



Detecting beer intake by unique metabolic patterns

Gözde Gürdeniz

PostDoc

University of Copenhagen

Department of Nutrition, Exercise and Sports



Alcoholic beverages ↔



Alcohol content

OR

Specific components

Assessment of intake
specific alcoholic
beverages



Self reported questioners

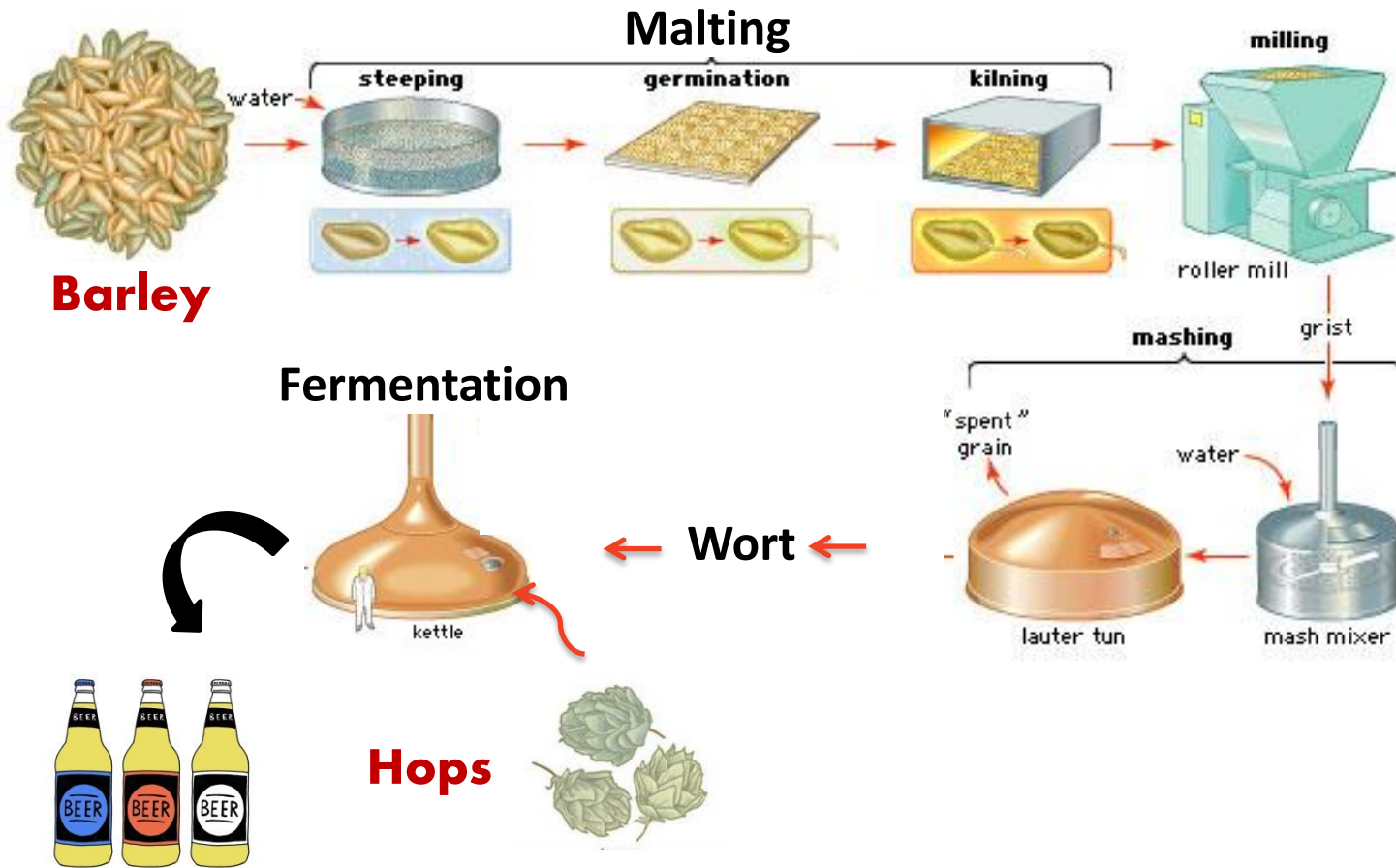
Biomarkers

Metabolomics

- Endogenous metabolites
- Beer components and biotransformation products



Beer components





Hops

flavoring and stability agent in beer

- Alpha-acids (AA)
Cohumulone, humulone and adhumulone
- Beta acids
- Flavonoids

Iso-xanthohumol³

Biomarker

Variable rate of biotransformation to 8-prenylnaringenin

Biomarker

Iso-alpha-acids (IAA)^{1,2}

Storage induced degradation

