Metabonomics in nutritional studies

–Metabolic effects of soybean and grapefruit consumption

Jon Gabrielsson

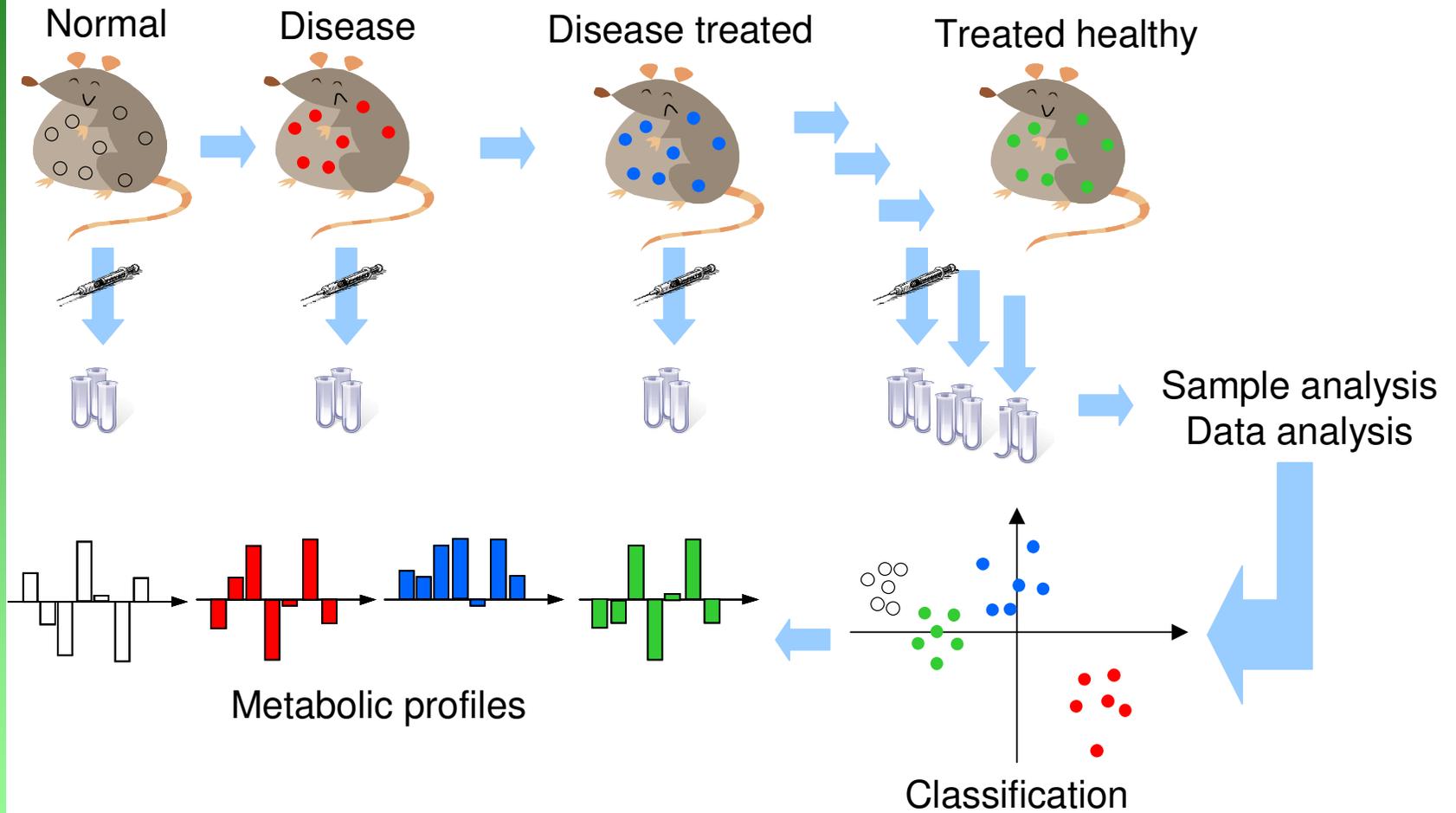AcureOmics AB

AcureOmics - Background

- Founded in September 2007
- Situated in Umeå, Sweden

- Core expertise
  - ❑ Chemometrics – the philosophy and its applications
  - ❑ Metabonomics – planning of experiments and multivariate data analysis in pharmaceutical R&D, diagnostics, etc

- Privately held company
  - ❑ Three parties with complementing expertises

- Has SME* status

*Small, Medium sized Enterprise

AcureOmics - Company profile

- A company with unique competence in the "omics" field of data analysis and interpretation, adding value to customers projects by increasing the information gained.
  - ❑ A contract research organisation (CRO) performing analysis according to customer specifications
    - ✓ AnaMar, Betagenon
  - ❑ Expand partnering/research collaborations for longer projects/assignments for developing diagnostic kits and target assays
    - ✓ Rheumatology clinic NUS, Gottfries Clinic AB, KPL Good Food Practice
  - ❑ Workshops/Courses directed to the field of "omics" related to biological systems and/or endpoints

Metabonomics at a glance

Normal Disease Disease treated Treated healthy

Sample analysis
Data analysis

Metabolic profiles

Classification

Functional foods study

- EU legislation extended to encompass health claims*
  - ❑ Claims regarding the nutritional or physiological effects of a product must be scientifically provable

- Nuclear Magnetic Resonance (NMR) spectroscopy to detect metabolism changes due to food supplement

