



Department of Infectious
Diseases and Public Health

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Intensive Feeding Increases Liver Ceramide Concentrations in Holstein Fattening Bulls

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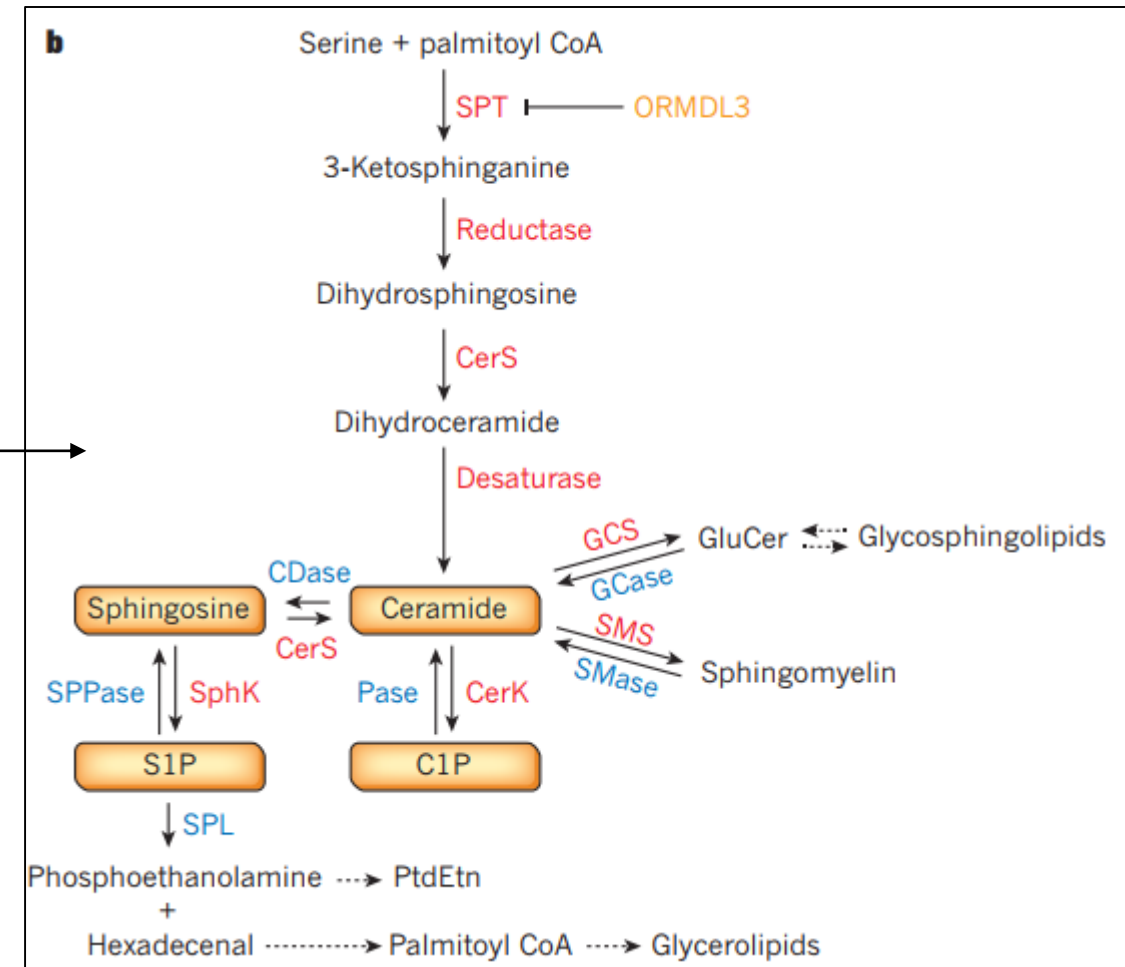
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Sphingolipid signalling

- Bioactive sphingolipid metabolites (Maceyka and Spiegel, Nature, 2014):

