

Changes in milk proteome and metabolome associated with cow health

22 August 2014, Kasper Hettinga



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Topics

- Proteomics & metabolomics methodology
- Two examples:
 - Negative energy balance
 - DGAT1 genotype



Proteomics & metabolomics methodology



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Why do we use -omics methods?

- Combining different -omics methods may lead to a more integrative view on underlying physiology. Better understanding of:
 - The physiology of cows - in relation to milk synthesis&secretion and animal health
 - The interlinkage between milk components and the newborn's immune system (calve/baby)



Which milk components are interesting?

Cow's milk contains:

- 3.5% protein
 - 2.7% casein
 - 0.7% serum proteins
 - 0.1% MFGM protein
- 5.4% small water-soluble components
 - 4.6% lactose
 - 1% minerals
 - 0.3% organic acids
 - 0.1% other solutes

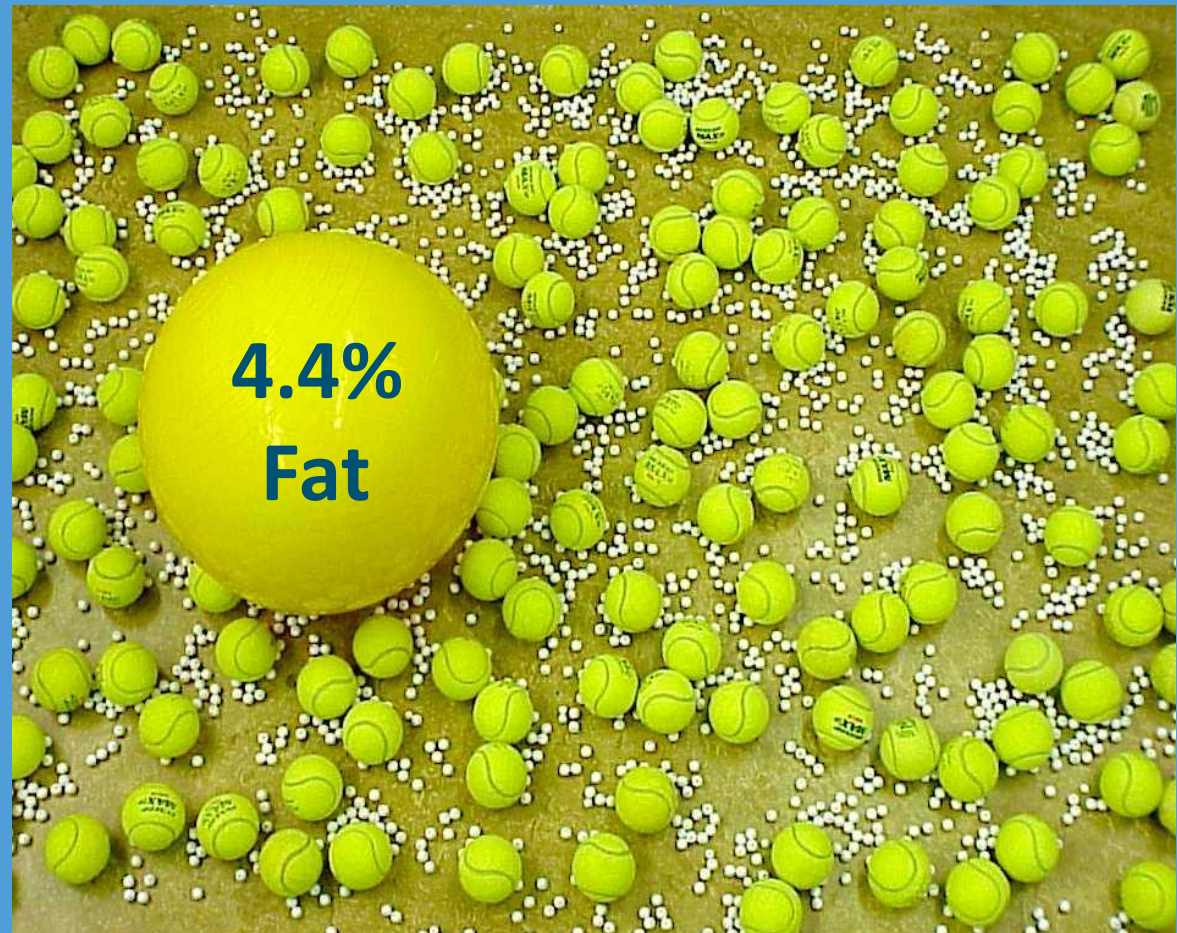


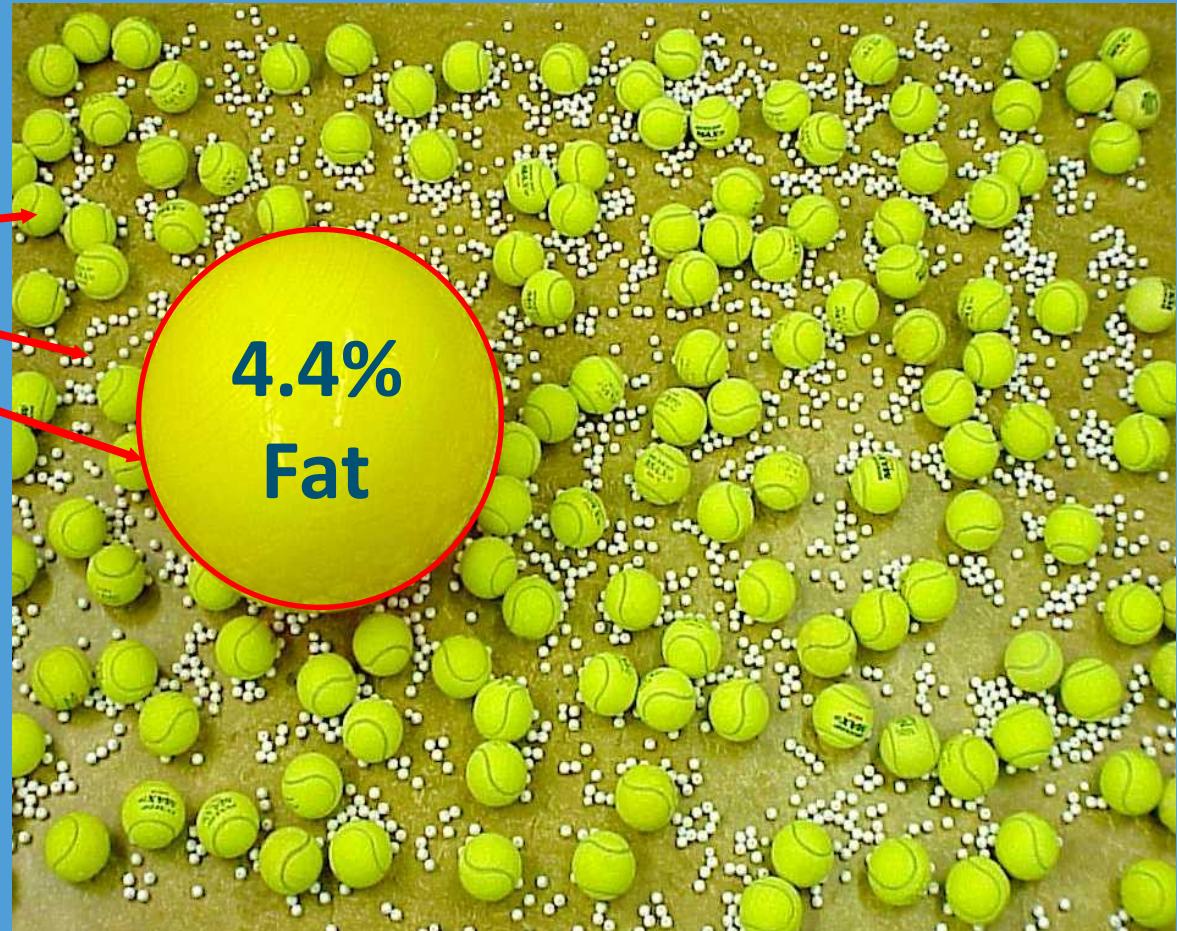
Image: Prof. Kees de Kruif



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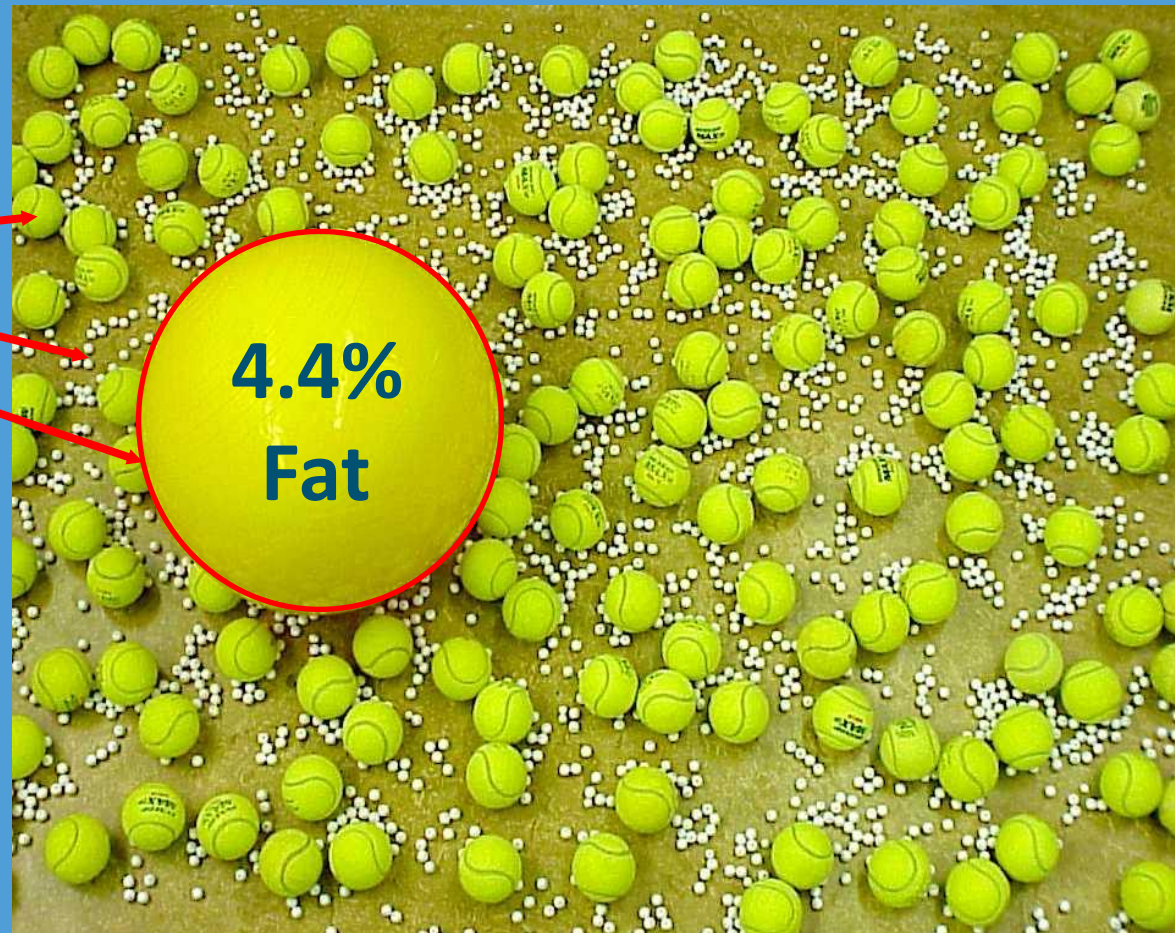
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Proteomics