- Goal: Effect of food supplement?
  - ❑ If any, what metabolites?
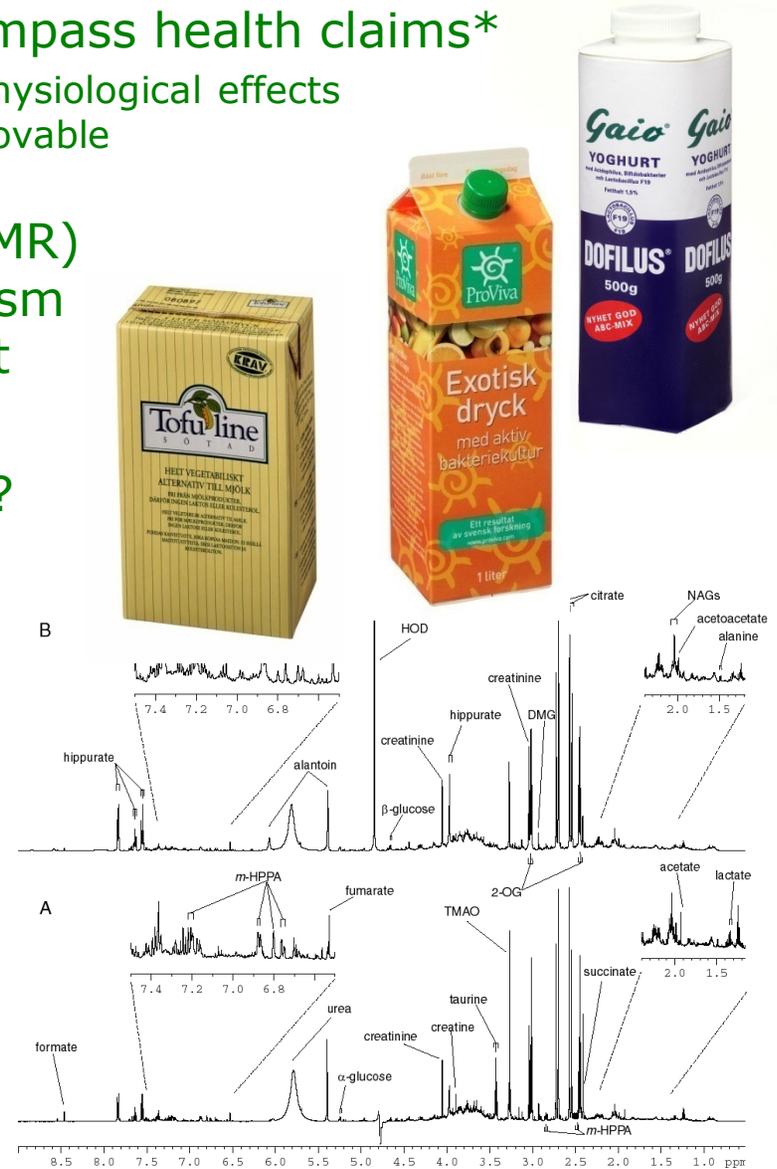
*1st July 2007, Health Claims Regulation [*Regulation (EC) No 1924/2006*

Study design

- Selection of healthy volunteers
  - ❑ 25 men were invited to a screening test, filled out questionnaire
  - ❑ Inclusion/Exclusion criteria included e.g. smoking, medication, dieting, health foods, BMI
 - ❑ 9 healthy individuals were selected for the study
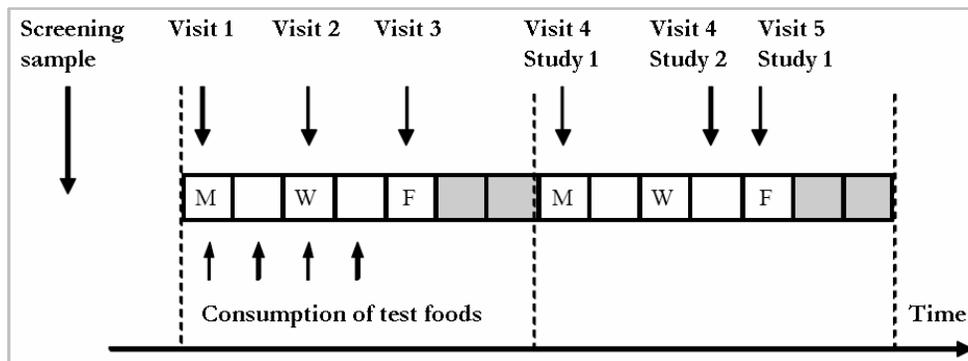
- ❑ Given prepared foodstuff for consumption
- ❑ Study performed on two occasions
  - May (study 1)
  - June (study 2)
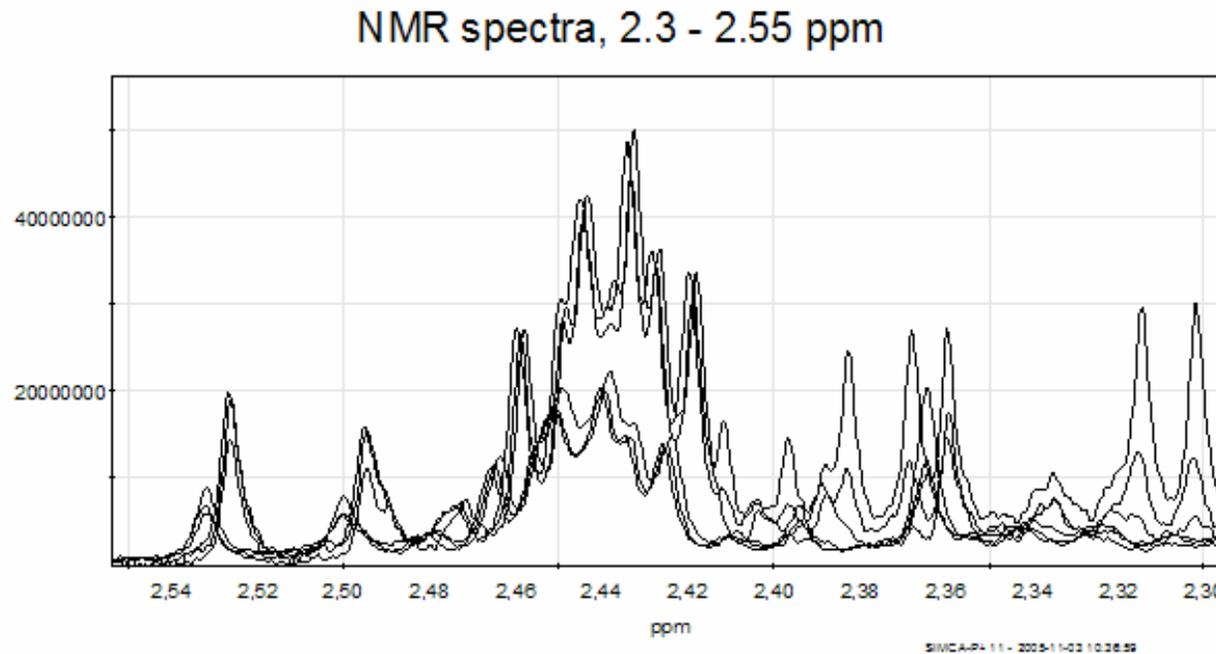- ❑ Multiple visits – document effect over time

Sample analysis

- 9 healthy individuals sampled over time, 47 samples in total
  - Bruker AMX-II 500MHz instrument
  - NMR shifts, K = 32 768 variables

- Specific analysis on subset
  - PCA* of questionnaire and screening test (BMI, age, clinical chemistry)
  - Four representative subjects were chosen according to multivariate design (MVD) for a deeper analysis of a few specific endogenous metabolites
  - For validation of metabolomics model

Multivariate design

Factorial design (2^2)

