DATA INTEGRATION AND PHENOTYPIC FLEXIBILITY: THE NUTRITECH STUDY

Suzan Wopereis

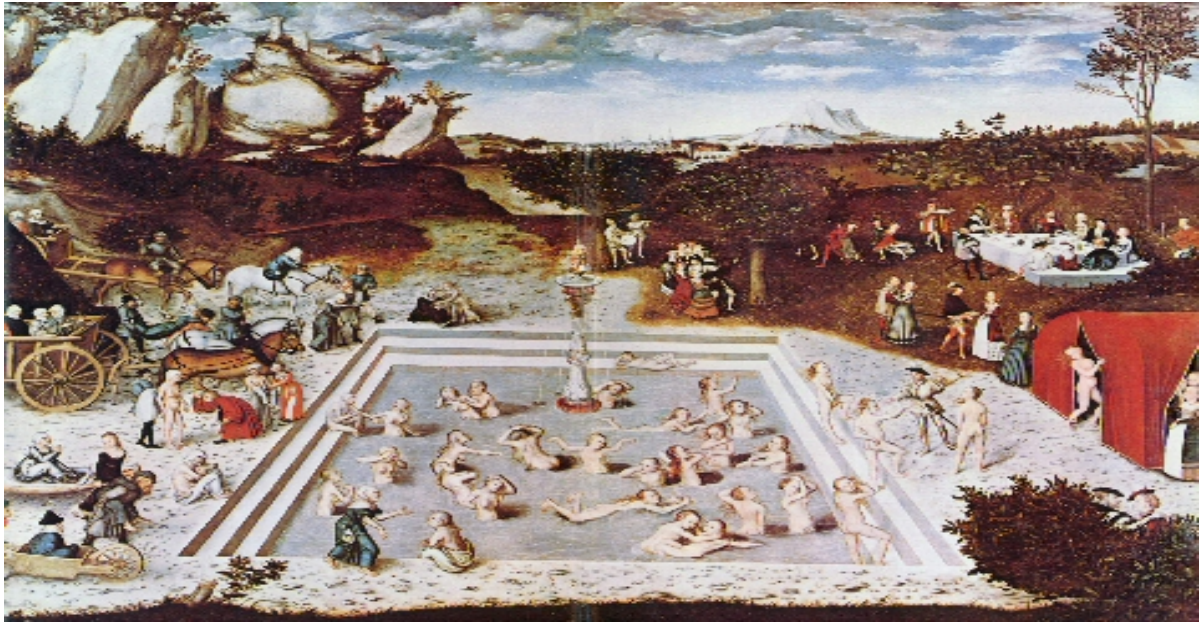
TNO innovation for life

My personal objective: identification of biomarkers of (optimal) health

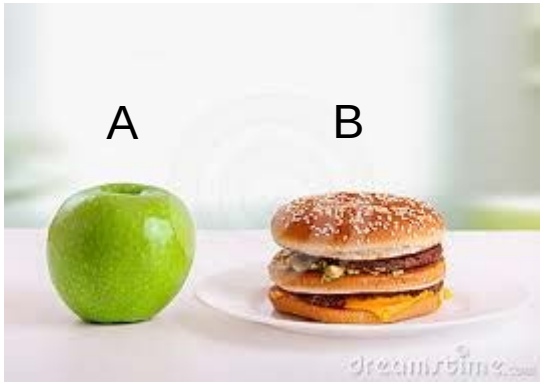
- Biomarker Definition:

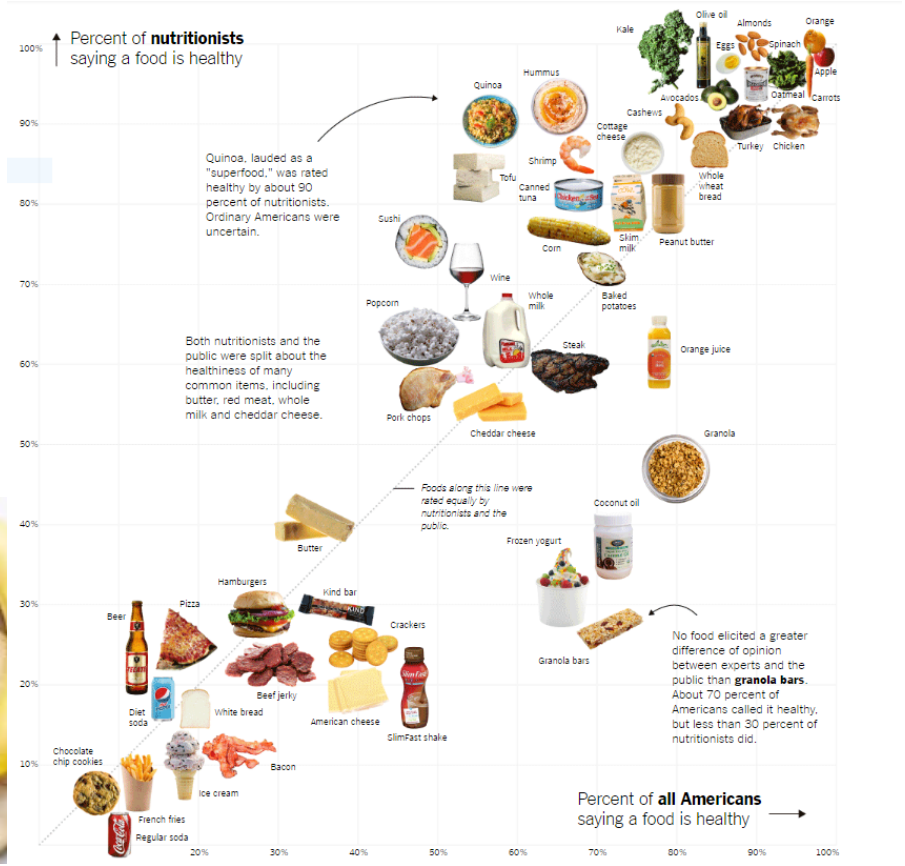
  'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or responses to an intervention'

  {Biomarkers definition working group, 2001 }



What is the definition of healthy nutrition?





Why is it so difficult to quantify health effects from food/nutrition?

Free living subjects, compliance

Target population is healthy

Interaction between nutrients

Subtle and long term effects

Multiple mechanisms

Multiple target tissues

Choice of reference

Inter individual variation

....ability to adapt and self-manage in the face of social, physical and emotional challenges

BMJ

BMJ 2011;343:d4163 doi: 10.1136/bmj.d4163

How should we define health?

The WHO definition of health as complete wellbeing is no longer fit for purpose given the rise of chronic disease. **Machteld Huber and colleagues** propose changing the emphasis towards the ability to adapt and self manage in the face of social, physical, and emotional challenges

Machteld Huber *senior researcher*[1], J André Knottnerus *president, Scientific Council for Government Policy* [2], Lawrence Green *editor in chief, Oxford Bibliographies Online—public health*[3], Henriëtte van der Horst *head* [4], Alejandro R Jadad *professor*[5], Daan Kromhout *vice president, Health Council of the Netherlands*[6], Brian Leonard *professor*[7], Kate Lorig *professor*[8], Maria Isabel Loureiro *coordinator for health promotion and protection*[9], Jos W M van der Meer *professor*[10], Paul Schnabel *director* [11], Richard Smith *director*[12], Chris van Weel *head* [13], Henk Smid *director* [14]

