Metabolite Profiling Unveils Nutrition Functionality

Innovative health-promoting food
International Workshop
French Embassy Berlin
September 29th, 2011

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Lead Scientific Affairs
Metanomics Health GmbH, Berlin
a BASF Group Company

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Paradigm Change in Biology by the Year 2000:

By 2000 - based on the publication of the human genome and the first plant genome in Nature - it became obvious, that gene function cannot be determined through gene sequence alone

And: genes alone would not be patentable!

By then metanomics GmbH, a BASF Plant Science joint venture, was already in the midst of its Core Project and ahead of competition in the determination of gene function in plants

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new dimensions in discovery

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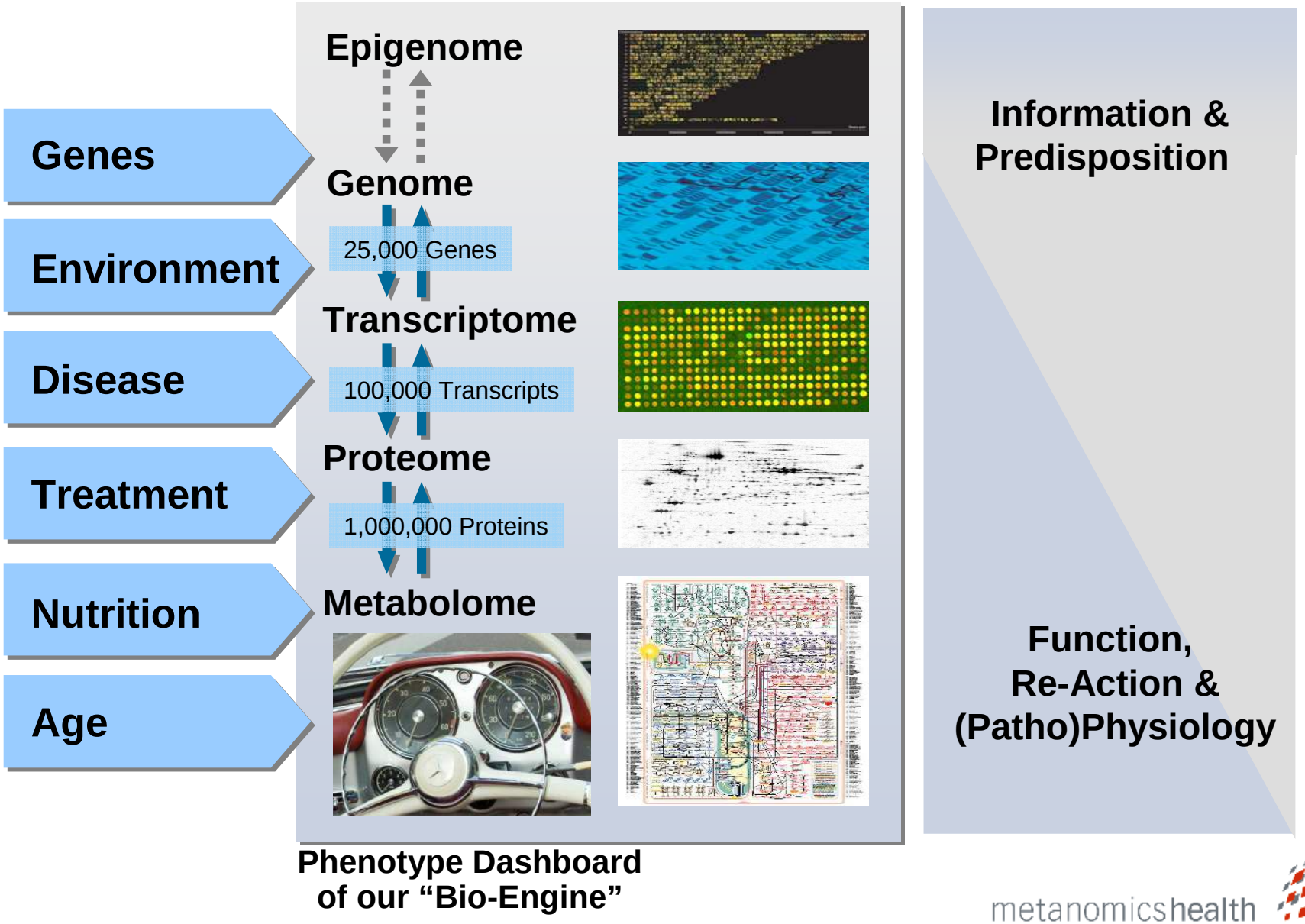
The Metabolome – Highly Responsive and Revealing



Phenotype Dashboard of our "Bio-Engine"

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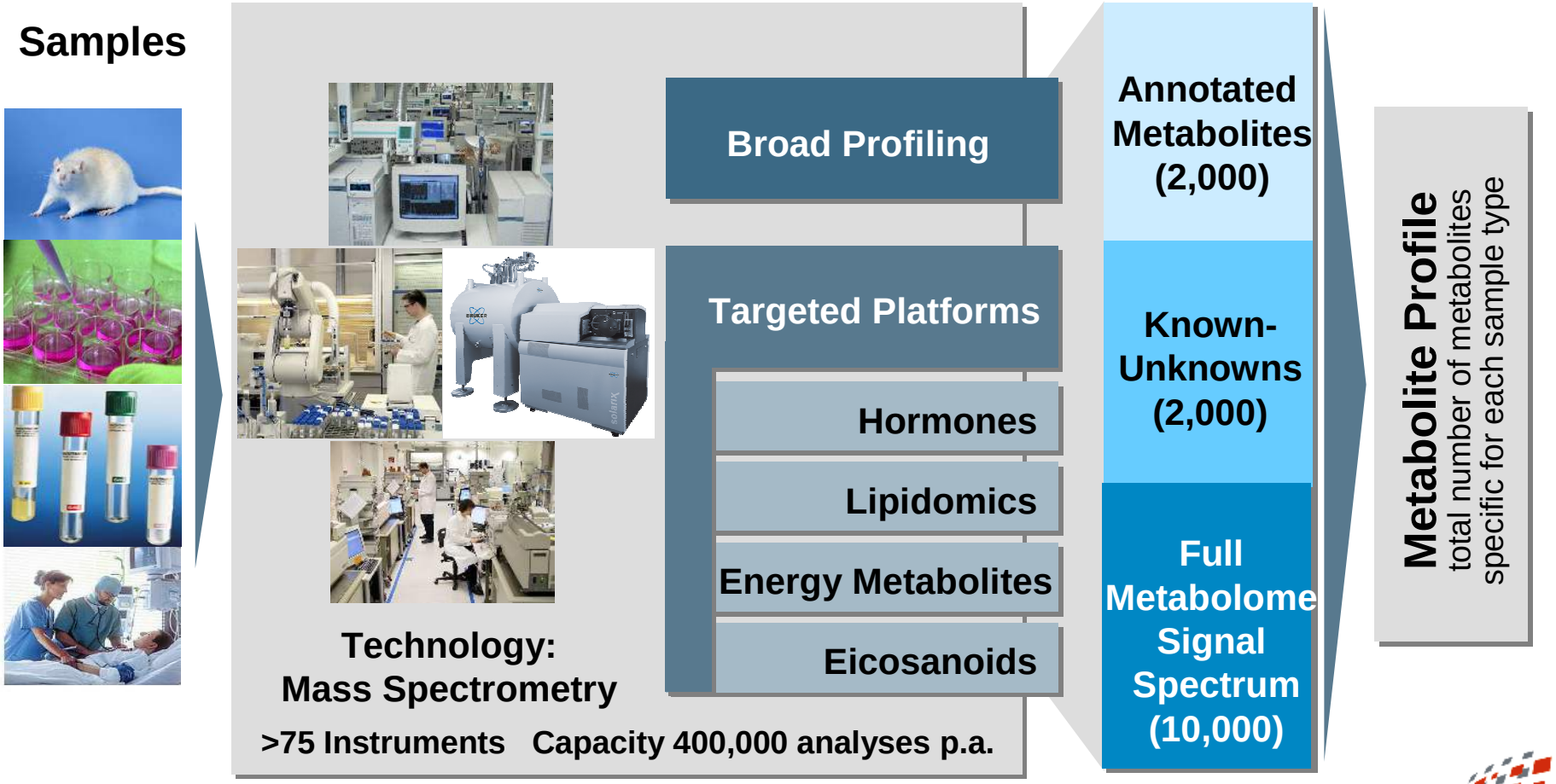
Metabolite Profiling Platforms

