**



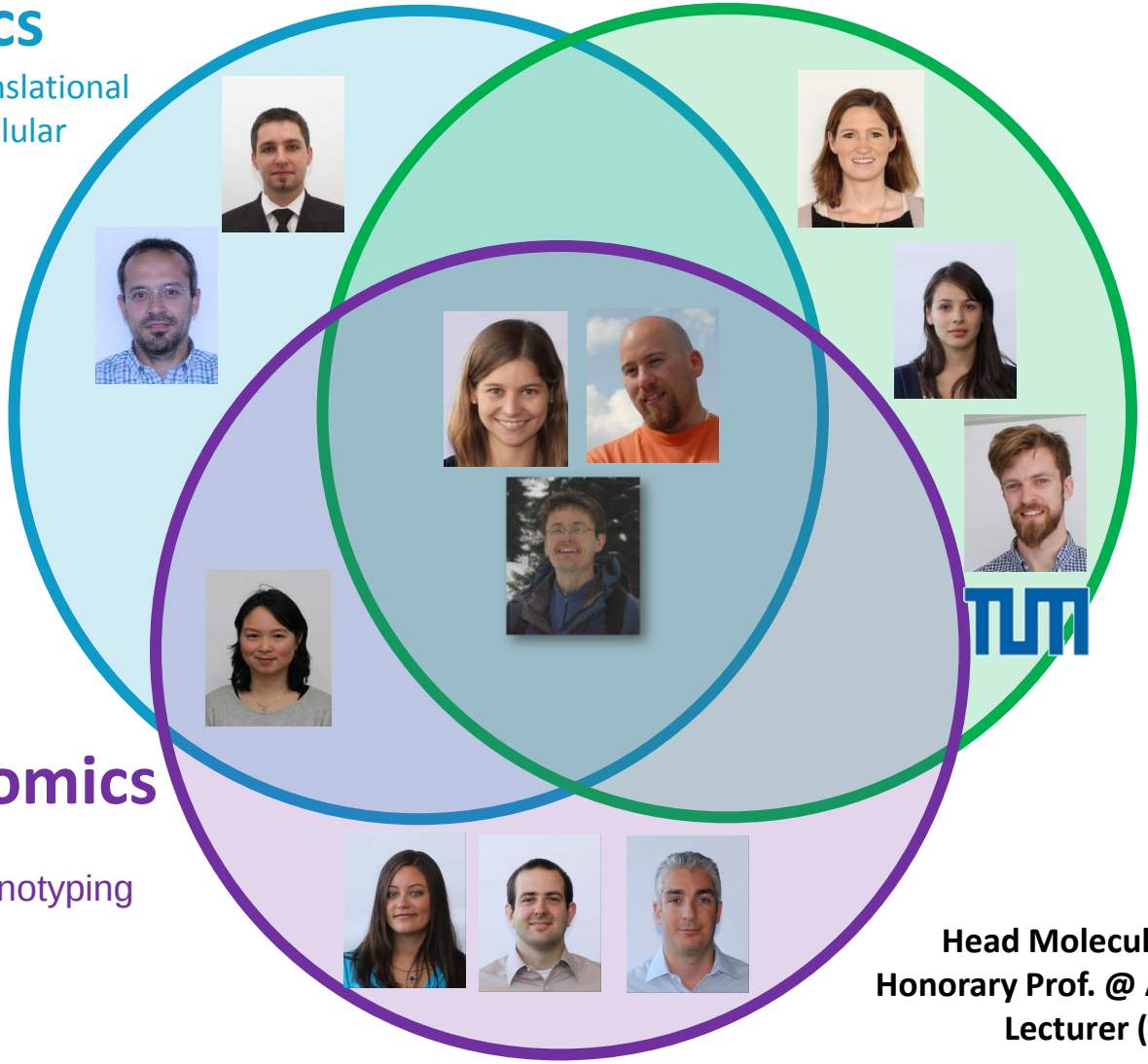
5. **We study health !**

Kussmann, Hager, Morine, Sonderegger, Kaput. Frontiers 2013
Kussmann, Kaput. Appl. Transl. Genomics 2014

Molecular Biomarkers – Platforms and Staff

Proteomics

- Clinical and Translational
- Molecular & Cellular
- Technology



Diagnostics

- Clinical and Translational
- Assay Development

Metabonomics

- Metabolism
- Molecular Phenotyping
- Technology

MARTIN KUSSMANN
Head Molecular Biomarkers @ NIHS
Honorary Prof. @ Aarhus Univ. Denmark
Lecturer (MER) @ EPF Lausanne

Molecular Biomarkers @ NIHS:

Where Genes and Environment intersect…

- EpiGenomics
- Transcriptomics

EpiGenotype

PREDISPOSITION
PROGRAMMING

PHYSIOLOGY
PATHOLOGY

Molecular Phenotype

- ***Proteomics***
- ***Metabonomics***
- ***Diagnostics***

- MicroNutrients
- Physical Activity
- Cognitive Activity

Diet and Lifestyle

ENVIRONMENT

Scope : Health Areas

Sebastiano Collino
Loïc Dayon

- **AGEING**

India Severin
Loïc Dayon
John Corthésy

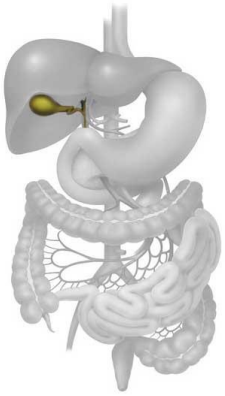
- **BRAIN HEALTH**

Longevity
Frailty
Muscle Health

Phenotyping
Cognitive Decline
Alzheimer's Biomarkers

Host-Microbe Metabolism
IBD

Juvenile Diabetes
DiOGenes
Circadian Biology



- **GASTRO-INTESTINAL HEALTH**

François-Pierre Martin

- **METABOLIC HEALTH**

François-Pierre Martin
John Corthésy
Loïc Dayon

Study Levels : Clinics → Analyses → Data

CLINICS

- Longevity
- Frailty
- IBD
- Diabetes
- Alzheimer's

ANALYSIS

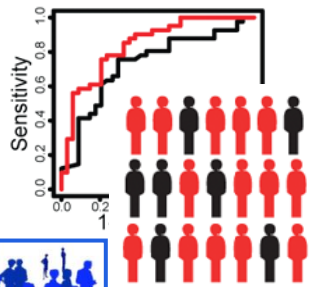
- Proteomics
- Metabonomics
- 1-Carbon Metabolism
- Diagnostics