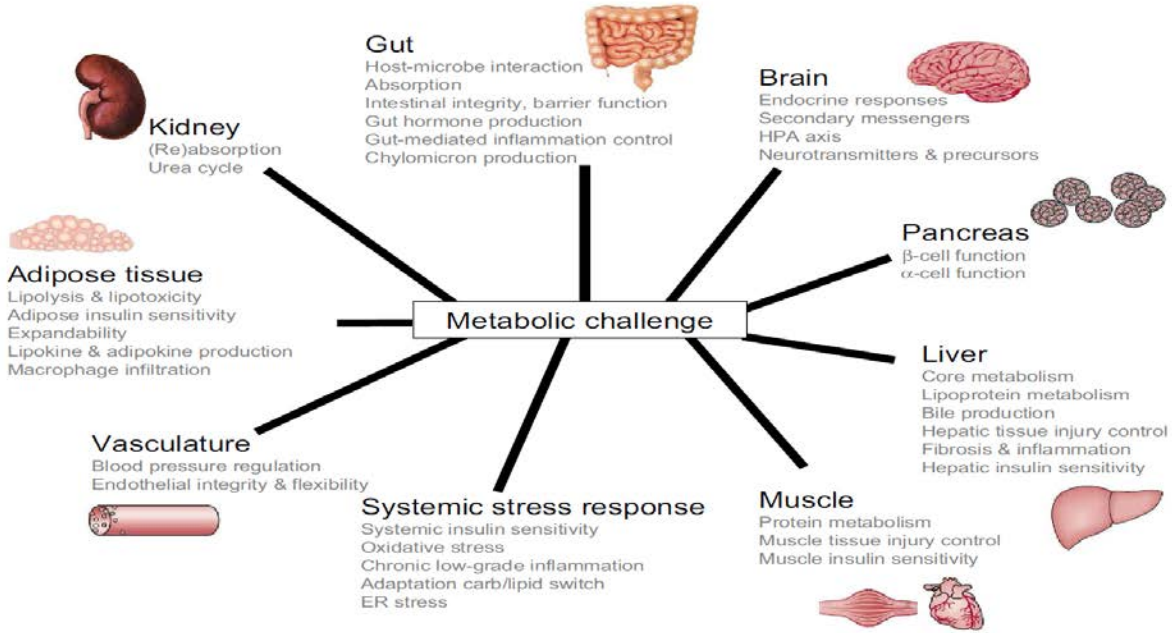
The challenge concept:
Study and quantification of the stress response curve

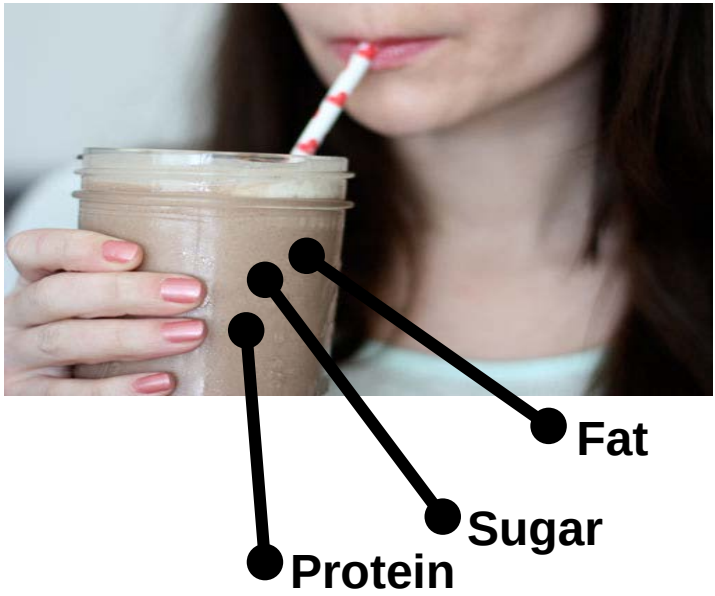
Fat

Sugar

Protein

Phenflex challenge test:





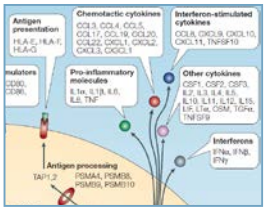
- 320 ml tapwater
- 60 grams palm olein
- 75 grams of glucose
- 20 grams of Protifar
- 0,5 gram / 20 droplets of artificial aroma

Genes Nutr (2015), 10:13

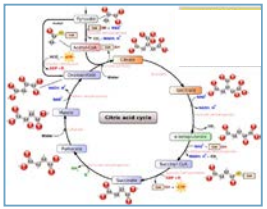
Time course studies to monitor PhenFlex challenge test response

Blood sampling at multiple time points after challenge, up to 10 hours

clinical chemistry



proteome



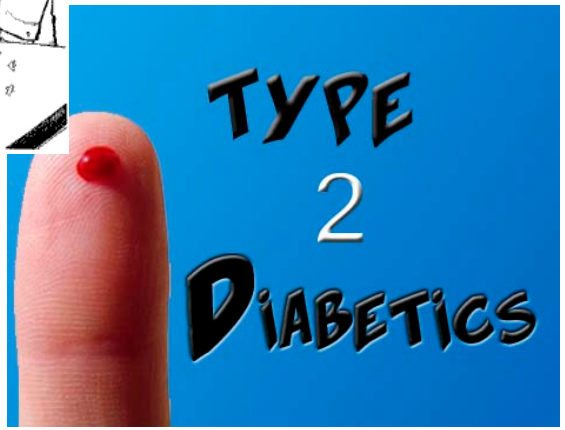
metabolome

transcriptome

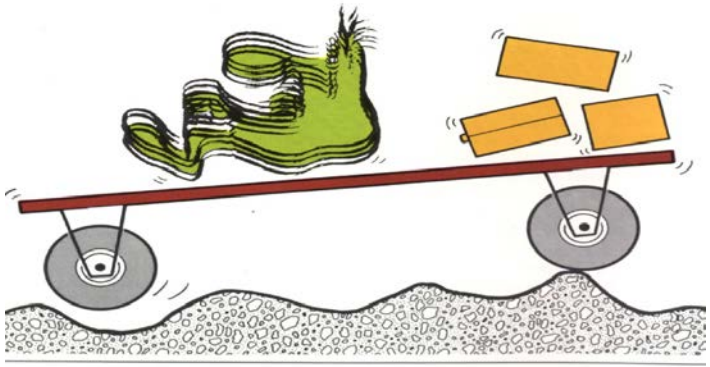
Measuring a total of ~150 different markers representing
~25 health related processes

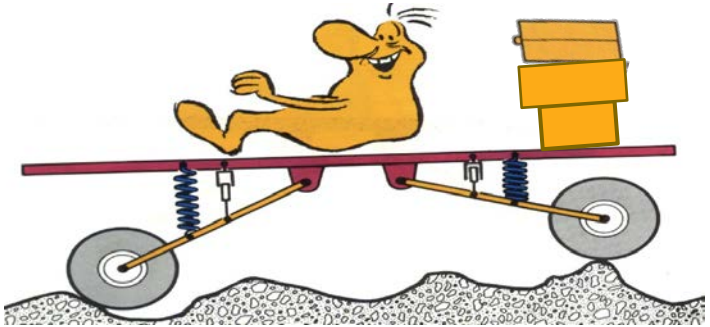

GAP


PHASE TWO: INTERPRETATION
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME