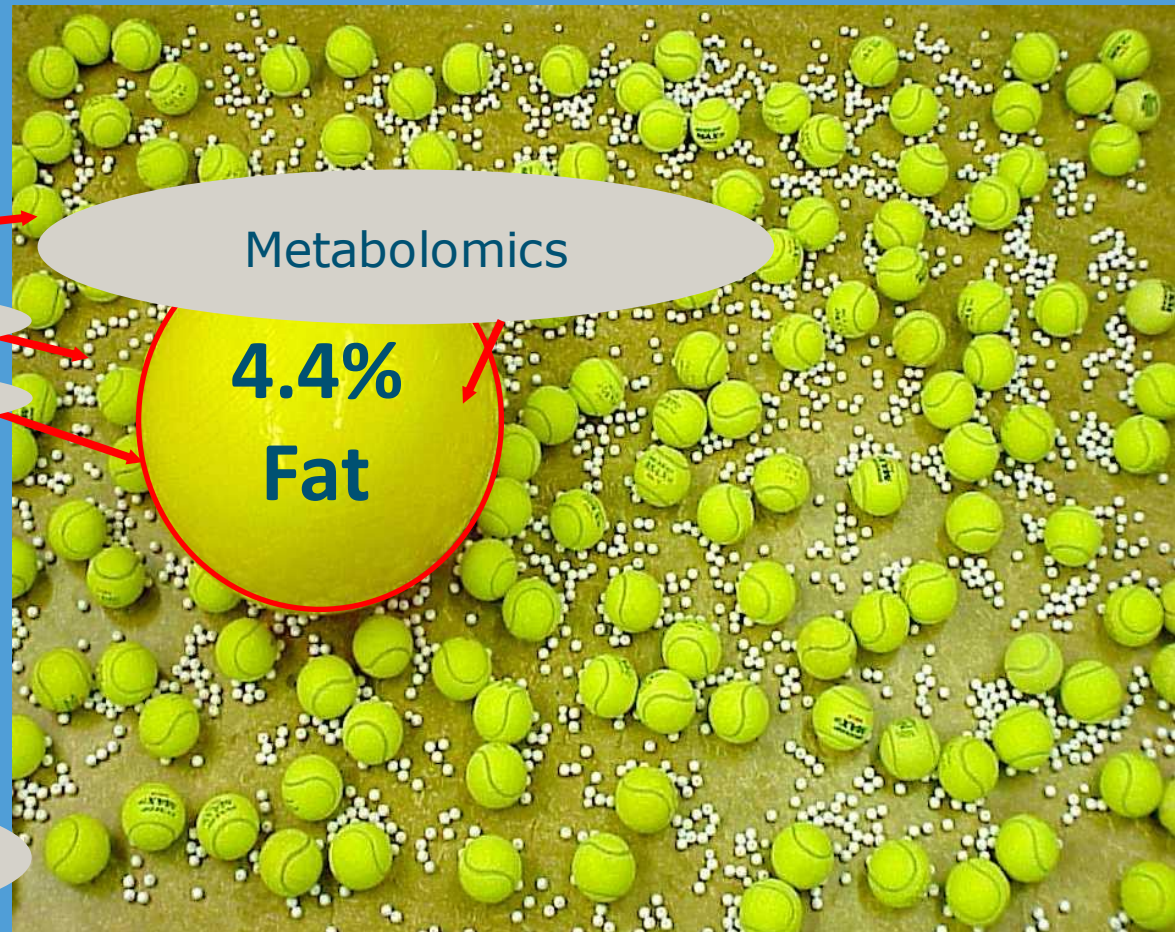
Proteomics

- 5.4% small water-soluble components

- ~~4.6% lactose~~

- ~~1% minerals~~

Metabolomics

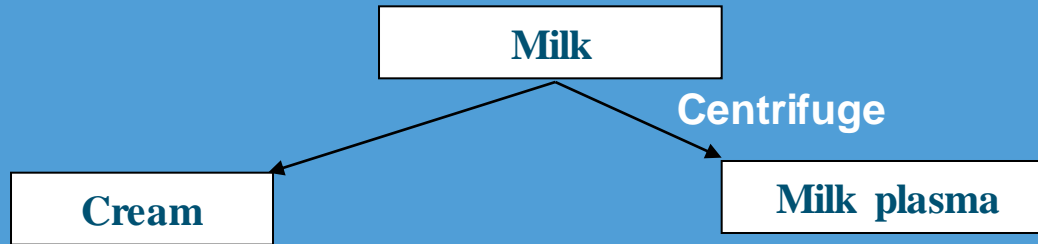


How to isolate these components?

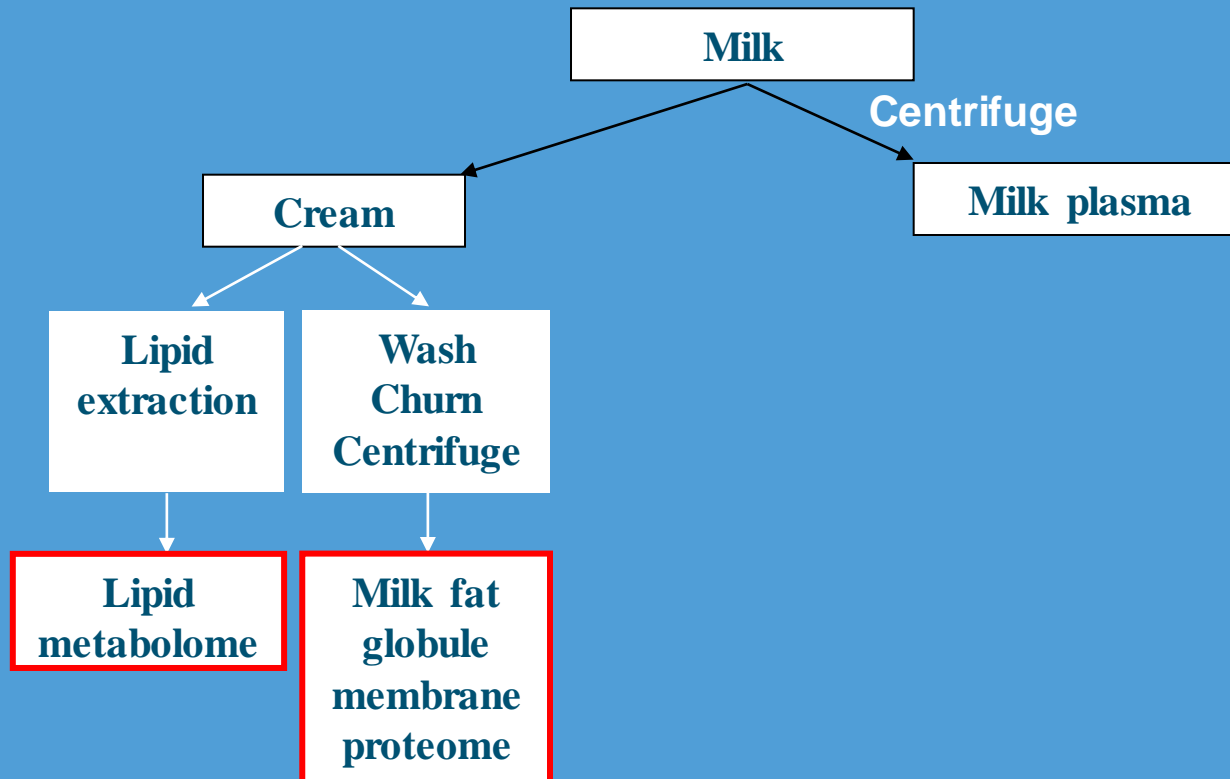
Milk



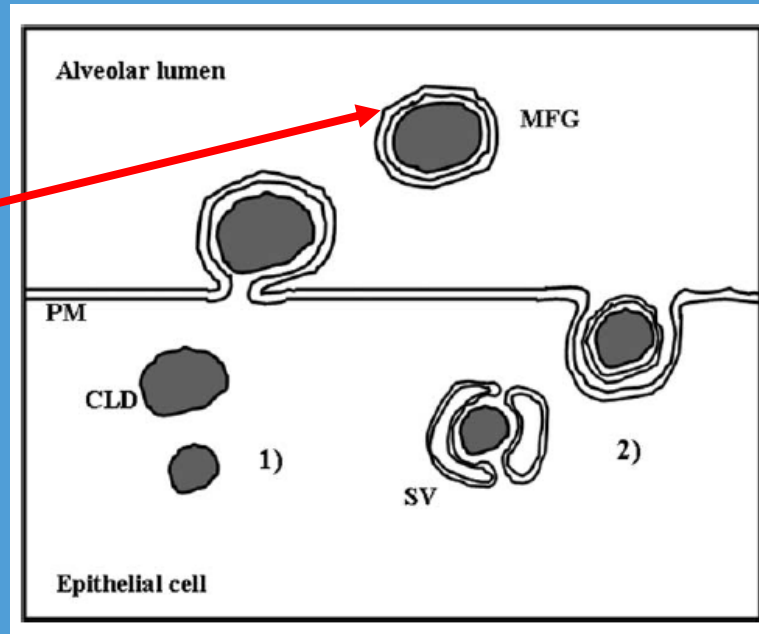
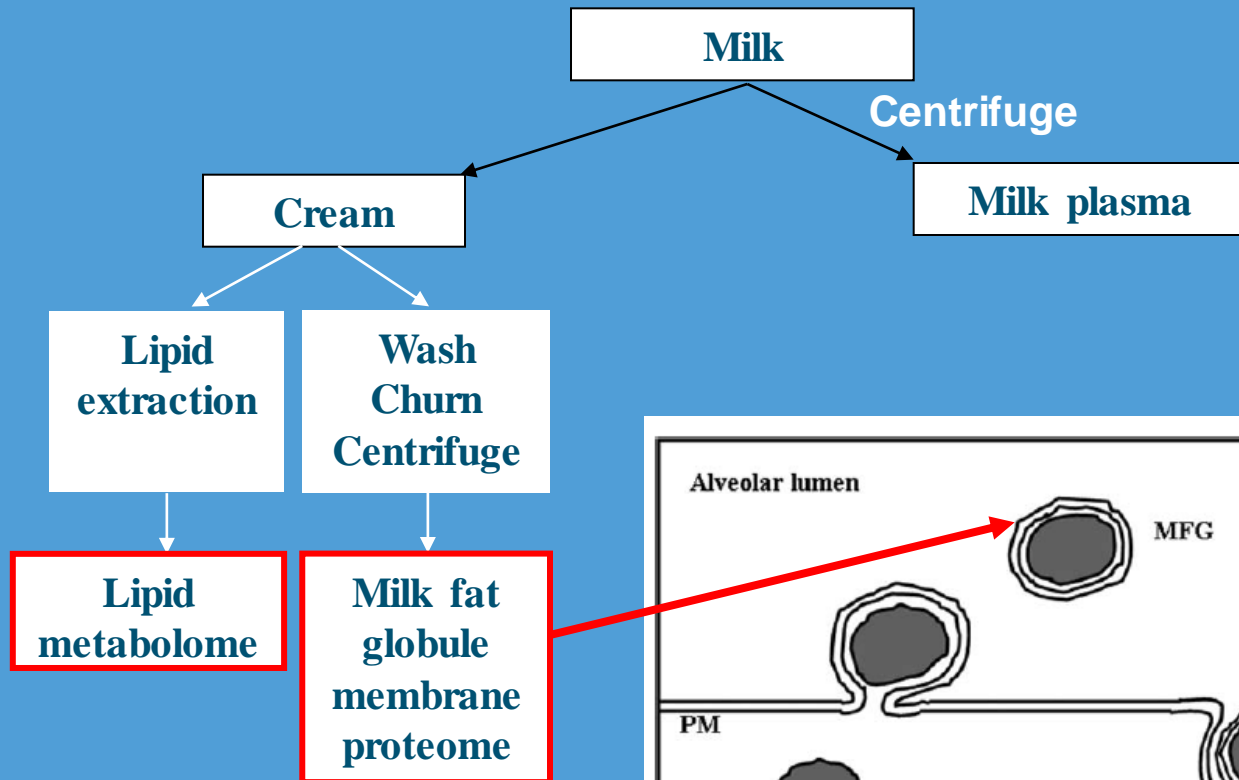
How to isolate these components?