Object	t1	t2
1	-	-
2	+	-
3	-	+
4	+	+

PCA score plot
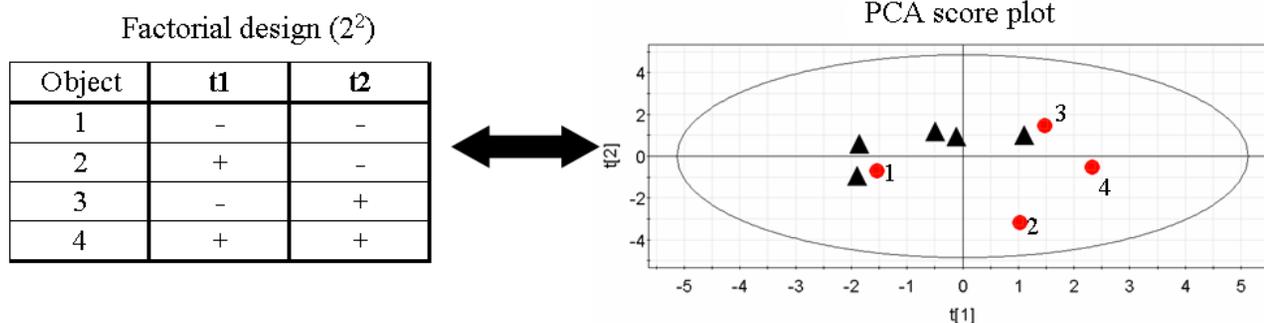
*Principal Component Analysis (PCA)