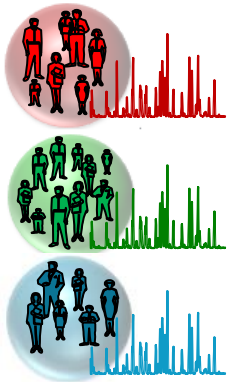
PROMETHEUS® Therapeutics & Diagnostics

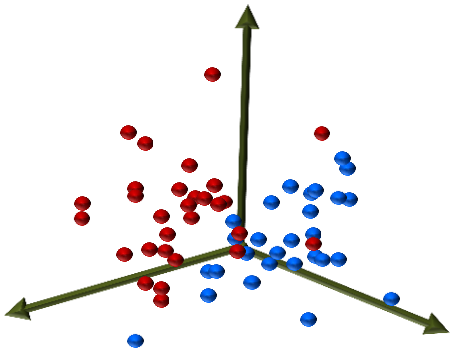
DATA

- Biomarkers
- Chemometrics
- Pathways
- Networks
- Omics Data Processing

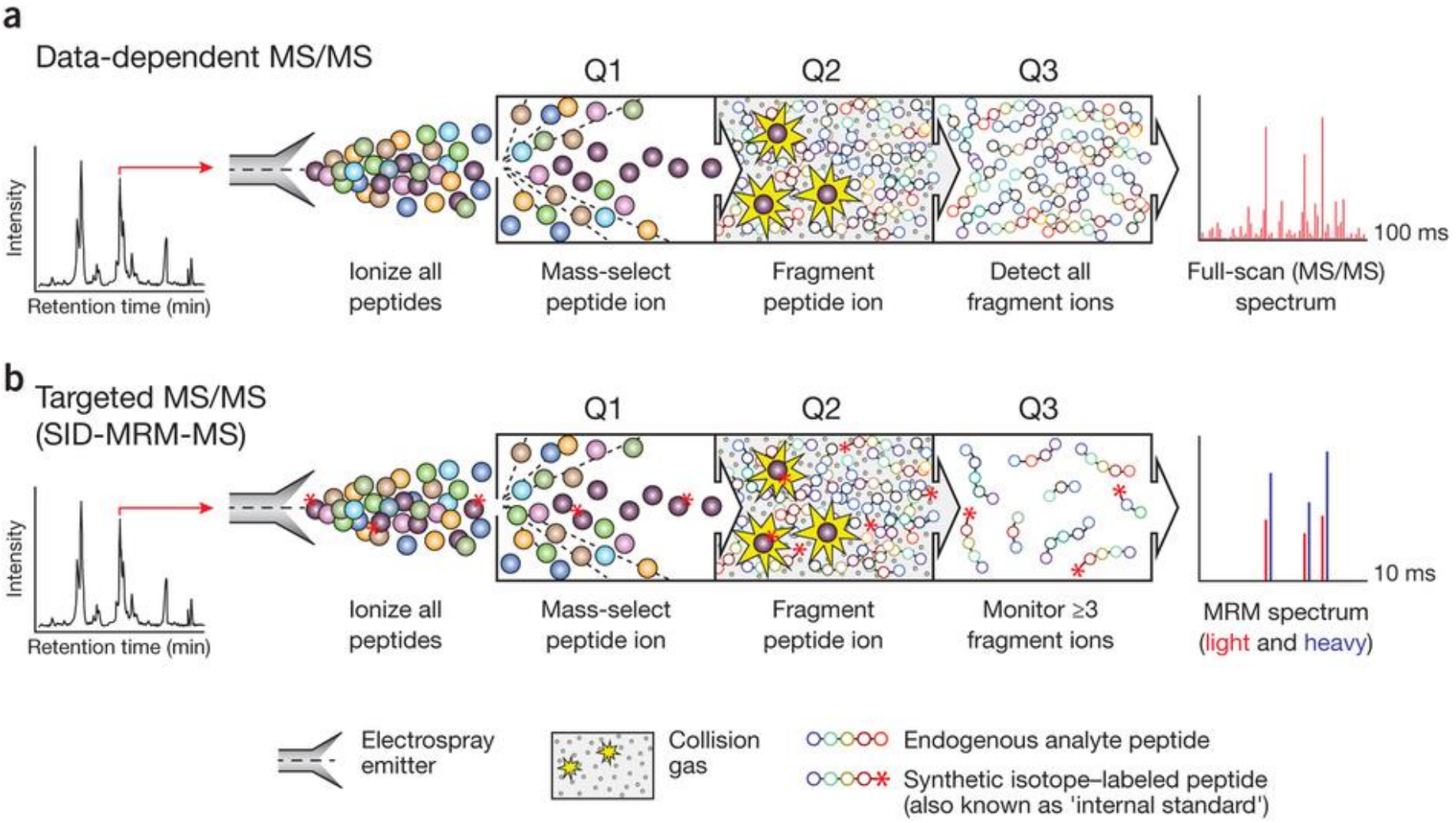
Ivan Montoliu
Ornella Cominetti





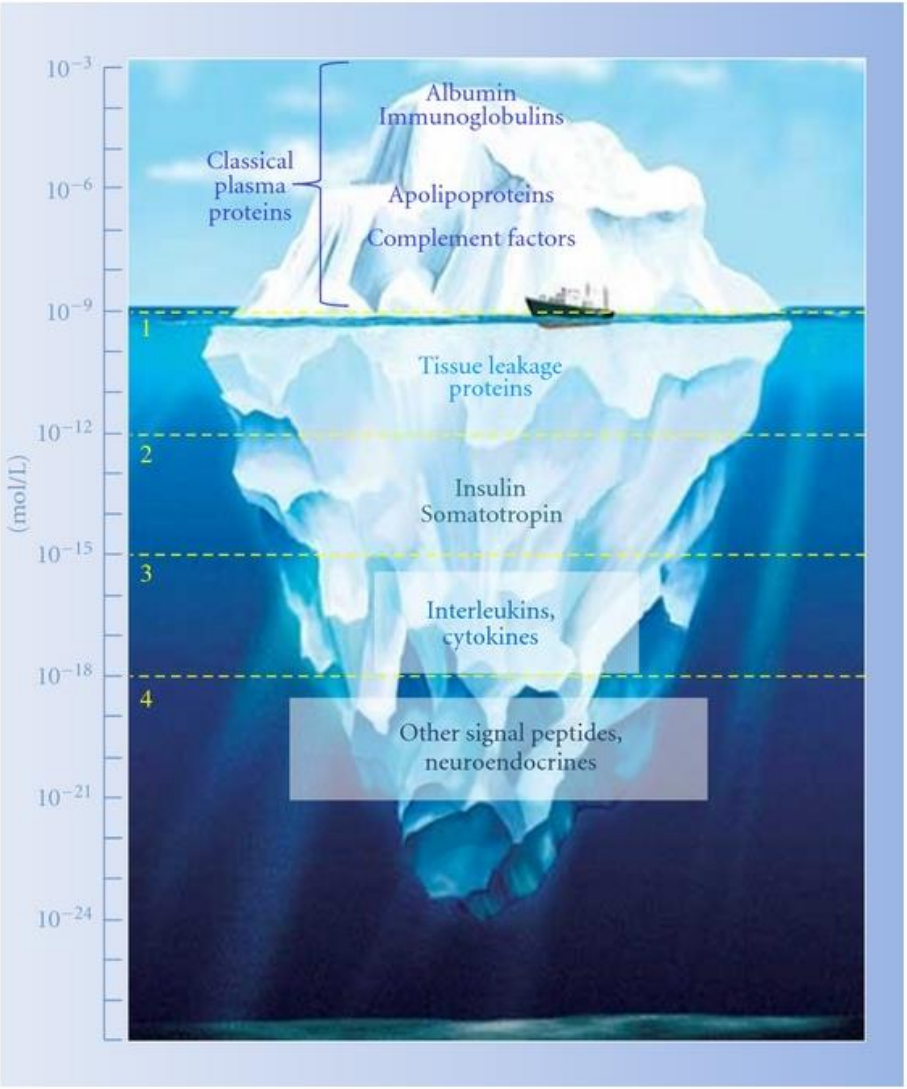


Proteomics: Discovery and Targeted/Validation



http://www.nature.com/doifinder/10.1038/nmeth.2309

Human Plasma Proteomics



Inez Finoulst, Martijn Pinkse, WilliamVan Dongen, Peter Verhaert, J. Biomed. Biotechnol. 2011;2011:245291

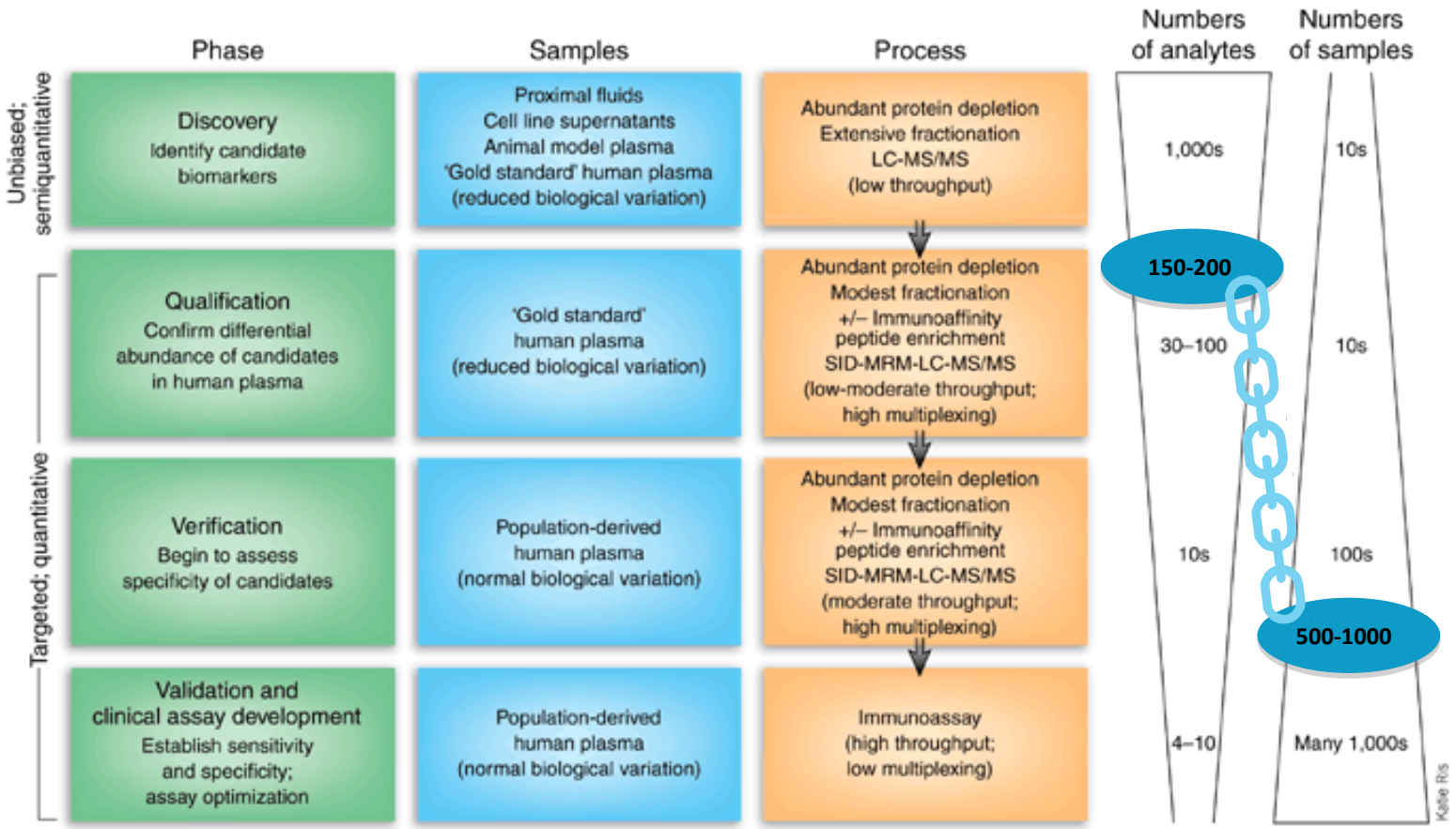
- Concentration range: 10-12 logs
- Abundant proteins
  represent > 99% of the total bulk
- Abundant tryptic peptides
  dominate MS analysis
- Many variants and modifications
  of few abundant proteins