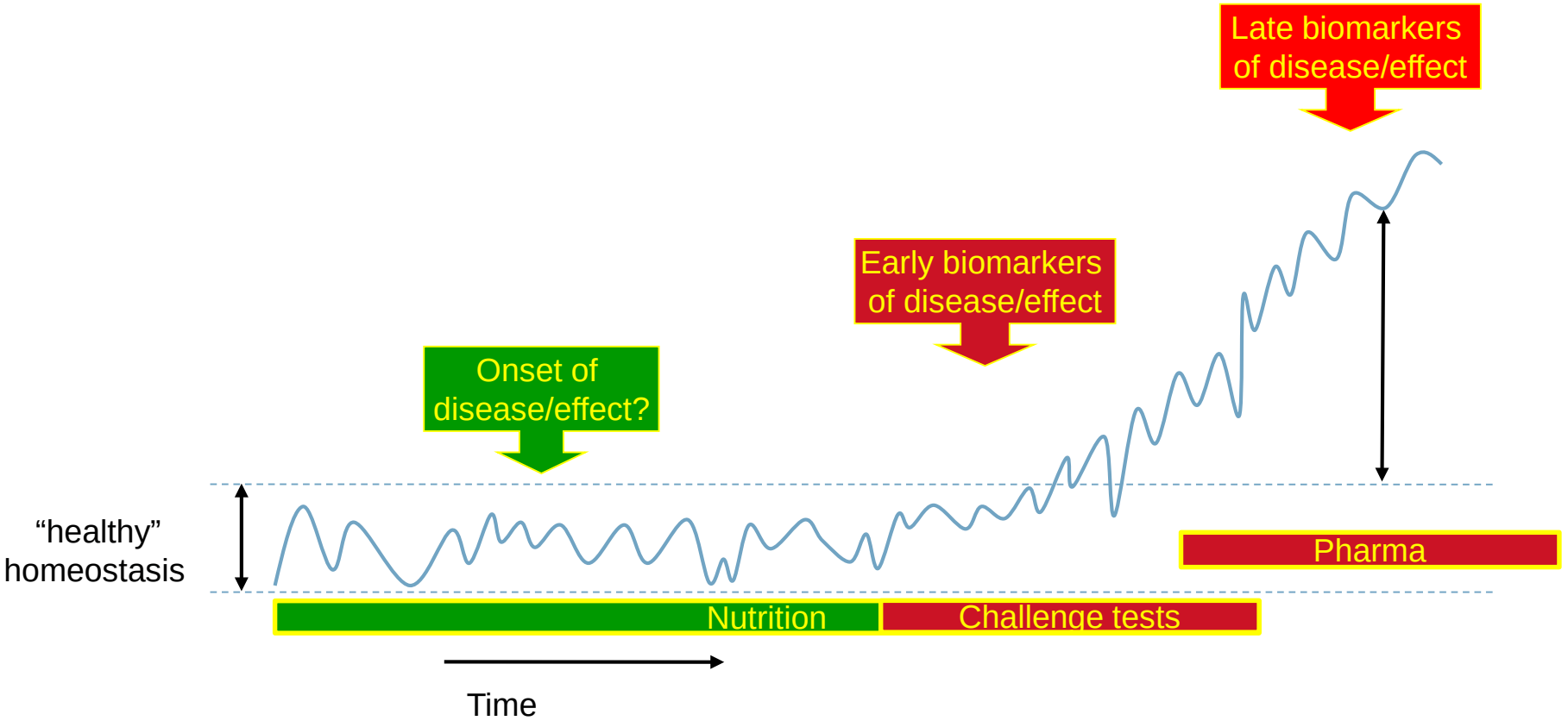

TYPE
2
DIABETICS

Phenflex challenge test with response markers as toolbox to test and evaluate the capacity of the shock absorber



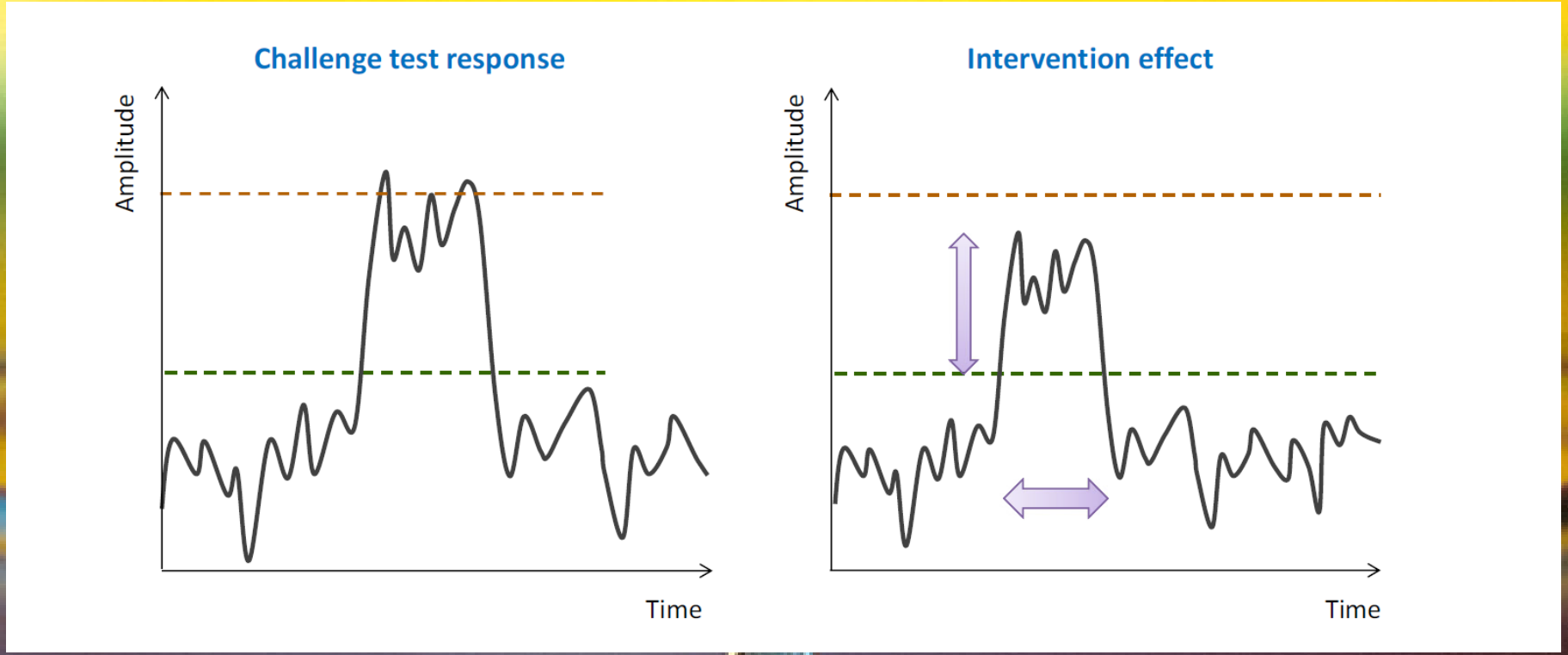


Resulting in the ‘next generation biomarkers’



Van der Greef (2005)

The challenge concept:
Study and quantification of the stress response curve



First publications that showed health effect of nutritional intervention by using a challenge test

- 36 overweight male elevated CRP
  5 weeks supplement mix
  cross-over design

  Bakker et al. Am J Clin Nutr. 2010; 91:1044-59.
  Pellis et al. Metabolomics. 2012; 8(2):347-359.
  Bouwman et al. BMC Med Gen. 2012; 6;5:1.

- 10 healthy male
  before and after 4 weeks overfeeding
  (1300 kcal/day extra)

  Kardinaal et al. FASEB J. 2015;29(11):4600-13.

- 18 MetS (male & female)
  12 weeks HMUFA diet before and after

  Cruz-Teno et al. Mol. Nutr. Food Res. 2012;56:854–865

- 29 healthy overweight middle-aged men
  double-blind crossover study
  effects of 4 wk high flavonol chocolate (HFC)
  vs normal dark chocolate (NFC)

  Esser et al. FASEB J. 2014;28(3):1464-73

Objective: identification of resilience markers of robustness

- A “biomarker of robustness” is defined as a marker for which an integrated analysis of its gene/protein and/or metabolite levels as well as available biological knowledge has provided sufficient evidence to reflect the health status of a physiologically relevant process

Nutritech FP7 EU project (2012 – 2016)

Application of new technologies and methods in nutrition research
– the example of phenotypic flexibility

1 core human intervention trial, executed at Imperial College London

- Analysis of multitude of parameters by the partners
  (new and existing technologies and methods)

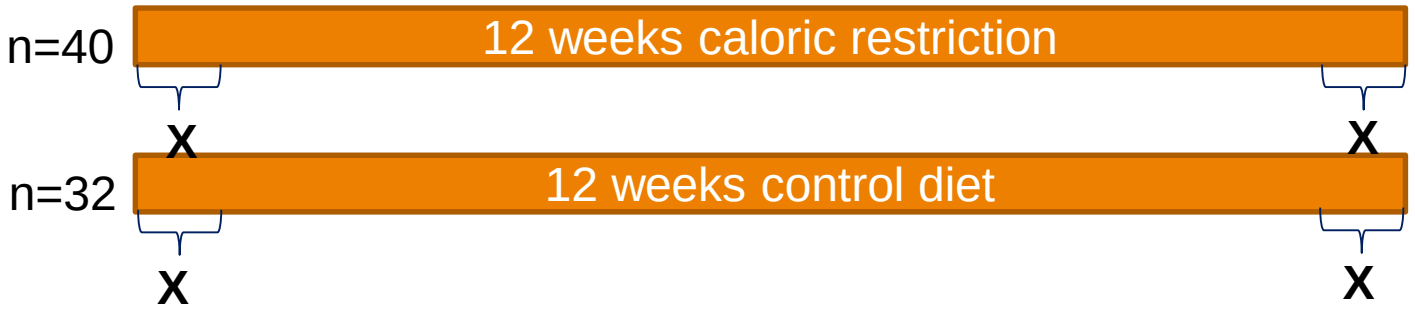
Goal of the study:

