“Impact of smoking cessation on the metabolic profile of former smokers"

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2015 SSPT CORESTA CONGRESS
Jeju Island, South Korea

4-8 October 2015
ST13

Overview

Theoretical section

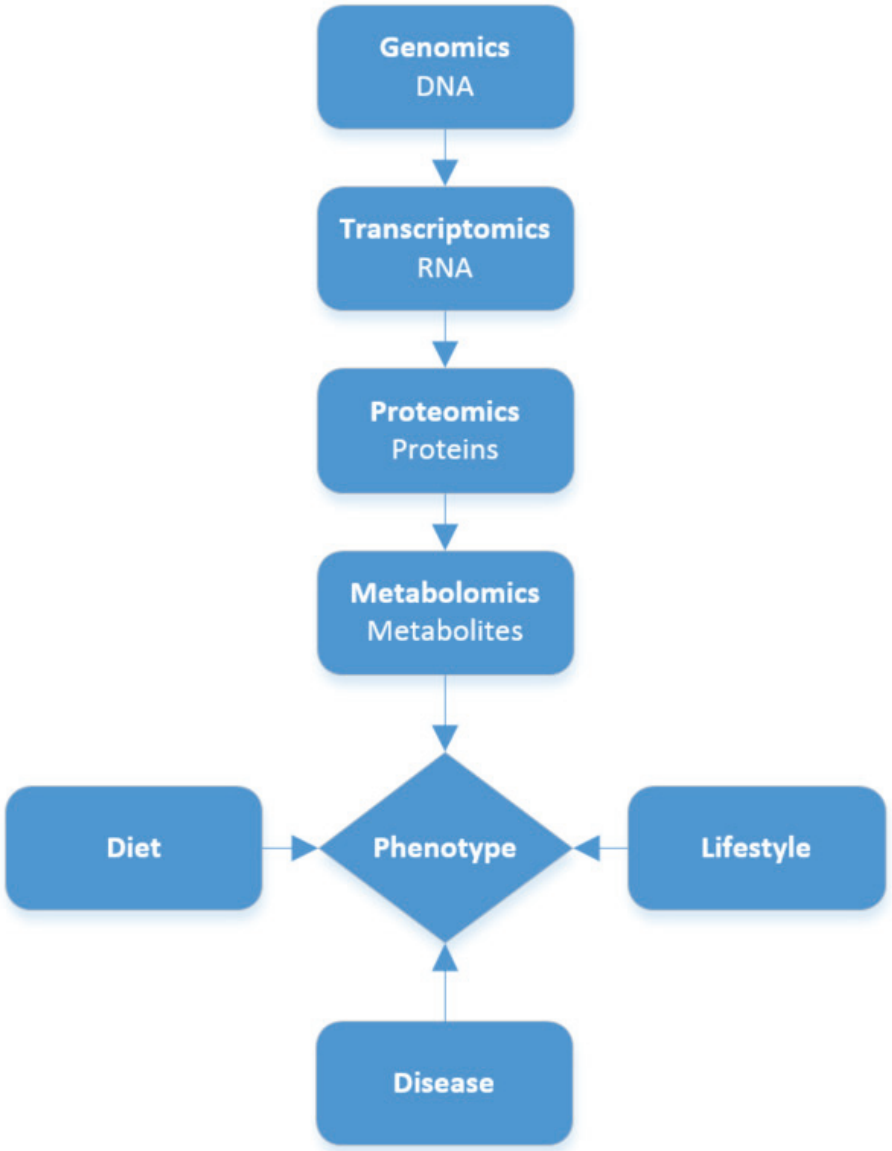
- Introduction to metabolomics
- Review of 1st metabolomic study at ABF
- Objectives

Experimental section

- Study design 2nd metabolomic study
- Compliance
- Plasma fingerprinting

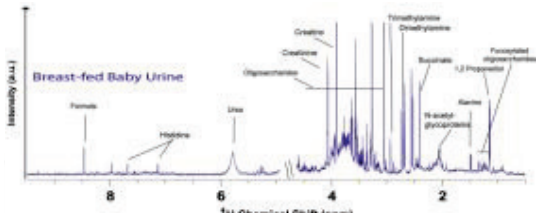
Summary

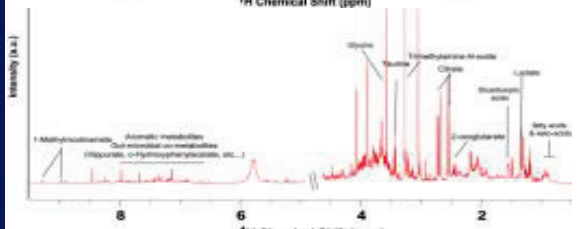
Cascade of ‘omics‘



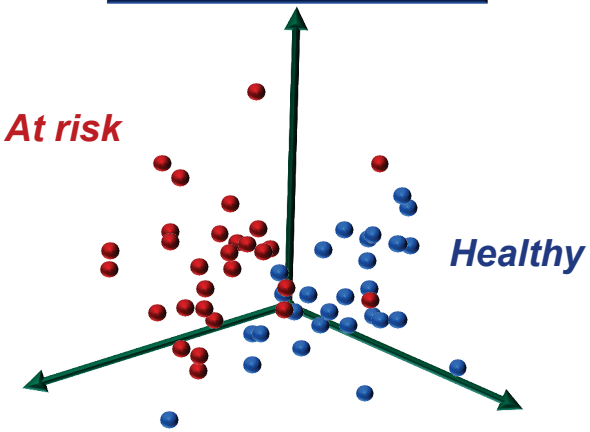
- The **metabolome** represents the entity of all ‘small’ molecules (< 1500 Dalton) and is most predictive of the phenotype of an organism.
- **Metabolomics** is the study of the entire set of small molecules in a biological sample.
  - Identification
  - Quantification
  - Validation

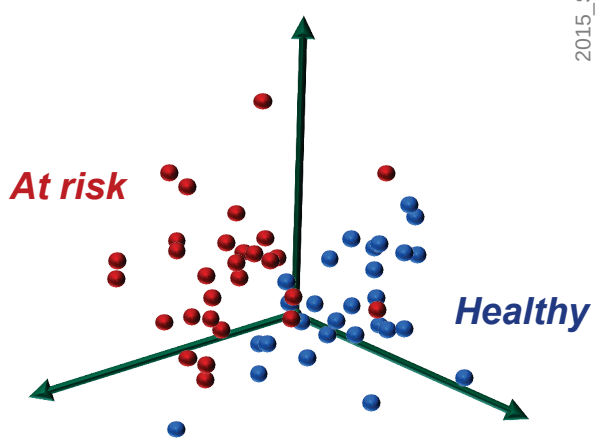
Why metabolomics?