Overview of all individuals/samples
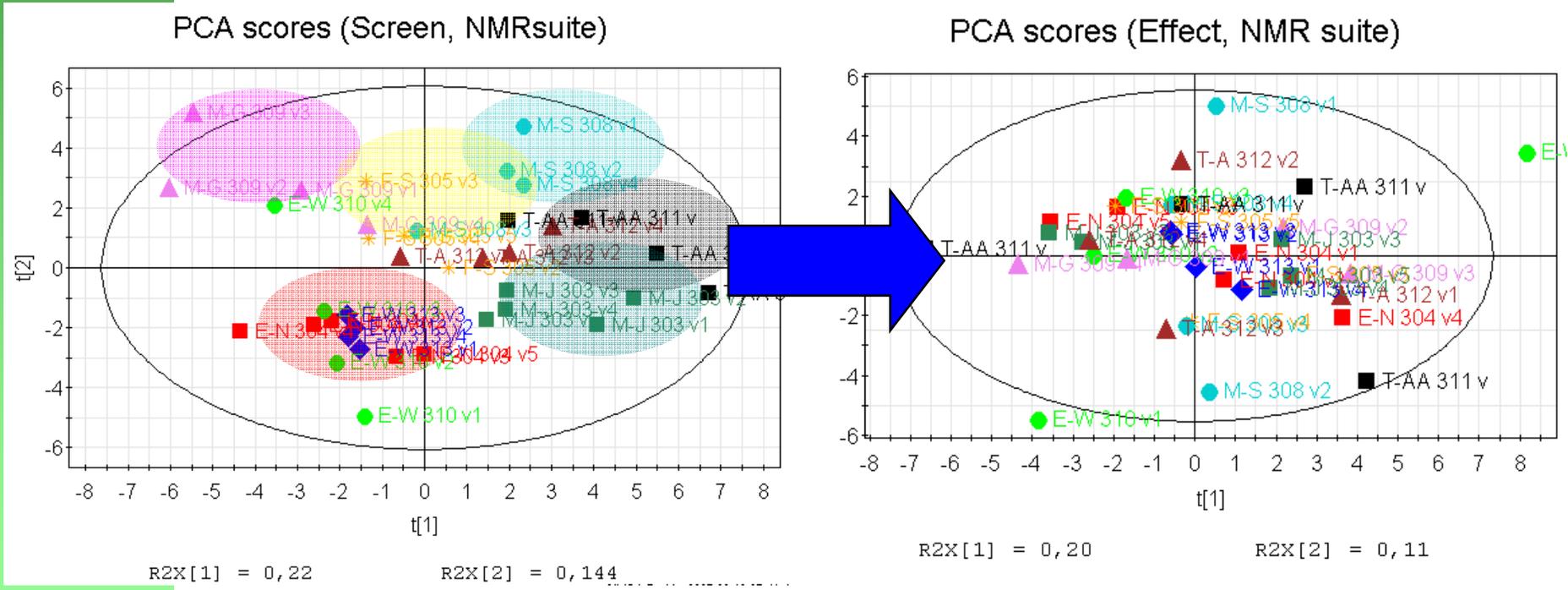

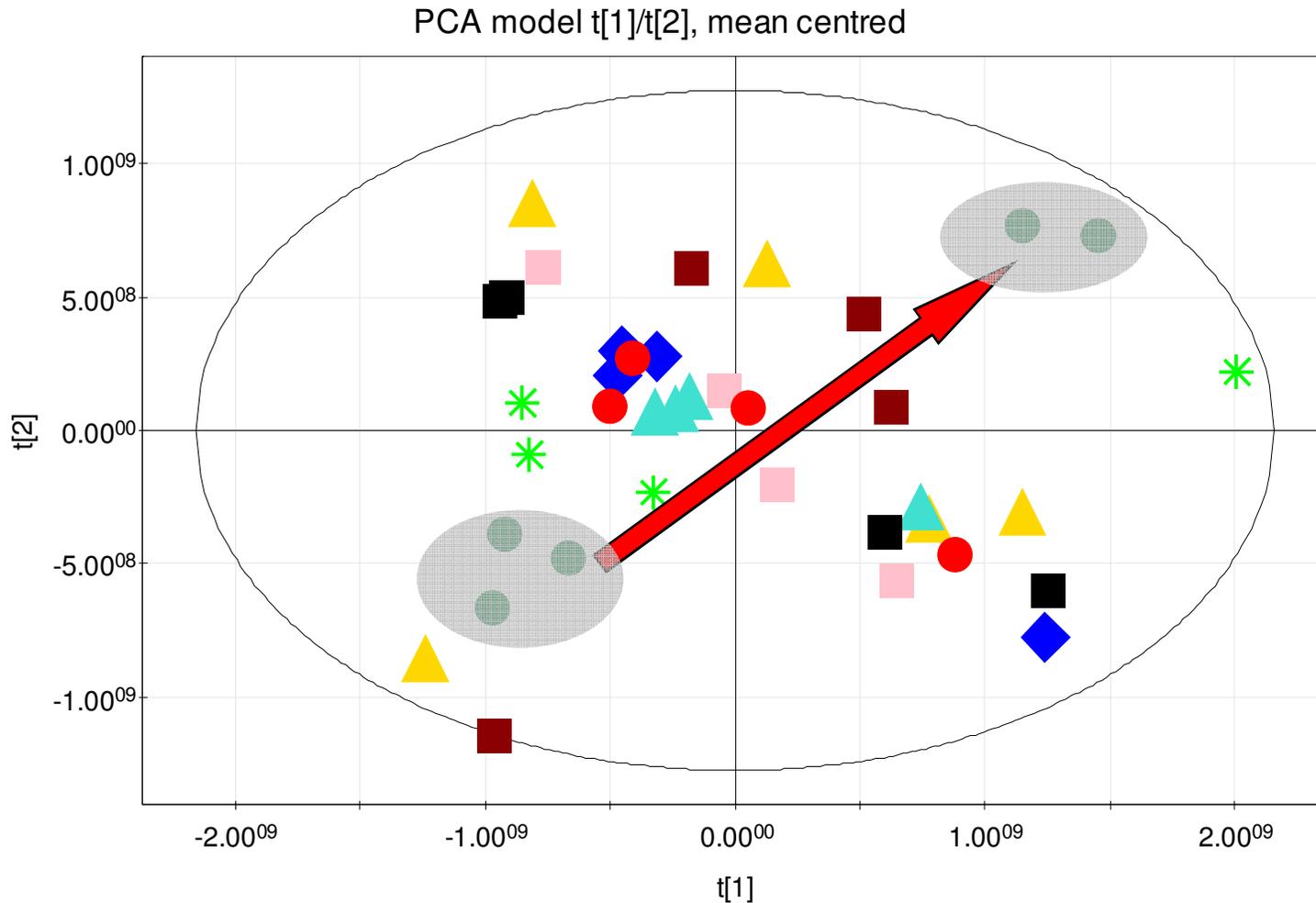NMR spectra, 2.3 - 2.55 ppm

Clear separation.
But ☹ … due to different sampling periods

Modelling dynamic metabolomic time series data

- Assumption 1: The metabolic baseline can be different over all individuals
- Assumption 2: The metabolic effect of the treatment is similar over all individuals
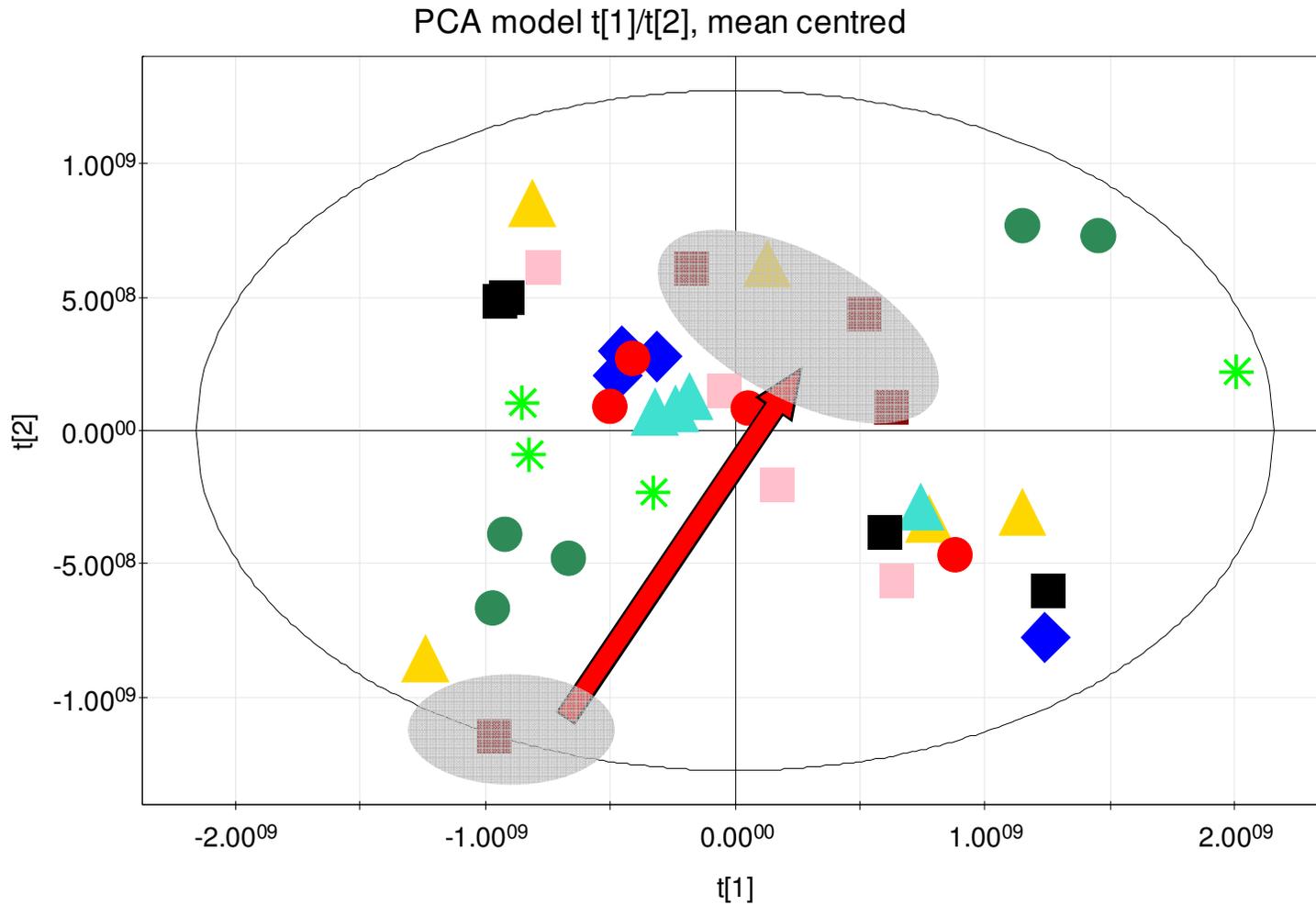

PCA scores (Screen, NMRsuite)

R2X[1] = 0,22   R2X[2] = 0,144

PCA scores (Effect, NMR suite)

R2X[1] = 0,20   R2X[2] = 0,11

Overview of all individuals/samples
... after pre-processing/filtering

PCA model t[1]/t[2], mean centred

R2X[1] = 0.662086   R2X[2] = 0.231252
Ellipse: Hotelling T2 (0 95

SIMCA-P 11 - 10/09/2008 10:01:19

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Individual metabolic profile, example, Individual 1

1. Identify direction of maximum change from pre-dose – PCA

2. Assign discrete values
   - One (1) for maximum effect
   - Zero (0) for other observations

3. Calculate OPLS-DA
   - Interpret
   - Validate

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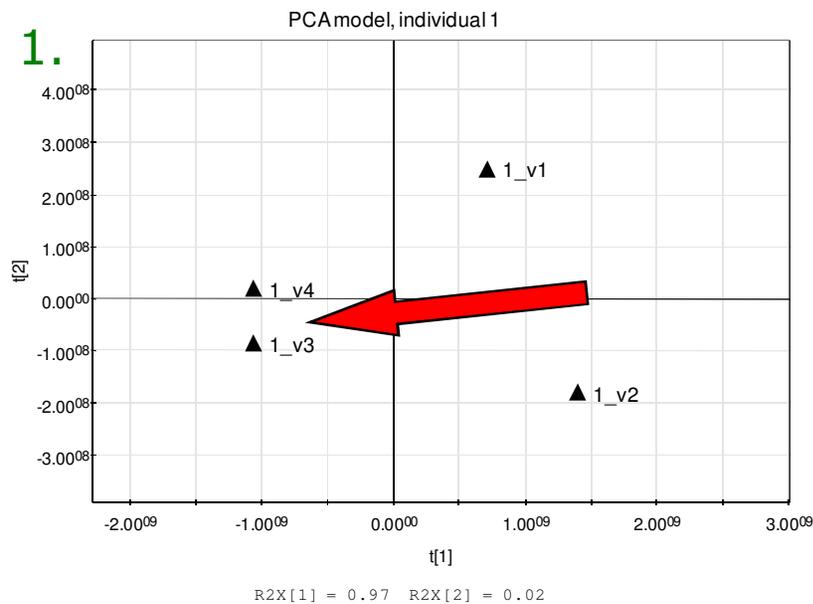# Individual metabolic profile, example, Individual 1

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   - ❑ Validate

1.