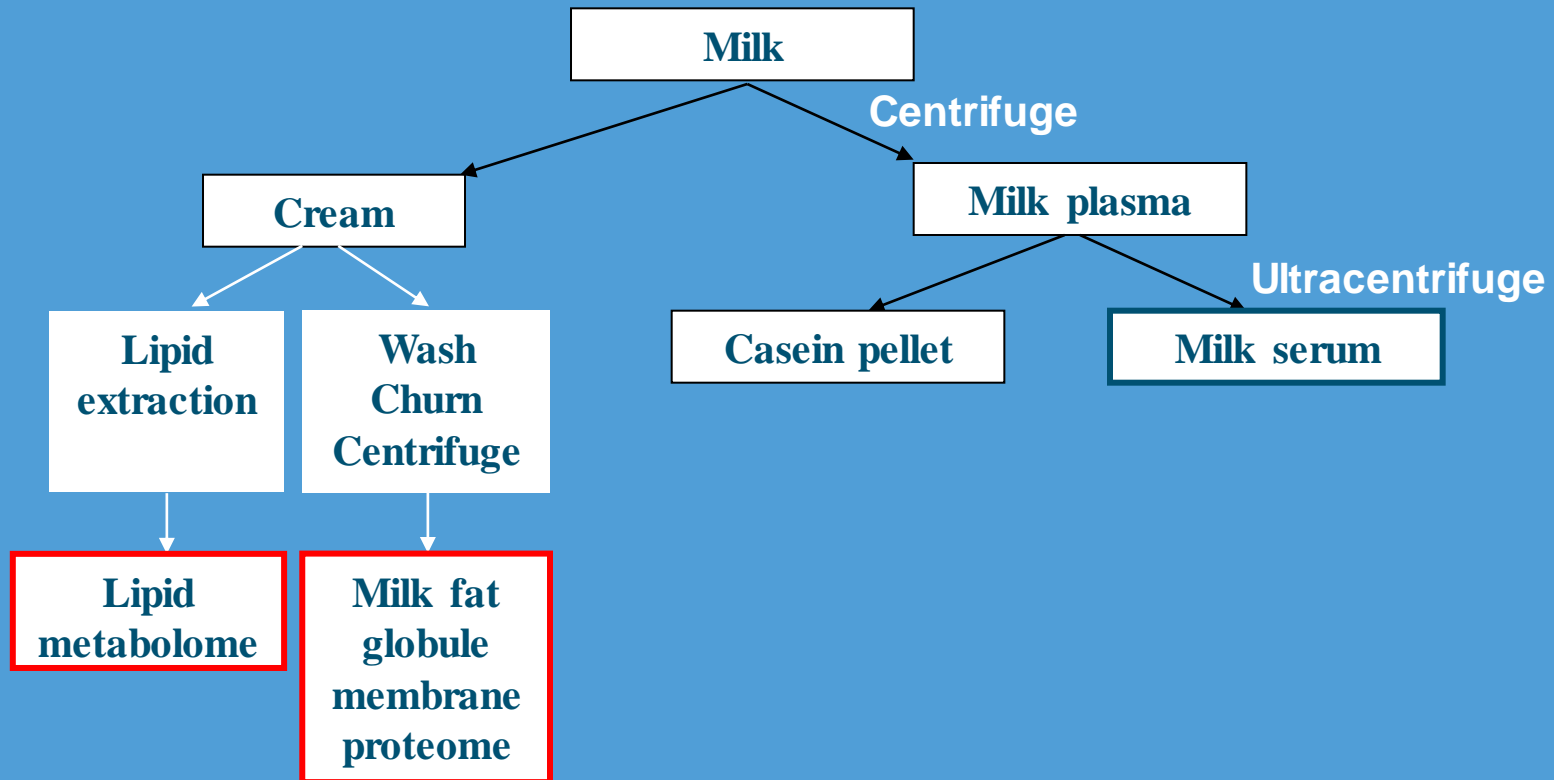
How to isolate these components?



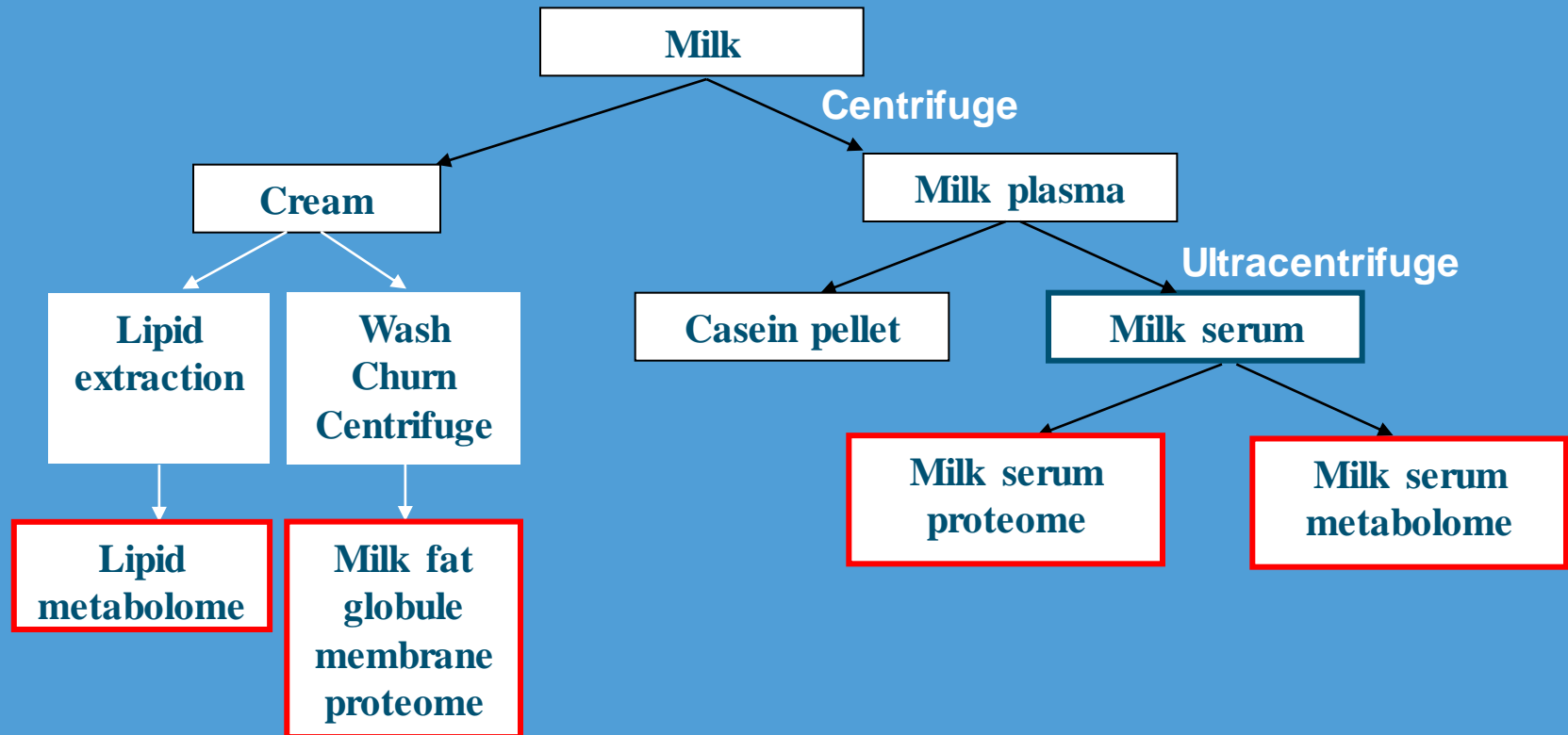
How to isolate these components?



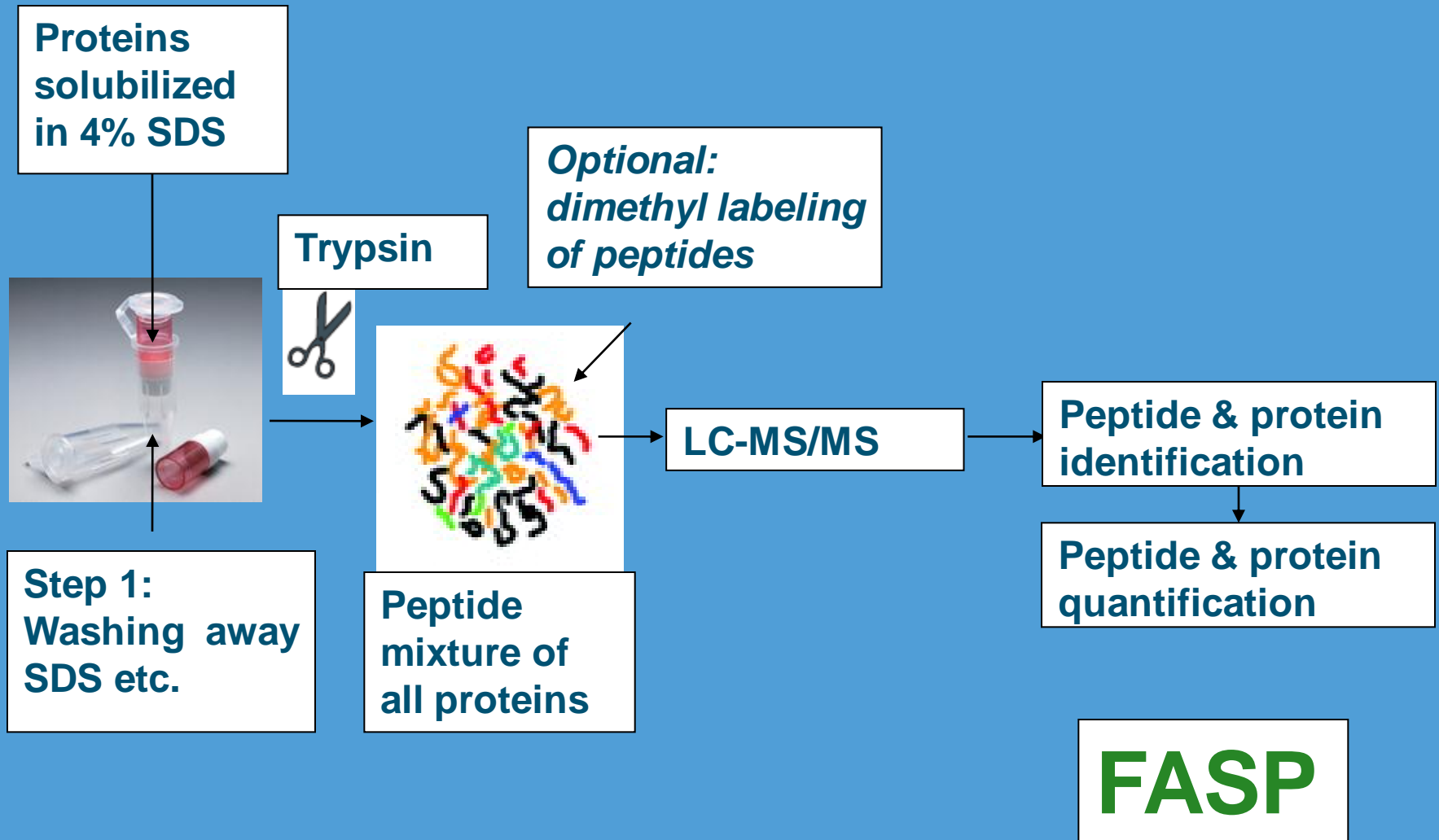
How to isolate these components?



How to isolate these components?