- Define set of practical biomarkers for health (maintenance, improvement)
- Evaluate mechanisms of ‘phenotypic flexibility’ as read-out for health status

TNO
Wageningen Un
TU Munich
Imperial Coll London
Un Coll Dublin
Un Oslo
Un Varna
Un Cordoba
NuGO
ISS
IMDEA
TUFTS
CSIRO
Un Alberta
Un Toronto
IARC
ILSI Europe
Un Auckland
Biocrates
Vitas
Biqualys
Paprika Analytics

Proof of Concept: Nutritech human intervention study

72 male and female volunteers,
age 50-65 years,
BMI 25-35 kg/m^2



X = 4-day assessment period:
OGTT, PhenFlex challenge, exercise challenge

Proof of Concept: Nutritech human intervention study

72 male and female volunteers,
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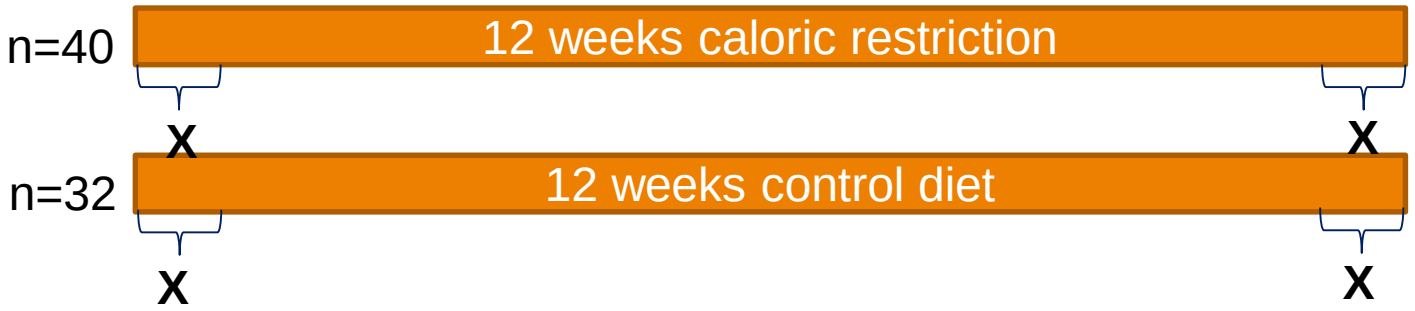
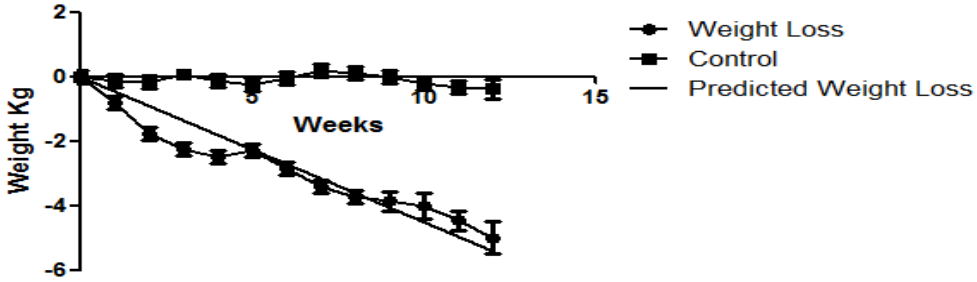
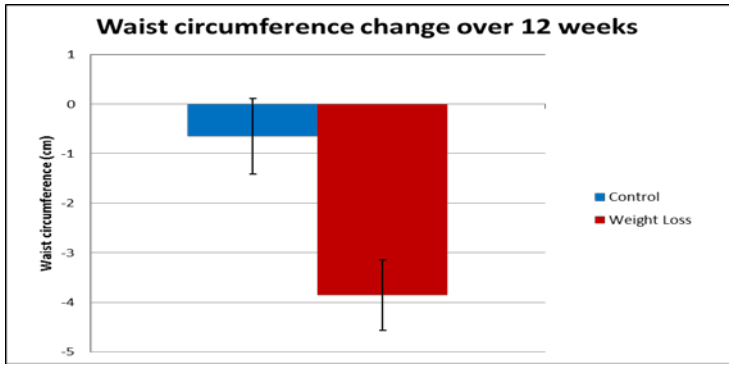
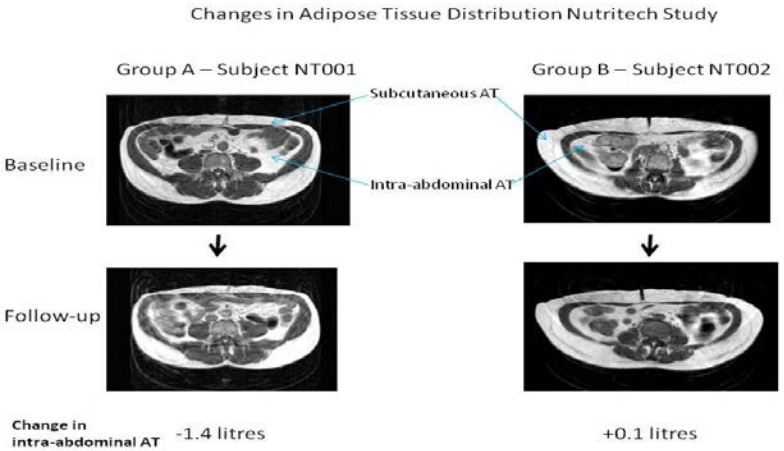


X = 4-day assessment period:
OGTT, PhenFlex challenge, exercise challenge

The intervention was successful in terms of weight loss







Billions of data points were collected:
>14000 plasma tubes and > 70000 Eppendorf/cryovials

Blood/plasma

- Clinical chemistry, Metabolic profiling, Inflammatory proteins

Cellular/tissue transcriptome

- PBMC, WAT and muscle biopsy

Faeces

- Microbiome analysis

Genetics

- whole exome sequencing, Analysis of genomic integrity

Body composition

- Weight, BMI, waist circumference, MRI data – fat content in different organs

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Phenotype database

Profile Logout

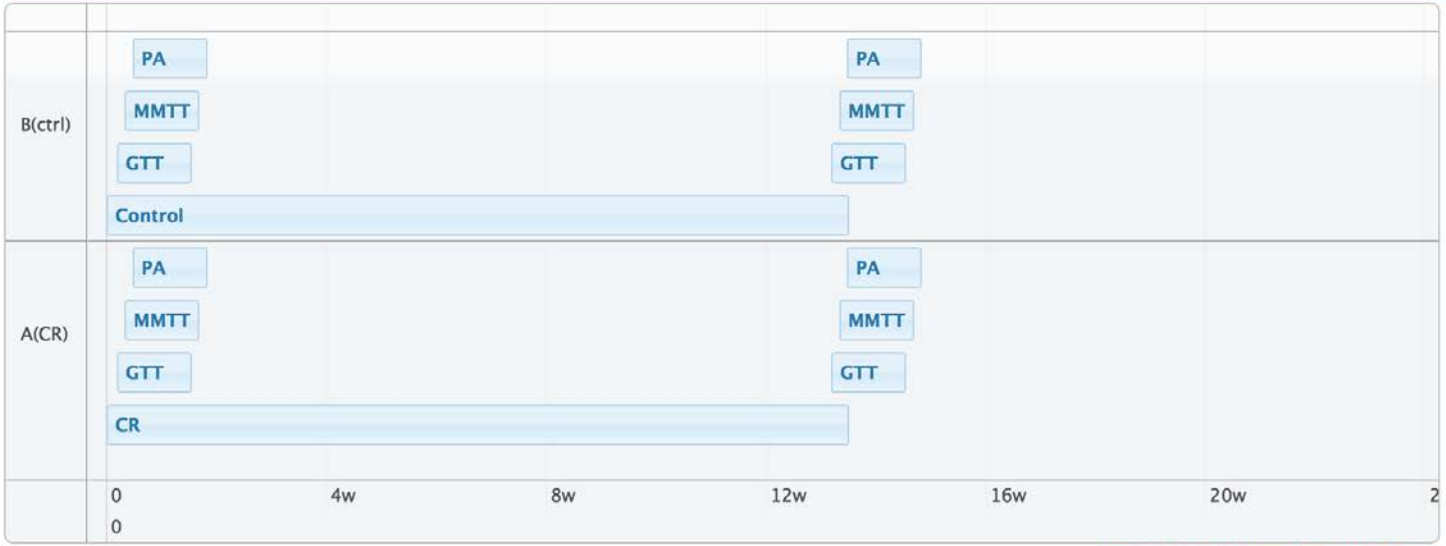
Home | Create | Import | Browse/Edit | Analyze | Export | Modules | Admin | Search term

Study [NutriTechHIS]

study / subjects / **design** / samples / assays

This page shows your study design

The study design consists of treatement types and sample types, grouped together in sample & treatment groups.
Double click on a subjectgroup or sample & treatment group to see details.