"Sample preparation is key and has been neglected".
Bruno Domon, EuPA 2013
7th Annual Conf. Saint-Malo, France, Oct. 2013

"We cannot do HT without sample preparation automation but nothing is available on the market".
Roman Zubarev, EuPA 2013,
7th Annual Conf. Saint-Malo, France, Oct. 2013

Dayon, Nuñez Galindo, Corthésy, Cominetti…Kussmann. J. Proteome Res. 2014 & 2015

Human Plasma Proteomics



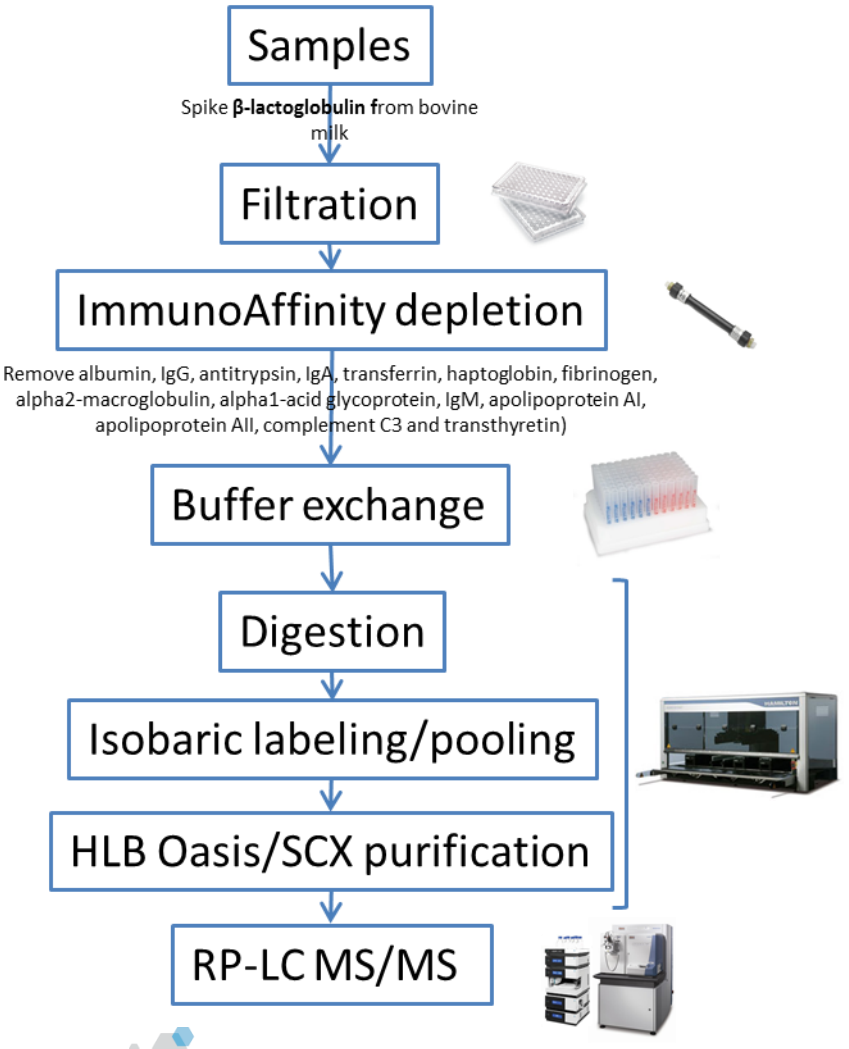
Nader Rifai, Michael A. Gillette, Steven A. Carr, Nature Biotechnology 24, 971 - 983 (2006)

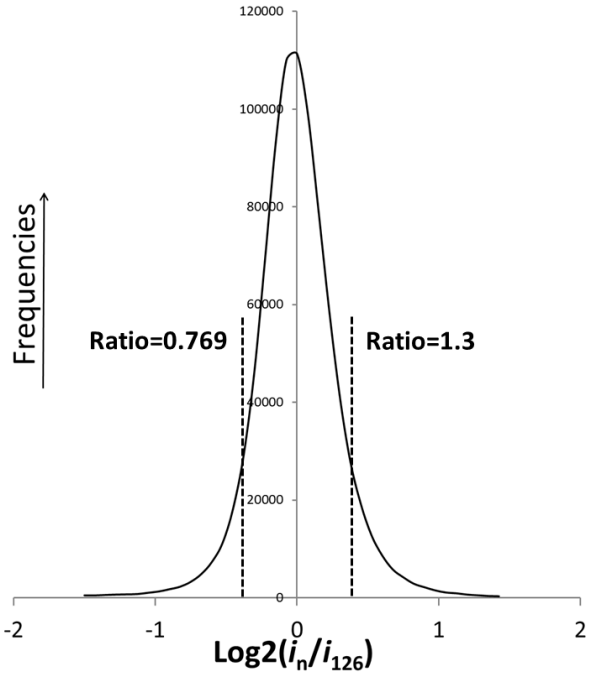
- Innovative biomarkers: discovery / verification / validation
- Small to higher number of samples for discovery

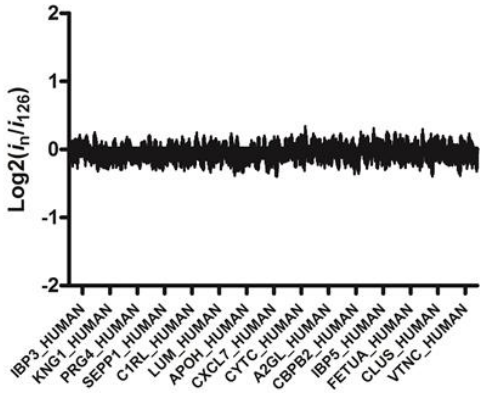
Dayon, Nuñez Galindo, Corthésy, Cominetti…Kussmann. J. Proteome Res. 2014 & 2015

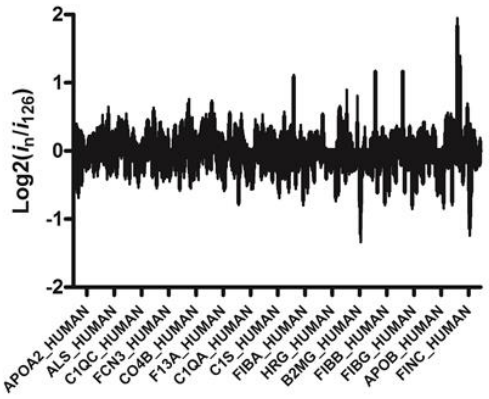
Human Plasma Proteomics Workflow

- 96 individual, identical samples on MTP-format









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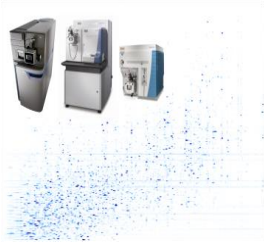
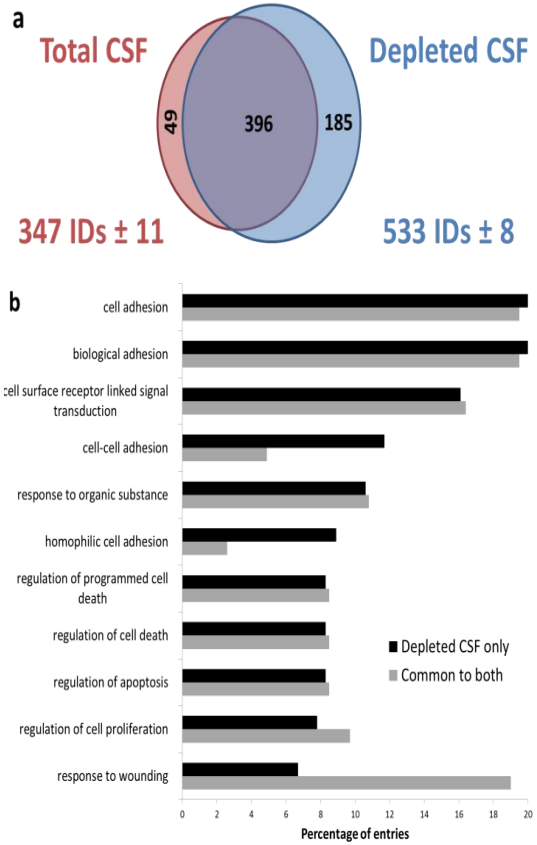
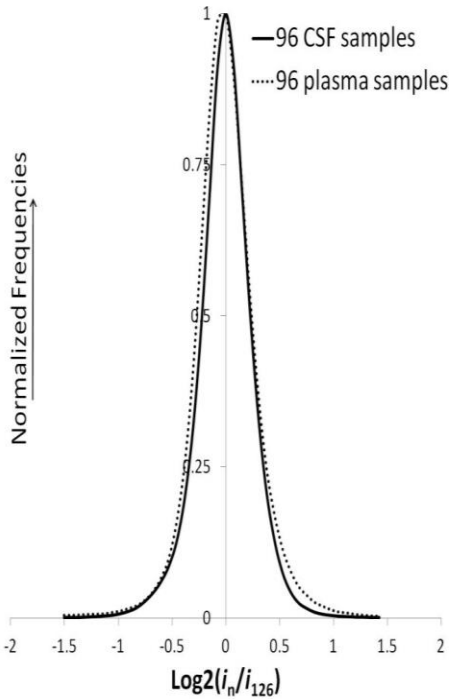
Dayon, Nuñez Galindo, Corthésy, Cominetti…Kussmann. J. Proteome Res. 2014 & 2015