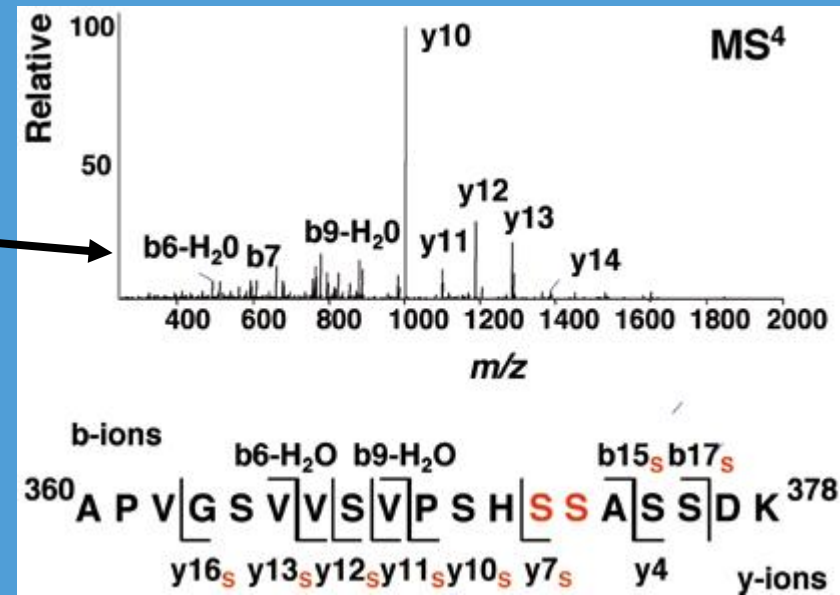
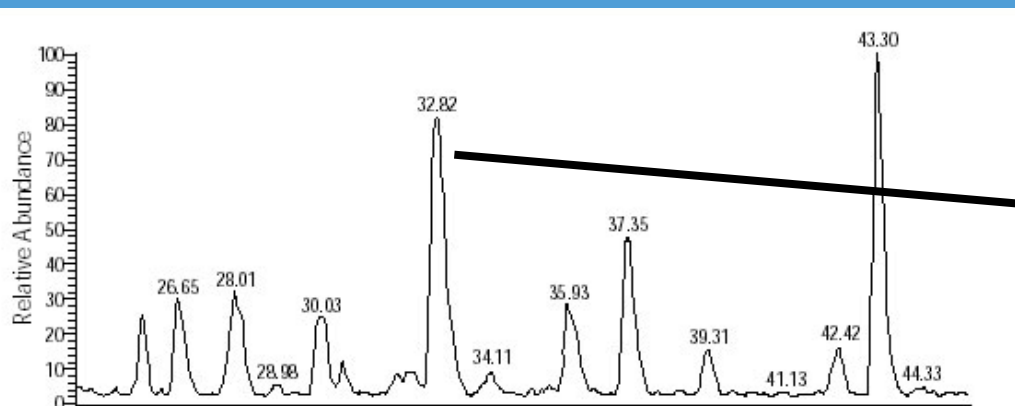


Proteomics method – sample prep



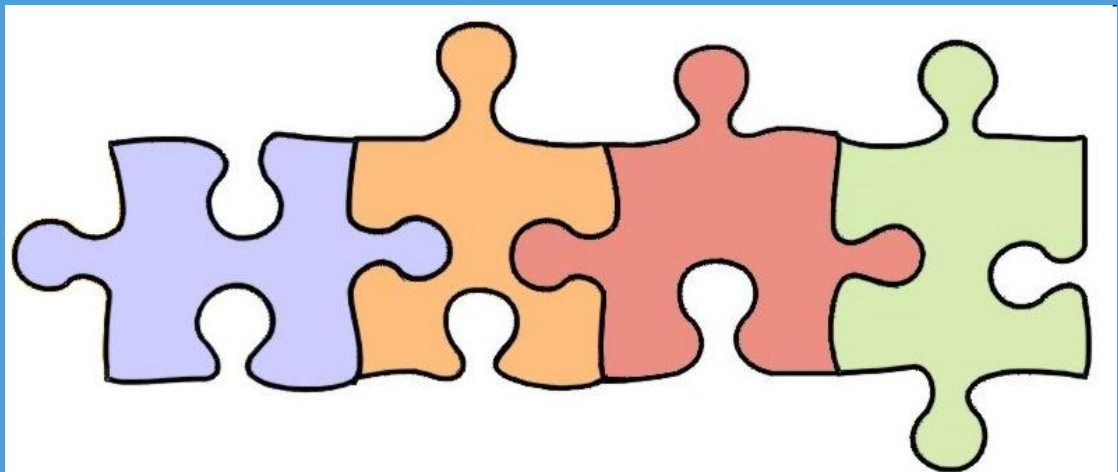
Proteomics method – LC/MSMS-analysis

- Result: chromatograms with thousands of peptides each
- Automatic peptide identification
 - High specificity of orbitrap-MSMS

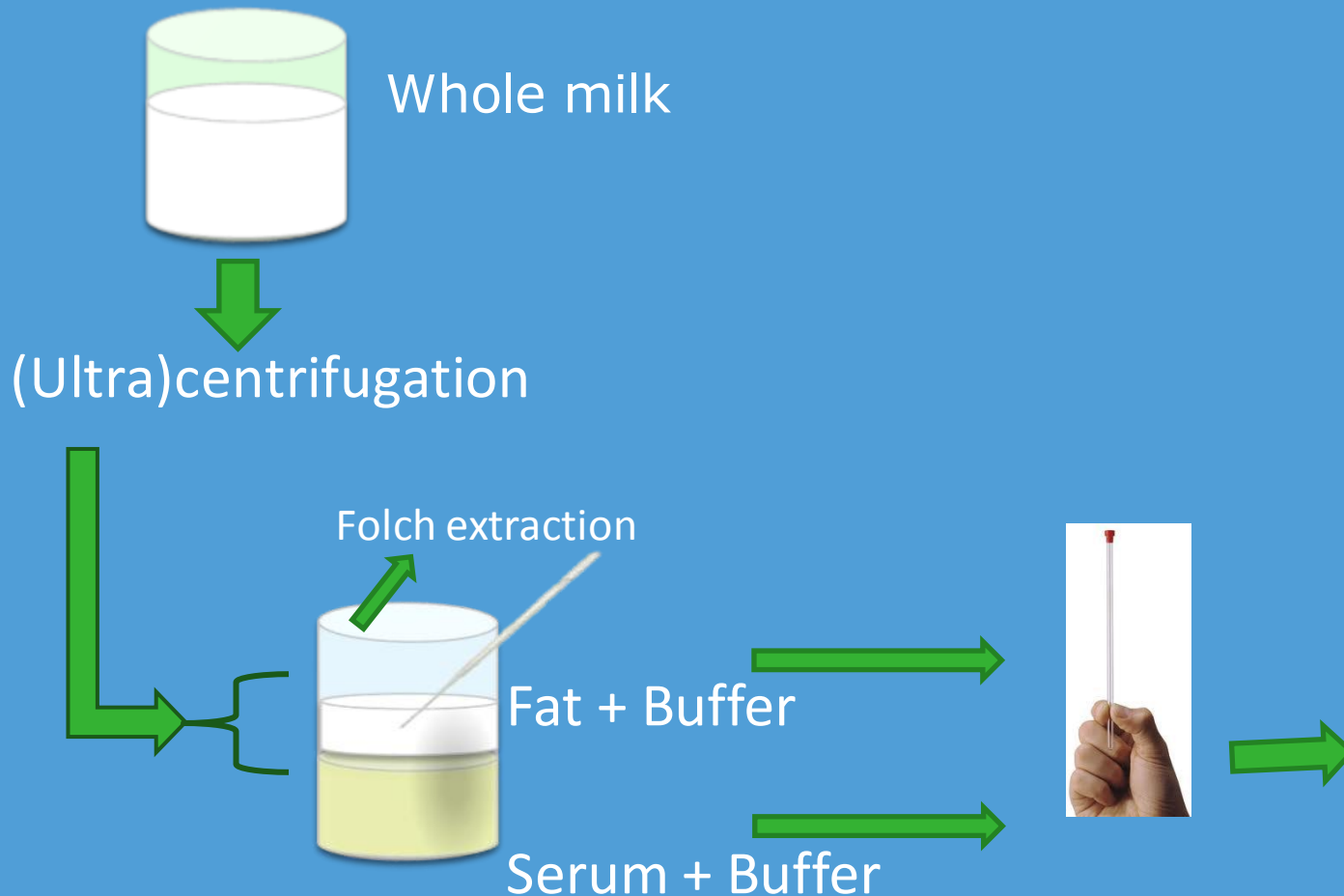


Proteomics method – protein identification

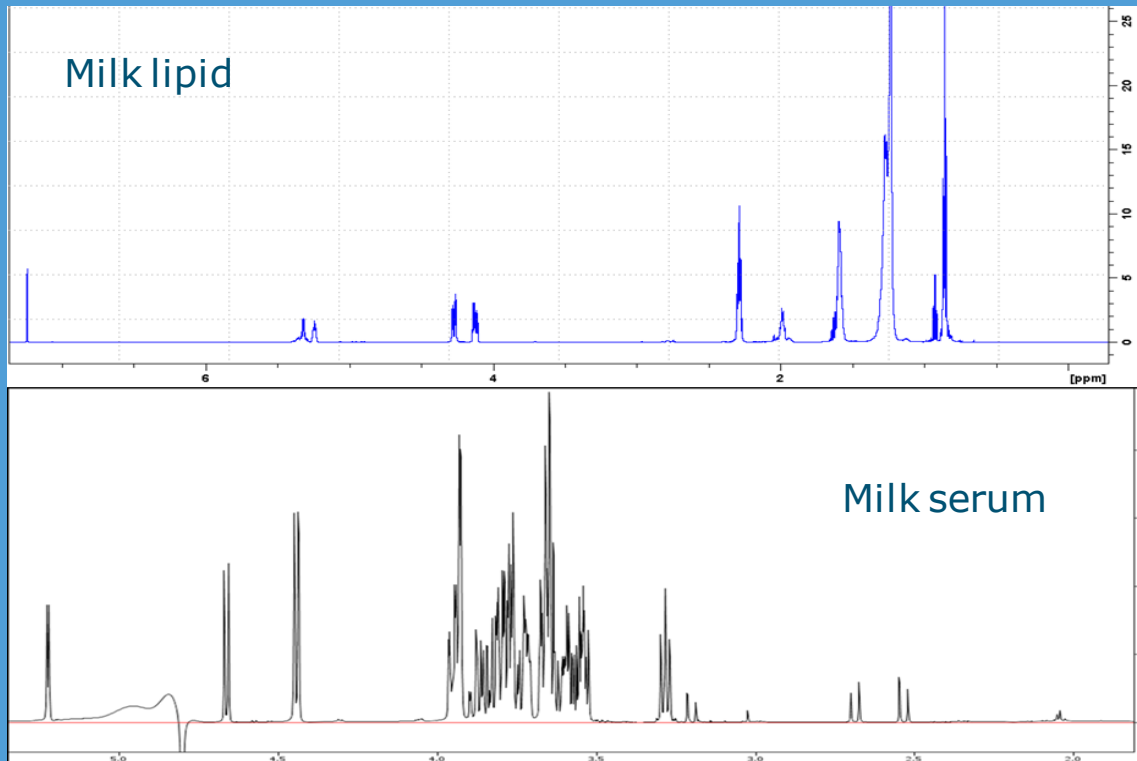
- Protein identification: database with amino acid sequences (>100.000 proteins):
 - Fit jigsaw pieces (peptides) in a jigsaw puzzle (protein)
 - Check for false-positive identifications
 - Known and “theoretical” proteins (information from the bovine genome)
- Protein quantification (label-free or dimethyl labeled)