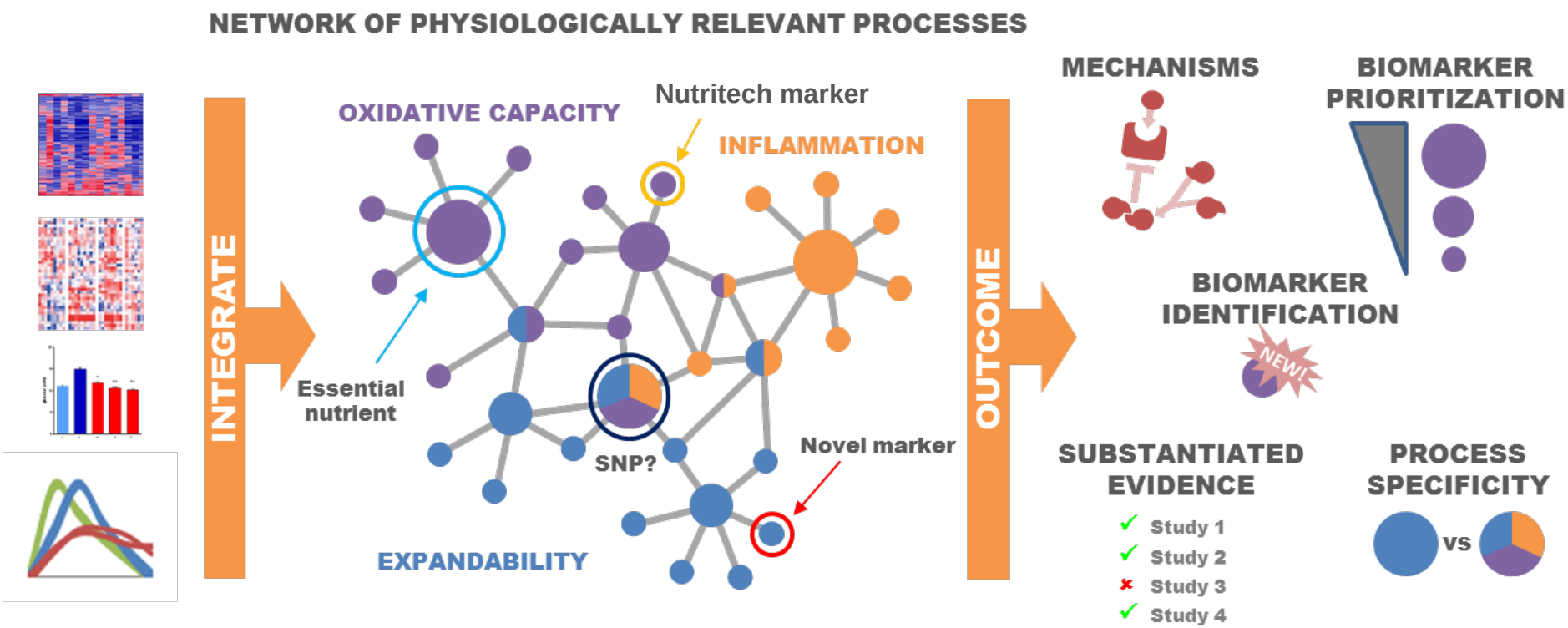


Readable scaled items | Time scaled items

Varna as data integration centre!

Varna as data integration centre!

Creation of Health Reference Networks for ‘Resilience’



Input for our health reference networks of ‘Resilience’ (1)

Prior knowledge from review paper on ‘nutrigenomics’ biomarker response to challenge tests

Genes Nutr (2015) 10:13
DOI 10.1007/s12263-015-0459-1

RESEARCH PAPER

Phenotypic flexibility as a measure of health: the optimal nutritional stress response test

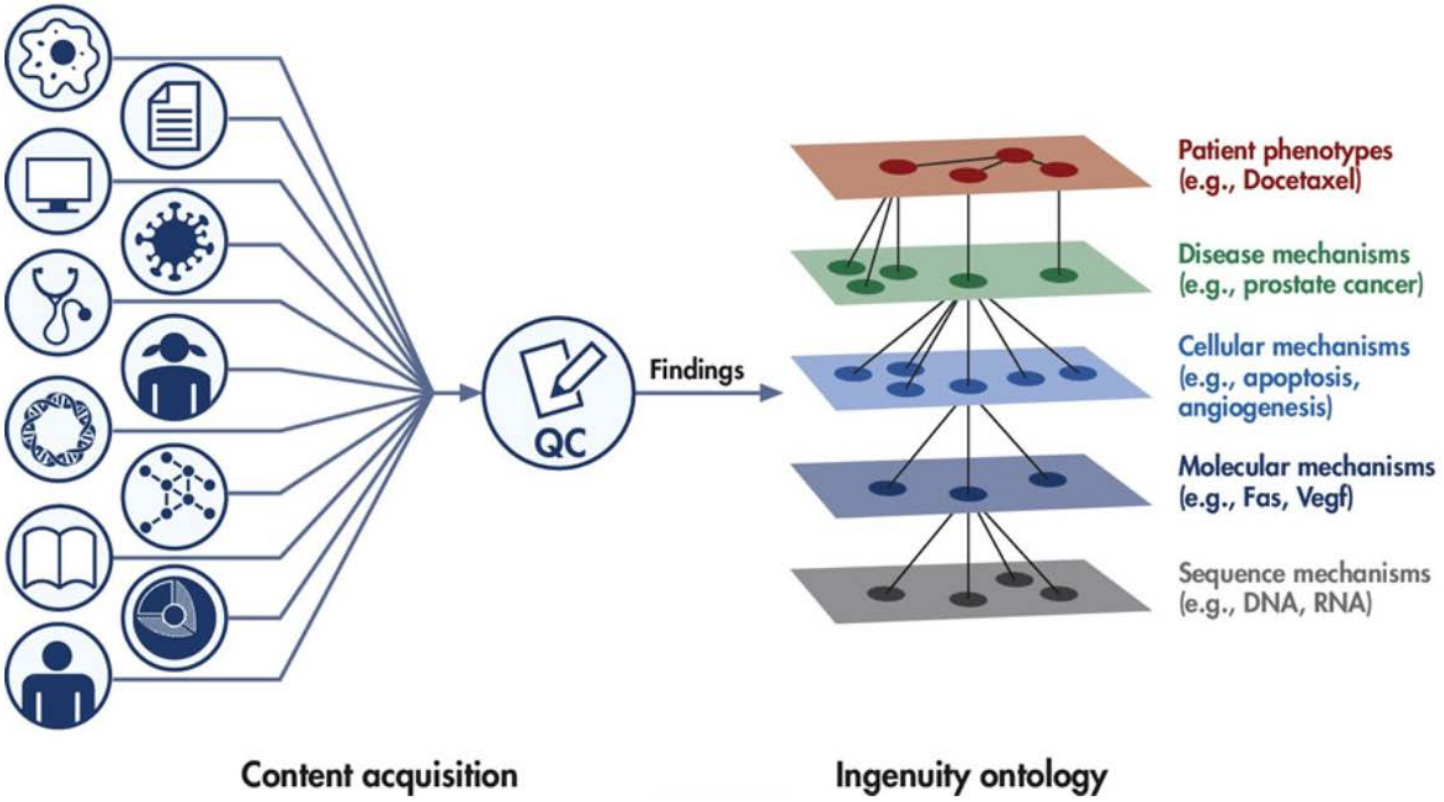
Johanna H. M. Stroeve[1] · Herman van Wietmarschen[1] · Bas H. A. Kremer[1] · Ben van Ommen[1] · Suzan Wopereis[1]

Received: 12 November 2014 / Accepted: 21 March 2015 / Published online: 21 April 2015