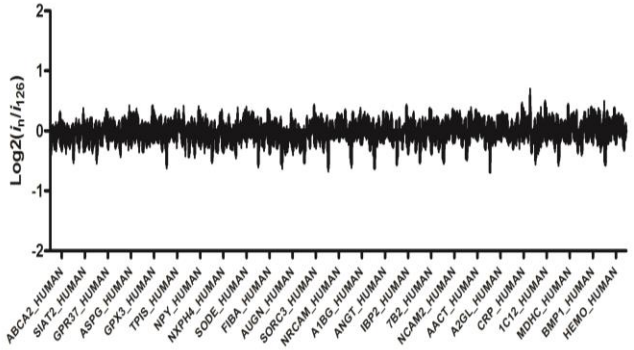
Human CSF Proteomics Workflow



- Automation of proteomics of human CSF
- Application to CHUV AD cohort







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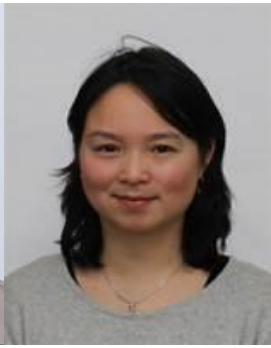
Nuñez Galindo, Kussmann, Dayon. Anal. Chem. submitted

NMR–/MS–based Metabonomics


Sebastiano Collino


Laeticia Da Silva


François-Pierre Martin


Seu-Ping Guiraud

NMR LC-NMR LC-NMR/MS

Rezzi/Collino/Martin/Kochhar/Nicholson et al. J. Proteome Res. 2007 + 2012. Anal. Chem. 2009, Trends Anal. Chem. 2013
Kussmann, Raymond, Affolter; J. Biotechnol. 2006, Curr. Opin. Biotechnol. 2008

Metabonomics: Clinical Screening and Discovery

Matrices: plasma, urine, saliva, synovial fluid, feces, cells , tissues…

Key features

High throughput:

5 min scan for urine, 15 min for plasma

Both solid and liquid state

Highly robust/reproducible:

CV: 0.02% across QC samples

0.04% across instruments

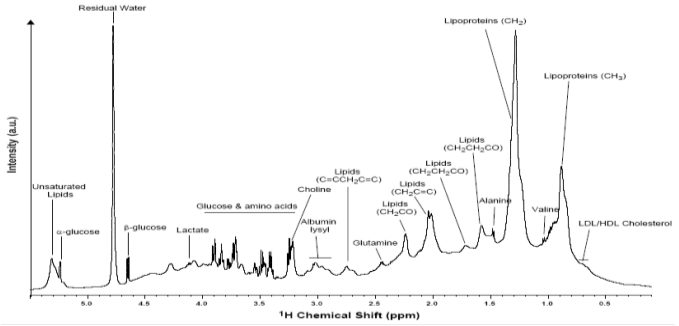
Provides information on:

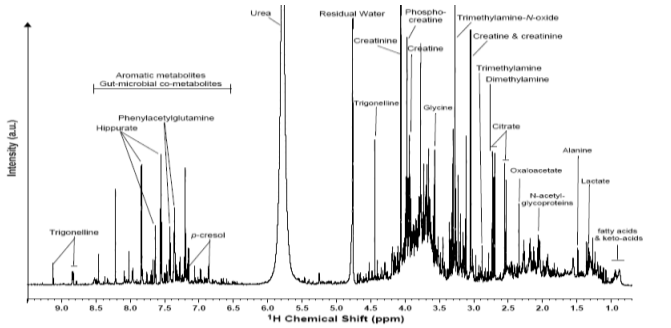
- Central E metabolism
- Amino acids
- Lipids (lipoproteins)
- Glucose
- Microbial metabolites

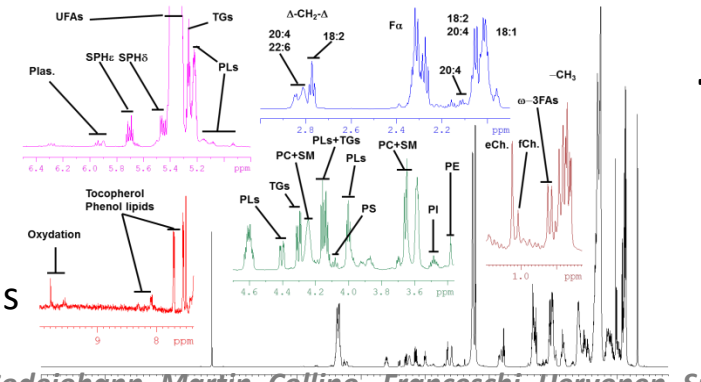
Provides guidance for subsequent targeted analyses

Metabolome coverage (*based on known metabolites*):

- **Urine: 209** (out of 605) compounds
- **Plasma: 50** compounds
- **CSF: 54** compounds







Da Silva, Godejohann, Martin, Collino…Franceschi, Hervonen, Spraul, Moco. Anal.Chem. 2013

Moco S, Colino S, Rezzi S, Martin FPJ. Ped. Res. 2013. Collino, Martin, Rezzi. J. Clin. Pharm. 2013

Rezzi, Collino, Goulet, Martin. Trends Anal. Chem. 2013

AGEING

Nestlē Institute of Health Sciences

What is ageing and how does it progress ?

Prenatal **Childhood** **Maturity**
Infancy **Puberty** **Reproduction** **Ageing**

Genes
Epigenetics
Maternal behaviours
Environmental factors
Family behaviours
Learned behaviours
Family environment
Biological factors
Genes/epigenetics
Fat mass / obesity
Family behaviours
Learned behaviours
Environmental factors

Environmental pressures

Biological ageing

Poor new adaptation

Previous maladaptation

Disease

Collino, Martin… Moco, Franceschi, Kussmann, Offord
Mech. Ageing Dev't 2013

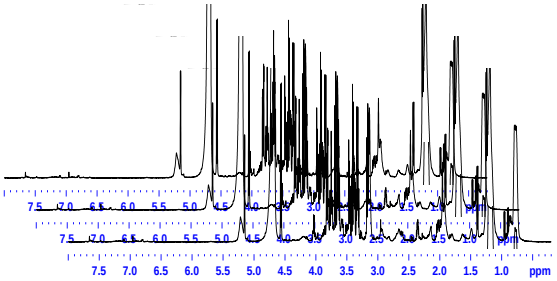
Ageing and Nutrition

- Role of nutritional deficiency in the development of frailty was suggested long ago. However, research in this area is only recent.
- 2%–16% of community-dwelling elderly are nutritionally deficient in protein and calories (Whitehead 1997). If mineral and vitamin deficiencies are included, malnutrition in persons older than 65 years may be 35% (Chandra 2002).
- Low energy and protein intake and low nutrients positively associate with frailty. Energy intake, serum Se, carotenoids and albumin levels are lower in frailty (Smit et al 2013). MMA: marker for available vitamin B12 (Mocchegiani 2010).
- Elderly become particularly vulnerable to compromised nutrient intakes.
  → Their diets should be nutrient-dense to ensure adequate intakes of e.g. n-3 fatty acids, protein, dietary fibre, B vitamins and other micronutrients such as iron, combined with low intakes of sodium.
- → Need better nutritional assessment tools for both successful diagnosis of malnutrition and development of appropriate and comprehensive treatment plans for frail (and others).

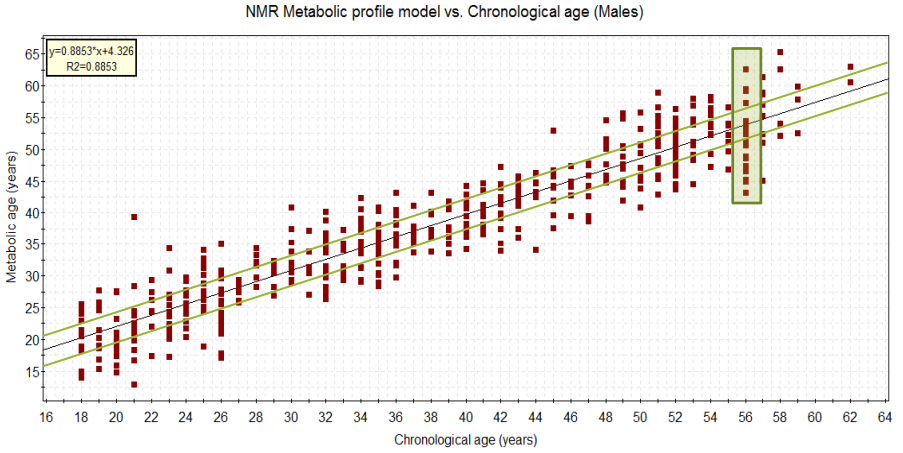
Metabolic phenotyping reveals differences between biological and chronological age