Metabolomics method – sample prep



Metabolomics method – ^1H -NMR



Metabolomics method – identification

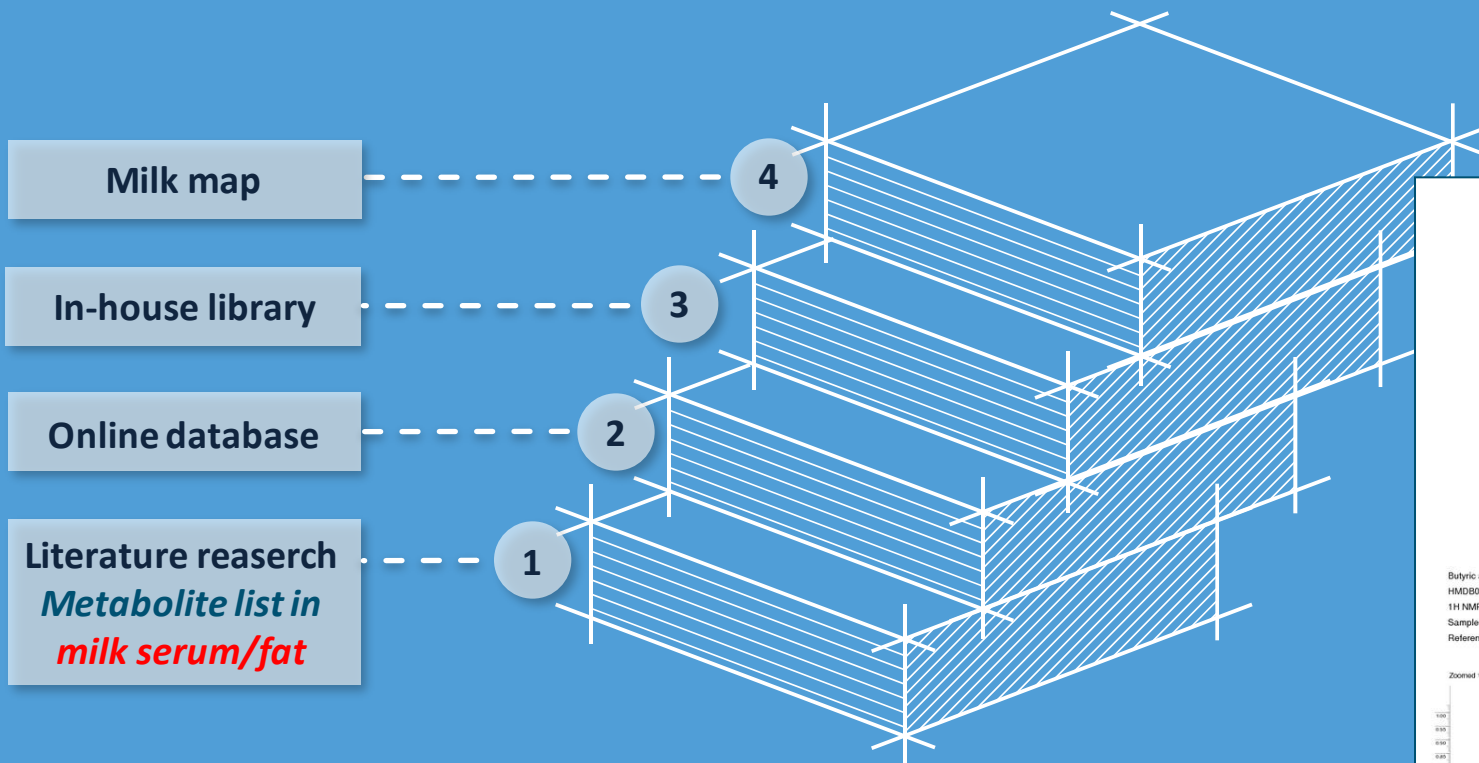


Table of Peaks

No.	Chemical shift (ppm)	Integration	Assignment
1	0.91	1.00	CH ₃
2	1.27	1.00	CH ₂
3	1.62	1.00	CH
4	1.92	1.00	OH
5	2.12	1.00	CH ₂
6	2.27	1.00	CH ₂
7	2.37	1.00	CH ₂
8	2.47	1.00	CH ₂
9	2.57	1.00	CH ₂
10	2.67	1.00	CH ₂
11	2.77	1.00	CH ₂
12	2.87	1.00	CH ₂

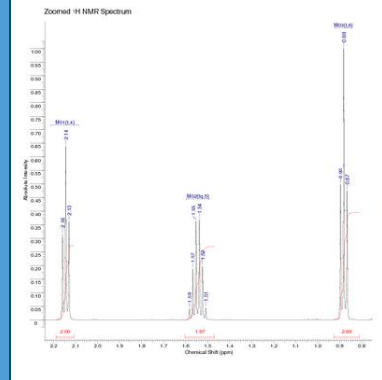
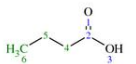
Table of Multiplets

No.	Chemical shift (ppm)	Integration	Assignment
1	0.91	1.00	CH ₃
2	1.27	1.00	CH ₂
3	1.62	1.00	CH

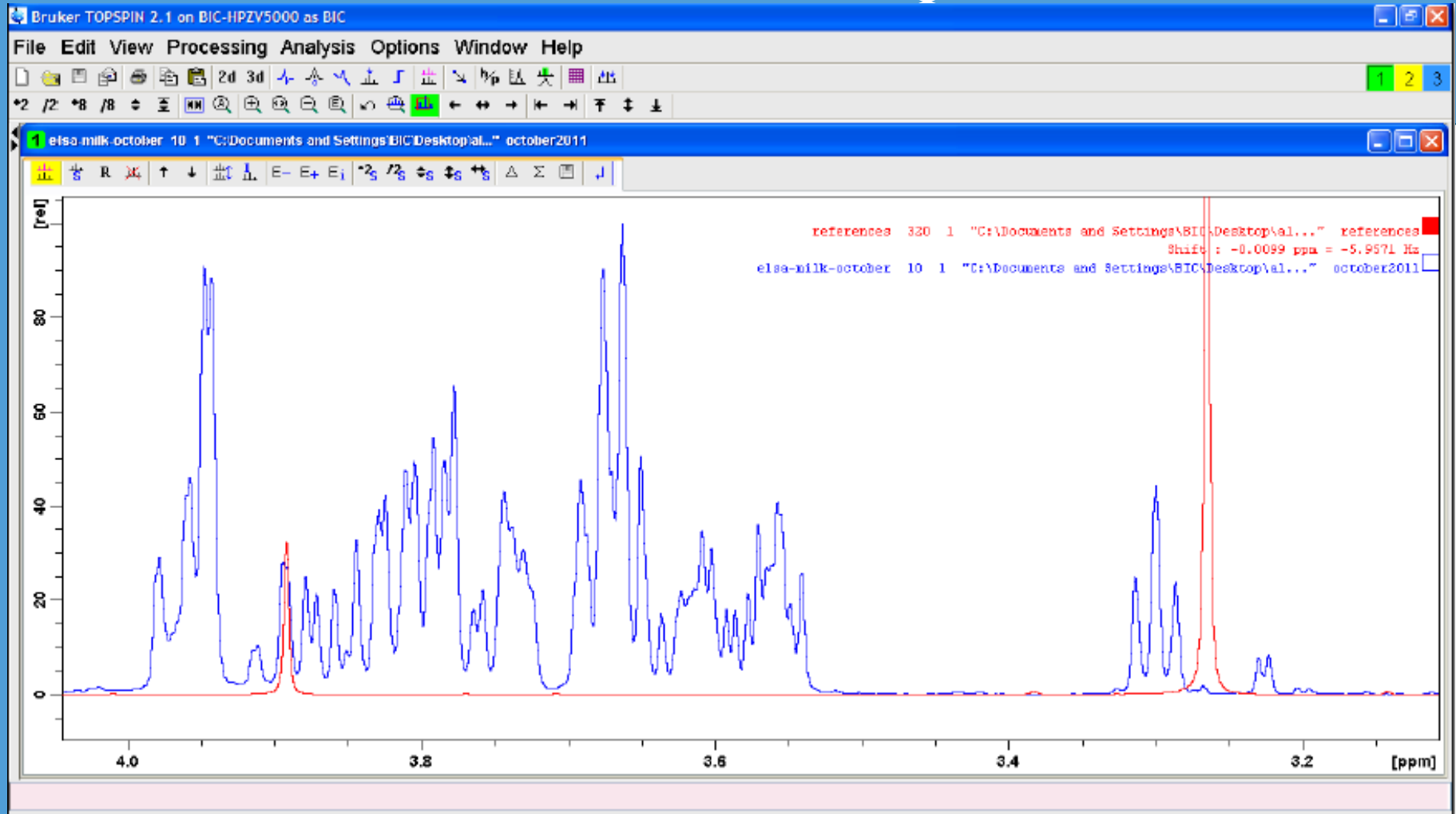
Table of Assignments

No.	Chemical shift (ppm)	Integration	Assignment
1	0.91	1.00	CH ₃
2	1.27	1.00	CH ₂
3	1.62	1.00	CH

Butyric acid
HMDB000039
1H NMR Spectrum : 500 MHz in H₂O
Sample : 50 Mm at pH 7.0 in H₂O
Referenced to DSS