Comprehensive Metabolite Profiling - small molecules (<1500 Daltons)



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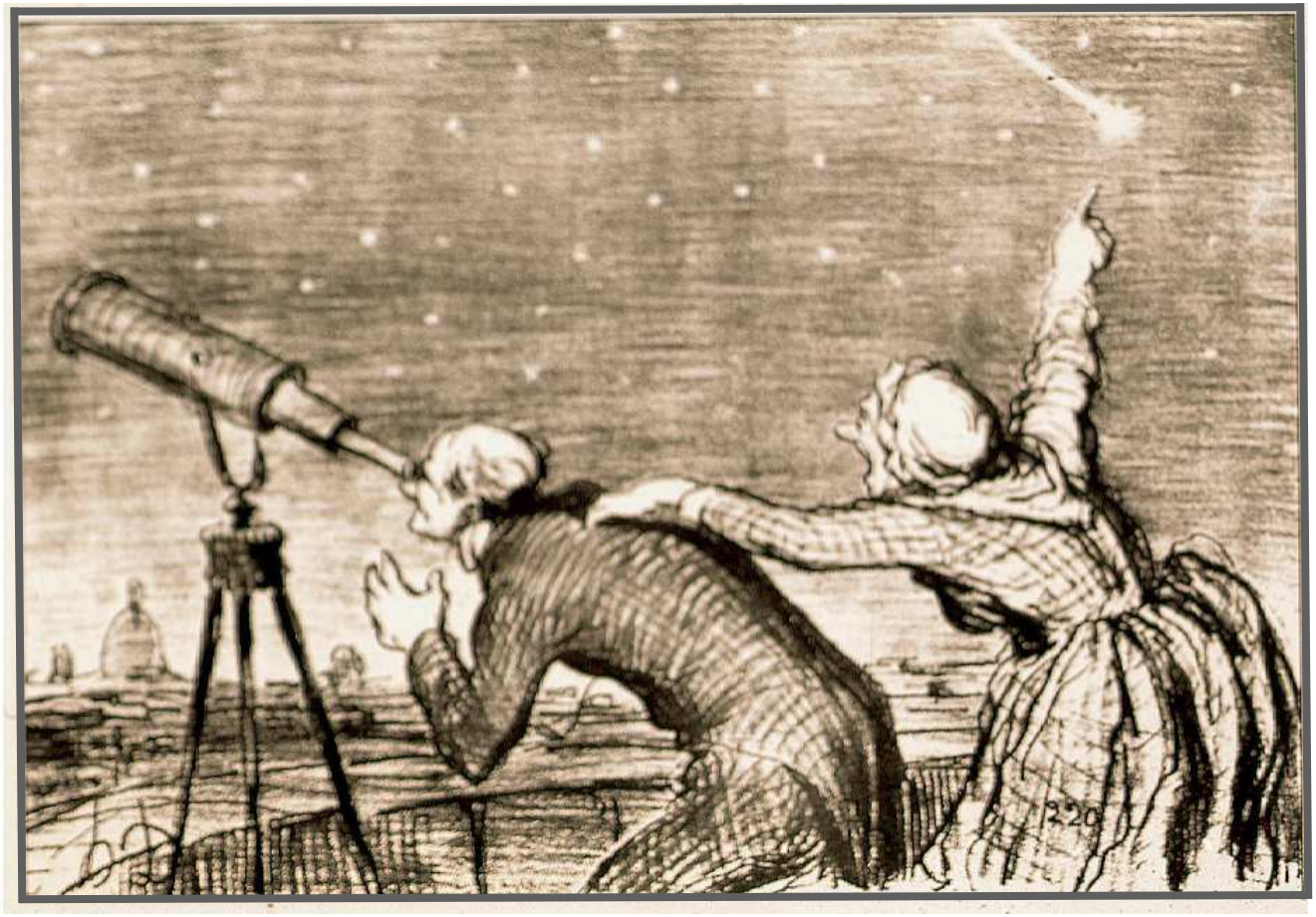
Targeted versus Non-targeted Profiling



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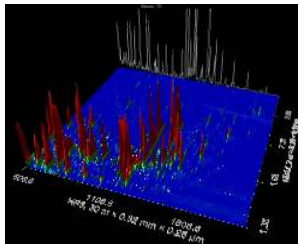
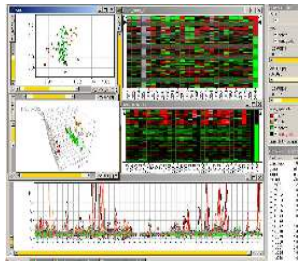
Method - Sample and Data Analysis

- Metabolite Profiling of samples via GC/LC - MS
- Correlate single or multiple metabolites to “golden standard”
- Prospective studies: Estimate performance for early detection
- Retrospective studies: Review changes prior to diagnosis



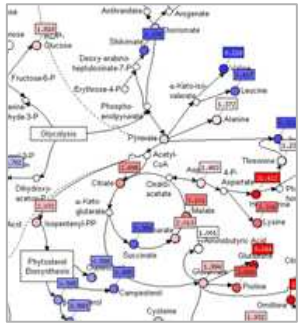
Statistical analysis

- ANOVA: each metabolite on its own concerning disease and confounders
- Multivariate classification
  (linear methods, decision trees; many metabolites)
- Feature selection methods (few metabolites)



Biomedical interpretation

- Review literature and compare findings to state-of-the-art
- Hierarchical network analysis
- Biochemical pathway interpretation