Abstract Nutrition research is struggling to demonstrate beneficial health effects, since nutritional effects are often subtle and long term. Health has been redefined as the biomarkers related to this subset of processes to the different challenge tests. Based on the obtained insights, we propose a nutritional stress test composed of a high-fat,

Input for our health reference networks of ‘Resilience’ (2)

Prior knowledge from The Ingenuity Knowledge Base



Input for our health reference networks of ‘Resilience’ (3)

Prior knowledge from metabolomics based biomarkers focusing on type 2 diabetes

Curr Cardiovasc Risk Rep (2015) 9: 12
DOI 10.1007/s12170-015-0440-y

LIPIDS (L PARNELL AND J ORDOVAS, SECTION EDITORS)

Metabolomics for Biomarkers of Type 2 Diabetes Mellitus: Advances and Nutritional Intervention Trends

Mireia Urpi-Sarda · Enrique Almanza-Aguilera · Sara Tulipani · Francisco J. Tinahones · Jordi Salas-Salvadó · Cristina Andres-Lacueva

Published online: 17 February 2015

Abstract Metabolic characterization of type 2 diabetes mellitus (T2DM) is crucial for the identification of individuals at risk for developing diabetes and T2DM-related vascular complications as well as for monitoring disease progression. The application of metabolomics to diabetes research may lead to the identification and discovery of diagnostic and prog- approaches. Several studies have demonstrated that tabolism of carbohydrates, lipids, and amino acids is erably altered in prediabetes and continue to vary course of T2DM progression. The identification of in ate metabolites involved in glycolysis, gluconeogen tricarboxylic acid cycle, lipolysis, and proteolysis h

Review | K SUHRE | *Metabolic profiling in diabetes* | 221:3 | R75–R85

Metabolic profiling in diabetes

Karsten Suhre[1,2]

[1]Department of Physiology and Biophysics, Qatar Foundation – Education City, Weill Cornell Medical College – Qatar, PO Box 24144, Doha, Qatar
[2]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

Correspondence should be addressed to K Suhre
Email karsten@suhre.fr

Abstract

Metabolic profiling, or metabolomics, has developed into a mature science in recent years. It has major applications in the study of metabolic disorders. This review addresses issues relevant to the choice of the metabolomics platform, study design and data analysis in diabetes research, and presents recent advances using metabolomics in the identification of markers for altered metabolic pathways, biomarker discovery, challenge studies, metabolic markers of drug efficacy and off-target effects. The role of genetic variance and intermediate

Key Words
- diabetes
- metabolism
- lipid
- insulin resistance
- glucose metabolism

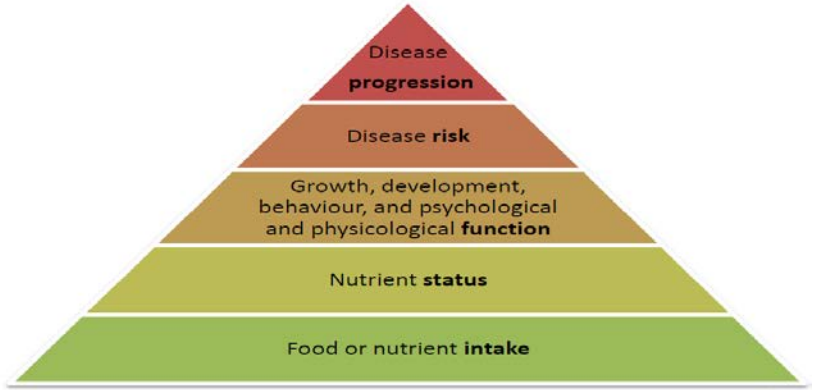
Input for our health reference networks of ‘Resilience’ (4)

Prior knowledge on scoring criteria to evaluate biomarkers

Nutrition Research Reviews, page 1 of 9 doi:10.1017/S0954422416000263

Improving selection of markers in nutrition research: evaluation of the criteria proposed by the ILSI Europe Marker Validation Initiative

Philip C. Calder[1]*, Alan Boobis[2], Deborah Braun[3], Claire L. Champ[4], Louise Dye[4], Suzanne Einöther[5], Arno Greyling[5], Christophe Matthys[6], Peter Putz[7], Suzan Wopereis[8], Jayne V. Woodside[9] and Jean-Michel Antoine[10]



Criteria to evaluate markers

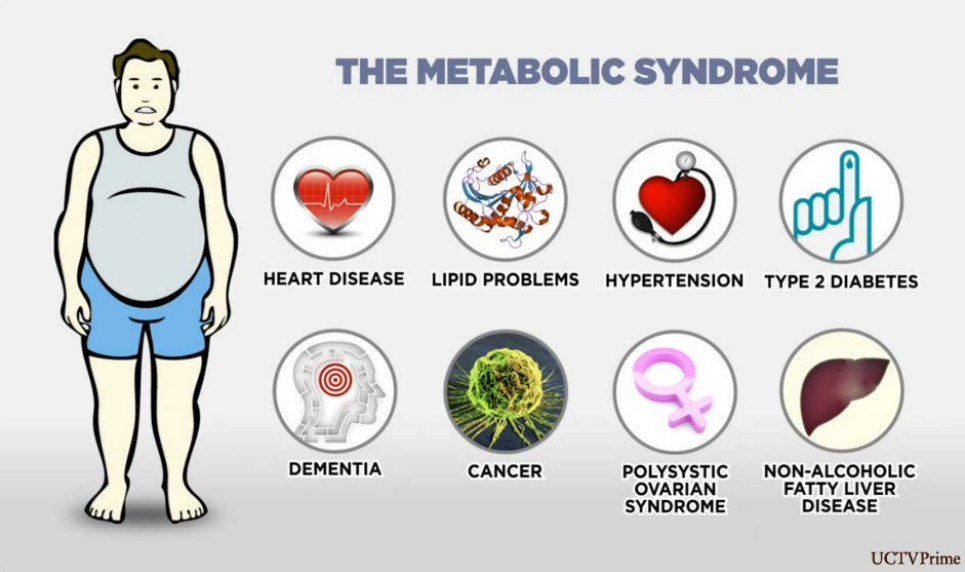
Specific field and related marker
- SCORING CRITERIA
  - Methodological aspects excluding study design
    - (Relevance of criteria can differ between different types and applications of markers)
    - Method should be validated according to recognised guidelines (please cite)
    - Appropriate* sensitivity
    - Appropriate* specificity
    - Reproducibility, accuracy, standardisation, stability (quality of the sample) and technical variation
    - Biological variation
  - Reflect/mark the study objective
    - A change in the marker is linked with a change in the endpoint in one or more target population(s)
    - Method should be validated according to recognised guidelines (please cite)
- ADDITIONAL INFORMATION
  - Relevance to nutrition research
    - What is considered as a normal range for healthy people?
    - What is a significant change (consider both biological and statistical)?
    - (might vary for different applications, e.g. epidemiological studies *v.* individual level)
    - Is there evidence that nutrition influences the marker? If so, what is the size of the effect reported?
    - Which other factors also have an effect on the marker? (if any)
  - Other relevant information
    - Are there experimental data where dietary intervention has not resulted in an anticipated change?

Conclusions
References

Goal: identification of resilience markers of robustness based on existing data by multi study analysis

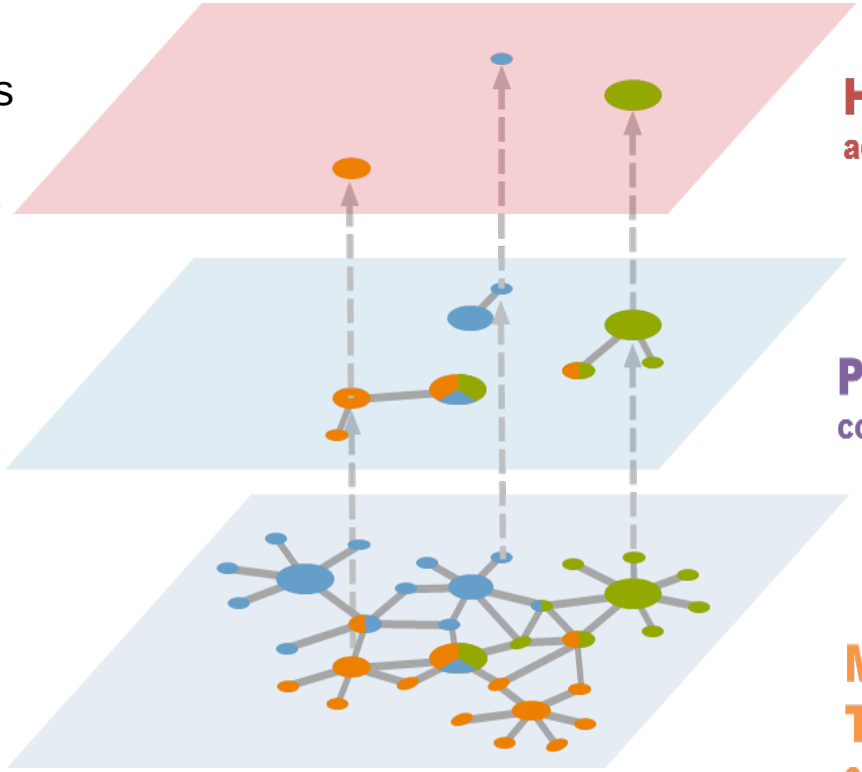
Creating the Metabolic Syndrome reference network of resilience