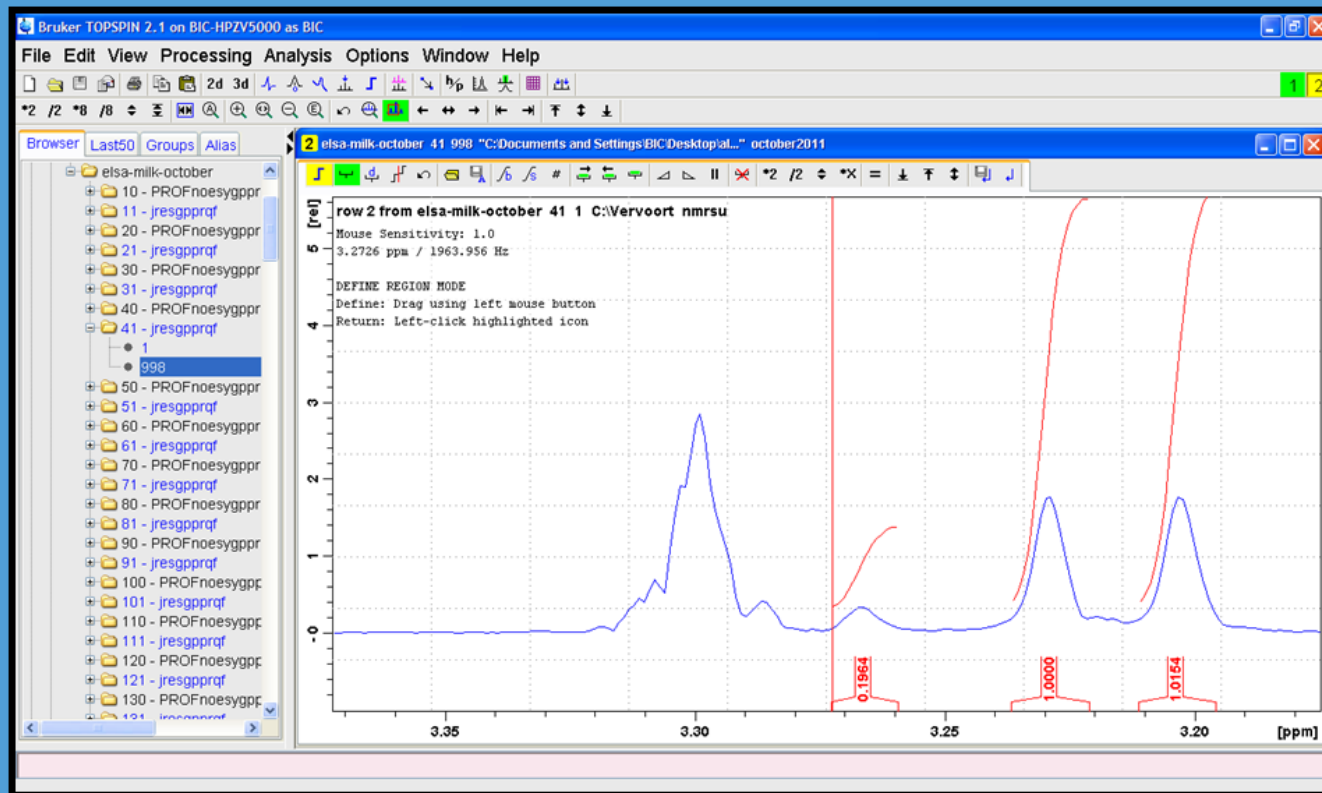


Metabolomics method – identification

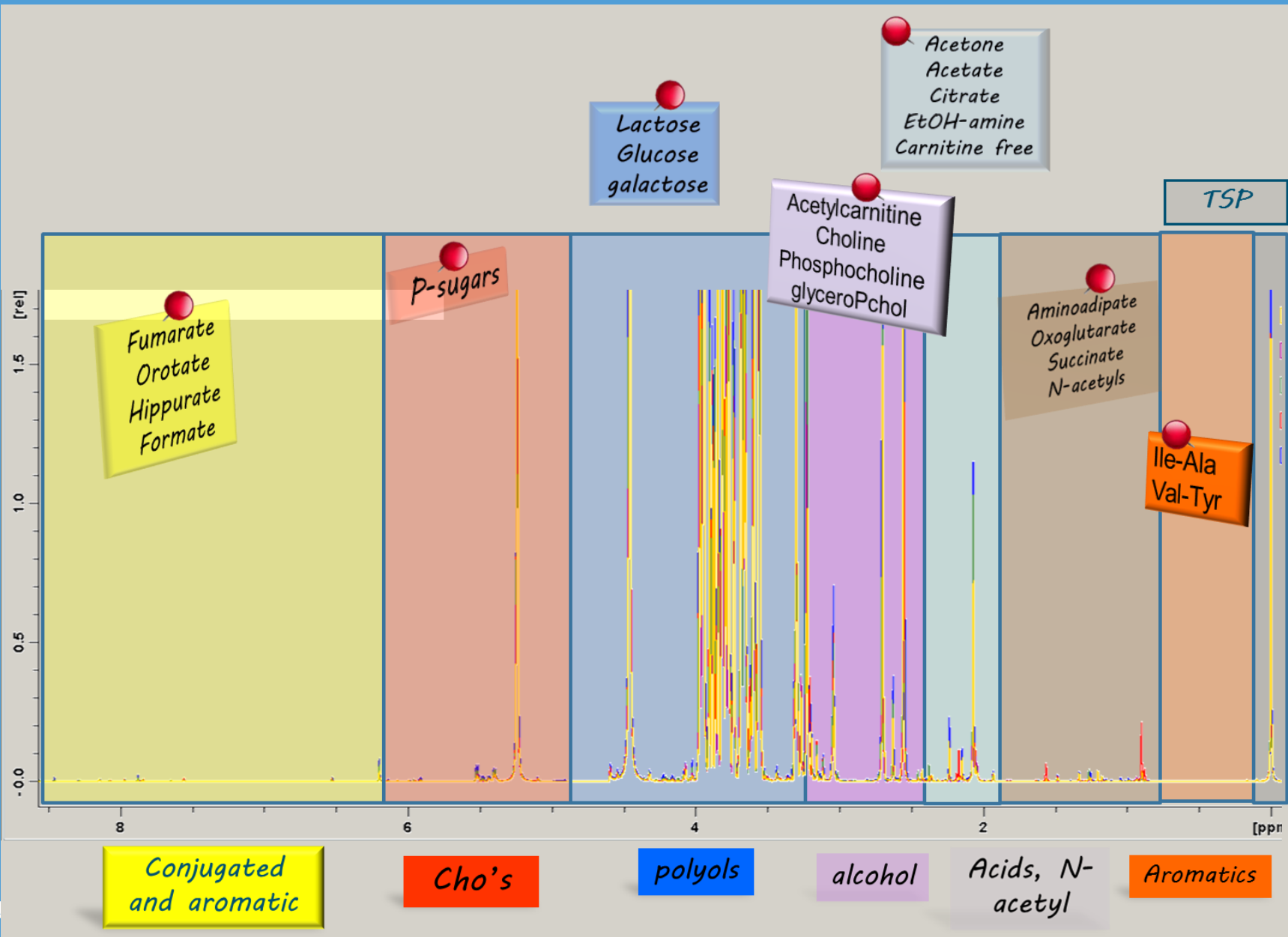


Metabolomics method – quantification

■ Spectra integration and relative quantification



Metabolomics method – milk serum map



Omics methods – time required

- Sample prep: 5-20 samples/day
- LC/MSMS or NMR analysis: 5-20 samples/day
- Primary raw data analysis: 20 samples/week
- Data interpretation: 20 samples/month

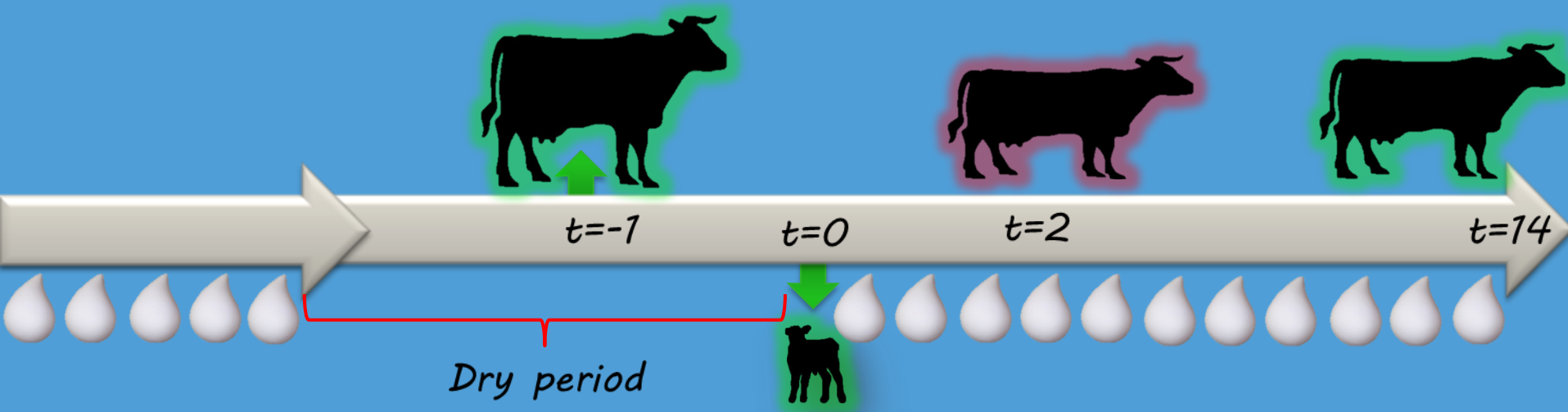


Example 1: Negative energy balance

J Lu, E Antunes Fernandes, et al
Journal of proteome research 12(7): 3288-96

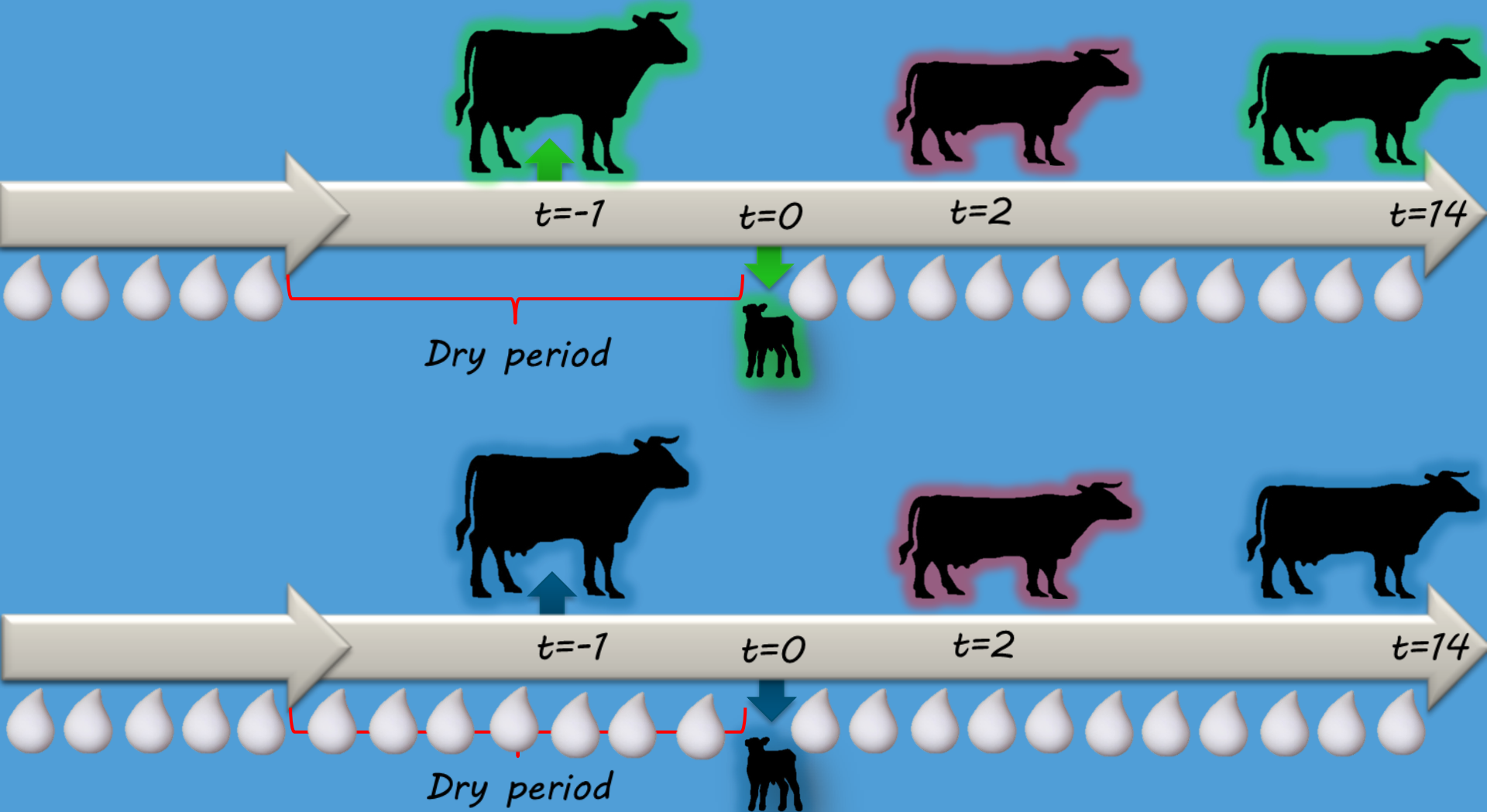


Negative energy balance (NEB) in dairy cows



- Body fat mobilization to compensate energy needs
- Higher susceptibility to diseases, metabolic disorders