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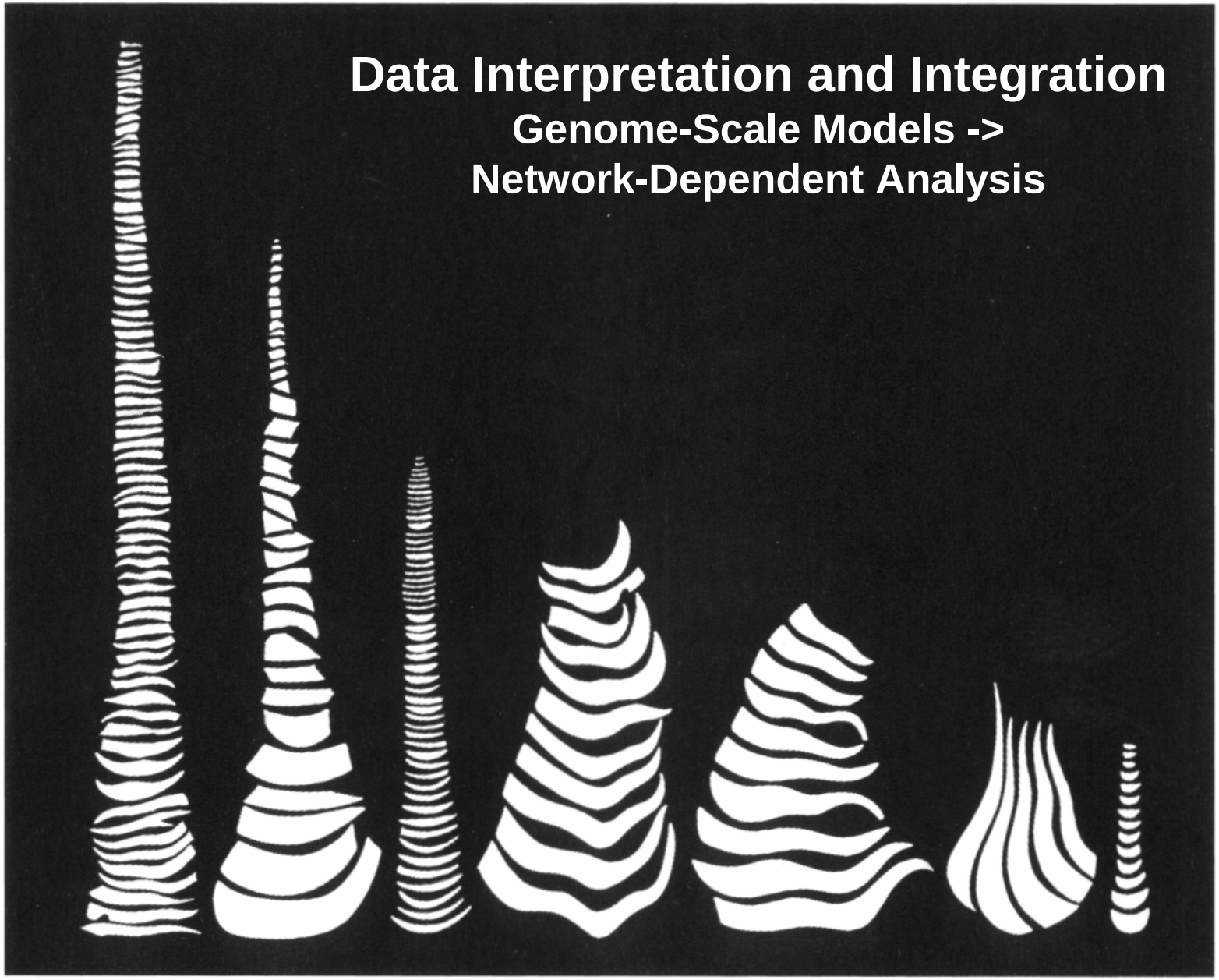
Data Interpretation and Integration

Genome-Scale Models ->
Network-Dependent Analysis



Ursus Wehrli, „Kunst Auf(zu)räumen“

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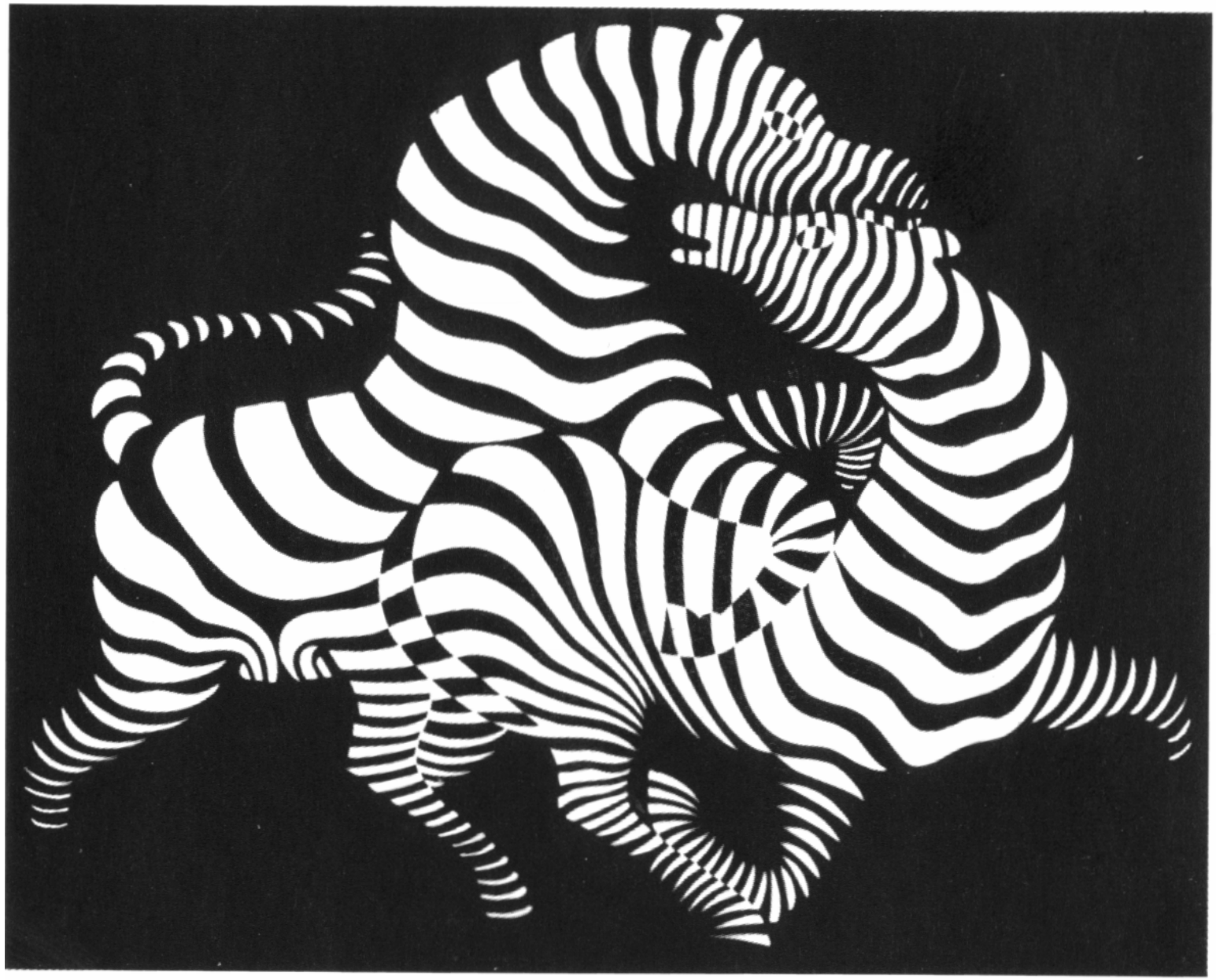