- Interaction with:
 - oxidative stress
 - inflammation
 - insulin resistance



- Ceramides involved in metabolic adaptation in transition cows (McFadden and Rico, JDS, 2019)

Metabolic effect of intensive feeding

- Intensive feeding: High energy diets to enhance production performance (faster, greater body weight gain)
- “Overnutrition syndrome” in transition cows (Janovick et al., JDS, 2011)

Hypothesis:

- High energy diet triggers metabolic alterations that correspond to a more inflammatory phenotype
- This is reflected in the liver by altered sphingolipid metabolism

Materials and Methods

- Holstein fattening bulls for beef production
 - Randomly assigned to 2 dietary treatment groups:
 - Intensive feeding (n=15): Corn- and grass-silage based diet \rightarrow 806.5 \pm 9.4 kg
+ 6 kg/day/animal concentrate feed
 - Control feeding (n=15): Corn- and grass-silage based diet \rightarrow 712.1 \pm 11.5 kg
 - Duration of treatments: Last 8 months of the fattening period
- Results:*
Final live weight:
vs.
(*mean \pm SEM*)
- Slaughter at 20 months of age \rightarrow collection of liver samples
 - Liver: central organ for sphingolipid metabolism, correlates with plasma profile

Metabolomics: Sphingolipid profiling

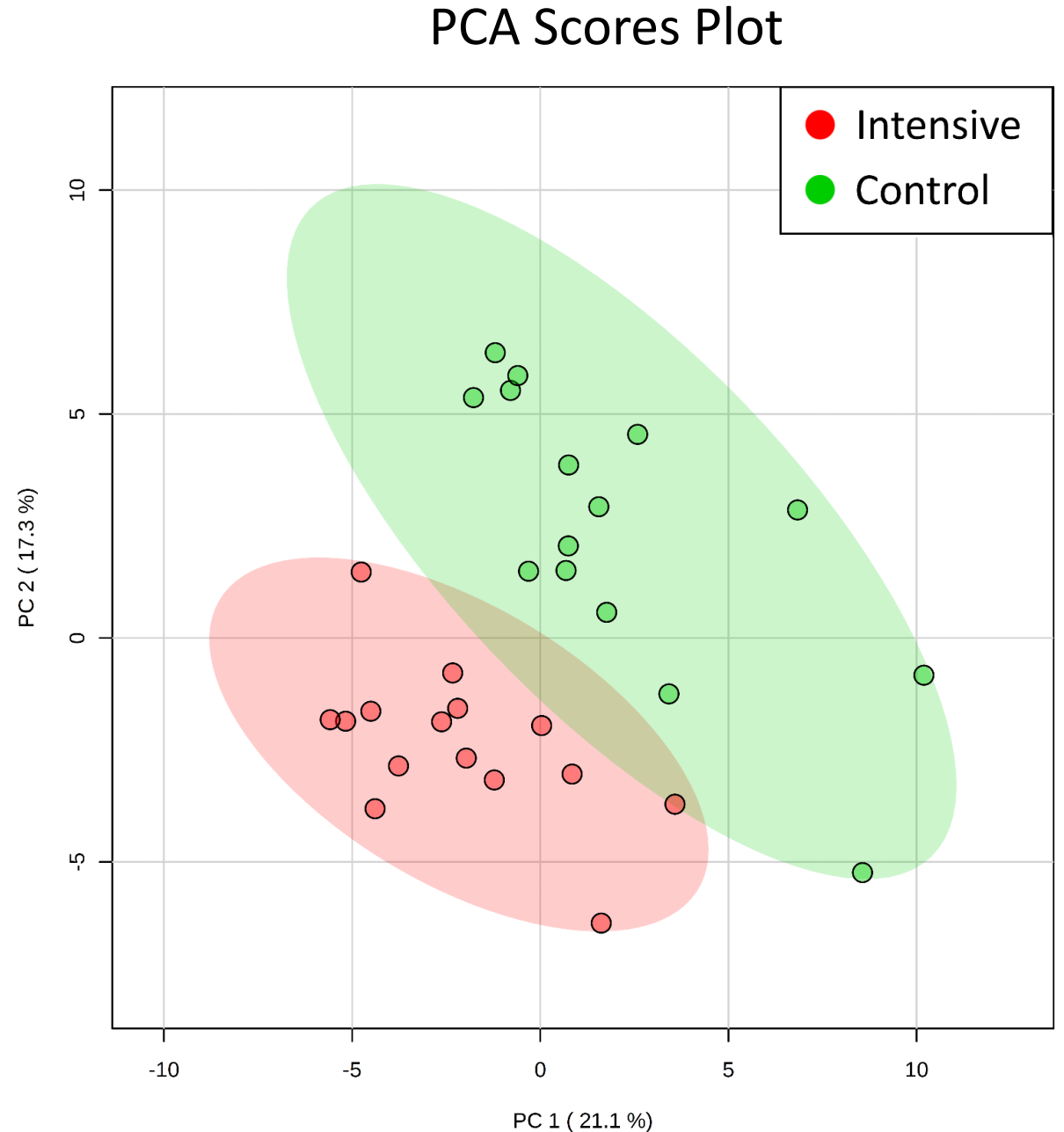
- Methanol-chloroform based lipid **extraction from liver** homogenates
- Liquid chromatography – tandem mass spectrometry (LC-MS/MS) analysis in positive- and negative-ion mode
- **77 sphingolipid species targeted**: concentrations determined by linear regression calibration curves based on standards
 - **Ceramides and dihydroceramides (24)**
 - **Sphingomyelins and dihydrosphingomyelins (19)**
 - **Ceramide- and sphingosine-1-phosphates (16)**
 - **Hexosylceramides (10)**
 - **Sphinganines and sphingosines (8)**
- Multivariate statistical analysis in MetaboAnalyst 4.0