Reduction of dry period improves NEB



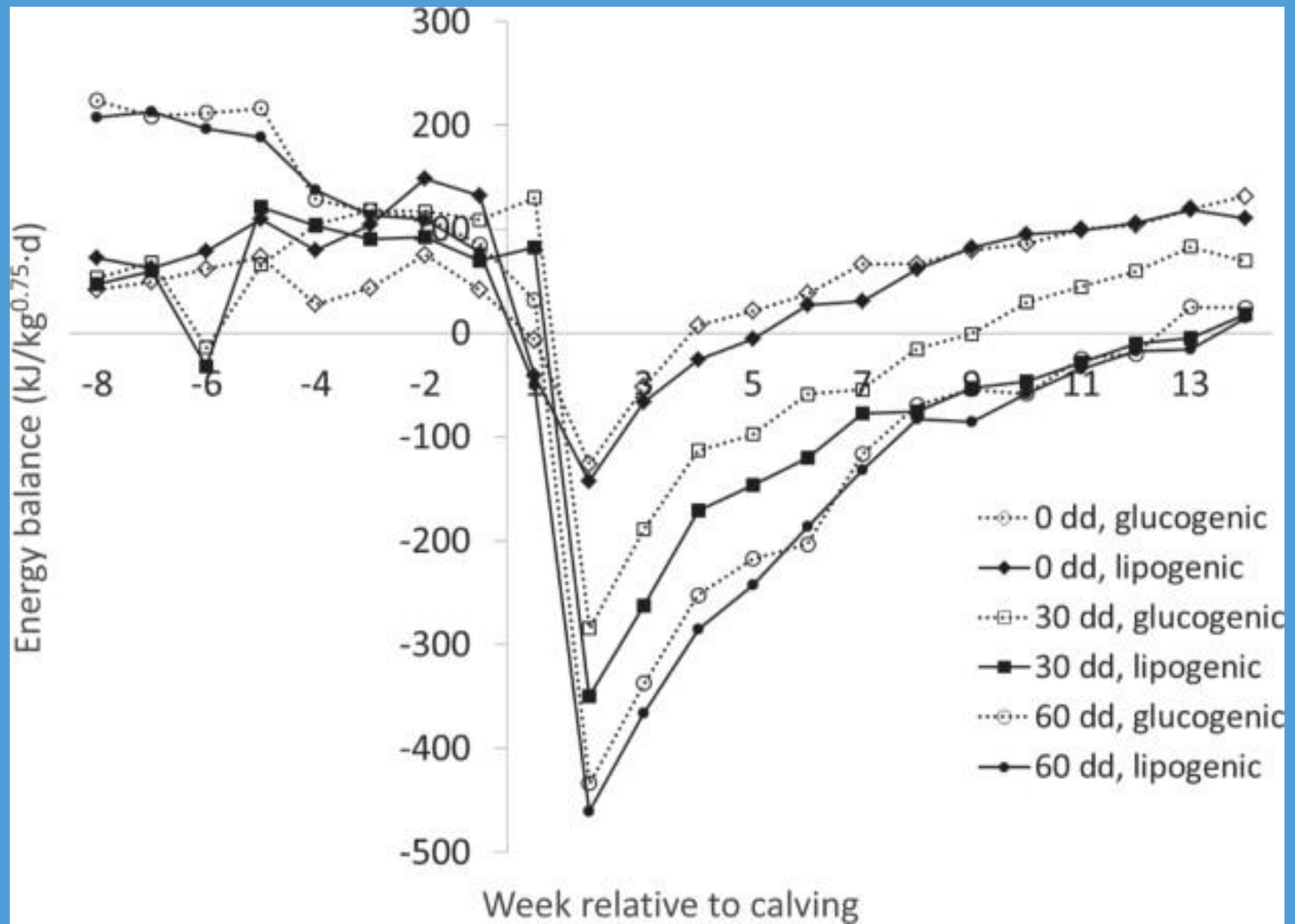
Work approach



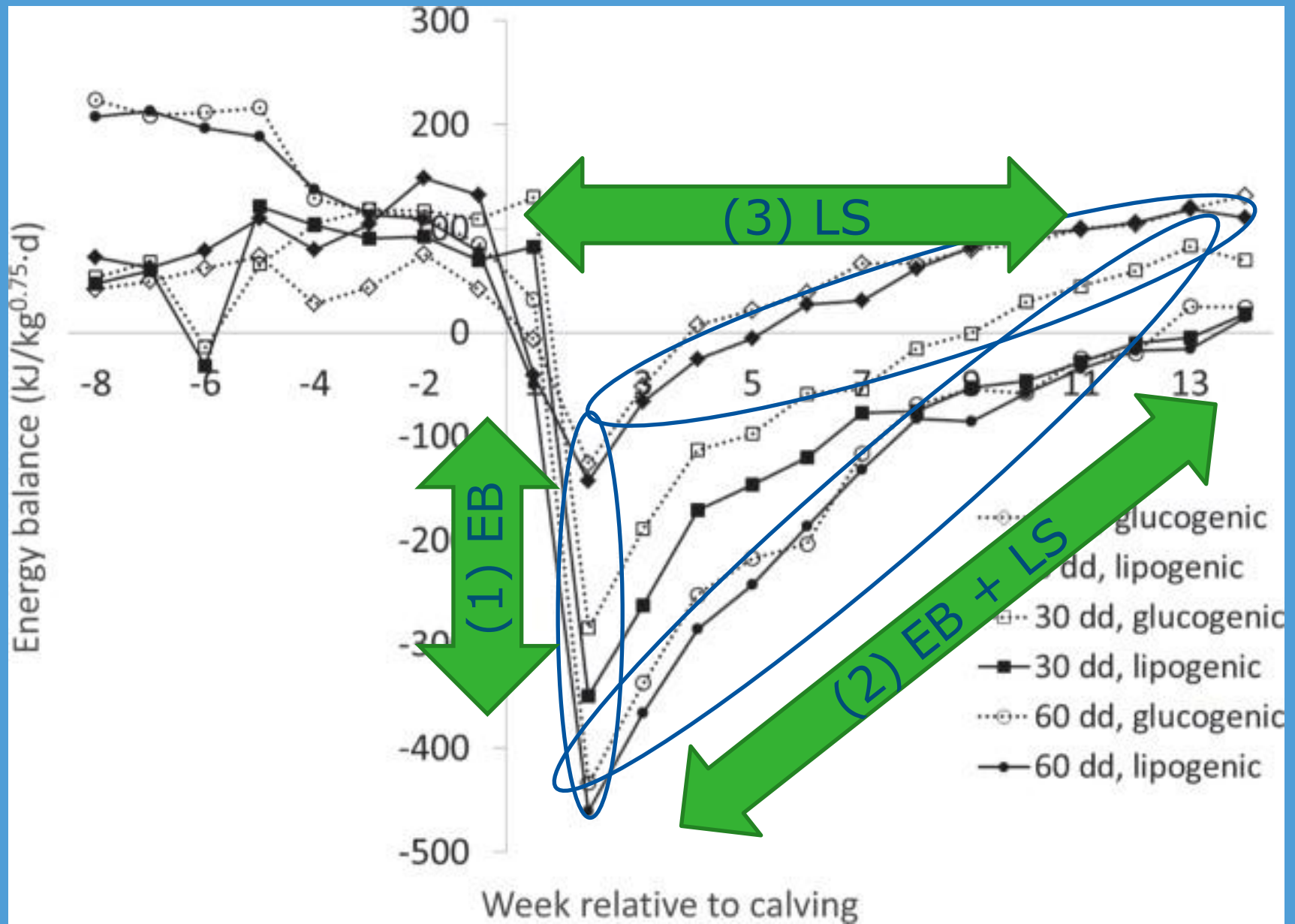
- Milk synthesis pathway
- ✓ Milk fat globule membrane (MFGM) to study proteins in mammary secretory cells (**proteomics - LC-MS/MS**)
- ✓ Milk metabolites related to energy balance of the cow (**metabolomics - $^1\text{H-NMR}$**)



Experimental design



Experimental design



Changes in proteome I

- Changes due to better energy balance

Changes in EB
0DP/60DP week2

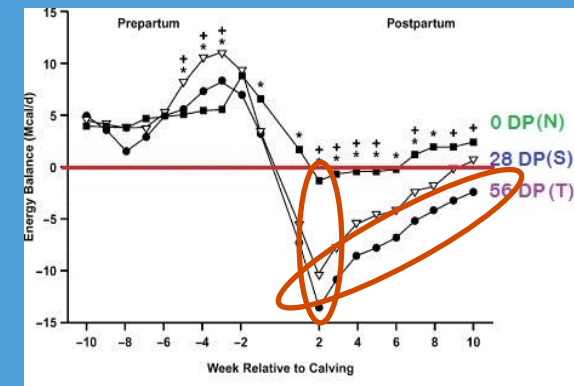
ALPL,
CRISP3,
RAB11B,
NPC2,
FOLR1,
Man8,
P4HB,
B2M.

STOM,
ENPP3,
ACSL1,
CYB5R3,
IDH1, LGB,
LALBA,
LPO, ALB,
LBP.

NSDHL, AGPAT6, LSS,
SLC34A2, RECS1,
ANKRD22, HSD17B7,
HSP90AA, S100A8,
ANSA2, HSPB1,
SAA3, HSPA8, LTF,
ANXA5, FGFBP1, GP2,
C3, UGP2, ANG1, IGJ,
PIGR, FGG, CD5L, IGK,
FGA, IG, FGB.

Changes in EB & LS
60DP Week14/week2

↑ Lipid metabolism
↓ Immune related proteins



Changes in proteome II

■ Changes due to progress of lactation

Changes in LS 0DP
week 14/week2

RAB6B,
SLC15A2,
RAB3D, CLU,
YWHAB,
ALOX12,
CAPN6, FASN,
HSP90AB1.

HSP90, AA1,
C3, IGJ,
IGL@, IGK.

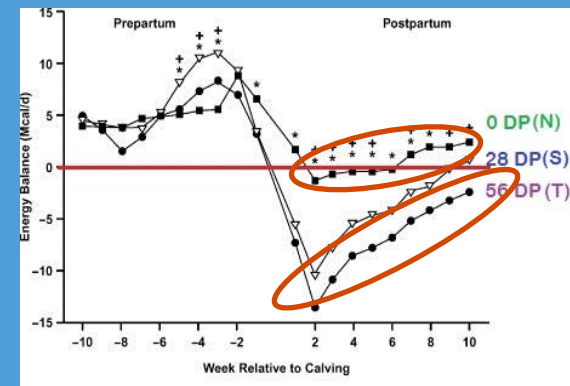
ACSL1, ENPP3, NSDHL,
AGPAT6, STOM, LSS,
SLC34A2, CYB5R3, RECS1,
ANKRD22, HSD17B7, IDH1,
S100A8, ANSA2, HSPB1,
SAA3, HSPA8, LTF,
ANXA5, LPO, FGFBP1, GP2,
C3, UGP2, ANG1, IGJ,
PIGR, FGG, LGB, LALBA,
ALB, CD5L, IGK, FGA,
LBP, IG, FGB.

Changes in EB&LS 60DP
week 14/week2

↓ Immune related proteins

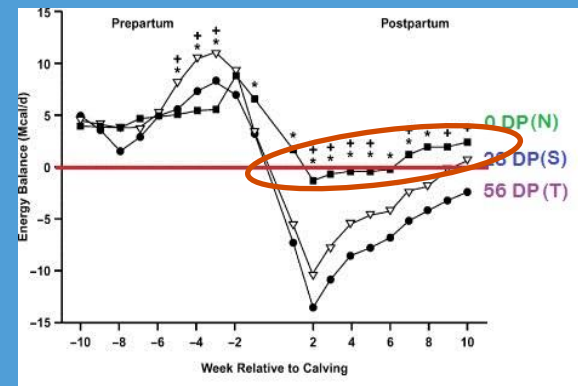
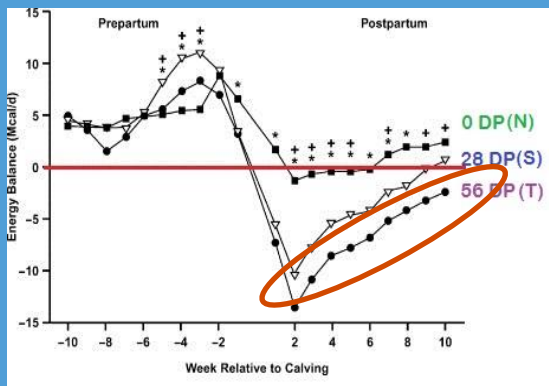
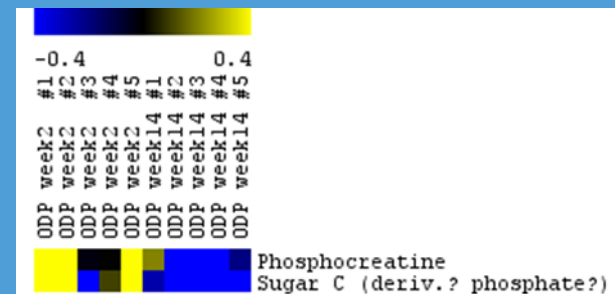
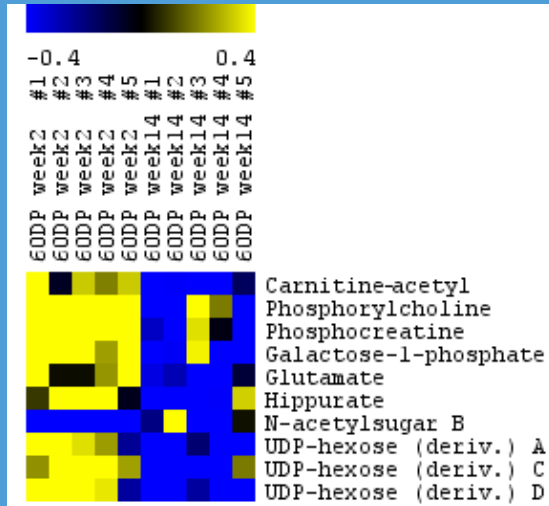


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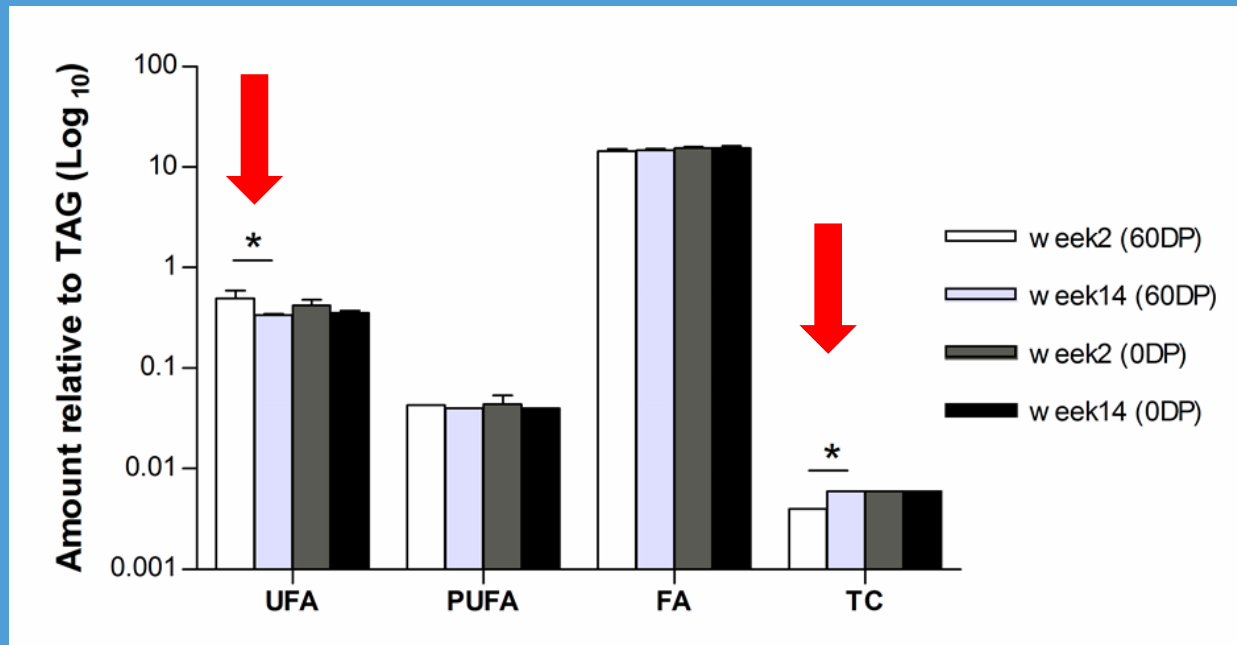


Changes in milk serum metabolome II

- Changes due to better energy balance and progress of lactation



Changes in milk lipid metabolome



- Severe NEB:
 - Increase in unsaturated fatty acids (UFA)
 - Decrease in cholesterol



Effect of NEB on mammary gland cell integrity

Galactose-1-phosphate in milk serum during NEB

- Sugar phosphates: intermediates of lactose synthesis: cell cytosol & Golgi apparatus → presence in milk → **leaking cells?**
- Stress of onset of lactation → apoptosis of epithelial cells in mammary gland?