1. Rodda, L.N., et al. (2015b). Forensic Sci Int. 250, 37-43.
 2. Rodda, L.N., et al. (2013). Anal Bioanal Chem. 405, 9755-9767.
 3. Quifer-Rada, P. et al. (2014) J Nut. 4, 484-488.



AIM

Metabeer

- Beer intake →
- to identify the plasma and urinary metabolites
 - to discover biomarkers



Pilotbeer

Validation of biomarker of beer intake
with small scale meal study



Metabeer - TEST DRINKS

Test Drinks	Iso-alpha-acids (mg/l)	Alcohol Content
Control drink (C) Sports Drink	x	x
Light/alcohol-free Beer (LB)	19.3	x
Regular Pilsner (RB)	22.9	4.6%
Strong Pilsner (SB)	38.3	8%

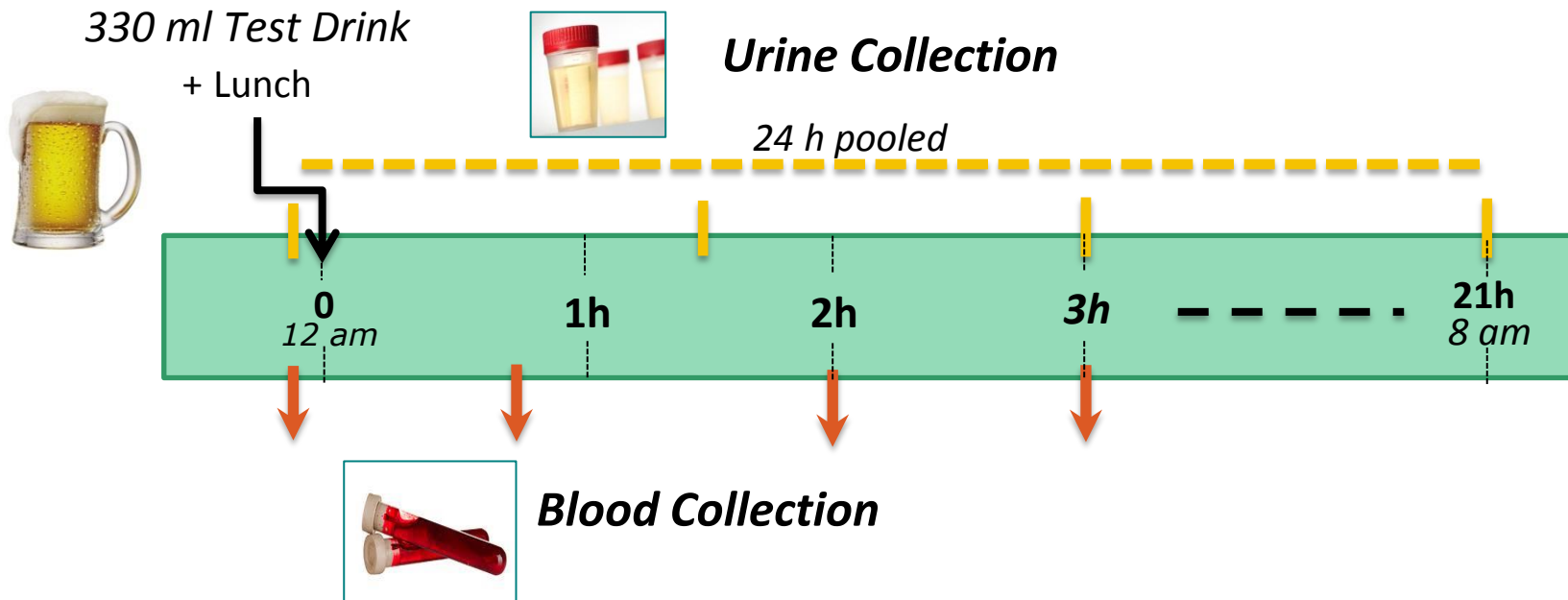


METABEER: STUDY DESIGN AND SUBJECTS

- ✓ 4-way, cross-over, single-blinded intervention study
- ✓ 18 healthy men and women in the age group of 18-60