PCA model, individual 1

R2X[1] = 0.97   R2X[2] = 0.02

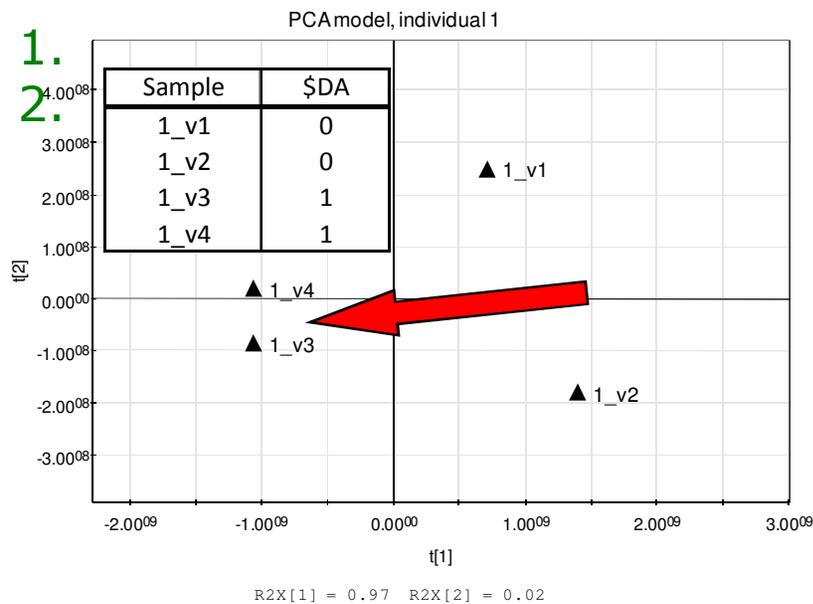# Individual metabolic profile, example, Individual 1

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3. Calculate OPLS-DA
   - ❑ Interpret
   - ❑ Validate

1.
2.

PCA model, individual 1

Sample	$DA
1_v1	0
1_v2	0
1_v3	1
1_v4	1

▲ 1_v1

▲ 1_v4

▲ 1_v3

▲ 1_v2

t[2]

t[1]

$R2X[1] = 0.97$ $R2X[2] = 0.02$
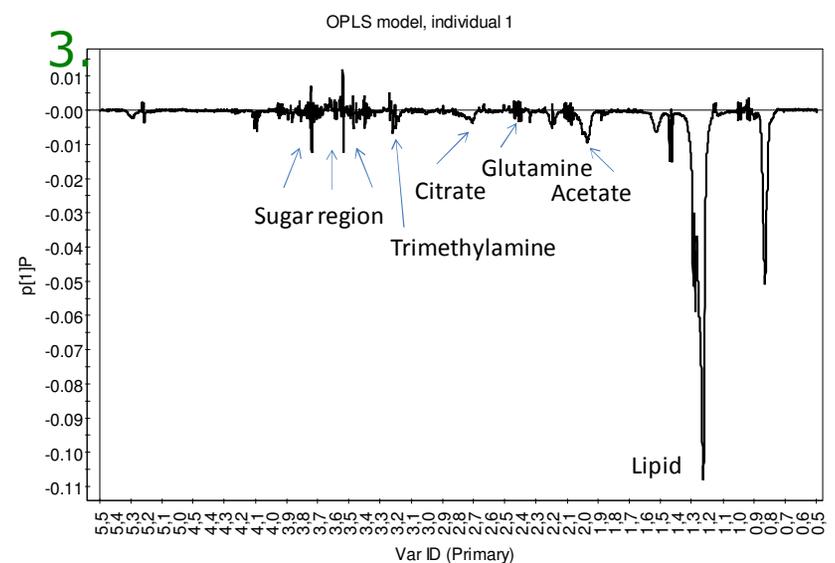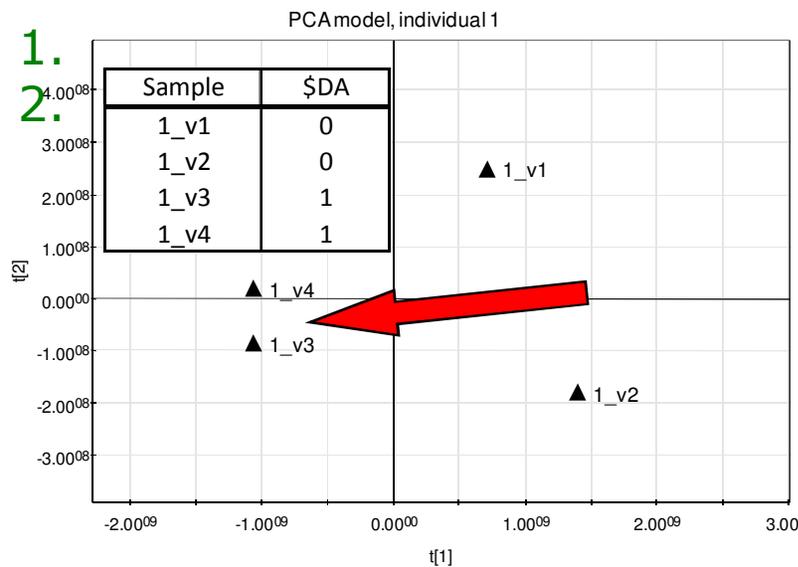
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1.
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PCA model, individual 1

Sample	$DA
1_v1	0
1_v2	0
1_v3	1
1_v4	1

▲ 1_v1

▲ 1_v4

▲ 1_v3

▲ 1_v2

t[2] axis: 4.00⁰⁸, 3.00⁰⁸, 2.00⁰⁸, 1.00⁰⁸, 0.00⁰⁰, -1.00⁰⁸, -2.00⁰⁸, -3.00⁰⁸

t[1] axis: -2.00⁰⁹, -1.00⁰⁹, 0.00⁰⁰, 1.00⁰⁹, 2.00⁰⁹, 3.00

3.