Effect of NEB on mammary gland immune response

Acute phase response during NEB

- Extended lipolysis → partitioning of NEFAs (blood) → changes in FA profile in milk (increase UFA)
- Stress associated with parturition: rapid increase in milk production → high efficiencies of transport, synthesis and secretion in mammary gland



Effect of NEB on mammary gland lipid raft structures

Lack of components in lipid rafts in cellular membrane system during NEB

- Decrease in enzymes and transport proteins related to cholesterol synthesis
- Lower cholesterol in milk lipids
- Impaired synthesis and transport of cholesterol into milk
- Decrease of organizational protein in lipid rafts (stomatin)
- Organizational differences in plasma membrane of mammary epithelial cells

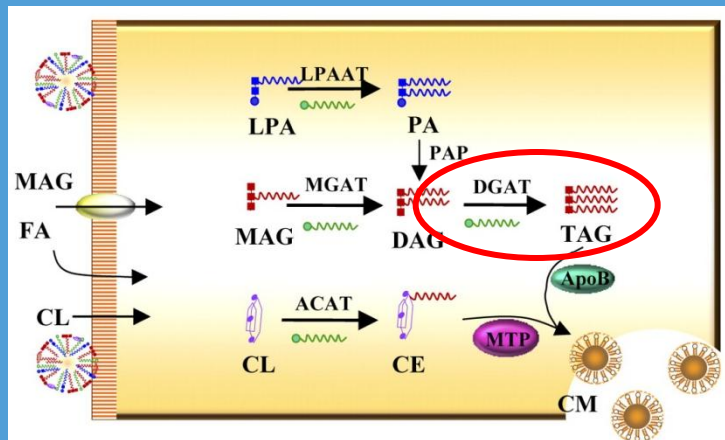


Example 2: DGAT1 genotype



Effect of DGAT1 on milk synthesis

- DGAT1 gene: Catalysis of the final step of triglyceride synthesis



DGAT1 K genotype:

↑ %fat,
↑ %protein,
↑ fat yield

↓ milk yield,
↓ protein yield

3 genotypes

AA



KA



KK



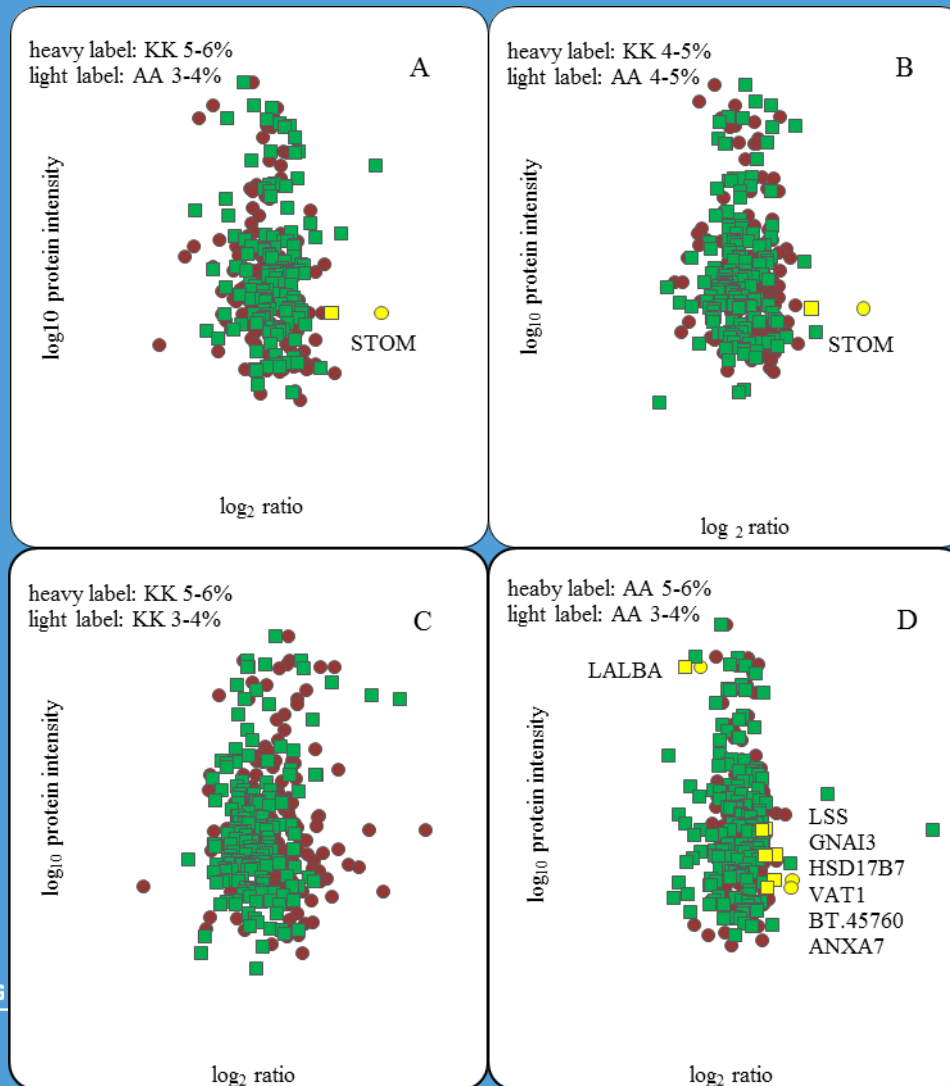
Experimental setup

Factors	polymorphisms & fat content	Poly-morphisms	fat content in KK	fat content in AA
Grp 1	5-6% KK	4-5% KK	5-6% KK	5-6% AA
vs	vs	vs	vs	vs
Grp 2	3-4% AA	4-5% AA	3-4 % KK	3-4% AA



Changes in milk fat globule proteome

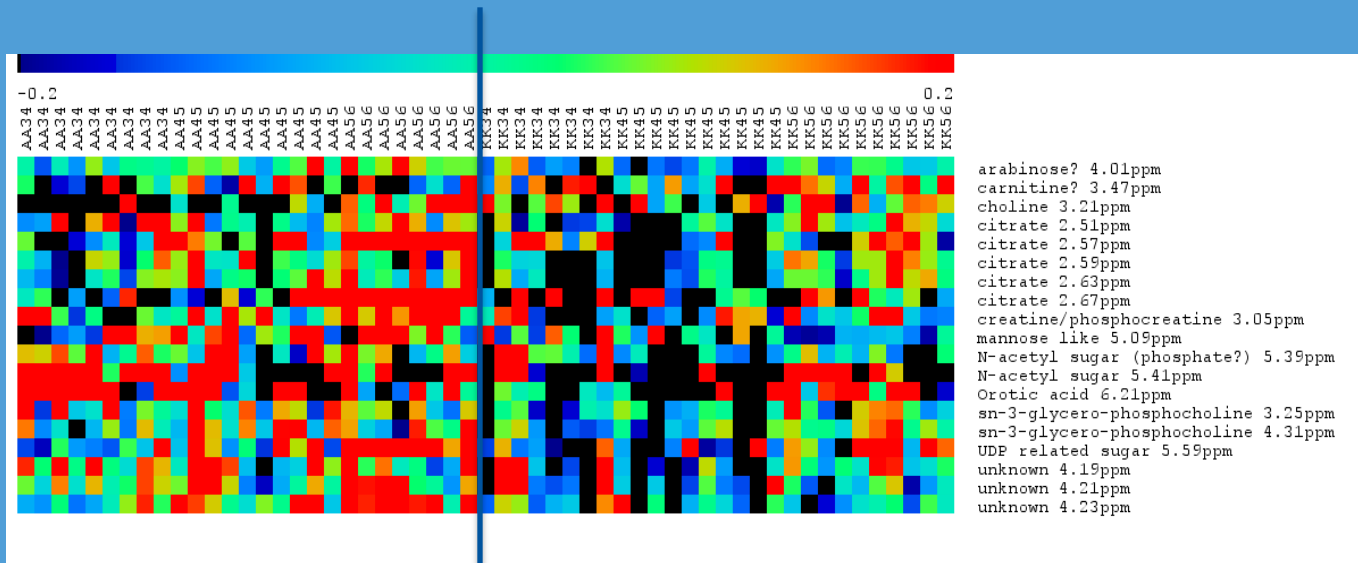
Proteomics results: KK: Stomatin[↑]



Changes in milk serum metabolome

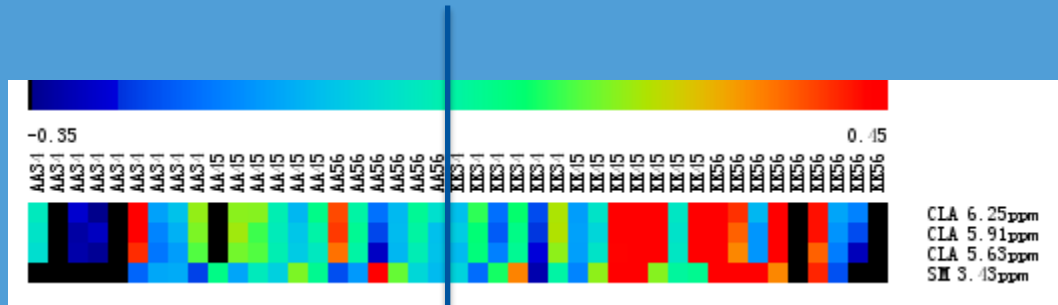
Milk serum proteome, in KK:

- Sugars indicative of leakage, citrate, (P-)creatine ↓
- Carnitine ↑



Changes in milk lipid metabolome

Lipid metabolome: KK: sphingomyelin↑, de novo synthesis↓



	C10:0	C12:0	C14:0	C4:0-	C15:0	C16:1	C17:0	C18:0	C18:1
				C14:0					trans11

KK	2.78	3.68	10.86	24.87	1.19	1.54	0.48	9.56	0.87
AA	3.03	4.18	11.92	26.47	1.08	1.32	0.44	8.73	0.70



Influence DGAT1 genotype on mammary gland & milk synthesis

In KK:

- Less indications of cell leakage
- More carnitine (involved in lipid metabolism)
- Less de-novo fat synthesis
- Increase of organizational protein in lipid rafts (stomatin & sphingomyelin) -> opposite effect to NEB
- This indicates a different cell architecture which may underlie the differences in milk composition



Conclusions & recommendations

These findings support the idea that combining information of different –omics technologies result in a better understanding of the physiology of lactating cows

For further research:

- Clarify the role of stomatin and galactose-1-phosphate in milk of dairy cows -> relate to what happens in the mammary gland epithelial cells and their membranes
- Assess the applicability of proteins/metabolites as biomarkers for cow physiology/cow health



Thank you for your attention!

Acknowledgements:

Elsa Antunes-Fernandes

Lu Jing

Ariette van Knegsel

Toon van Hooijdonk

Jacques Vervoort

and many MSc students

kasper.hettinga@wur.nl



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