Ursus Wehrli, „Kunst Auf(zu)räumen“ (Vaserely, Zebrès)

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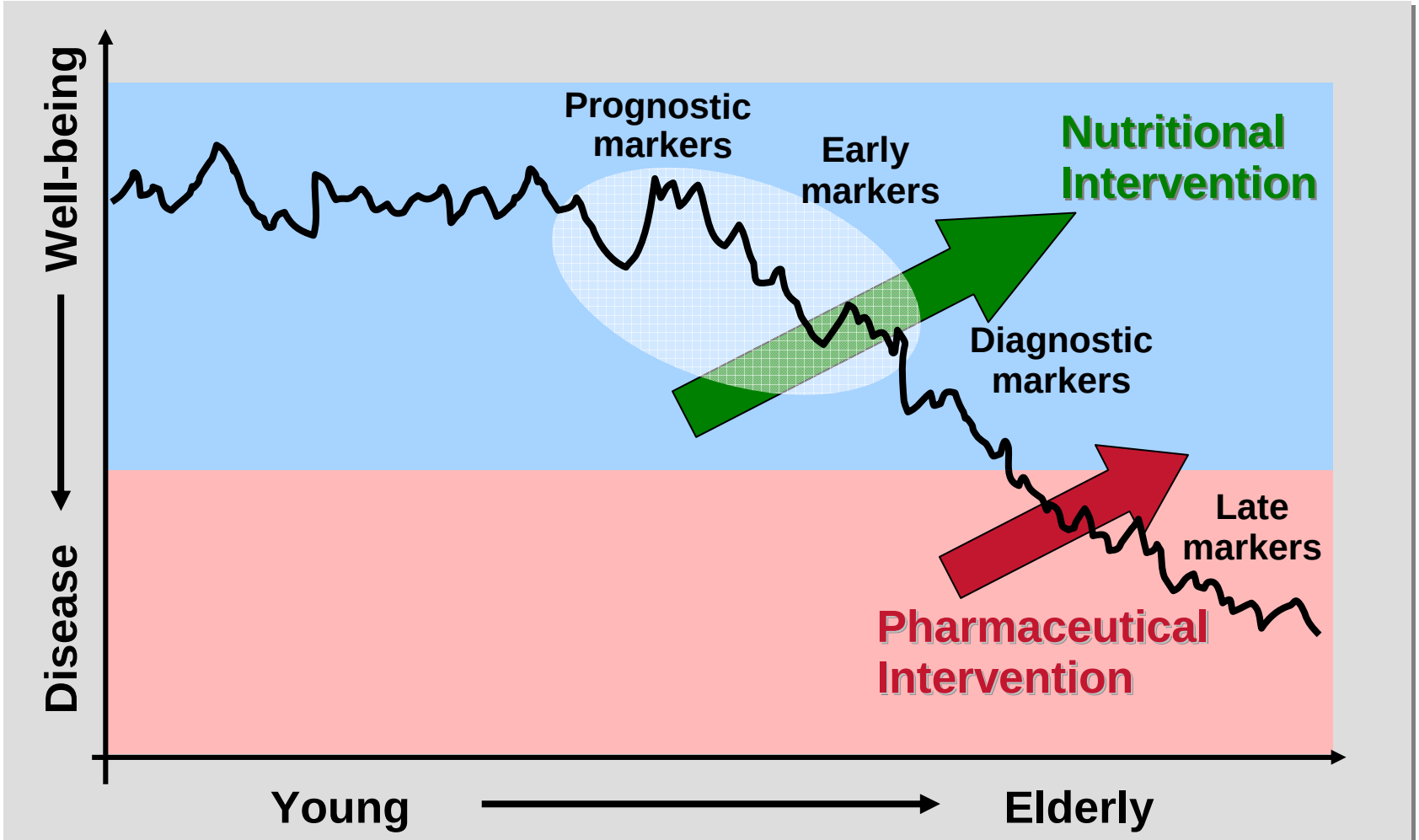
Metabolic Balance Throughout Life



There is no more clear separation between pharma and nutrition
Earlier diagnosis is increasingly important to prevent progression of/to disease
Importance of nutritional intervention is rapidly increasing

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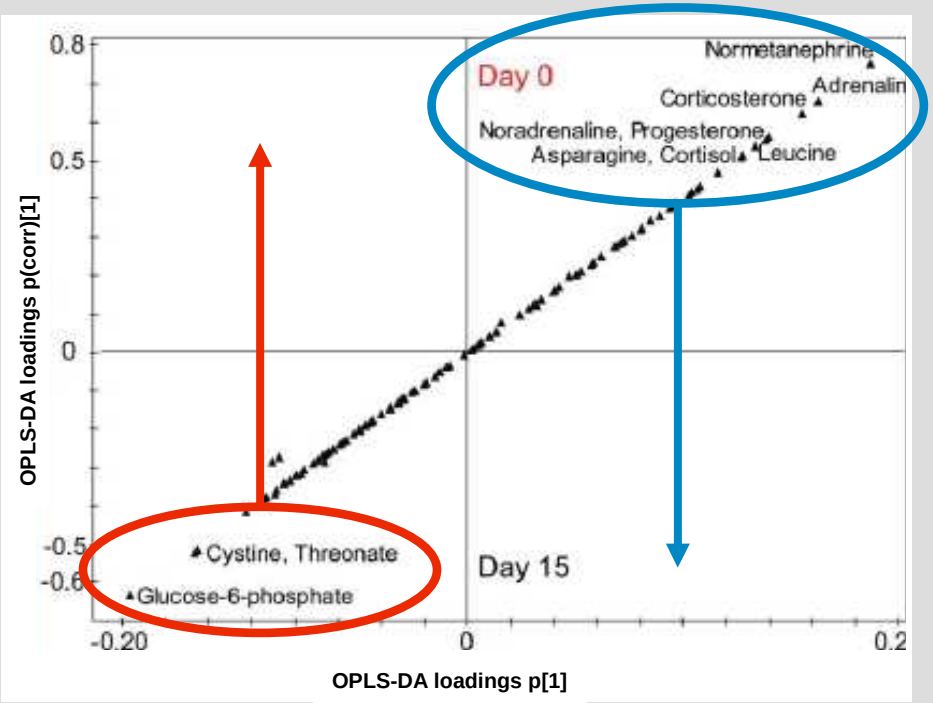
… Stress Relief Through Chocolate - Metabolic Effects

Metabolic effects of 15 days of regular dark chocolate consumption (40 g / d)



Nestlé – Metanomics Health

- Chocolate has long been associated with effects on mood in humans
- Several components in chocolate act psychoactive and affect stress, mood and chocolate craving