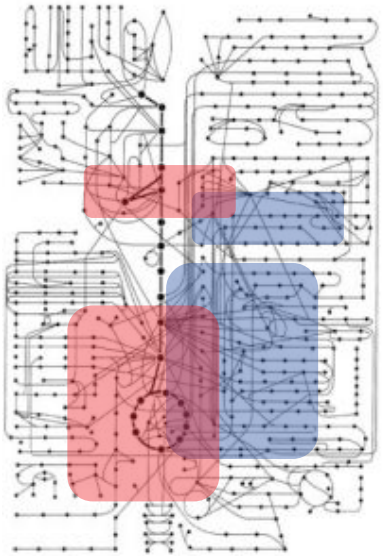


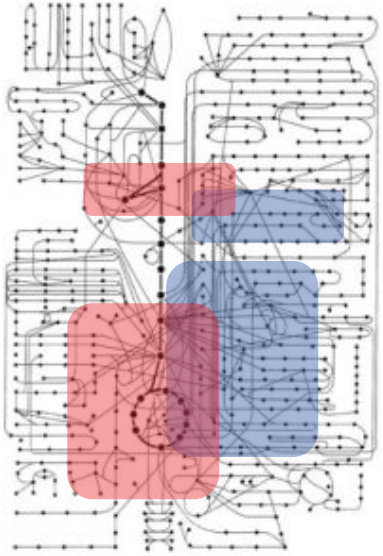


BIOMARKERS



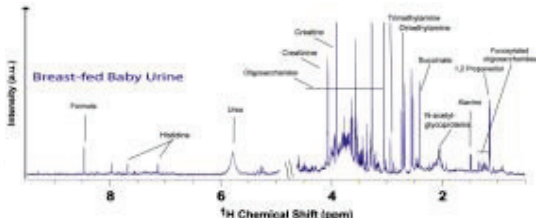


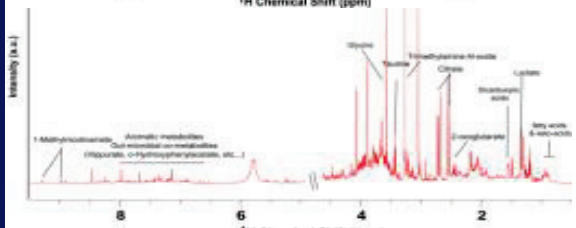


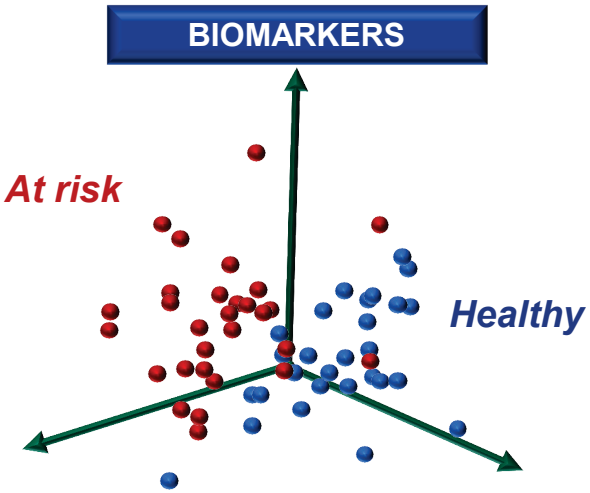


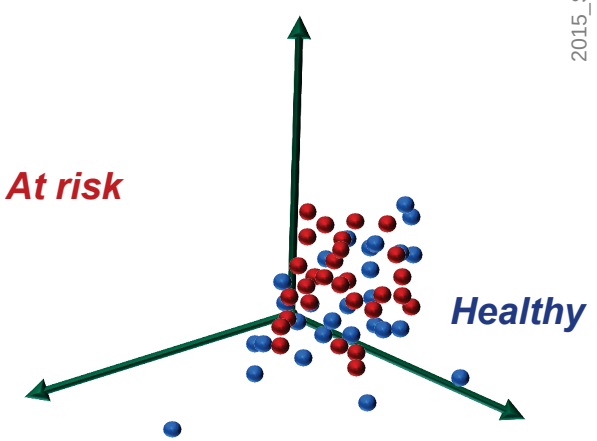
Differences in metabolic pathways

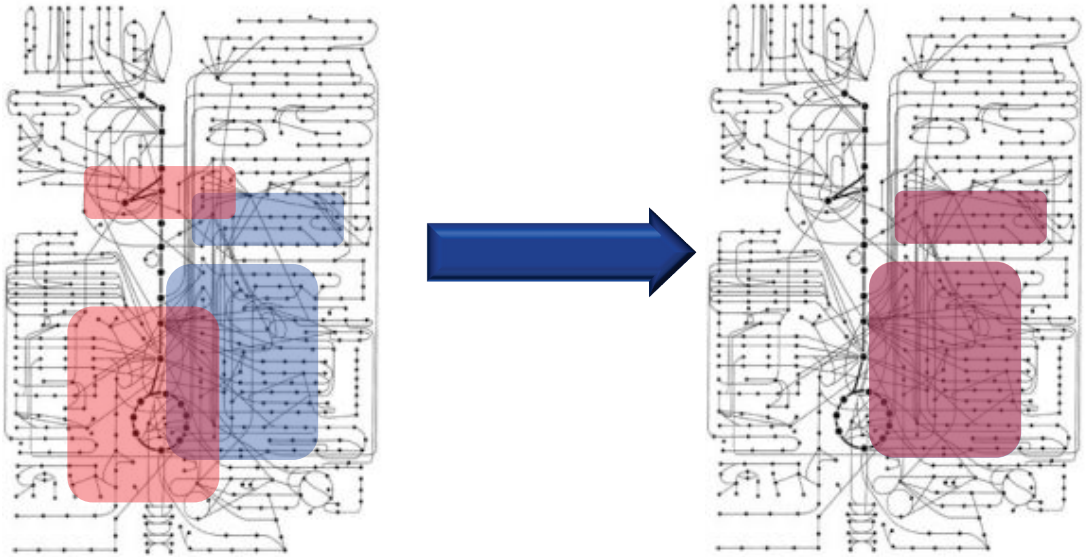
Why metabolomics?