OPLS model, individual 1

Sugar region
Citrate
Trimethylamine
Glutamine
Acetate
Lipid

p[1]P axis: 0.01, -0.00, -0.01, -0.02, -0.03, -0.04, -0.05, -0.06, -0.07, -0.08, -0.09, -0.10, -0.11

Var ID (Primary)

R2X[1] = 0.92
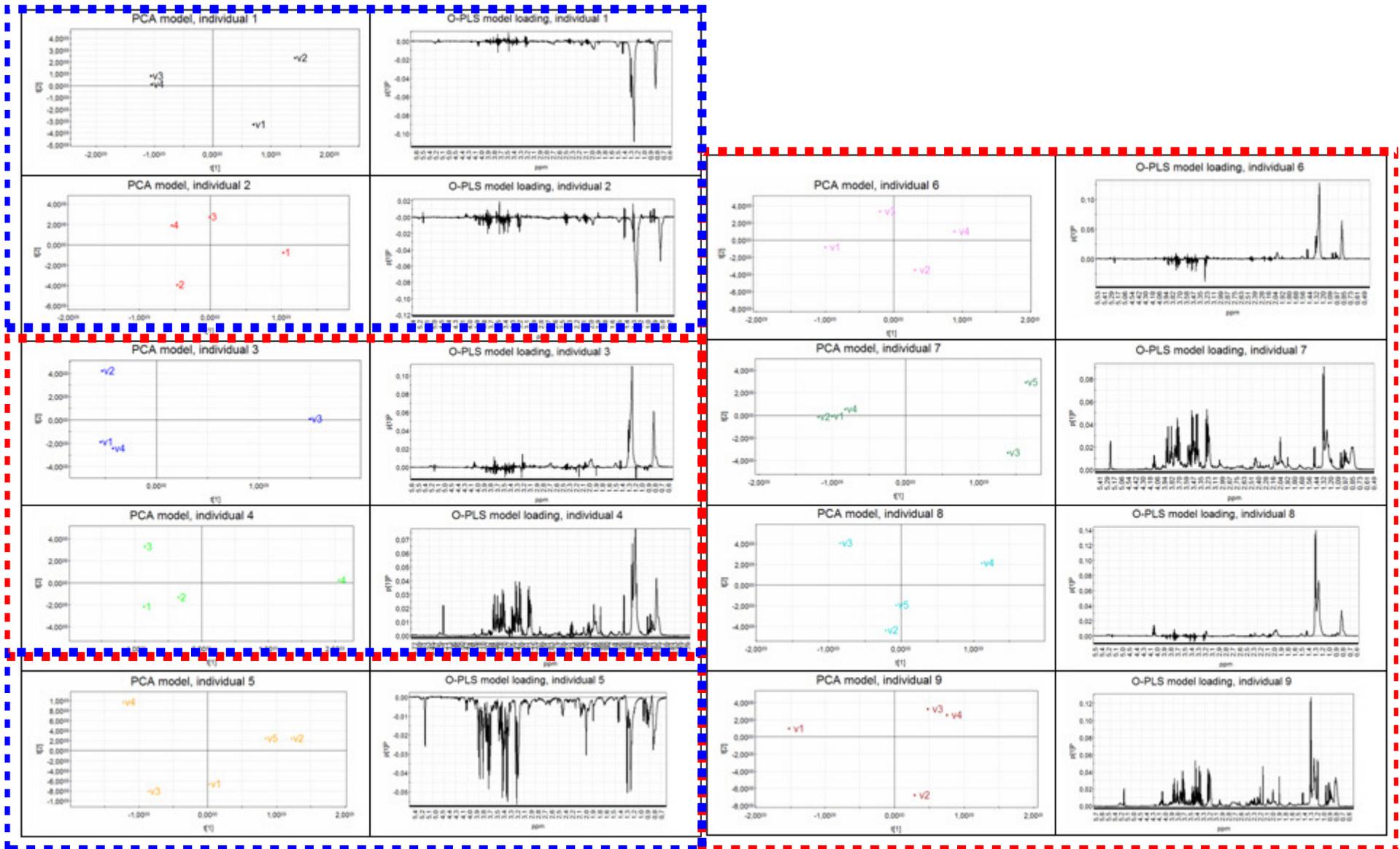
SIMCA-P 11 - 11/09/2008 11:13:11

*Bylesjö, M.; Rantalainen, M.; Cloarec, O.; Nicholson, J. K.; Holmes, E.; Trygg, J.,
OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification.
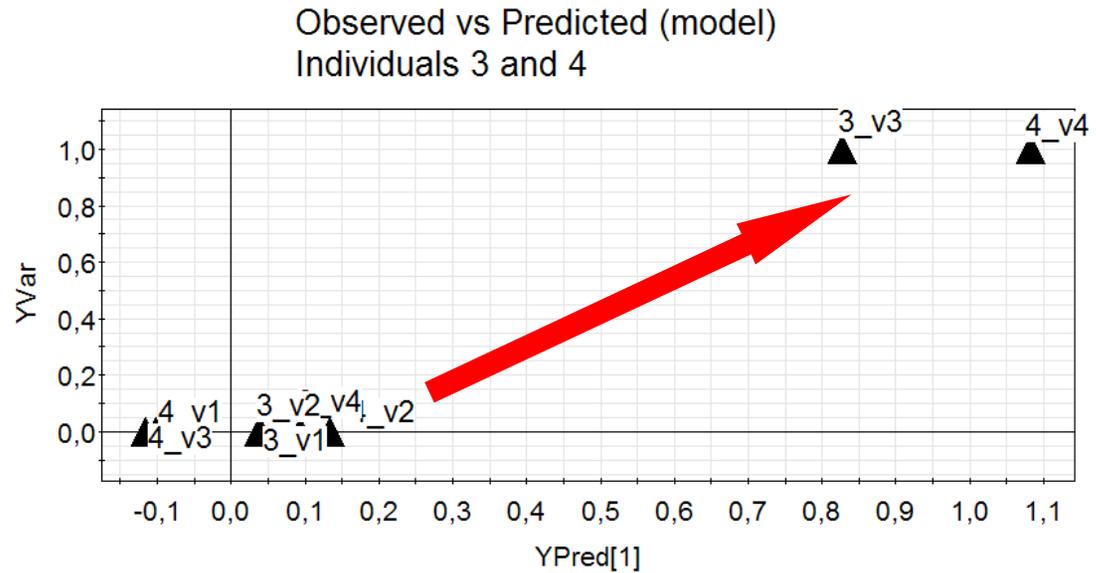*J. Chemometrics*, 20, 341-351 (**2006**).