Results

- Subjects with higher stress showed different energy homeostasis, hormonal metabolism and gut microbial activity
- Dark chocolate reduced stress associated energy and hormone metabolites mainly in healthy males
- 2 week consumption of dark chocolate reduced stress levels as indicated by reduced urinary cortisol and catecholamines

Martin et al. 2009, J. Proteome Res. 8: 5568-79

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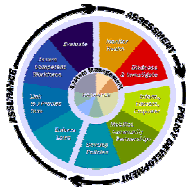
Metabolite Profiling and Nutrition



- **Applications in Nutrition**
  - **Ingredient Mechanism of Action**
    - Increasing mechanistic understanding
    - Identifying underlying biological pathways
  - **Toxicology/MetaMap^{R} Tox**
    - Early safety prediction and classification of toxicity
    - Understanding toxicity mechanisms
  - **Substantiation of Health Claims**
  - **"Classical" Biomarkers**
    - Early prognosis and diagnosis
    - Surrogate efficacy markers
    - Ingredient response prediction
    - Disease progression
  - **Bioprocessing/Fermentation**
    - Optimization of media formulations
    - Metabolic engineering of expression systems
    - Manufacturing process optimization

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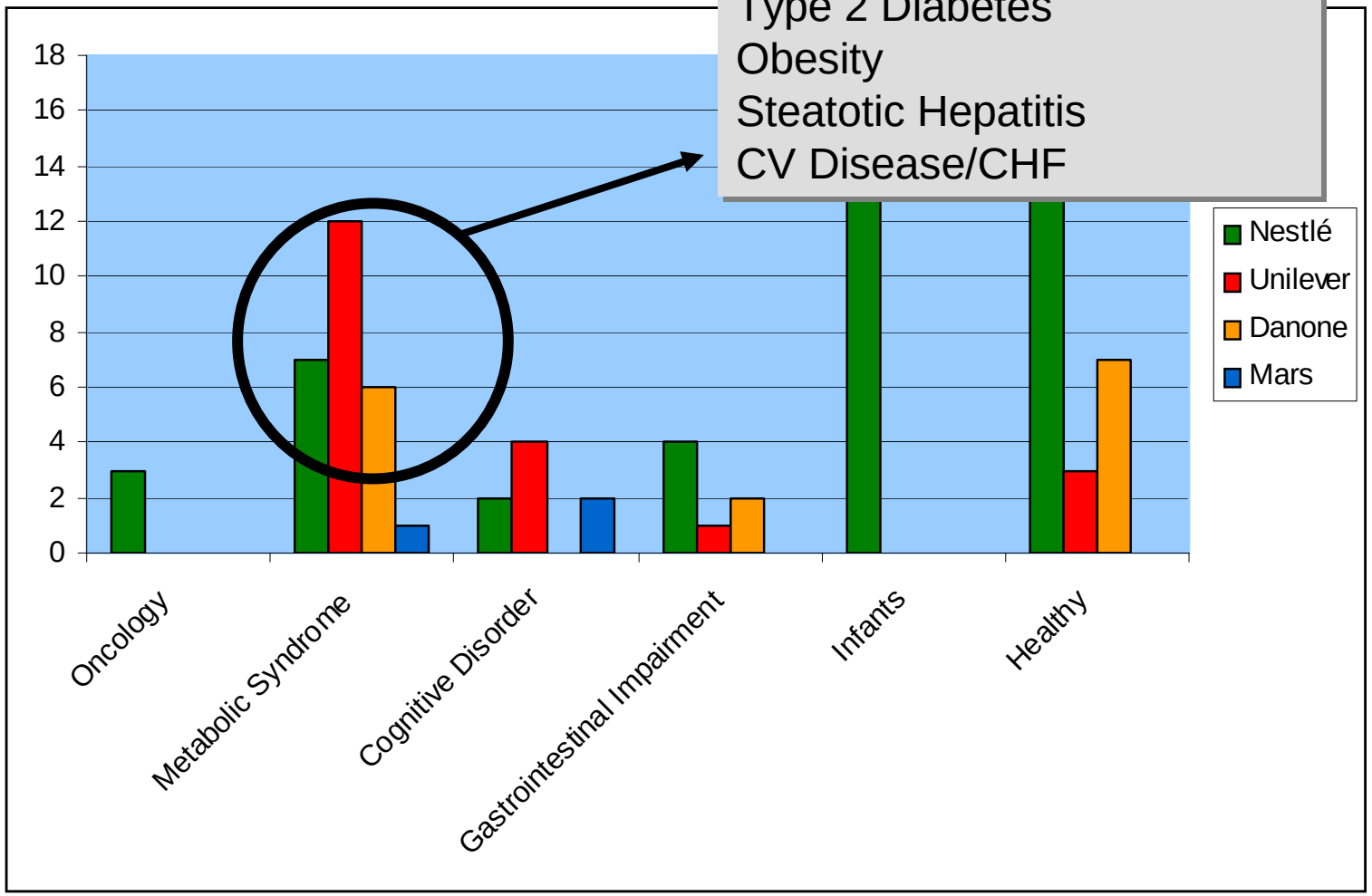
Key Nutrition Development Fields

Clinical Trials 2001-2011 *(source*

MTXH Biomarker Program:
Type 2 Diabetes
Obesity
Steatotic Hepatitis
CV Disease/CHF