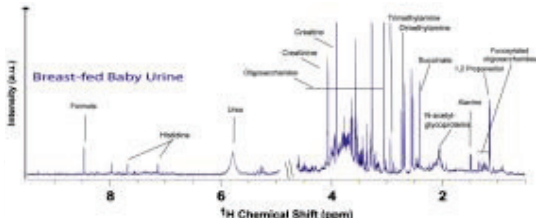


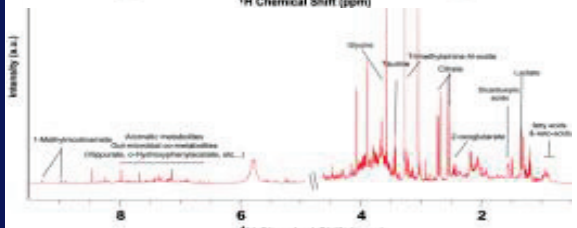


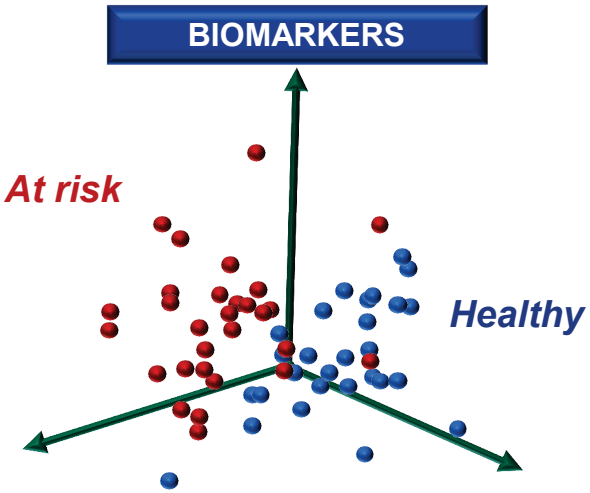


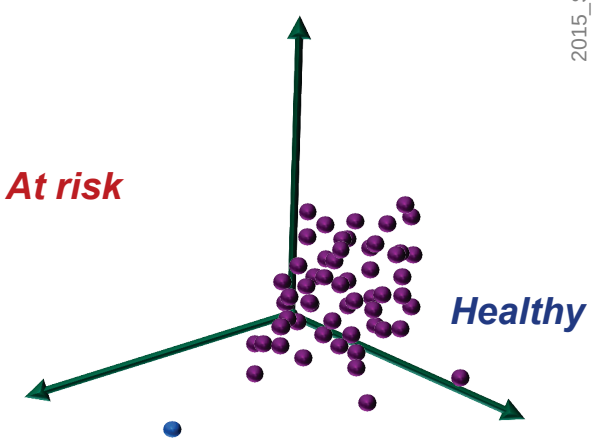


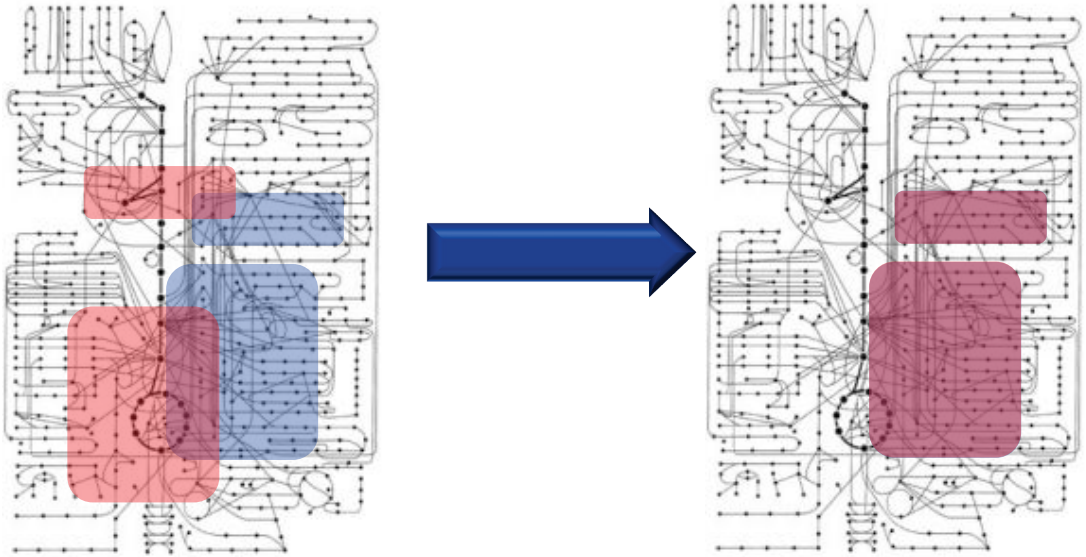
Why metabolomics?











Metabolomic strategies

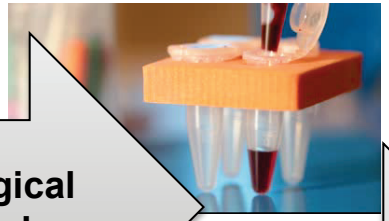
Metabolomic fingerprinting:

- Untargeted screening and identification of as many metabolites in a sample as possible
- ideally identification of the related metabolic pathways.

Metabolomic profiling:

- Identification and quantification of a selective number of predefined metabolites, which are normally related to a specific metabolic pathway.

GC-TOF-MS: Analytical workflow for the untargeted biomarker identification



Biological Sample

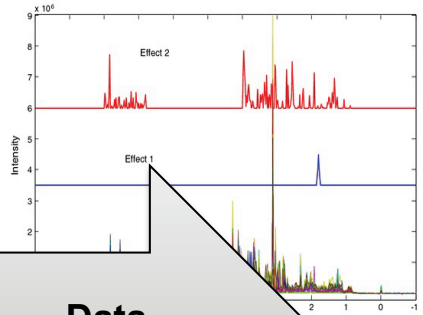
Urine,
Plasma,
Saliva

Sample clean-up

- IS-spiking (d_4-*tt*MA)
- Urea digestion (only Urine)
- Protein precipitation
- Methoximation, Silylation

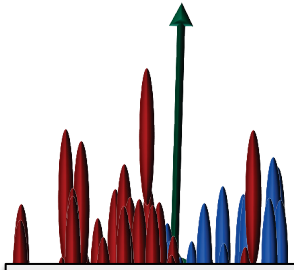
MS Analysis

- GC-TOF-MS (Almsco)
- GC: Dimethyl-polysiloxane (30m x 250µm i.d.)



Data processing

- Baseline correction ProtoTOF (Almsco)
- Mass detection, peak alignment *(Mzmine)*
- Normalization to IS



Statistical analysis & target hit identification

Statistics: PLS-DA
Mann-Whitney-U test, fold change
Identification:
Deconvolution,
Databases (NIST, Golm)
Reference compounds

Mueller et al. JPR, Dec 2013.
Müller et al. JCB, Mar 2014.