Test Day

No alcohol consumption 2 days prior



Sample and Data Analysis

UPLC-QTOF



Data Analysis

- ASCA to isolate effect of test drinks
- PLS-DA to extract metabolites associated with beer intake
- VIP



Sample and Data Analysis

UPLC-QTOF



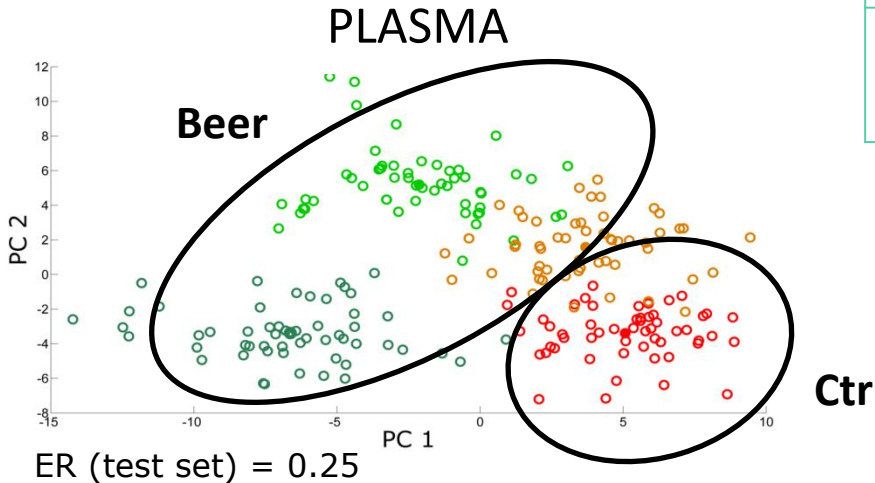
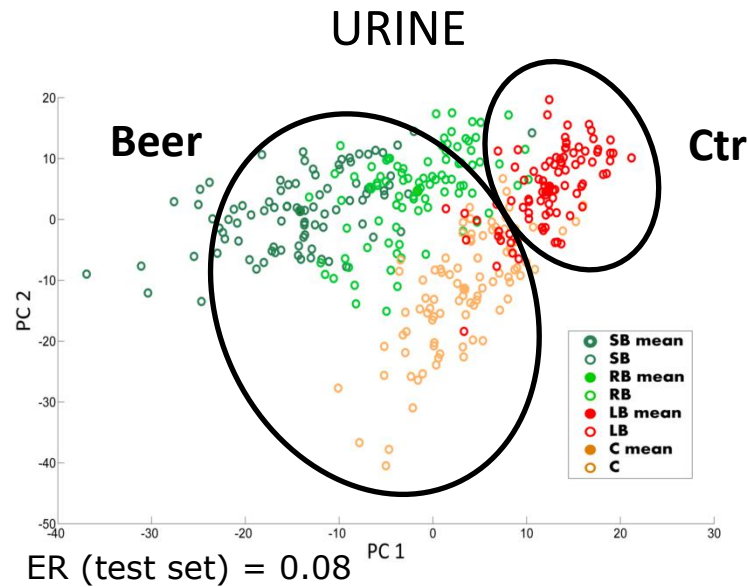
- Test beers
- Hops
- Beer Wort



- **Hops**
(present in hops and beer)
- **Barley/malting**
(present in wort and beer)
- **Fermentation**
(present in only in beer)
- **Human metabolism**
(not present in beer, hops or wort)