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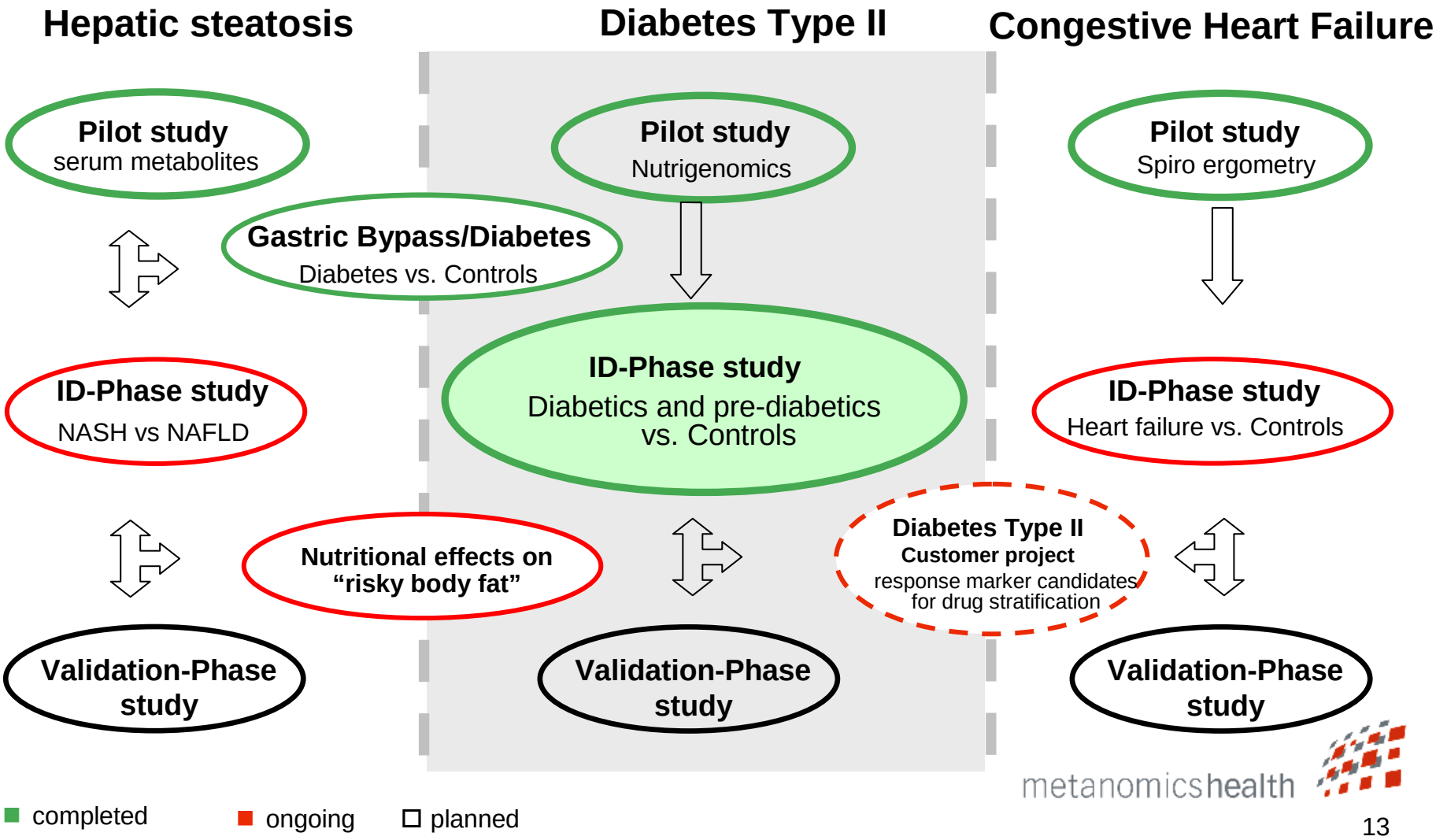
The Challenge – Metabolic Syndrome

Overview of studies to identify diagnostic and interventional targets

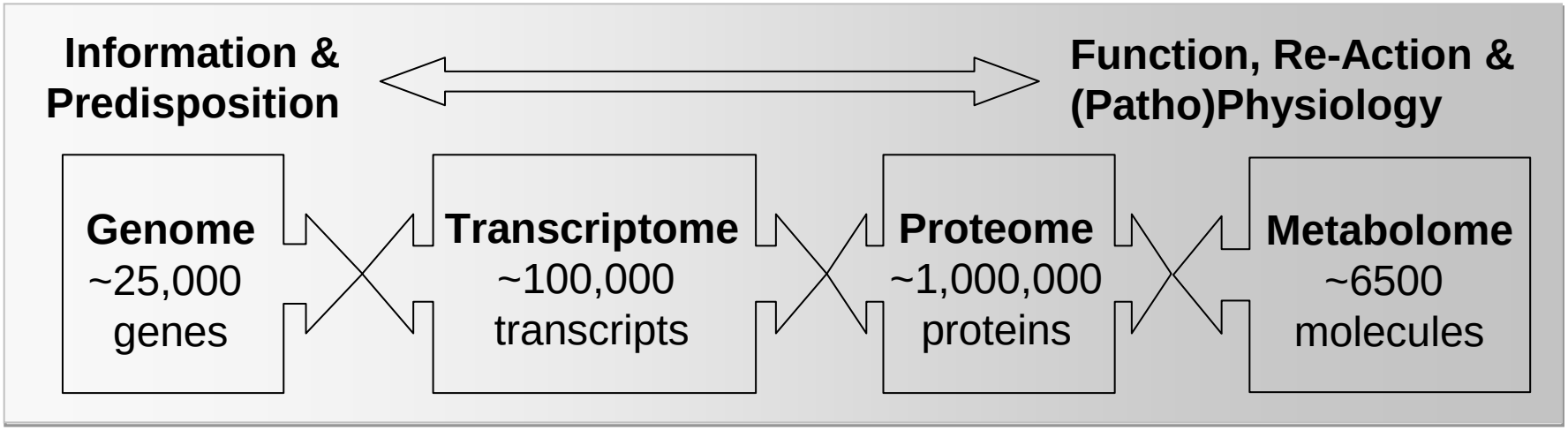


Metabolite Profiling and Diabetes

- Diabetes is caused by complex interactions of genes and environment
- Individual genes are contributing very little to the risk of disease
- Dysfunction of multiple organs (muscle, adipose, hepatic glucose production)

→ **Metabolite Profiling provides an integrated biological status**

→ **Net result of genomic, transcriptomic and proteomic variability**



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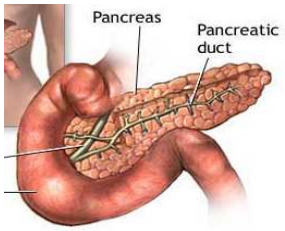
Diabetes Type 2 – ID Phase Study Algorithm



87,033 blood donors

↓

60,859 Findrisk (FR) forms

↓

4,241 high risk
(FR>12 & HbA1c>5.6)

↓

789 performed OGTT

↓

Matched (age, sex, BMI)

↓

478 subjects
(prospective sample)

↓

242 subjects
4 retrospective samples from
(bloodbank 0, -1.5, -3 and -6 years)

Goal: Biomarkers for early detection

Multicenter Case/Control study

- Cases: new and untreated diabetes
- Controls: non-diabetic blood donors
  - Pre-diabetics (impaired glucose tests)
  - Non-diabetics (normal glucose tests)
- Prospective plasma collection
  - Fasting glucose and OGTT
- Retrospective plasma collection
  - samples up to 6 years ago (non-fasting)

Metabolite profiles of 1440 samples

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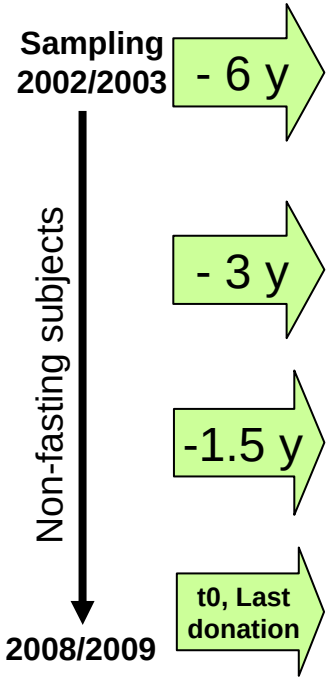
Retrospective Samples – Change Over 6 Years



	Fold change	Significance
Glucose	0.99	NS*
1,5-Anhydrosorbitol	0.87	p = 0.021
Mannose	1.20	p = 0.005
Glyoxylate	0.89	p = 0.31*
Glucose	1.09	p = 0.087*
1,5-Anhydrosorbitol	0.90	p = 0.098*
Mannose	1.13	p = 0.048
Glyoxylate	1.35	p = 0.008
Glucose	1.10	p = 0.063*
1,5-Anhydrosorbitol	0.85	p = 0.006
Mannose	1.24	p < 0.001
Glyoxylate	1.20	p = 0.10*
Glucose	1.14	p = 0.013
1,5-Anhydrosorbitol	0.80	p = 0.001
Mannose	1.25	p < 0.001
Glyoxylate	1,44	p = 0.002