Recap: Main finding from Metabolomics study 1

- *A diet controlled clinical study with 25 smokers and 25 non smokers was conducted*
- *We successfully established a GC-TOF-MS metabolomics fingerprinting platform*

Plasma and urine:

- *Mainly alterations in fatty acid, amino acid and energy metabolism*

Saliva:

- *Mainly alterations in tyramine, purine, lipid and energy metabolism*

Objectives of study 2

- *Clinical study: smoking cessation*
- *Untargeted metabolomic fingerprinting by GC-TOF-MS*
  - *In plasma, saliva and urine*
- *Identify altered biochemical pathways and biomarkers for smoking cessation*
- *Establish targeted methods (e.g.: fatty acids, amino acids ,...)*

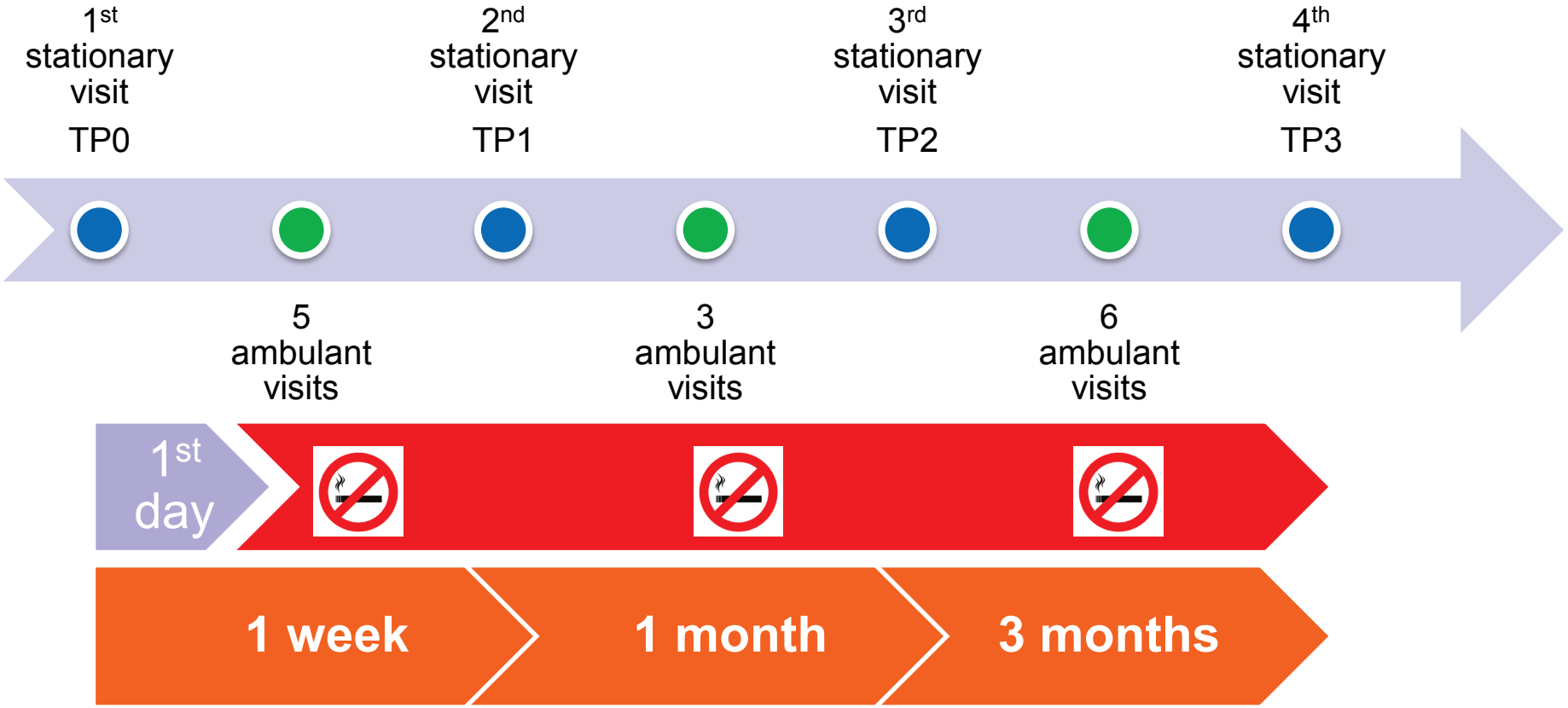
Study design of smoking cessation study

- *60 healthy volunteers*
  - *Male: age 20-50*
  - *BMI 18-29*
  - *Smoker: >15 cigarettes / day during the last year*
  - *Strong intention to stop smoking*

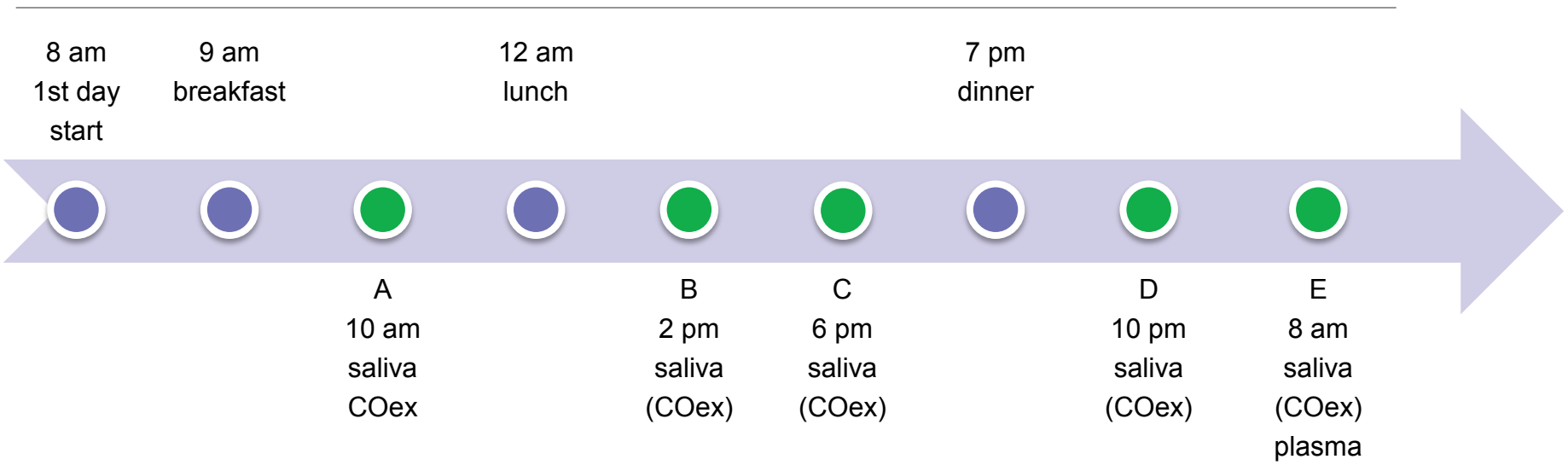
- *3 months:*
  - *Study start (first group in): January 14th 2015*
  - *Study end (last group out): June 23rd, 2015*