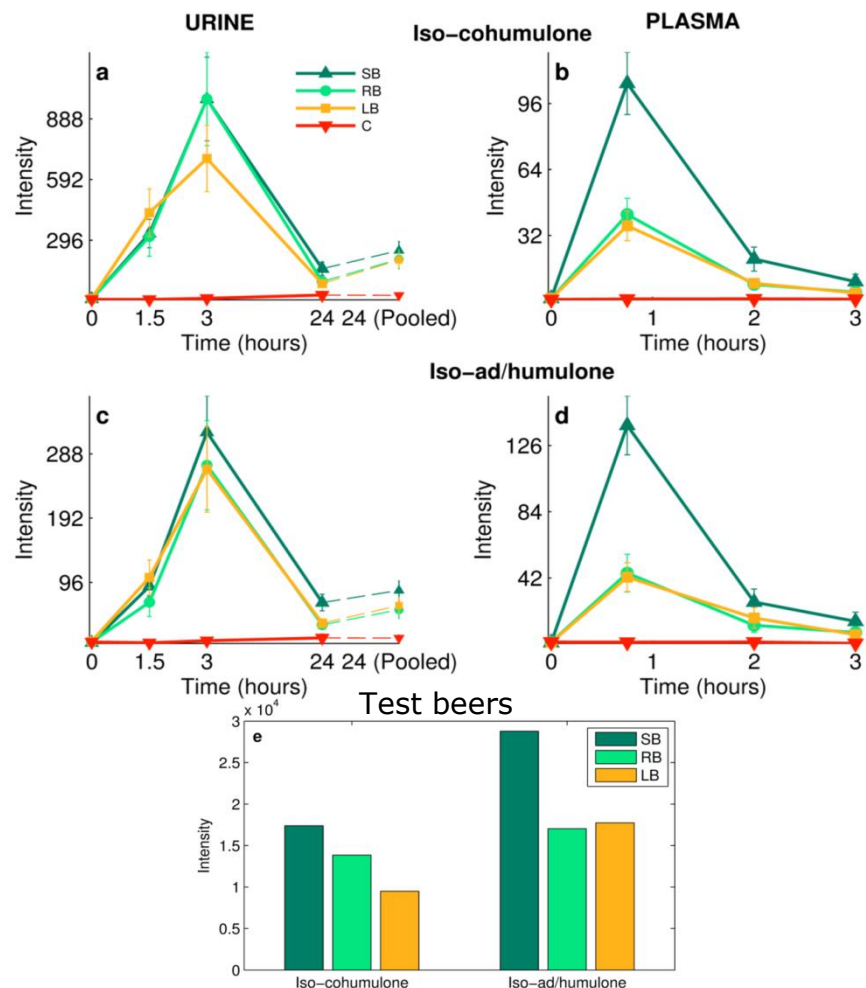
Metabolites Associated with Beer Intake



	Plasma	Urine
Total number of features	1503	8935
Beer markers	6	52
Hops	2	15
Barley/malting	4	15
Fermentation	-	2
Human metabolism	-	20



Hops originating metabolites



Iso-alpha-acids (IAAs)

- Early excreted metabolites
- Beer specific
- Detected in both plasma and urine

Can we use IAAs as biomarker of beer intake??