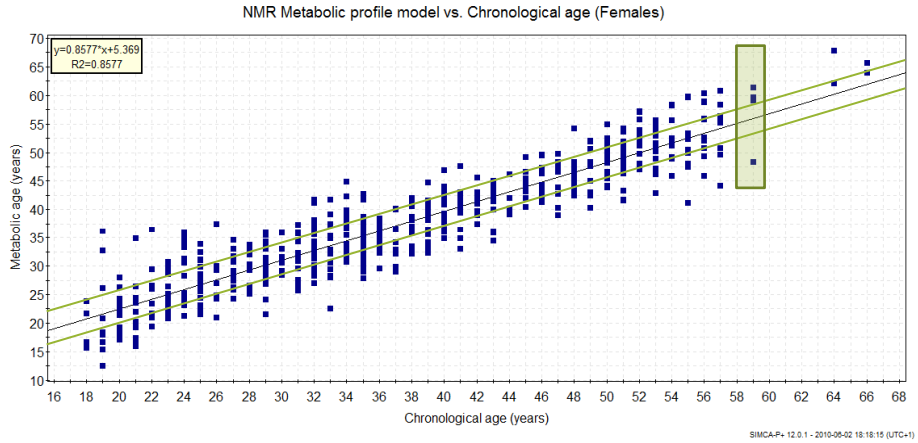
Plasma samples from n = 3'700 ageing males and females aged 18 to 67

NMR profiling



Statistical modeling (PLS)





The observed metabolic distributions suggest that chronological age differs from metabolic (biological) age

Collino, Martin… Moco, Franceschi, Kussmann, Offord
Mech. Ageing Dev't 2013

Longevity (centenarians) as a model of healthy ageing

Factor	Centenarians	Elderly	Young	Pvalue
BMI, *kg/m²*	24.2⁺3.8 (13.3-31.2)	26.9±4.6 (16.7-54.7)	22.1±2.0 (18.3.24.6)	a(***), b(***)
HOMA, *μU/mL*	1.77±1.1 (0.20-23)	2.81±2.57 (0.20-28.9)	n/a	a(***), n/a
Diabetes[1], *n*	1	25	n/a	n/a
Cholesterol, *mg/dl*	185.0±32.7 (112-264)	201.0±37.2 (5-335)	162.3±28.4 (133-207)	a(***), b(***)
Triglycerides, *mg/dl*	114.4±46.1 (60-283)	129.9±65.7 (44-530)	71.7±32.1 (28-143)	a(*), b(***)
HDL, *mg/dl*	48.2±13.1 (25-99)	55.2⁺20.4 (20-147)	51.8±8.7 (38-66)	a(**), b(**)
LDL, *mg/dl*	105.6±35.1 (75-165)	118.7±45.7 (23.8-199)	89.8±51.5 (49-144)	a(**), b(***)
CRP, mg/L	5.0±5.3 (0.28-28.2)	2.7±3.6 (0.11-25.7)	0.72±0.4 (0.28-2.08)	a(***), b(***)
A-SAA, *μg/ml*	437.6±483.7 (15.5-851)	149.1±204.6 (0.01-186)	n/a	a(***), n/a
IL-6, *pg/ml*	46.9±41.6 (7.5-225)	35.4±54.9 (0.28-28.2)	20.3±17.5 (2.70-28.2)	a, b(***)
IL-8, *pg/ml*	20.9±20.8 (6-71)	22.72±27 (2.3-100)	19.3±13.3 (4.4-46.6)	a ,b(*)
IL-10, *pg/ml*	3.93±4.3 (0.6-19.9)	6.07±15.4 (1.5-20)	2.38±2.58 (0.80-3.80)	a,b(***)
TNF-alpha, *pg/ml*	23.5±4.3 (0.40-113)	49.1±153.1 (0.1-80)	18.5±28.5(5.80-65.5)	a,b(***)
MMSE[2]	20.4±7.04 (1.3-30.3)	27.3±1.3 (1.3-31.0)	n/a	n/a

Our centenarians are lean, youthful-looking, energetic, independent, and have low rates of heart disease and diabetes

Centenarians appear to be capable of neutralizing/diminishing the deleterious effects of low-grade, chronic inflammation, characteristic of the aging process

Franceschi et al. 2007

Healthy ageing involves the **interaction** between **genes**, the **environment**, and **lifestyle** factors, particularly **diet**

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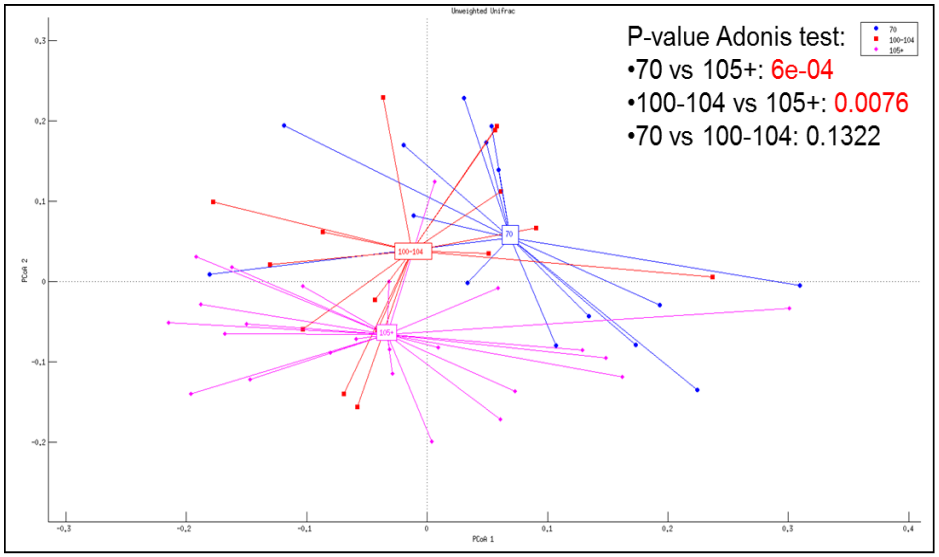
Rampelli S. et al, Aging 2013, Montoliu et al, Aging 2014, Collino et al. MAD 2014 Santoro et al. MAD 2014, Garagnani et al. Biomed research 2014, Cominetti et. al. 2014, AgroFoodTech

Human Longevity

Most comprehensive genotype, microbiome, and phenotype database

	Milano **	Bologna *	Calabria ***	TOTAL	Mean Age (± std)	Male (N)	Female (N)
105+	29	33	20	**82**	105.51 ± 1.72	18	64
Offspring	28	22	13	**63**	69.83 ± 7.24	22	25
Controls	17	16	14	**47**	71.56 ± 8.02	26	37
TOTAL	74	71	47	**192**			

NGS-based gut microbiota fingerprint



Unweighted unifrac, PCoA

Diversity analysis of gut microbiota composition resulted in significant segregation of 105+ from other groups

Whole Genome Sequencing

	Mean	Standard deviation
Number of sequenced fragments	1,783,333,790.43	57,589,952.91
% mapped reads	92.65	0.85
Percent coverage (%) > 1 X-fold	98.16	0.76
Percent coverage (%) > 10 X-fold	97.64	0.74
Average coverage depth	110.03	2.87
# SNPs	3,605,779	31,319.02

- **Developed and applied novel statistical genetic, bioinformatic, and data mining algorithms**

✓ **Genetic variants for pathogenesis, risk and protection**

✓ **Distribution of variants for single genes**

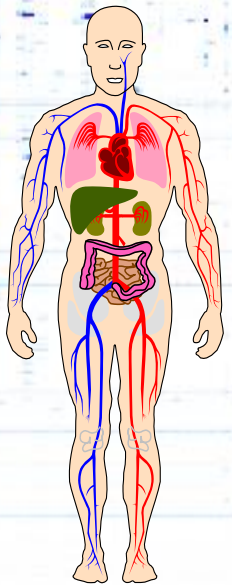
Nestlē Institute of Health Sciences

UNIVERSITÀ DEGLI STUDI DI VERONA

ALMA MATER STUDIORUM A.D. 1088 UNIVERSITÀ DI BOLOGNA

Sebastiano Collino, Laetitia Da Silva

METABOLIC HEALTH



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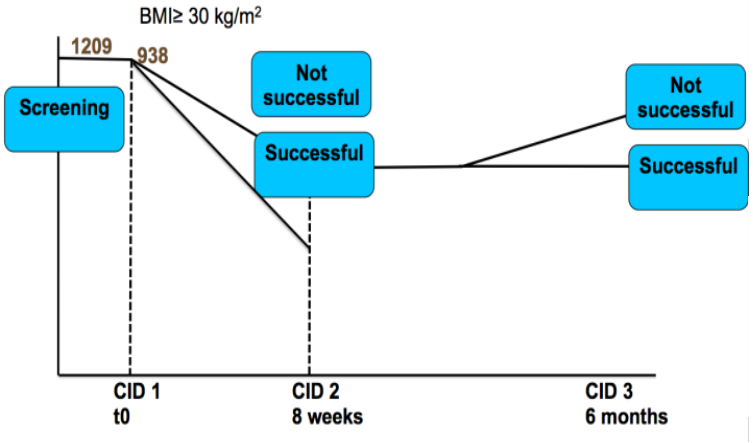
DiOGenes

- DiOGenes stands for Diet, Obesity, and Genes
- EU program incl. weight loss/maintenance in obese
- NIHS questions:
  Can we predict success in weight loss/maintenance at baseline ?
  Who needs which diet to succeed ?