Metabolic profiles for each individual
Assumption: the effect of foodstuff treatment

Prediction of effect using NMR profile

Observed vs Predicted (model)
Individuals 3 and 4

Model

Prediction
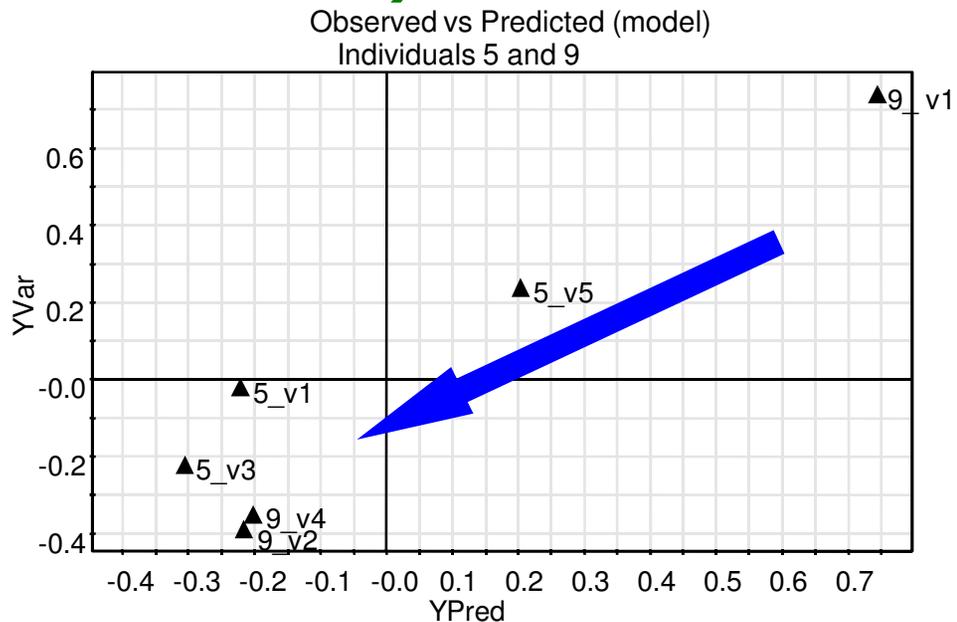
Observed vs Predicted (Prediction set)
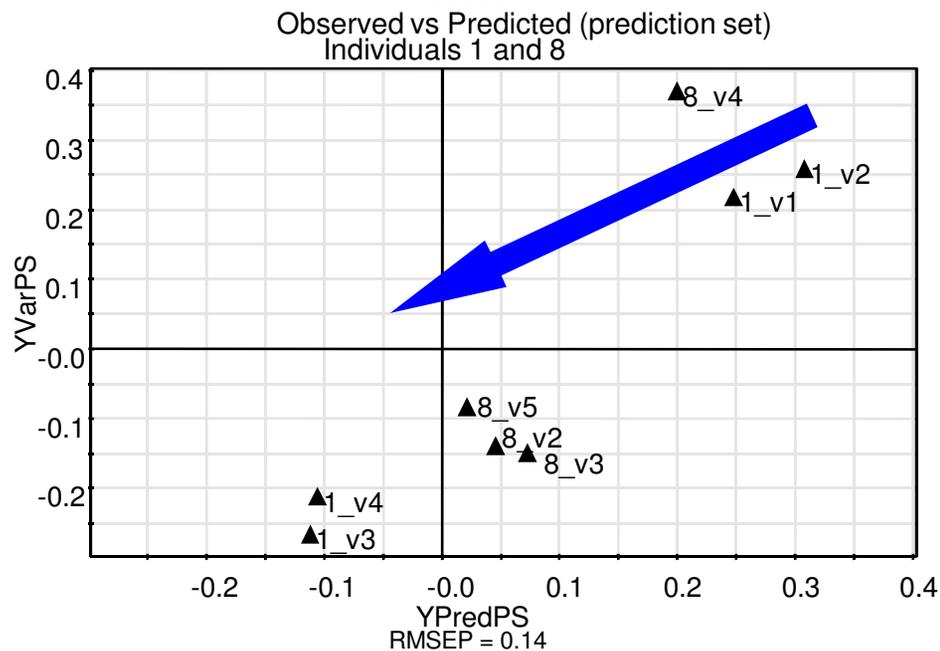Individuals 6,7,8,9
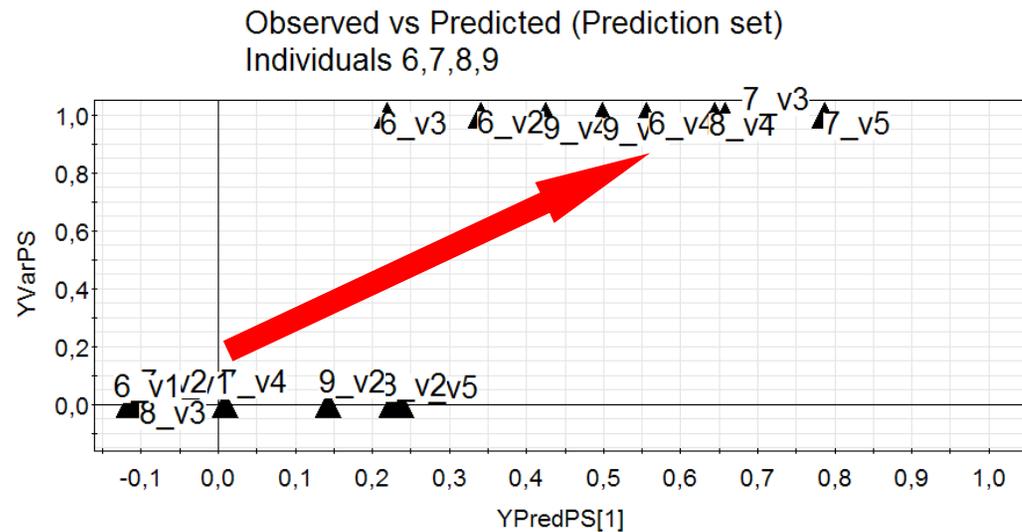
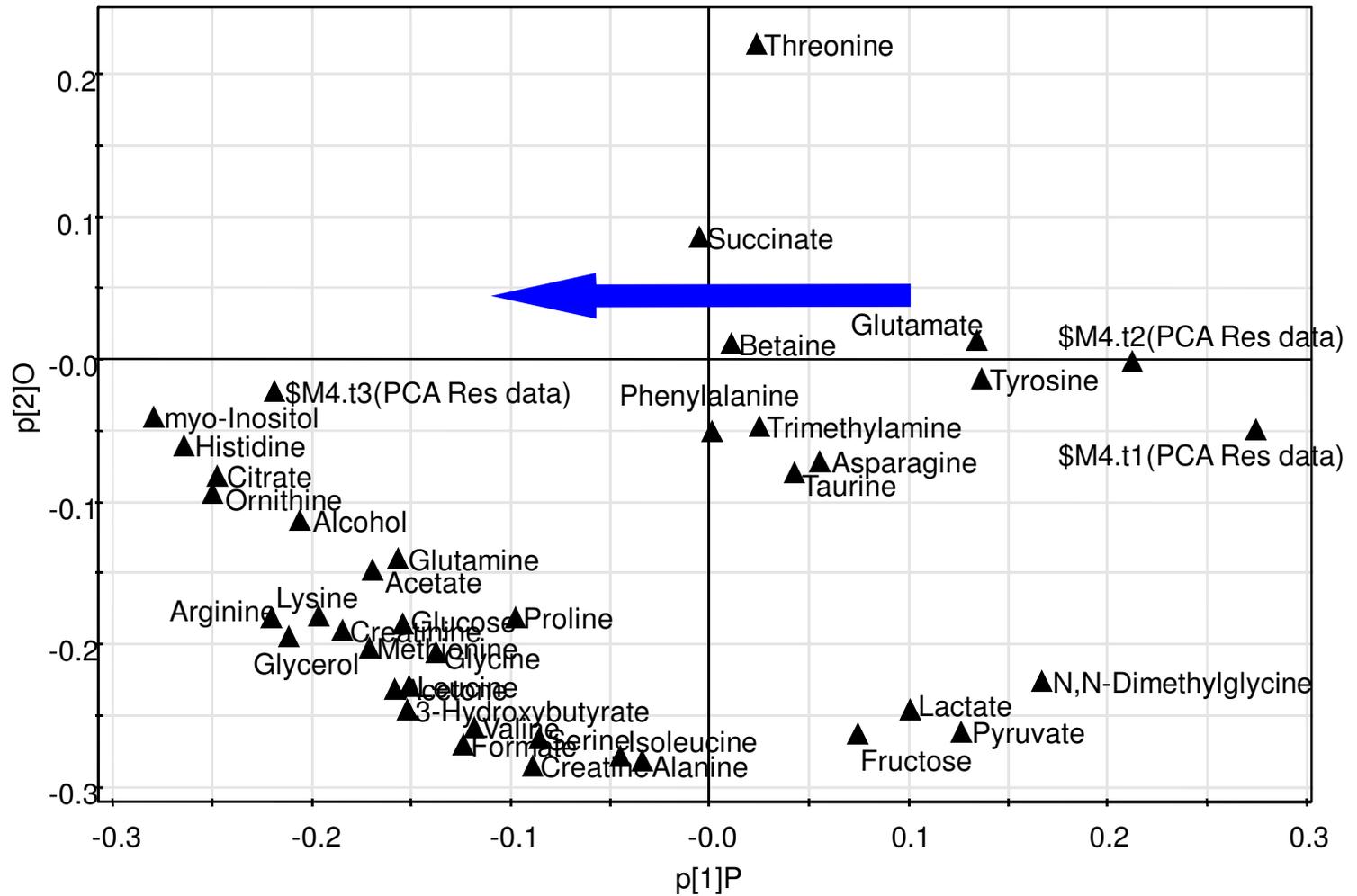# Prediction of health effect in endogenous metabolites ("biomarkers")
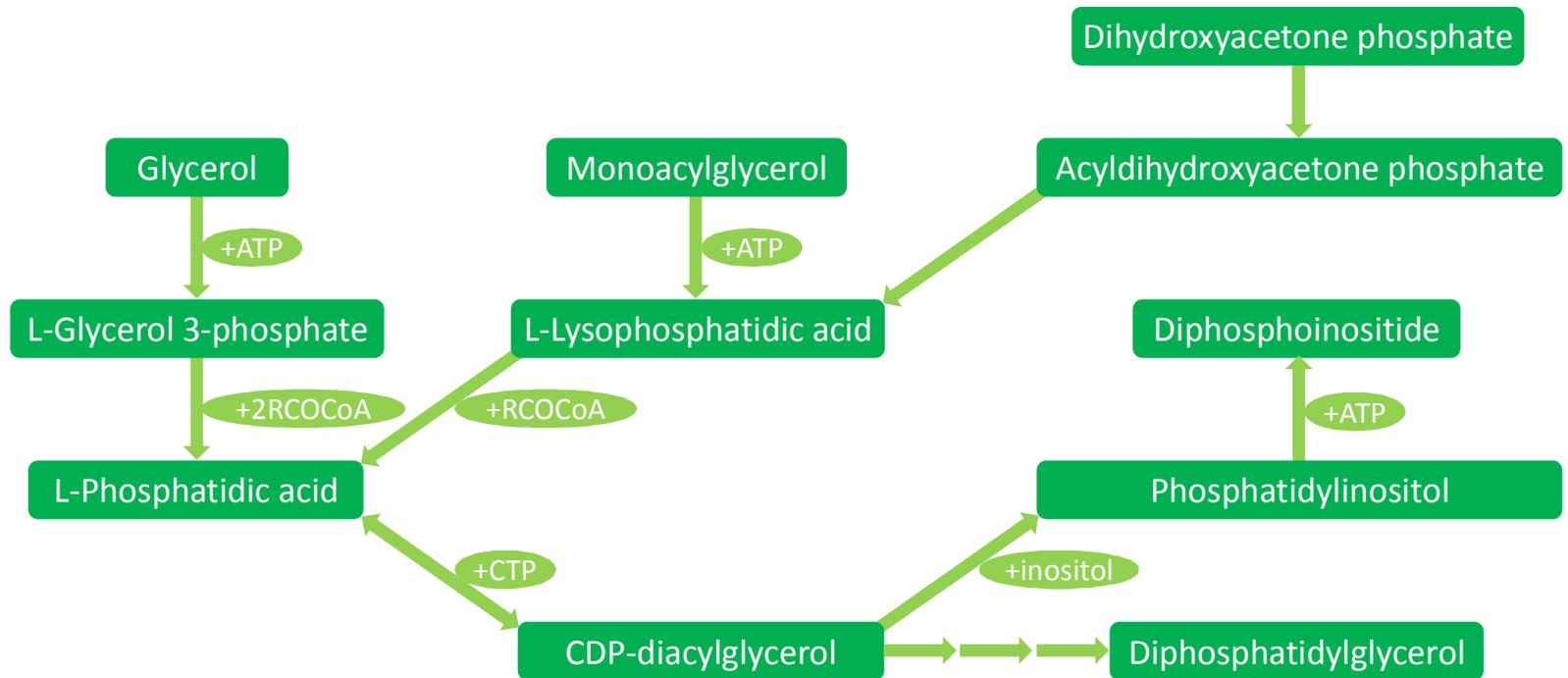
Model

Prediction

Analysis of health effect in endogenous metabolites ("biomarkers")

R2X[1] = 0.31   R2X[2] = 0.35

Metabolic pathways of important endogenous metabolites

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Conclusions and Discussion

- Increase in myo-Inositol
  - ❑ Shows the study subjects have actually conformed
- Decrease in triglycerides
  - ❑ Observed effect over time
- …Health effect of food supplement established!

- Usefulness of MVD and dynamic sampling demonstrated
  - ❑ Selection of representative subjects in heterogeneous material
  - ❑ Allows for identification of slow and fast responders

- Possibilities for development of functional foods
  - ❑ Documented proof of physiological health effect
  - ❑ Target identification

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- Per Lek, Acure Pharma
- Thomas Moritz, UPSC
- Chenomx Inc.
- Tor Ny, UBI

Questions or comments?

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