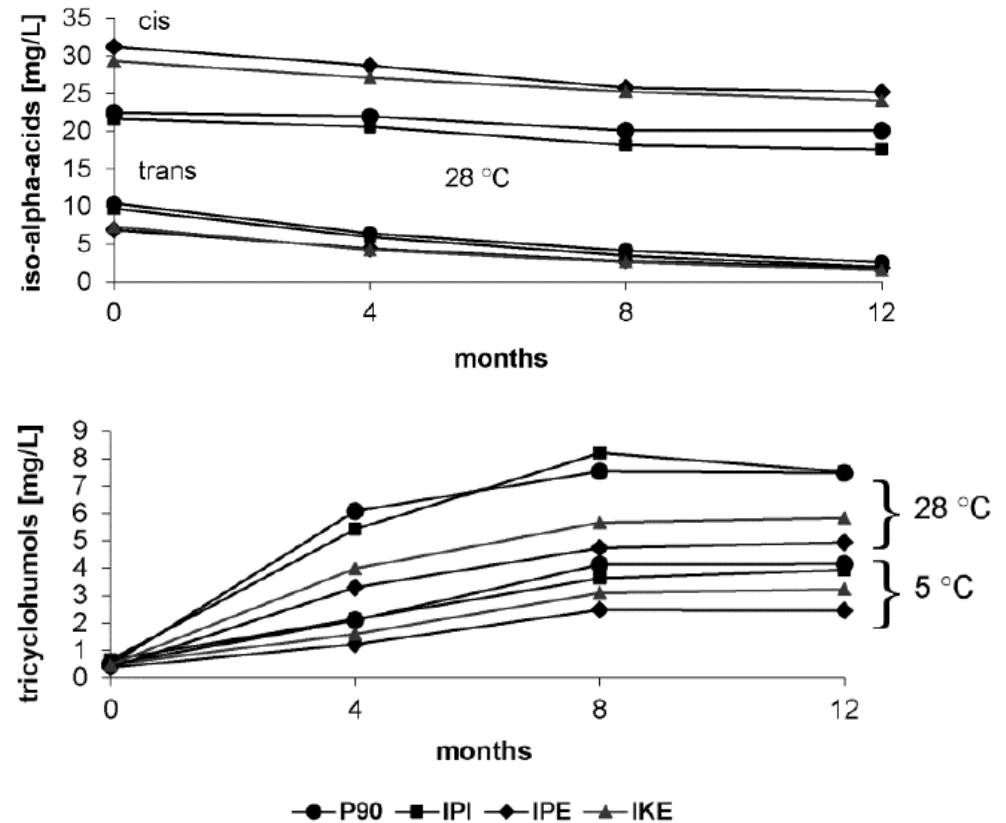


Hops originating metabolites

Storage induced degradation of IAAs

- Tri- and tetra-cyclohumols
- Hydroxylated humulones

Effect of storage on
iso-alpha-acid concentrations



Human metabolism associated metabolites

- NO or CH₂O conjugate of IAAs
- NO₂ or CH₂O₂ conjugate of IAAs
 - Cysteine conjugates
- Phase II metabolites



**Specific to
beer
intake**



Barley/Malting and Fermentation originating metabolites

m/z	RT	Annotation	Suggested Compound (identification level)	Biofluid	Source
365.10	0.88	[M+Na] ⁺	Maltose ²	Urine	Malting/Mashing
225.08	1.93	[M-H] ⁻	Pyroglutamyl proline¹	Urine	Heat treatment
227.10		[M+H] ⁺		Plasma	during malting
182.08	0.78	[M+H] ⁺	Tyrosine ¹	Plasma	Barley
132.10	0.91	[M+H] ⁺	Iso/Leucine ¹	Plasma	Barley
166.12	1.13	[M+H] ⁺	Hordenine ¹	Urine	Barley
230.05	1.01	[M-H] ⁻	N-methyl Tyramine sulfate²	Urine	Barley
232.06		[M+H] ⁺			
161.04	1.79	[M-H] ⁻	2-ethyl malate²	Urine	Fermentation

*identification level



Single Biomarker of Beer Intake

Specificity Stability

Hops originating
metabolites



Barley/malting and fermentation
originating metabolites



Aggregated beer biomarkers

Representative of beer raw materials and production process.

Criteria for selection :

- Identified metabolites
- Significant up-regulation after beer intake



Selected metabolites for the aggregated beer biomarker

Compound	Source	Specificity
IAAs + Tricyclohumols	Hops	✓
N-methyl Tyramine Sulfate	Barley	✗
Pyro-glutamyl Proline	Barley/malting	✗
2-ethyl malate	Fermentation	✗