The **Metabolomics** Innovation Centre

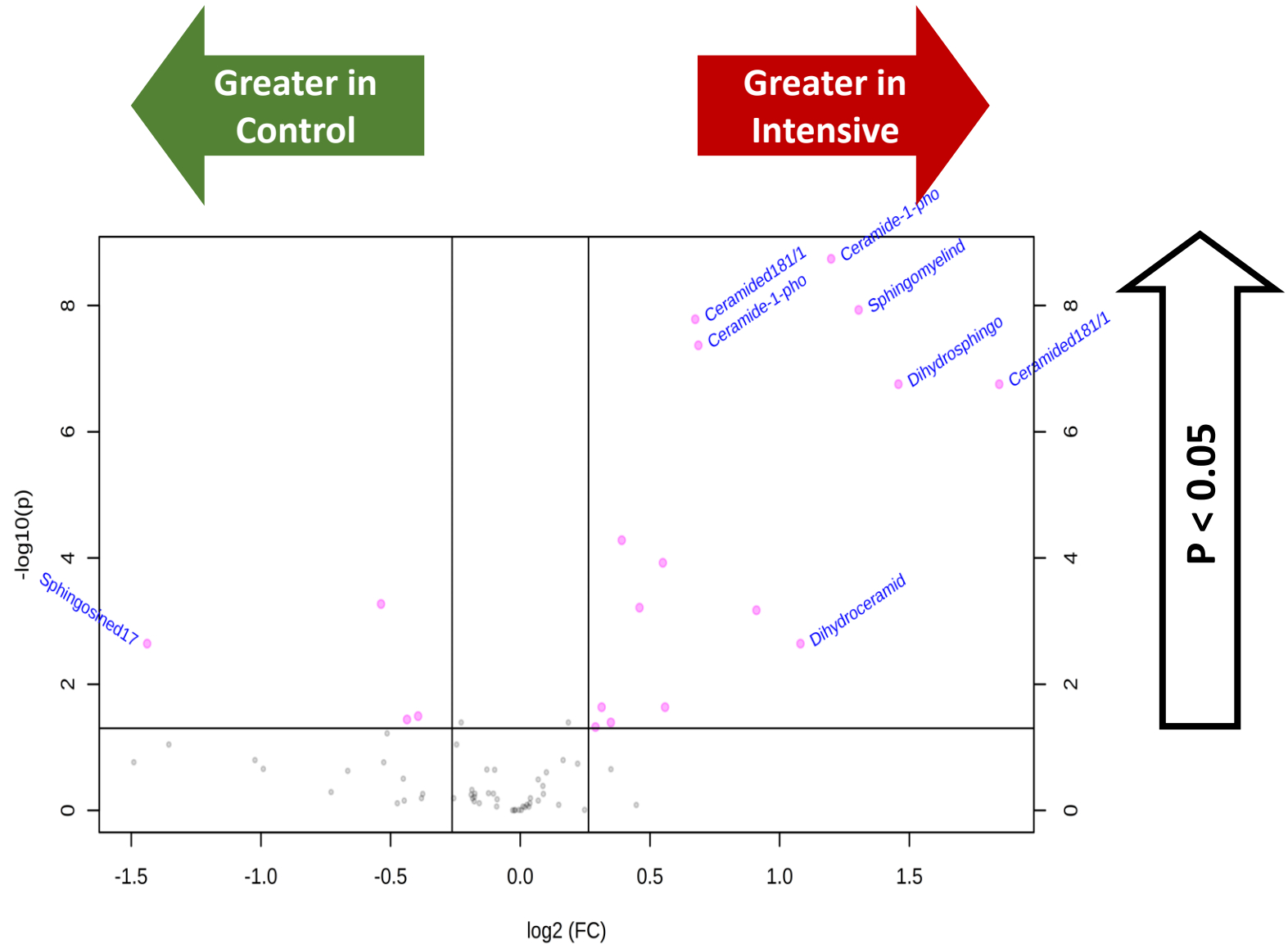
Liver sphingolipid profiles

- Concentrations of 77 sphingolipids (n=15 per treatment)
- Principal component analysis (PCA): Liver sphingolipid concentration data showed separation according to dietary treatments



Significant metabolites

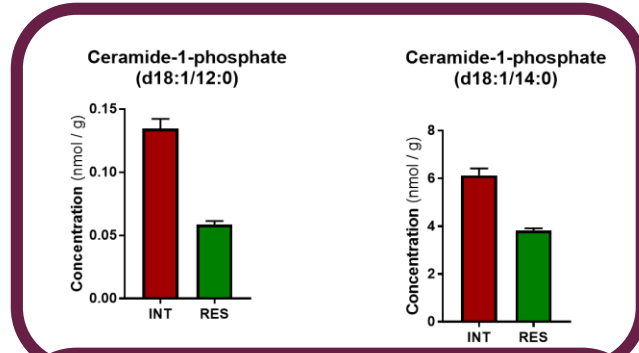
- Volcano plot:
Filtering significant metabolites
(FC > 1.2 and FDR-adjusted P < 0.05)
- 15 lipids greater in
“Intensive” group
- 4 lipids greater in
“Control” group



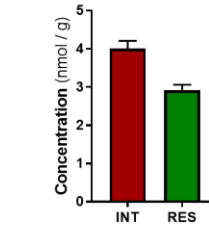
Volcano plot

Greater concentration in “Intensive” group

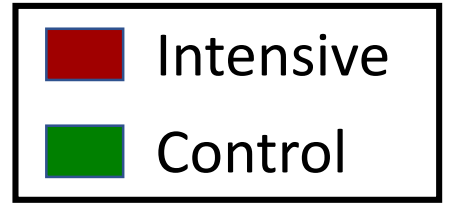
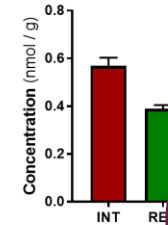
Ceramide-phosphates:



Dihydroceramide-1-Phosphate (d18:0/16:0)