1) Select studies & data-sets in which ‘similar’ challenge test was performed and different ‘markers’ were measured in response to challenge test
2) Diagnose subjects on basis of medical criteria whether they have metabolic syndrome according to WHO criteria
3) Look for ‘response’ biomarkers that are identified in multiple studies or across studies



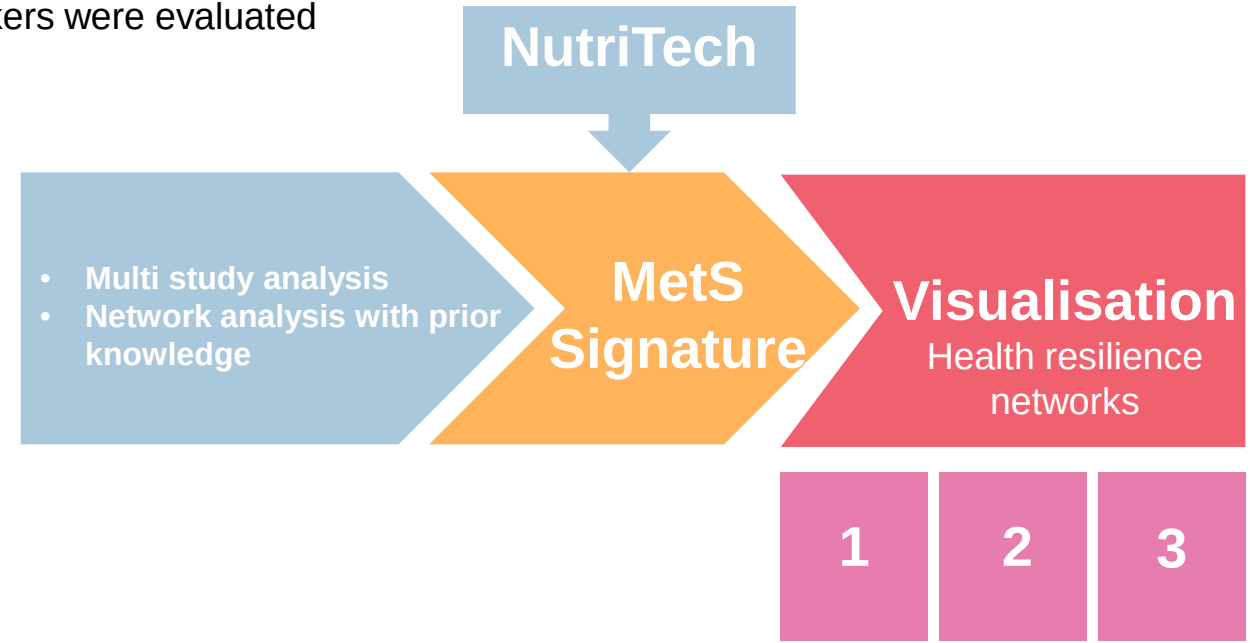
Multiple level visualization

- The view exists on multiple levels
  - Uses ontological information
  - Levels can be independently visualised
- First and most basic: molecular
- Higher level views aggregate nodes according to function or health domain

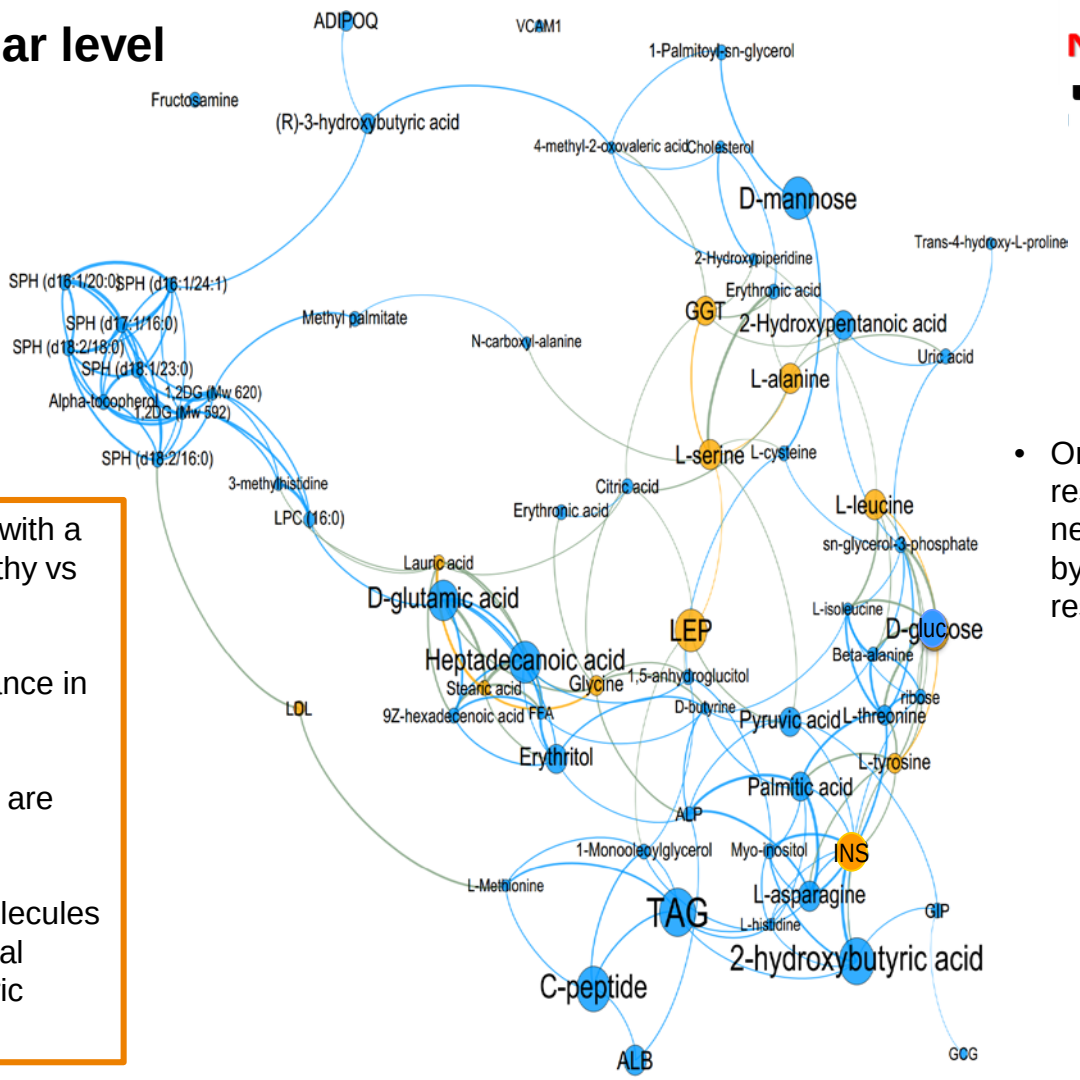


Workflow

- 4 studies having a total of 195 subjects
- 117 healthy vs 78 MetS
- All studies used a PhenFlex like challenge test
- A total of n=400 markers were evaluated