VALIDATION of aggregated beer biomarker PILOTBEER study

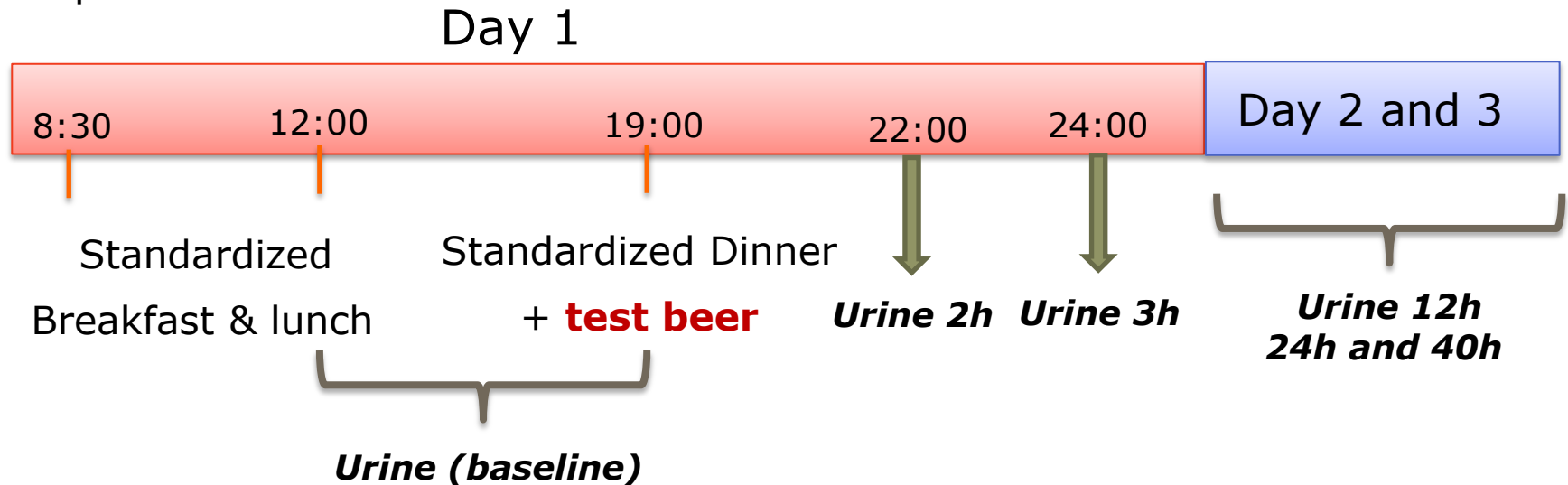


PILOTBEER : Study design

Cross-over study
4 healthy subjects (age 28-60)

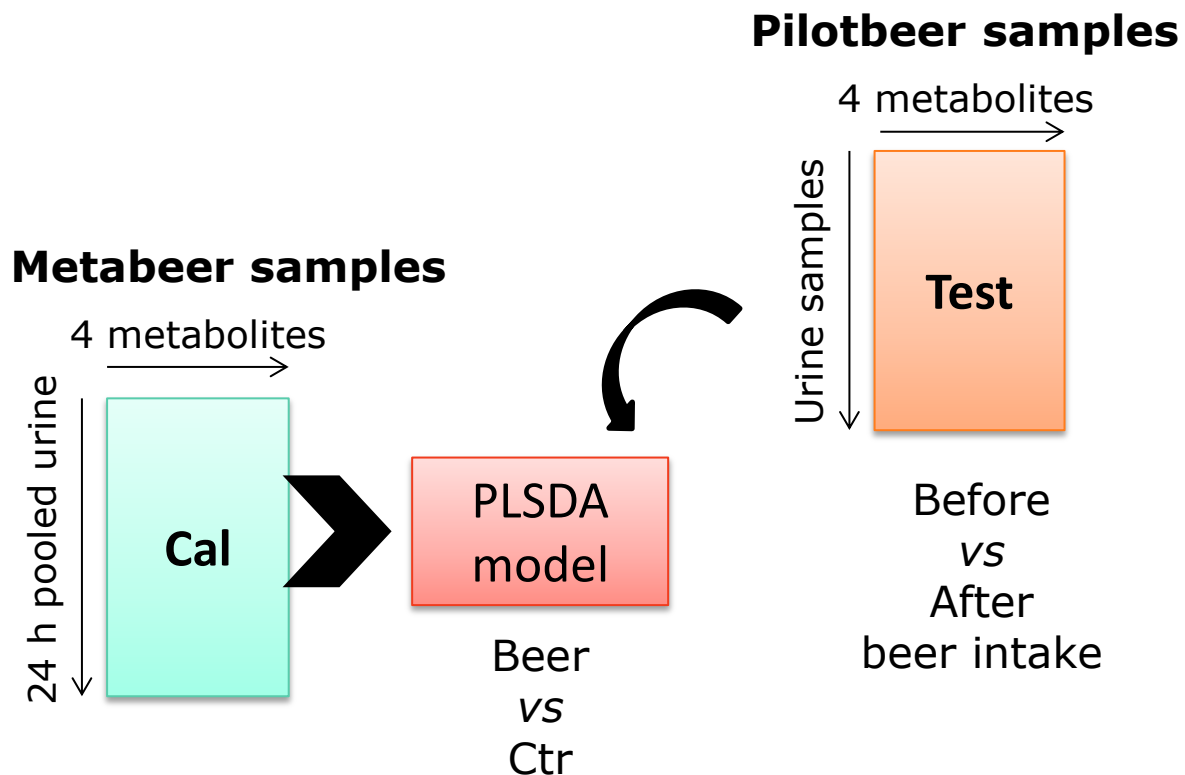
*two days
before*
No alcohol
consumption