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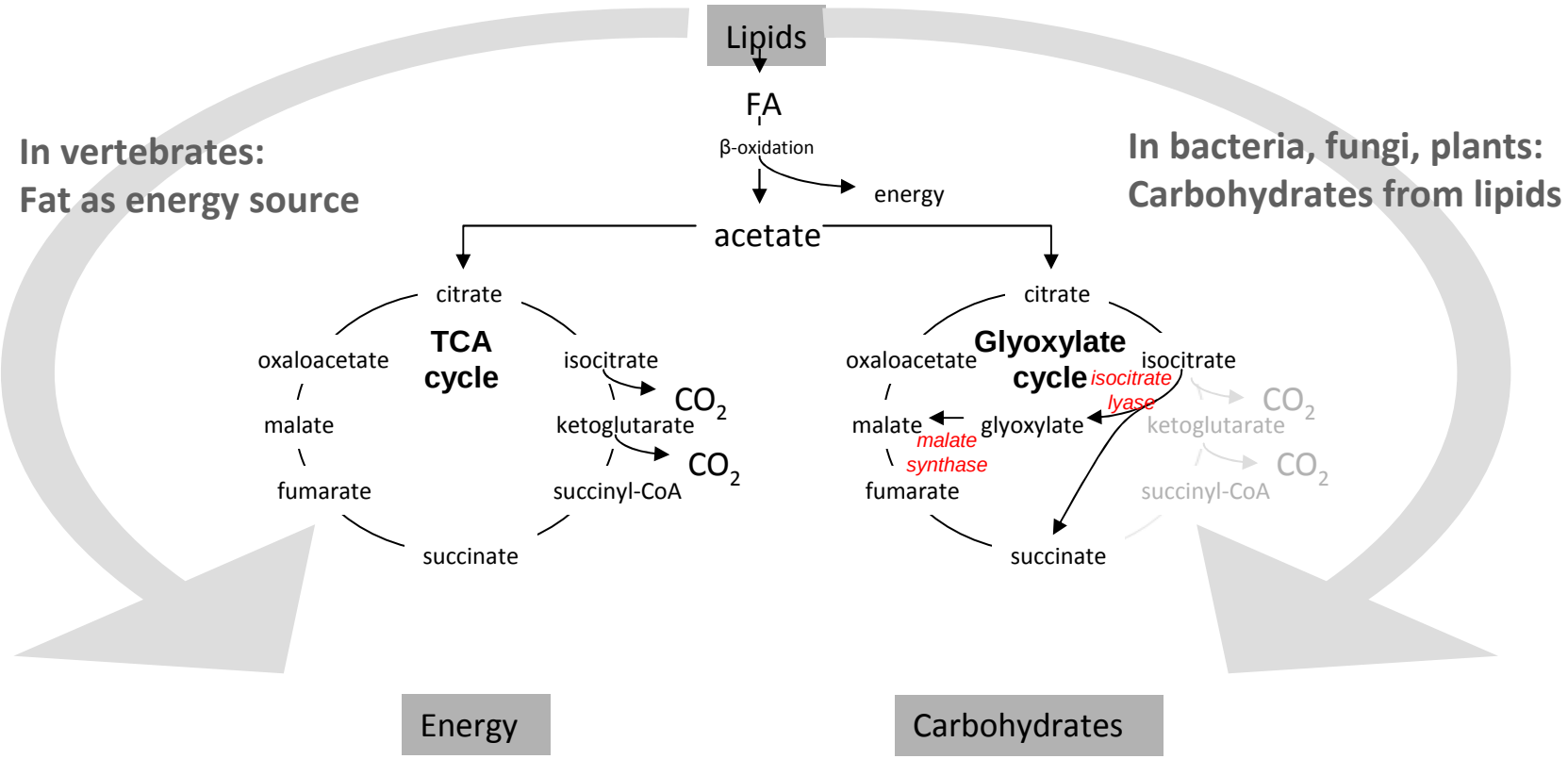
*Assumed significance of p > 0.05 was not achieved

Glyoxylate Pathway

- Part of the citrate cycle creates energy from fat
- Pathway is well-described in bacteria and plants
- Humans lack key enzymes in the liver to produce glyoxylate



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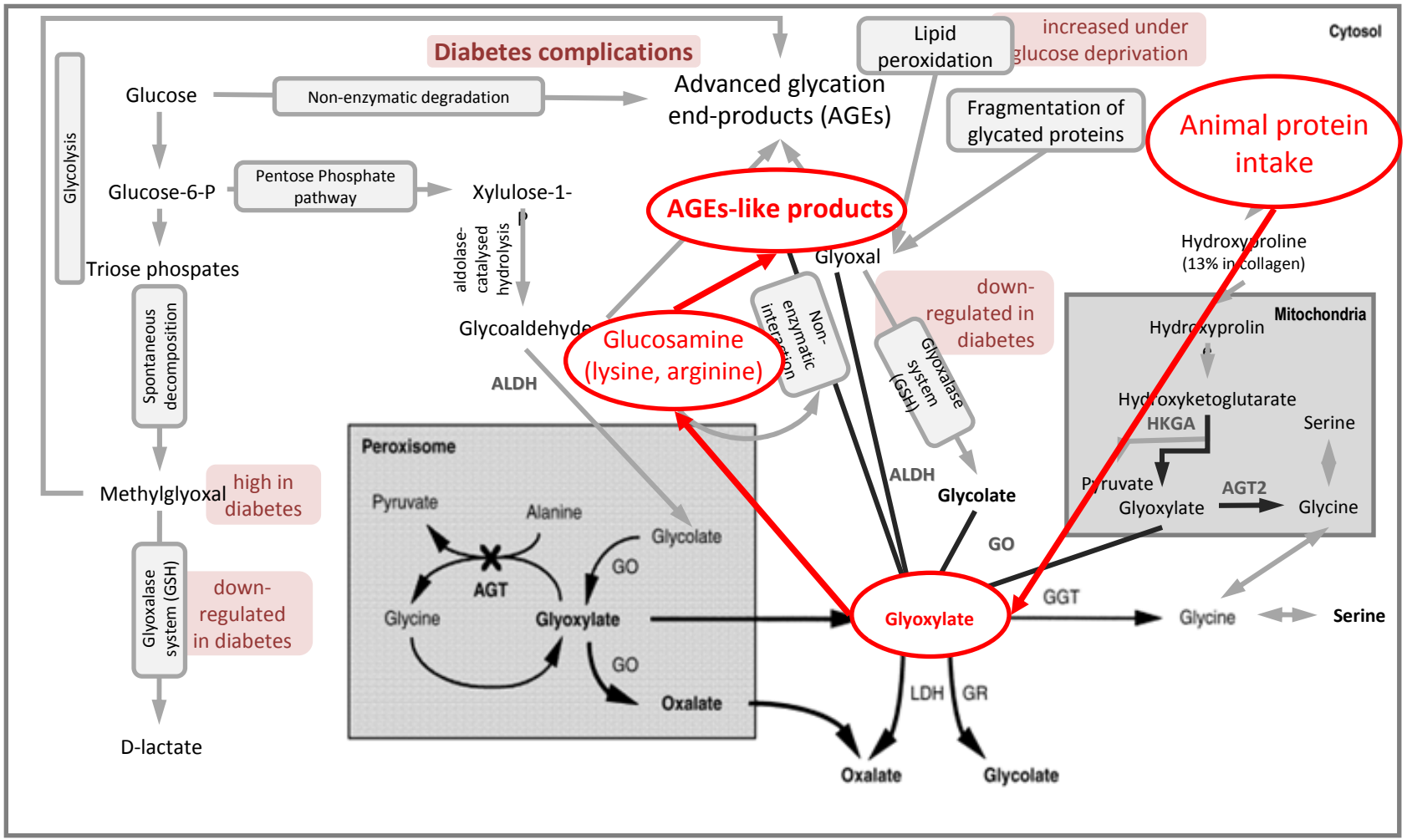
Gyoxylate is turned into Advanced Glycation end products (AGEs) via a non-enzymatic interaction