Result at molecular level



LEGEND

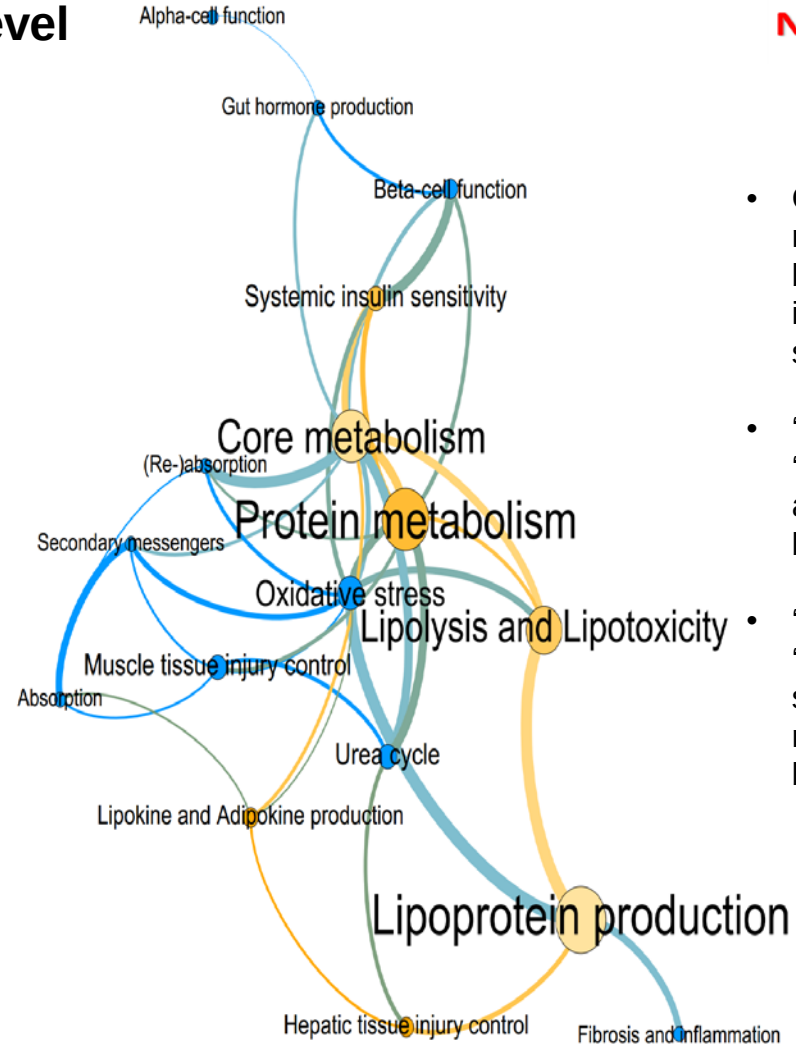
- Nodes represent molecules with a differential resilience in healthy vs MetS
- Node size indicates significance in multi-study analysis
- Layout is meaningful: nodes are arranged by similarity
- Orange nodes represent molecules that also showed a differential resilience by NutriTech caloric restriction intervention

- Only part of the MetS resilience reference network was modulated by Nutritech caloric restriction intervention

Result at biological process level

LEGEND

- Size shows amount of molecules in biological process
- Edge thickness shows similarity between biological processes
- Orange coloring = significantly altered molecules related to biological process by Nutritech caloric restriction intervention
- The higher the intensity of color the higher percentage of molecules from biological process are significantly altered by Nutritech caloric restriction

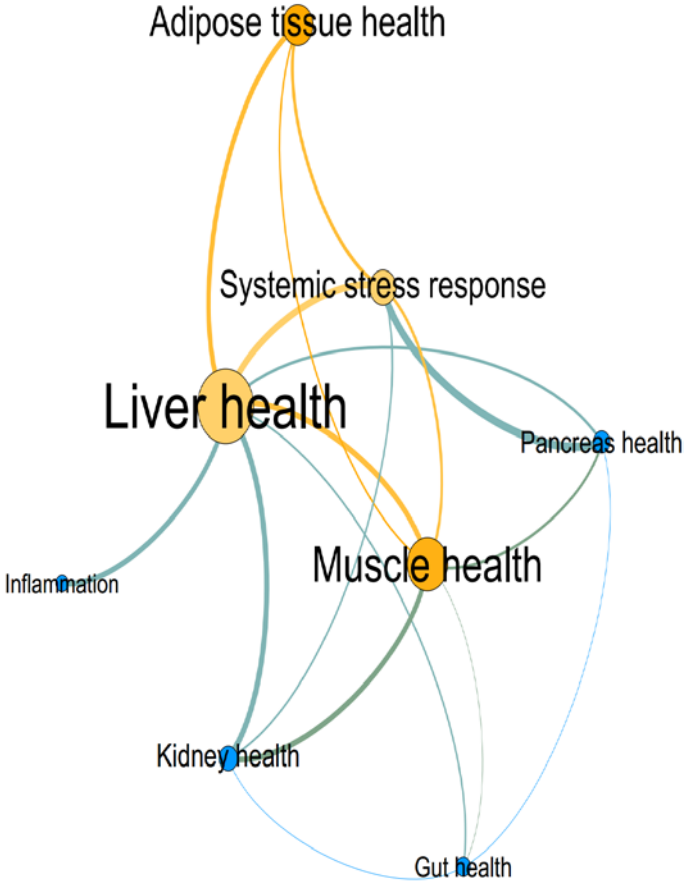


- Caloric restriction modulates several biological processes involved in metabolic syndrome
- ‘Hepatic injury’ and ‘lipokine production’ are modulated strongly by caloric restriction
- ‘protein metabolism’ and ‘systemic insulin sensitivity’ are modulated moderately by caloric restriction

Result at tissue or health domain level

LEGEND

- Size shows amount of biological processes in tissue or health domain
- Edge thickness shows similarity between molecules in tissue or health domain
- Orange coloring = biological processes related to tissue or health domain are significantly modulated by Nutritech caloric restriction intervention
- The higher the intensity of color the higher percentage of biological processes related to tissue or health domain are altered by Nutritech caloric restriction



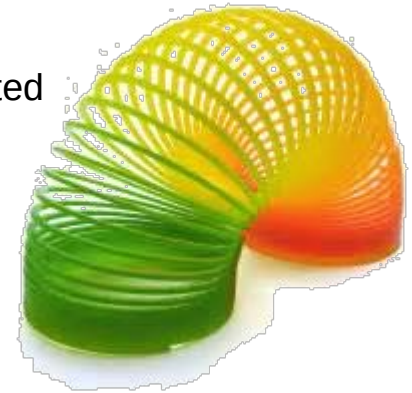
- Biological processes related to adipose tissue and muscle are modulated most by Nutritech caloric restriction

Conclusions

- We were able to create the Metabolic Syndrome reference network of ‘Resilience’ by doing a multiple study analysis and combining this with prior knowledge, thereby allowing the identification of resilience markers of robustness. The multiple layer visualisation helps to simplify the ‘hairball’ at the molecular level into the involvement of biological processes and tissues/health allowing the comprehensively characterization of metabolic syndrome as well as a systems view on this pre-stage of disease.

- This Metabolic Syndrome reference network of Resilience was helpful in the biological interpretation of the results from the Nutritech intervention study. It provided clinical relevant context to relatively unknown nutrigenomics based markers and to the relatively new PhenFlex challenge test responses. It enabled to summarize relatively scattered results into biological meaningful results

- Phenotypic flexibility signature related to metabolic syndrome is partly modulated by Nutritech caloric restriction intervention. Caloric restriction affected multiple biological processes especially related to lipid and protein metabolism as well as liver health.



Take home message

- For diagnosis of health effects of nutrition we need resilience markers of health rather than biomarkers of disease
- These are “multi-biomarker” panels representing defined and accepted health-related processes, that can be modulated with the new PhenFlex challenge test that can lead to next generation of biomarkers
- The PhenFlex challenge discriminates between different states of health and allowed the substantiation of nutritional intervention effects, which could not be found in the traditional way.
- Coming to evidence based substantiation of health effects from food and nutrition structured knowledge & data capture is crucial!
- In this way we can quantify and interpret scattered and subtle health effects of nutrition!

A movie to explain the concept

› phenotypic flexibility movie

Acknowledgements

The Nutritech family!

Special thanks to:

Tim van den Broek
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Sabina Bijlsma
Ruud Boessen
Heleen de Weerd
Carina de Jong-Rubingh
Age Smilde
Margriet Hendriks
Jildau Bouwman


THANK YOU FOR YOUR
ATTENTION
TNO innovation
for life