1. Base line
2. Weight loss period (800 kcal/day)
3. Weight maintenance

≈ 1'000 subjects
> 4'000 samples
> 7'000 variables (clinical, food diaries, behavior, molecular)

Quantitative Multivariate Analysis

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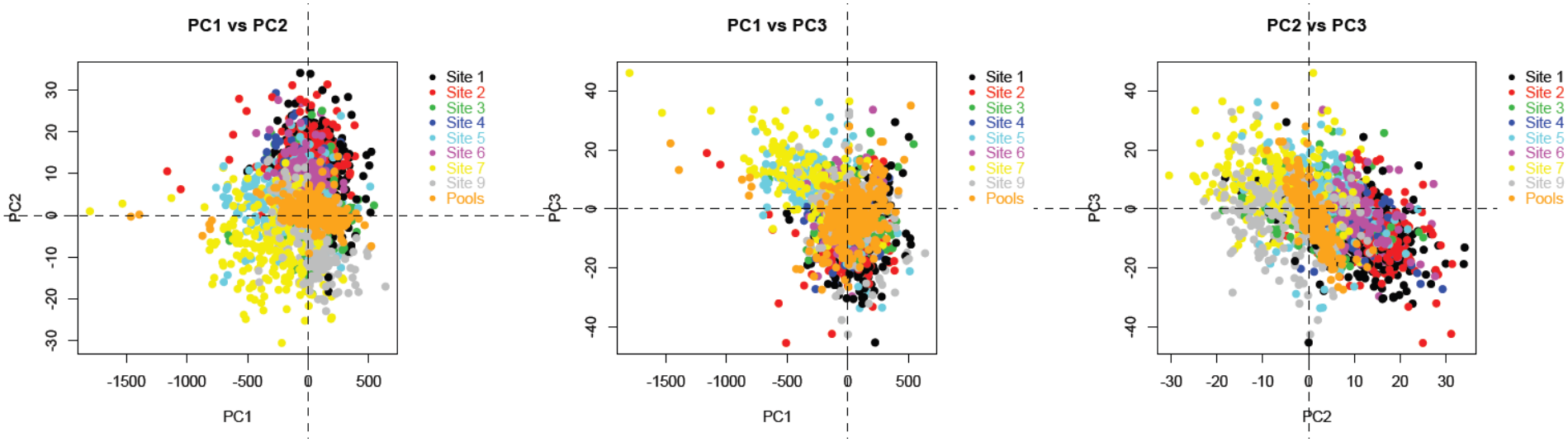
www.diogenes-eu.org

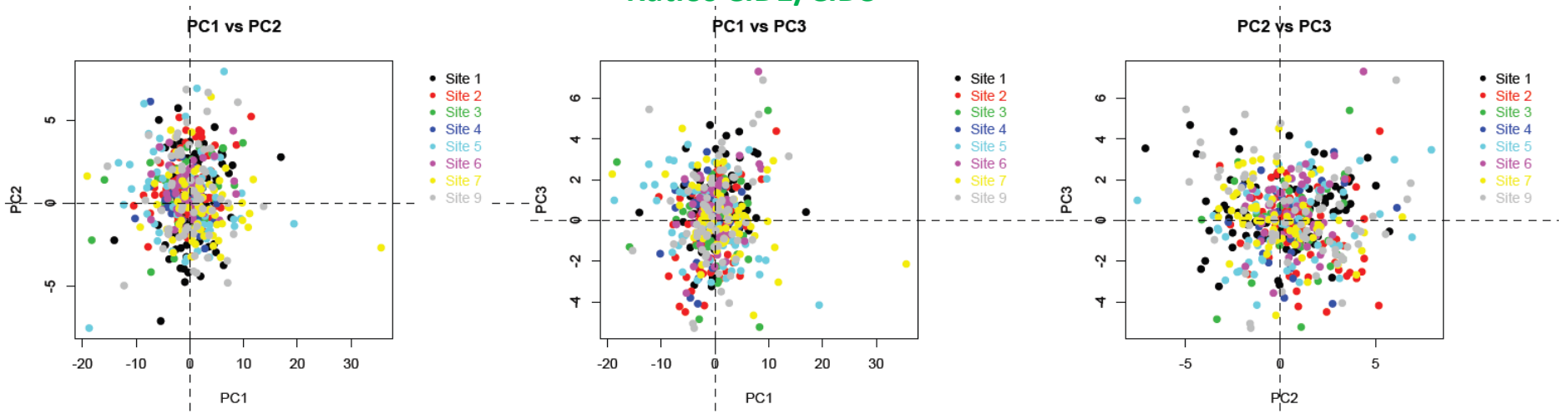
Larsen *et al.*, *N. Engl. J. Med.*, 2010, *363*, 2102–2113