Test Drinks	Iso-alpha Acids (mg/l)	Alcohol Content
Low hops Pilsner	8	7-8%
High hops Pilsner	31	4.8%



Sample and Data Analysis

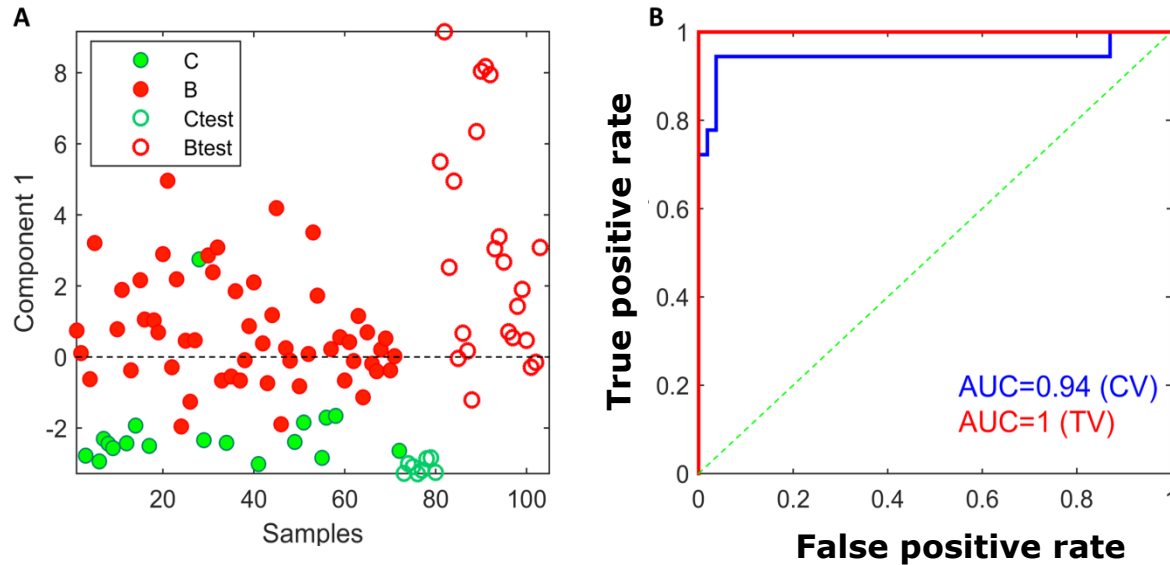
UPLC-QTOF



Results : Validation

Metabeer : Pooled 24 h urine

Pilot beer : Urine collected up to 12 h.



Pilot beer : urine collected 12 to 24 h after beer intake are wrongly classified as controls.



metabolites associated with beer intake were already excreted at 12 h



Conclusions

- Beer intake is associated with increase in a number of metabolites in plasma and urine.
- The metabolites are grouped according to their origin: hops, wort, fermentation, and human metabolism.
- The majority of beer-specific compounds are originated from hops, but many of these are chemically unstable limiting their usefulness as single biomarkers.
- Wort and fermentation products, in combination with one or more hops-specific marker provided a biomarker model for compliance.
- Biomarker model based on a set of three or four of these signature biomarkers was able to completely discriminate between samples collected after beer intake in an independent study with high sensitivity and specificity.



Limitations/Future Perspectives

Validation study was a controlled study with low number of subjects and limited beer types

Further interventions with other types of beers (stout, ale, wheat, rice, etc.) is required to test whether additional markers are needed.

The sensitivity and specificity of the aggregated biomarker of beer intake still needs to be validated in an observational study.





**THANK YOU FOR
YOUR ATTENTION**

University of Copenhagen
Lars Ove Dragsted

Calsberg Research Laboratory
Morten Georg Jensen
Lene Bech
Erik Lund

Technical University of Denmark
Sebastian Meier

Laboratory Technicians (University of Copenhagen)
Ummuhan Celik
Sarah Fleischer Ben Soltane



ALL PARTICIPANTANTS OF THE STUDIES