- *After first day subjects have to quit smoking*
- *4 x 24 hour stationary visits*
  - *Controlled diet*
- *Several ambulant visits to assess compliance of the subjects*

Study timelines



Study design: stationary visits (N=4)



- 24 h-urine sample from each subject generated from the 3 fractions
- EDTA-plasma: cooled vacutainer, immediately centrifuged 4°C, frozen with dry ice
- Saliva: modified unstimulated spitting method by *Navazesh, ANN NY ACAD SCI, 1993.*

Compliance measurement

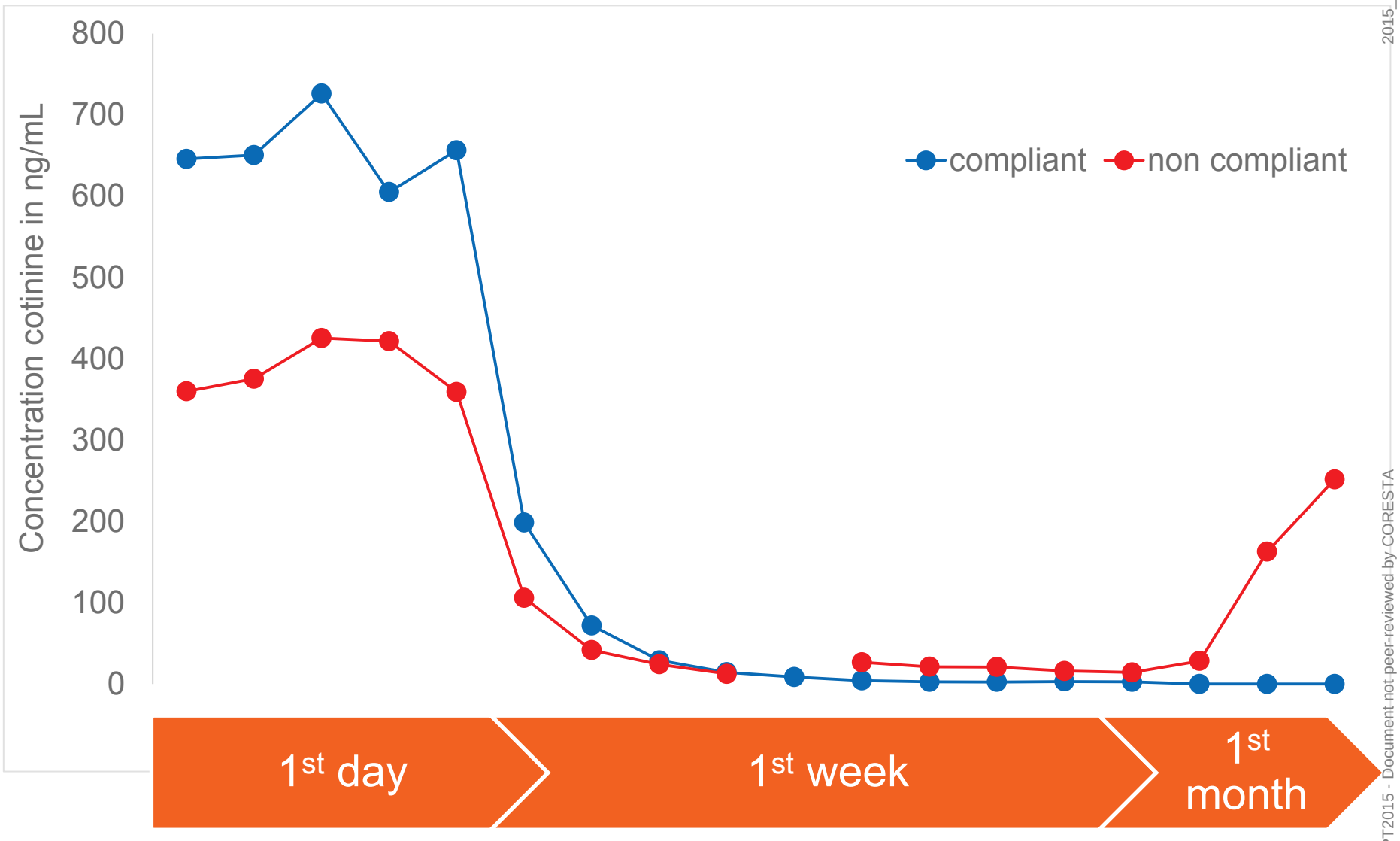
Biomarkers for nicotine and tobacco product consumption:

- *Carbonmonoxide in exhaled breath (COex)*
- *Cotinine in saliva*
- *Urinary cotinine for confirmation*

Study exclusion criteria

- *COex > 5 ppm*
- *Salivary cotinine > 15 ng/mL*
- *Urinary cotinine > 50 ng/mL*