Proteomics and clinical center effect

Individual samples

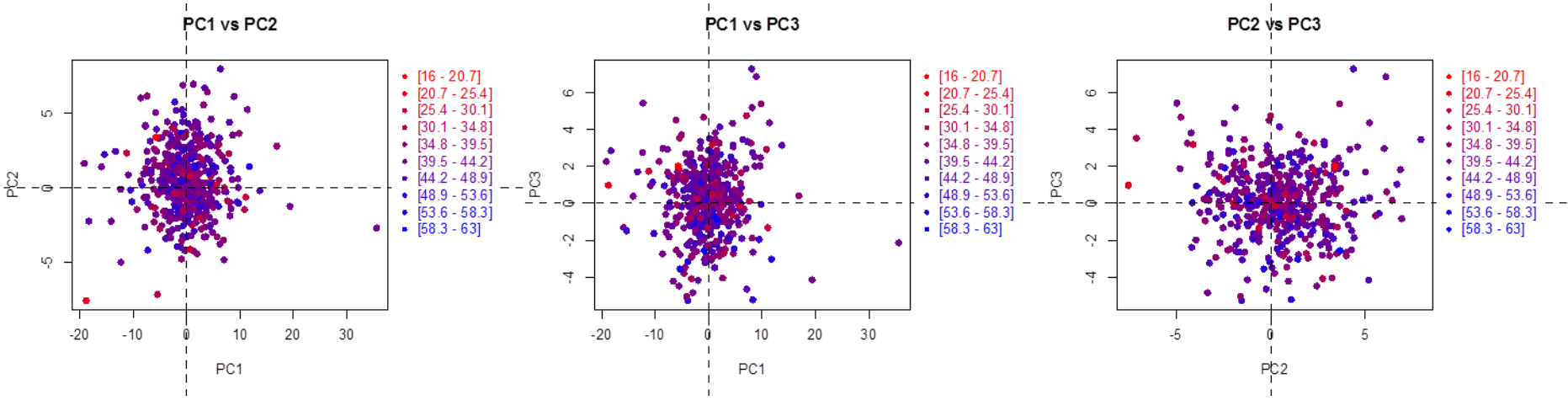


Ratios CID1/CID3

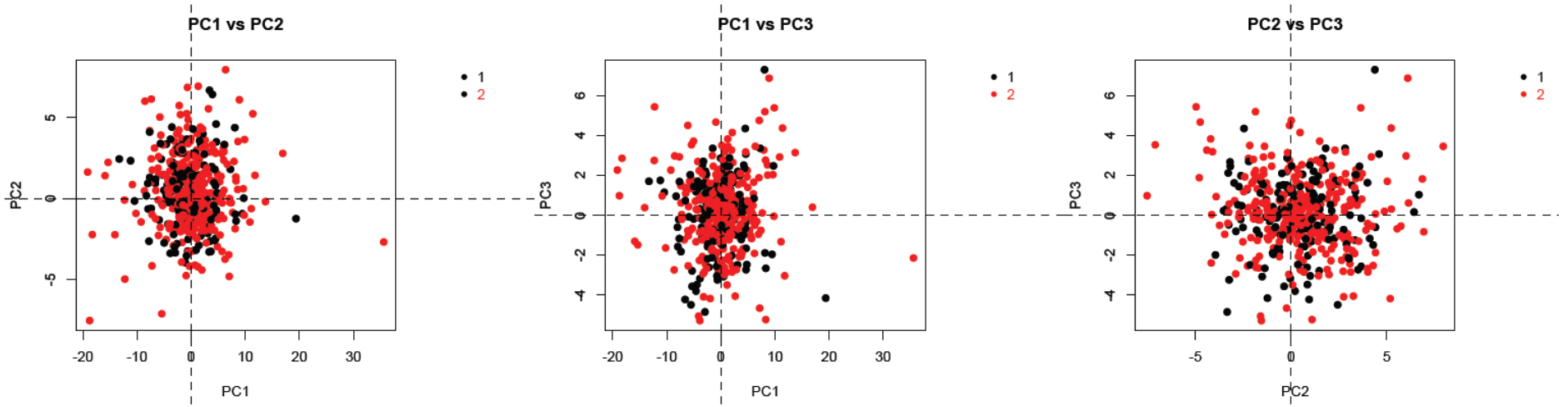


Proteomics and clinical variables

Age

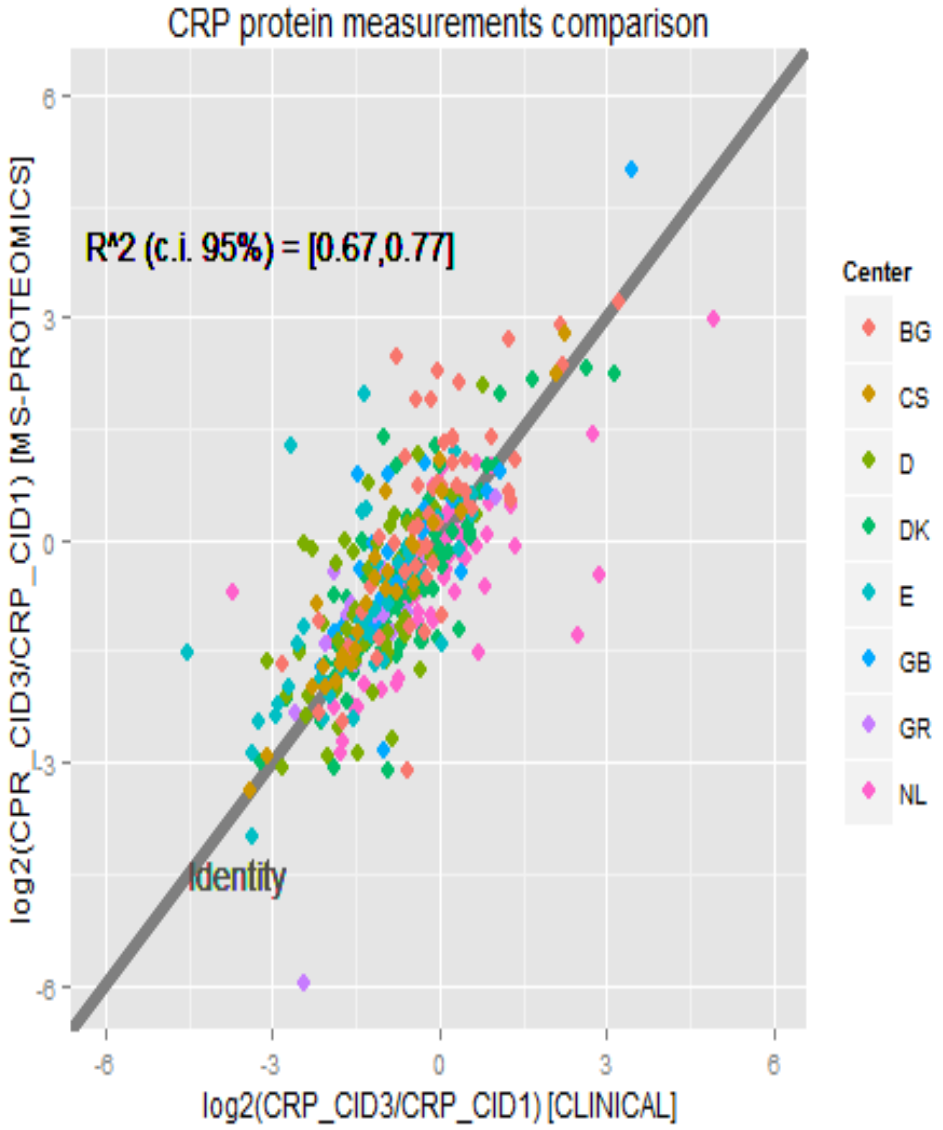


Gender



Validation: C-Reactive Protein

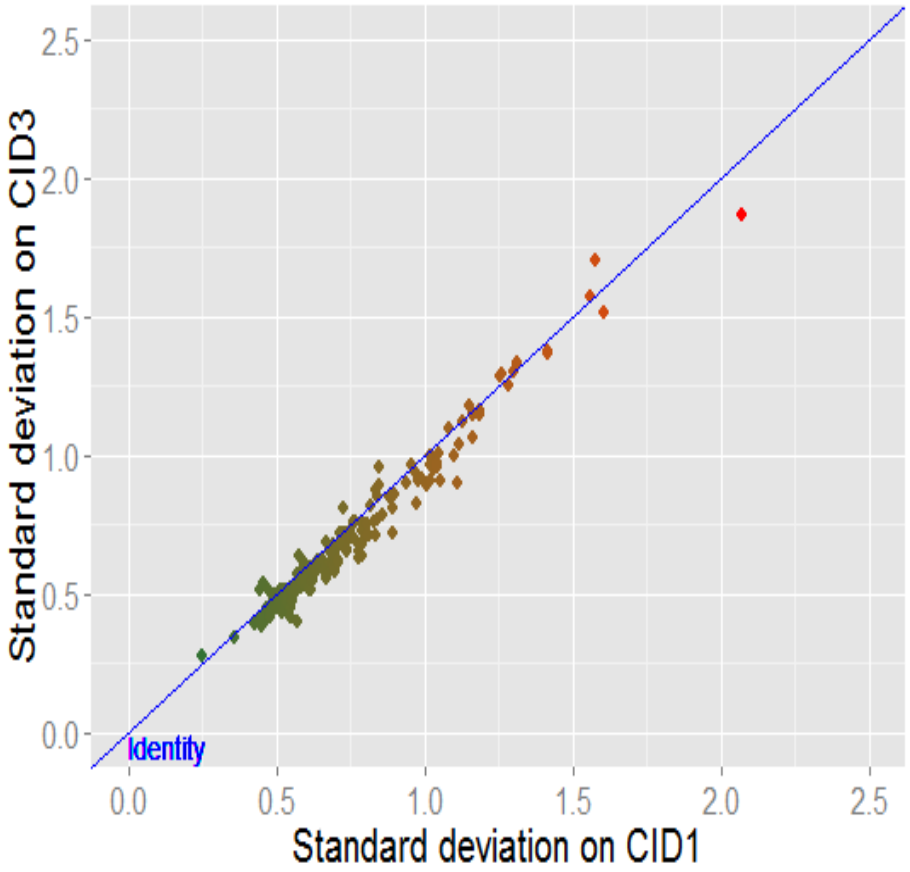
- Measured in clinics
- Measured by MS-proteomics
- Comparison of: $log_2\left(\frac{CRP@CID3}{CRP@CID1}\right)$
- Good correlation between techniques



Variability of the proteins

- Protein variability is consistent across the time points.
- Technical variability is smaller than biological variability.
- Low-variability proteins can be used for normalization
- Complement C1r shows potential to be used as an internal protein standard in human plasma

Protein variability in CID3 and CID1

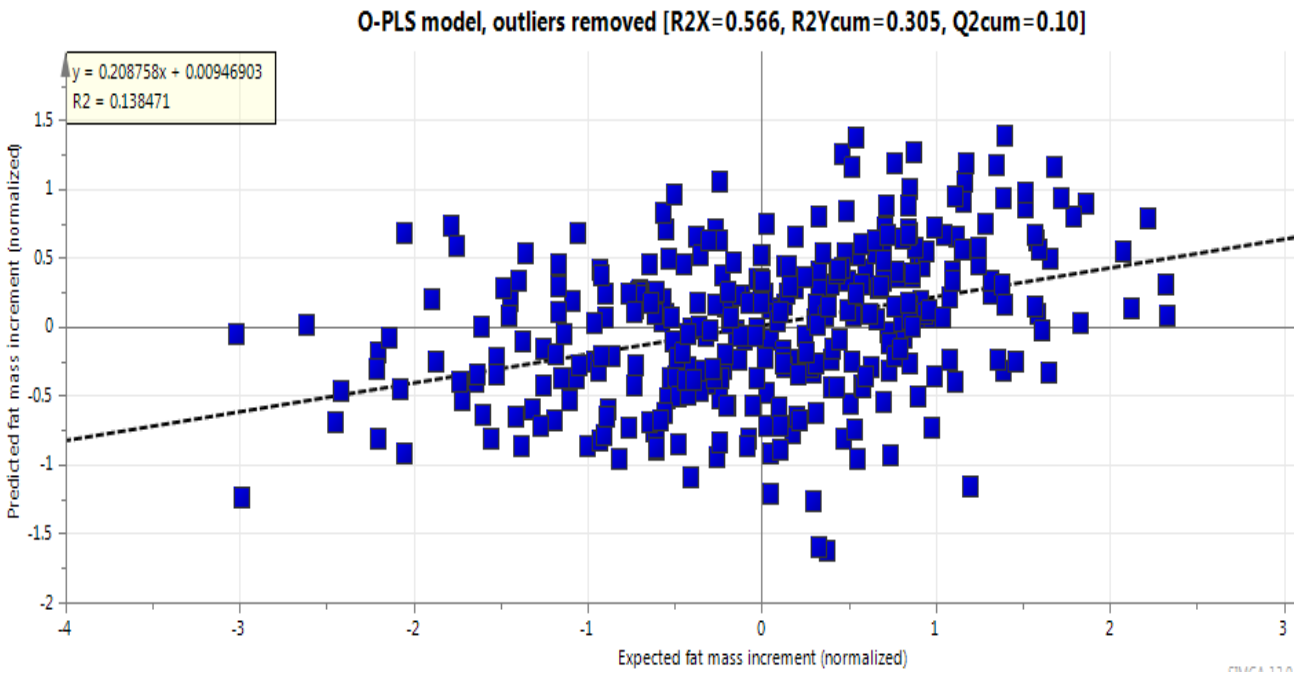


Differential expression and fat mass increment relation

- 46/183 proteins were significantly differentially expressed between timepoints
- Welch t-test on the fold change
- Multiple testing correction

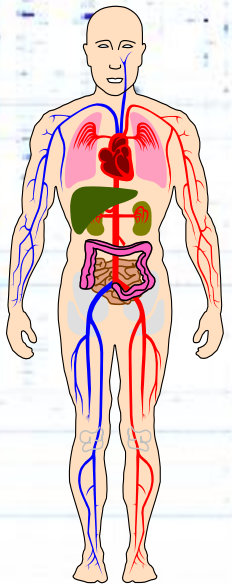
$$\text{Fold Change} = \log_2\left(\frac{protein\ expression\ CID3}{protein\ expression\ CID1}\right)$$

- Fat mass change relation to proteins
  - Multiple proteins involved
  - Large biological variability



Nestlē Institute of Health Science

PAEDIATRIC METABOLIC HEALTH



Nestlē Institute of Health Sciences

Juvenile Diabetes

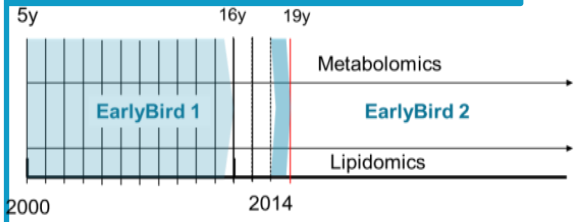
Background

- How do diet, lifestyle and environment interact with genes and metabolism during childhood and adolescence ?
- How does that determine health in child- and adulthood ?