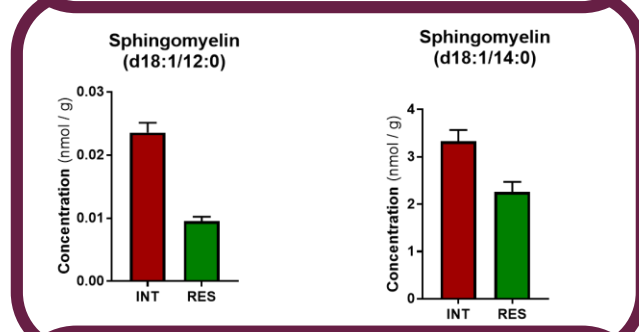


Dihydroceramide-1-Phosphate (d18:0/20:0)

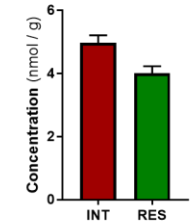


Chain lengths:
14:0 and 12:0
Consistently greater
in “Intensive”

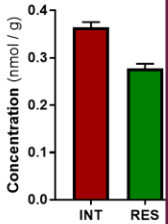
Sphingomyelins:



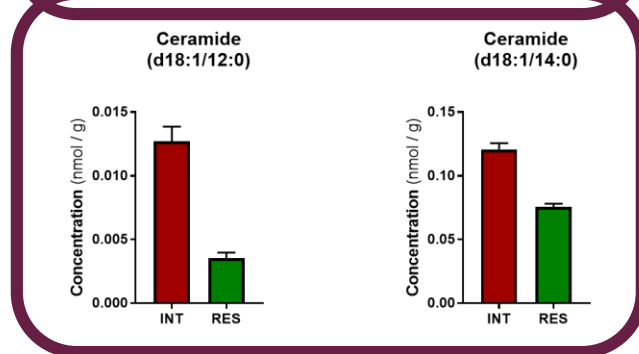
Sphingomyelin (d18:1/18:1)



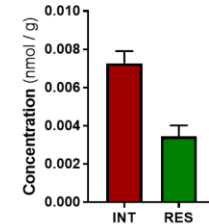
Sphingomyelin (d18:1/20:1)



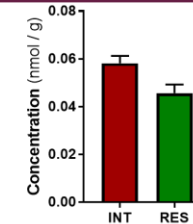
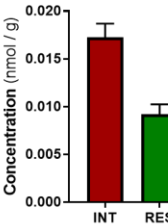
Ceramides:



Dihydroceramide (d18:0/12:0)

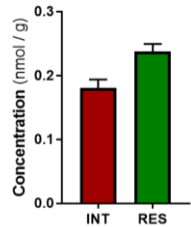


Dihydroceramide (d18:0/14:0)

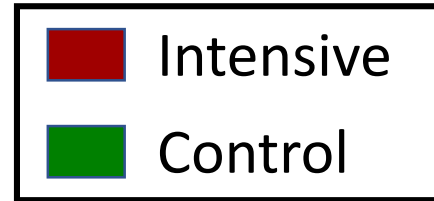


Greater concentration in “Control” group

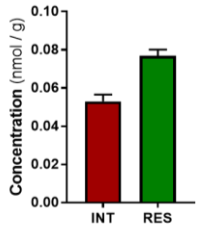
Ceramide-1-phosphate(d18:1/28:0)



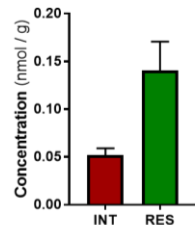
Ceramide-1-P (d18:1/28:0) – very long chain



Ceramide (d18:1/17:0)

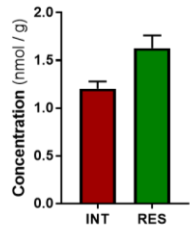


Sphingosine (d17:1)



Ceramide (d18:1/17:0)
and Sphingosine (d17:1) – odd chain

Sphingomyelin (d18:1/18:2)