Compliance

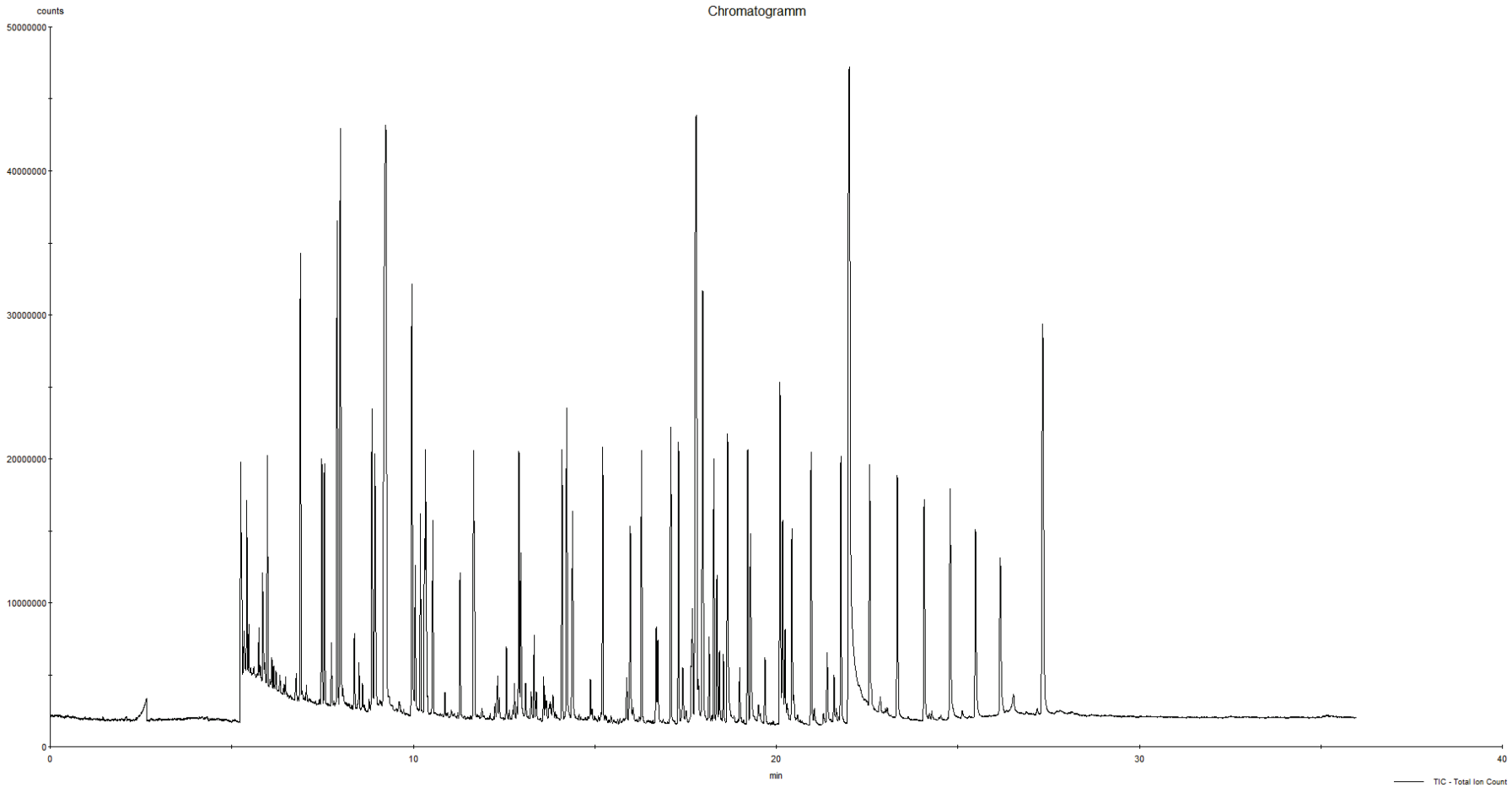


Study completion

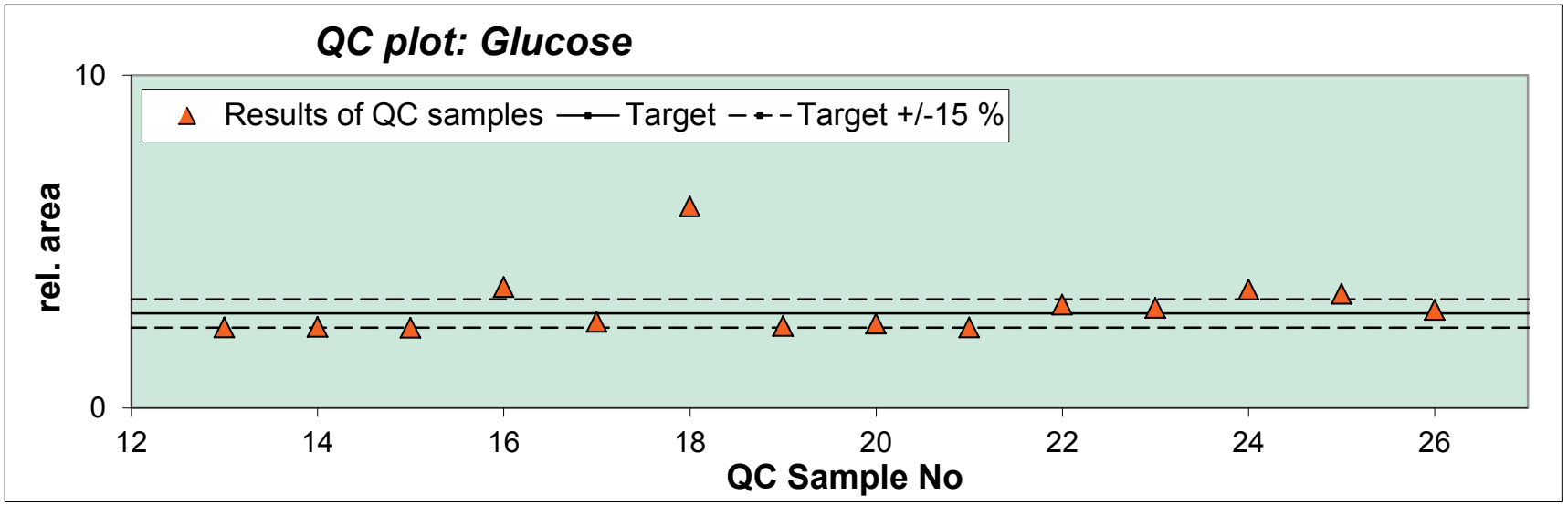
Included subjects:	60
Drop outs:	21
Compliant subjects	**39**

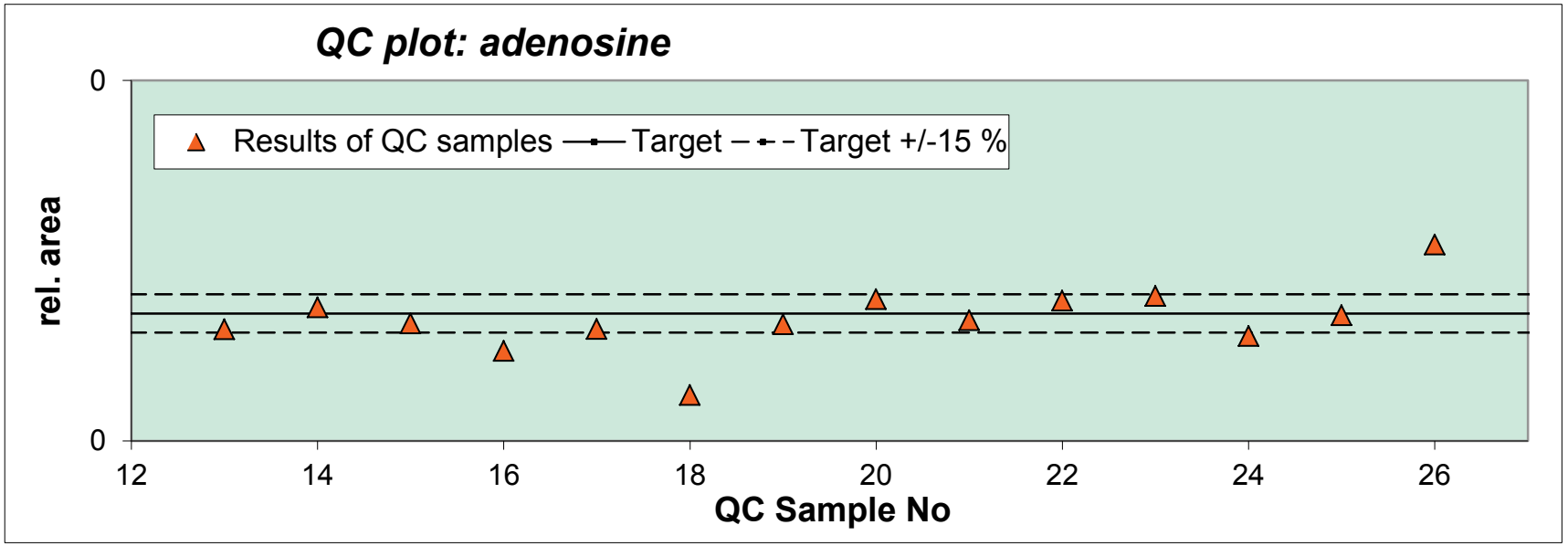
Dropout reasons:	Number
➢ Protocol violation (e.g. too high COex)	4
➢ Started smoking (self reported and/or detected)	12
➢ Withdrawn study agreement	3
➢ Missed visit	2