AGT, alanine:glyoxylate aminotransferase
GGT, glutamate:glyoxylate aminotransferase
GO, glycolate oxidase
GR, glyoxylate reductase

LDH, lactate dehydrogenase
ALDH, aldehyde dehydrogenase
HKGA, 4-hydroxy-2-ketoglutarate lyase

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Perspectives for Intervention

High animal protein intake (eating meat) is linked with an increased risk of diabetes and overall mortality *

→ Eat less meat

Hypothesis

The association of meat consumption with diabetes is mediated via the glyoxylate pathway

*Ann Intern Med. 2010;153:289-298
Diabetologia 2009, v.52 #11: 2277-2287
Diabetes Care September 2004 vol. 27 no. 9 2108-2115

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Metabolite Profiling - Applications for Nutrition Research

Consumer Nutrition

Medical Nutrition/DS

Companion Animal Feed

Lifestock Feed

- Mechanistic studies for new ingredients/new formulations (e.g. mode of action, underlying pathways, impact on gut microflora)
- New health claims supported by metabolic footprint
- Science-based nutrition recommendations/composition
- Biomarkers serving as surrogate intervention endpoints
- Early biomarkers indicating disease onset and disease progression
- Supporting development of personalized diet and lifestyle

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Sincere thanks to ….

- Our academic and clinical cooperators
- Our pharma and nutrition partners
- Our dedicated and enthusiastic staff at Metanomics Health and metanomics

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Two Companies with one Common Focus

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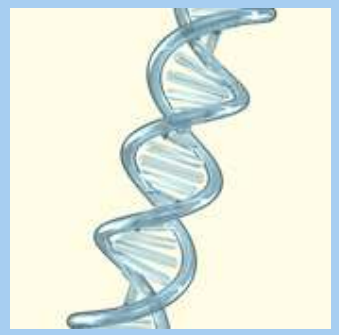
new dimensions in discovery

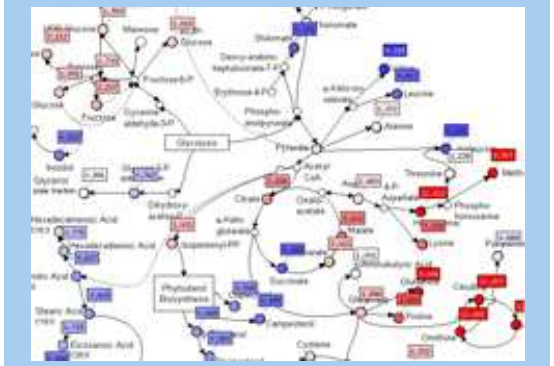
Plant Biotechnology

- founded 1998
- BASF Company
- Focus on plant biotechnology
- ca. 1/3 dedicated to pharma, diagnostics and nutrition
- Most comprehensive metabolite profiling platform worldwide
- >75 mass specs (GC-MS, LC-MS/MS etc.)
- Integrated IT infrastructure
- Largest database for gene functions in plants



GENOME

METABOLOME



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Healthcare

- founded 2003
- BASF Company
- Biomarker research for pharmaceutical, nutrition and diagnostics companies
- Proprietary biomarker identification and validation program in various indications
- Analytical work and data interpretation performed by metanomics
- Presence in Europe, NAFTA and Japan

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Prospective Samples: 2-Metabolite Performance

Diabetes vs. Healthy – prospective data without Glucose

Diagnostic performance estimate determined strictly on test data

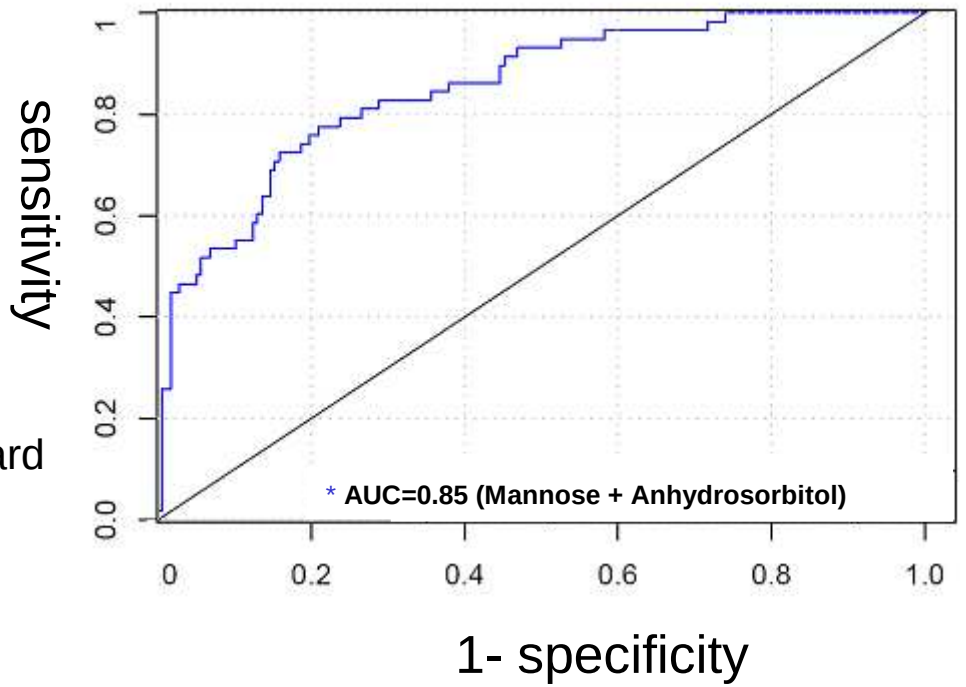
Performance

- 58 cases vs. 177 controls (fasting)
- 2 metabolites (excluding glucose)
- AUC ~ 0.85

Note:

- Glucose (t0 OGTT) used as gold standard
- Biomarkers for clinical use may include glucose → Impact on performance

ROC curve for 2 metabolites



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