Earlybird Cohort:

- Longitudinal cohort study
- 300 children and their parents
- Measurement every 6 months/12 months from 5-19 years

Science



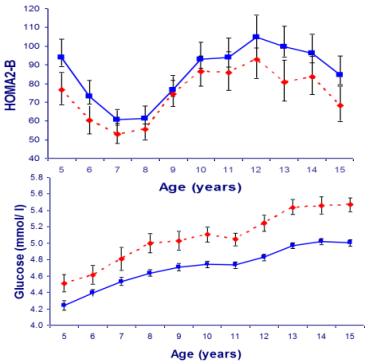
Describe:

- Normal childhood and puberty
- Origins of childhood weight gain and IR
- Impact on long-term risks of diabetes and cardiovascular disease.

Unique longitudinal database across childhood:

- Body composition
- Dietary intake
- Clinical and metabolic status
- Energy expenditure

Deliverables



- Establish relationships between genes, metabolism and childhood glucose metabolism
- Findings in mothers of IFG children suggest that β cell defect may be transmissible (gestational and/or genetics)
- Metabolic, anthropometric and nutritional characterization across childhood

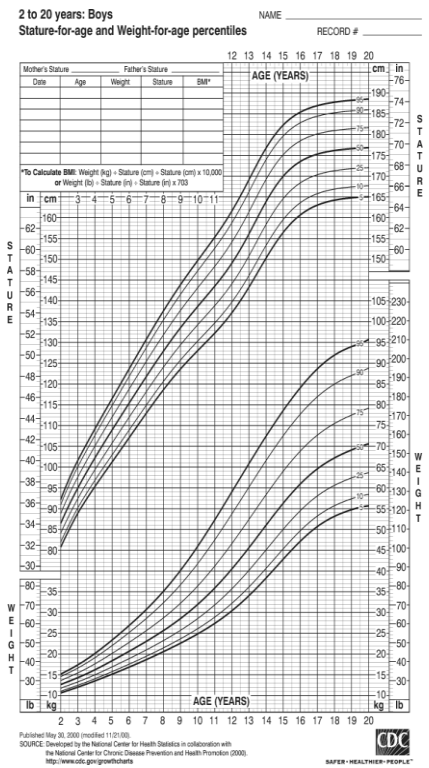
François-Pierre Martin

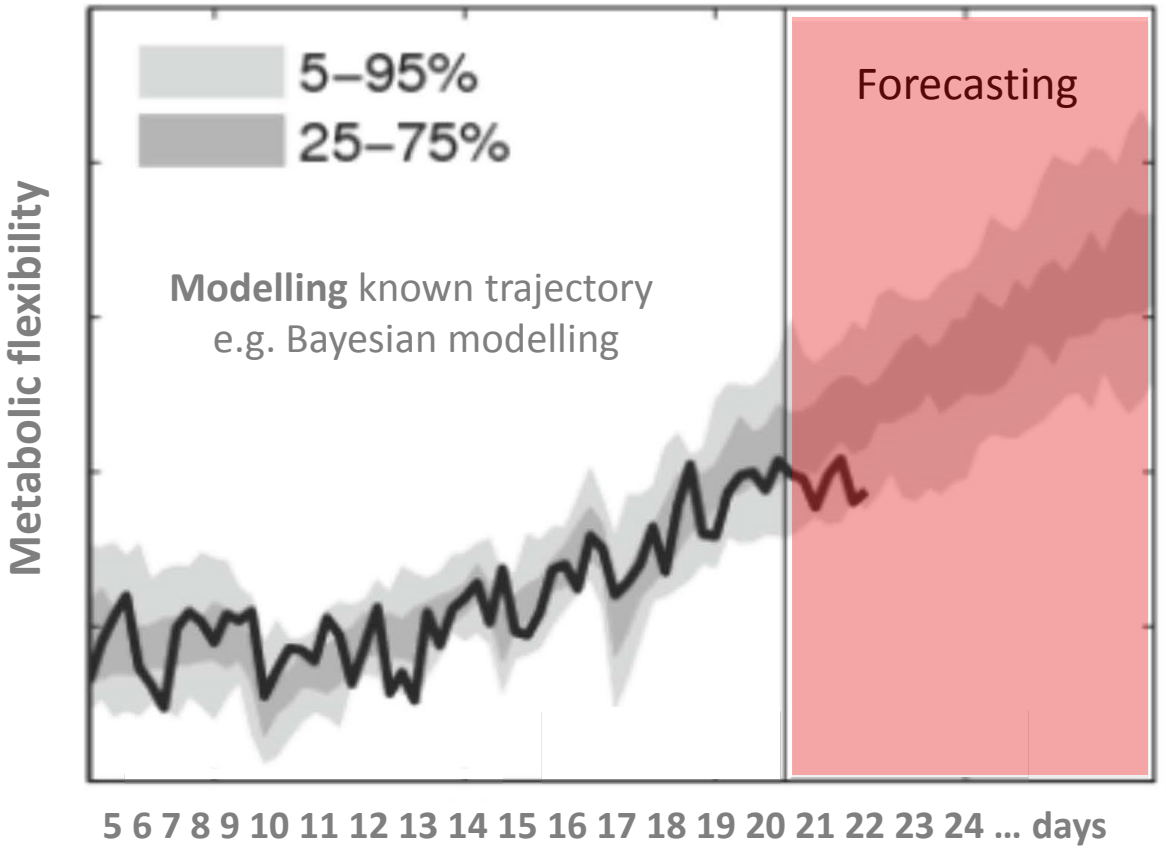
EarlyBird DIABETES Insights

PENINSULA MEDICAL SCHOOL
UNIVERSITIES OF EXETER & PLYMOUTH

Forecasting metabolic trajectories, disease risk and childhood physiology

Growth curve reference





Cominetti, Martin. Agro FOOD Industry 2014

Nestlē Institute of Health Sciences *Sperisen, Cominetti, Martin. Frontiers Mol. Biosci. 2015*

KEYSTONE SYMPOSIA
on Molecular and Cellular Biology

Human Nutrition, Environment and Health (T1)

October 14-18, 2015 China World Hotel Beijing, China

Scientific Organizers: Martin Kussmann, Hannelore Daniel and Jacqueline Pontes Monteiro

Supported by the Bill & Melinda Gates Foundation

Global Health Travel Award Deadline: May 12, 2015 / Abstract & Scholarship Deadline: June 16, 2015 / Abstract Deadline: July 14, 2015 / Discounted Registration Deadline: August 13, 2015

Vijayalakshmi Varma, National Center for Toxicological Research, FDA, USA
Adipogenesis and Adipocyte Response to Fructose: A Characterization Using Systems Biology Approach

Aldons J. Lusis, University of California, Los Angeles, USA
Animal Models for Complex Human Traits

Patrick J. Stover, Cornell University, USA
One-Carbon Metabolism

Short Talk Chosen from Abstracts

Poster Session 1

FRIDAY, OCTOBER 16

Human Nutritional and Lifestyle Interventions

Hannelore Daniel, Technische Universität München, Germany
Characterizing Normal Human Metabolism

Ben van Ommen, TNO, Netherlands
Diet, Systems Flexibility and My Optimal Health

Jacqueline Pontes Monteiro, Universidade de São Paulo, Brazil
The Genomics of Micronutrient Requirements

Arne Vernon Astrup, University of Copenhagen, Denmark
Human Dietary Interventions to Probe Metabolic Health

Short Talk(s) Chosen from Abstracts

Capturing and Monitoring Human Individuality

Speaker to be Announced

Michael Snyder†, Stanford University School of Medicine, USA
Longitudinal Omics in Humans

Richard Weiss, Viocare, Inc, USA
Self-Monitoring of Diet and Lifestyle

[illegible] and the Gut Brain Axis

Juan B. Ochoa†, Nestlé HealthCare Nutrition USA, USA
Personalized Clinical Nutrition for the Critically Ill

Peter Gluckman†, University of Auckland, New Zealand
Perinatal Nutrition and Maternal Health

Short Talk Chosen from Abstracts

Poster Session 3

SUNDAY, OCTOBER 18

Global Nutrition and Sustainability

Jim Kaput, Nestlé Institute of Health Sciences, Switzerland
Genetic, Cultural and Environmental Variability and Nutrient Needs

Zulfiqar A. Bhutta, Hospital for Sick Children, Canada
Maternal and Child Nutrition: Global Challenges and Solutions

Ted Bianco†, Wellcome Trust, UK
Sustainable Nutrition

Shawn Baker†, Bill & Melinda Gates Foundation, USA
Global Nutrition

Short Talk Chosen from Abstracts

Meeting Wrap-Up: Outcomes and Future Directions (Organizers)

Joint Session with Grand Challenges and Keystone Symposia

MONDAY, OCTOBER 19

Departure

华大基因 BGI

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