Plasma fingerprinting chromatogram



Plasma fingerprinting – Quality assurance

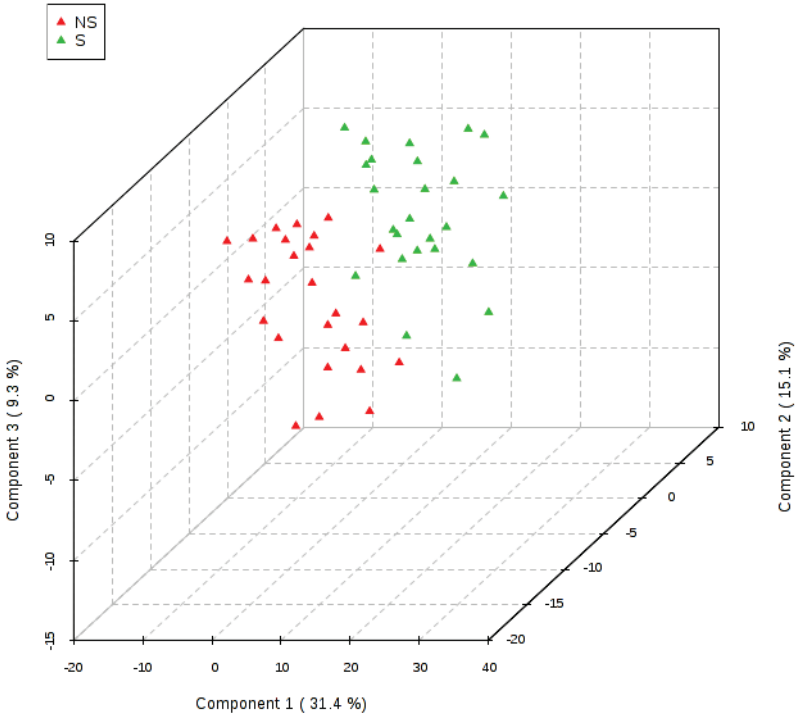




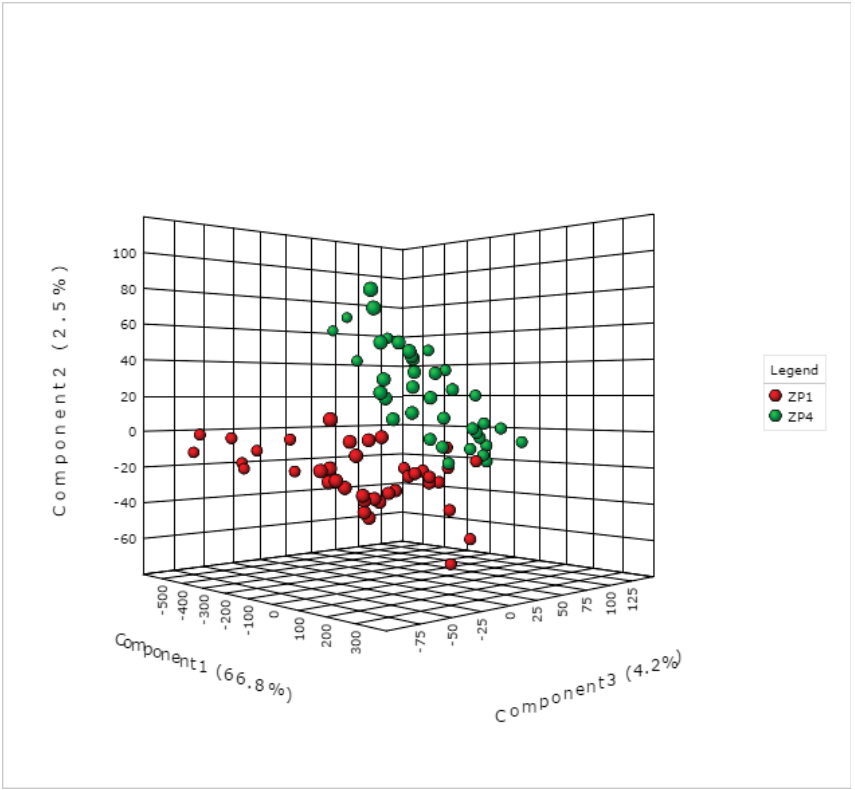
Fingerprinting data processing workflow

Plasma Group separation PLS-DA

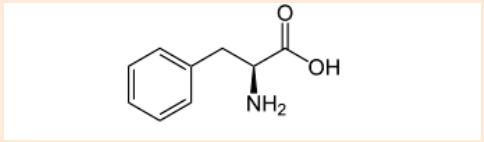
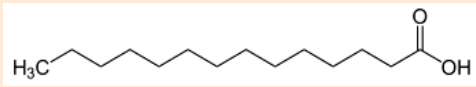
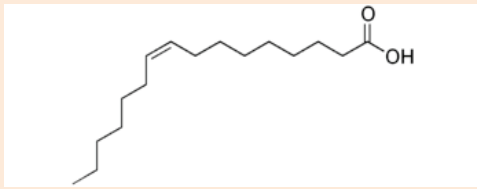
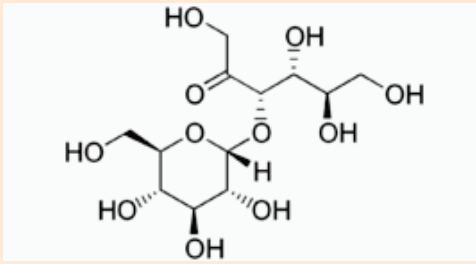
Study 1 Plasma S/NS



Study 2 Plasma TP0/TP3



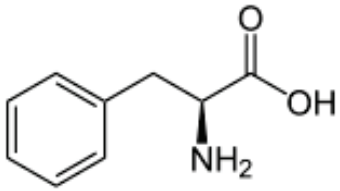
Plasma – Target hits TP0 vs TP3

Compound	Significance	Fold change (TP0/TP3)	Identification	Stucture	
L-Phenylalanine	*	5,7 ↑	Mainlib		Amino acid
Tetradecanoic acid	*	5,2 ↑	Mainlib		Fatty acid (saturated)
Hexadecenoic acid	*	4,9 ↑	Mainlib		Fatty acid (mono-unsaturated)
D-Turanose	*	5,6 ↑	Mainlib		Disaccharide

*p < 0.05 Mann-Whitney-U Test

Plasma – L-Phenylalanine

General information:



- Essential amino acid
  - precursor for the amino acid tyrosine
    - precursor of catecholamines (neurotransmitters)
      (tyramine, dopamine, epinephrine and norepinephrine)

Pathways described:

- Phenylalanine and Tyrosine Metabolism
- Transcription/Translation

Possible reason for increase in smokers / hypothesis:

- Inflammatory effects caused by smoking[1] could decrease efficency of Phenylalanine hydroxylase[2]
  - Reduced degradation of Phenylalanine → higher level

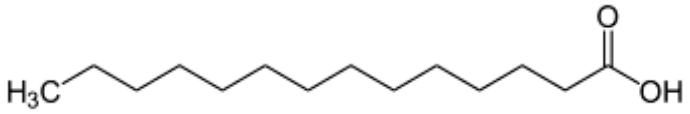
1) van der Vaart,Thorax, 2004

2) Murr et al, J Amino Acids, 2014

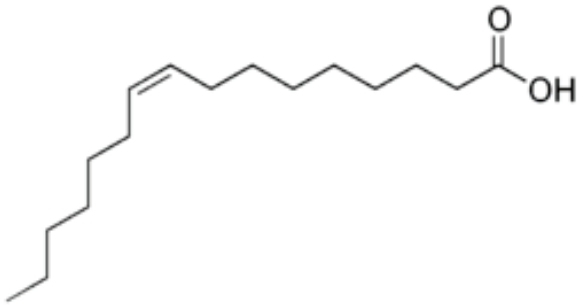
Plasma – Fatty acids

General information:

- Saturated fatty acid: FA 14:0



- Mono unsaturated fatty acid: FA 16:1

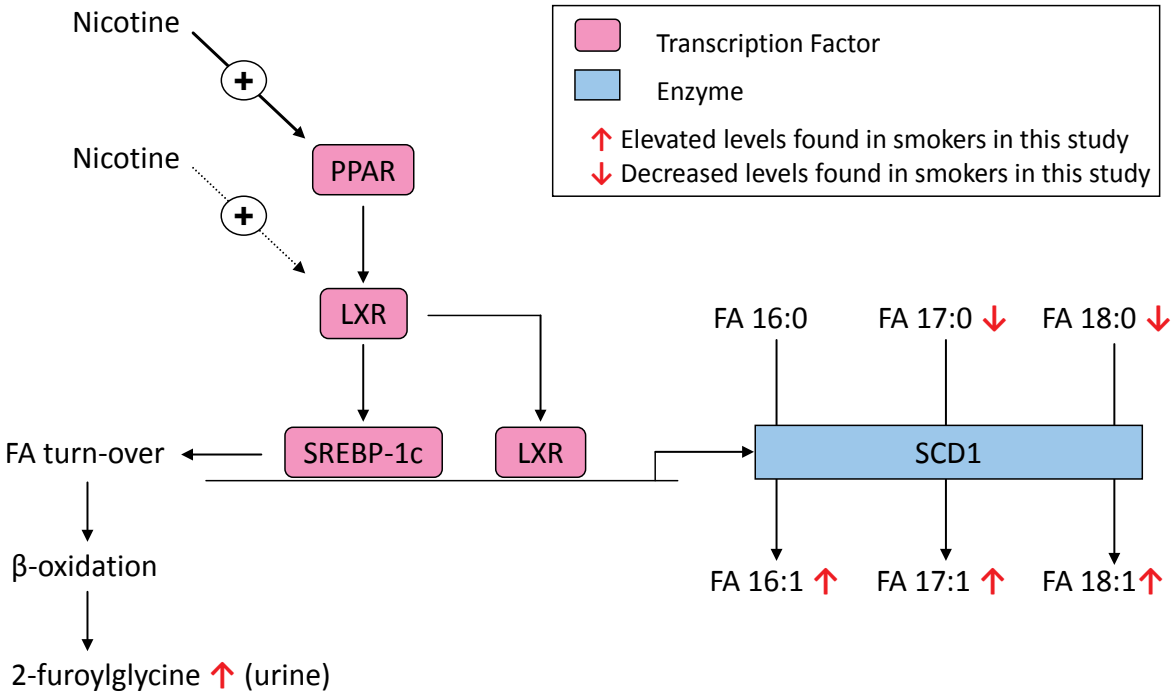


Possible reason for increase in smokers / hypothesis:

- Altered fatty acid metabolism of mono unsaturated fatty acids
  - also found to be increased in study 1 (fingerprinting and targeted approach)
  - up-regulation of stearoyl-Coenzyme A desaturase 1 (SCD1) [1,2,3]

1) Hodson et al. Prog. Lipid Res. 2013
2) Yanagita et al. Cell. Immunol. 2012
3) Amoruso et al. Life Sci. 2007

Plasma – Hexadecenoic acid



- Hypothesis for the emergence of increased levels of MUFAs in plasma of smokers.
- Nicotine leads to a transcription factor activated increase of the enzyme stearoyl Co-enzyme desaturase 1 (SCD1), which catalyzes the desaturation of saturated fatty acids to MUFAs.
- 2-Furoylglycine was found to be increased in smokers' urine and might be generated via a nicotine triggered activation of the transcription factor SREBP-1c, resulting in an elevated FA turn-over by β-oxidation.
- LXR: liver X receptor. Sterol regulatory element binding protein-1c: SREBP-1c.

Mueller et al. Diss. Apr. 2014.

Summary

- ✓ Diet controlled clinical study with 60 subjects (followed over 3 months) successfully conducted
- ✓ COex and cotinine for compliance measured
  - ✓ 39 compliant subjects, 21 drop outs
- ✓ Plasma metabolomic fingerprinting measured
  - ✓ 4 potential target hits identified
  - ✓ Primarily fatty acid and amino acid metabolism affected

Acknowledgement

Sponsor:

Imperial Tobacco Group

ABF

- Michael Goettel
- Prof. Dr. Gerhard Scherer
- Dr. Nikola Pluym
- Dr. Daniel Mueller
- ABF Team

Thank you for your attention

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