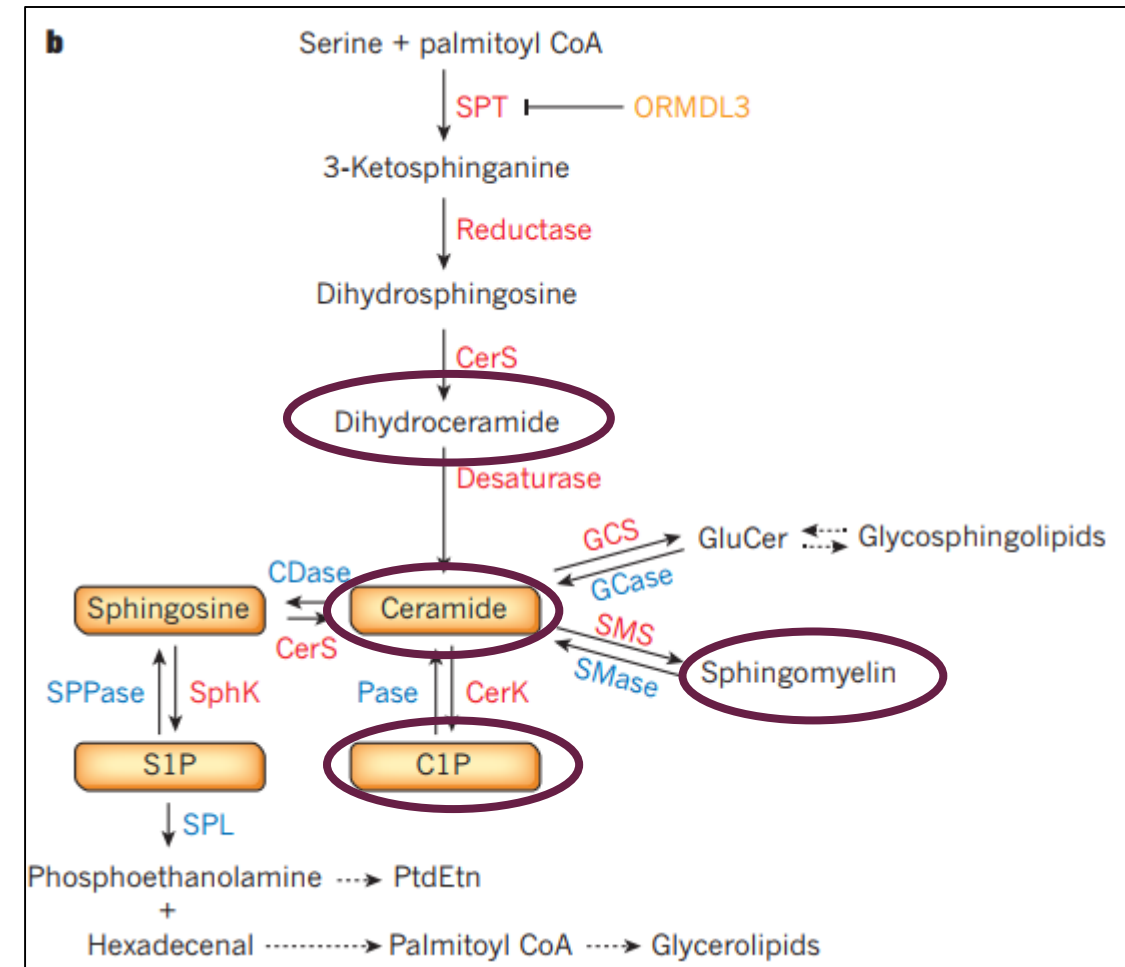
Sphingomyelin (d18:1/18:2) – double unsaturated

Uncommon
sphingolipids:

Metabolic function
yet to be explored

Sphingolipids greater in “Intensive” group

- Chain length 12:0 and 14:0
- Ceramide: antagonises insulin action in transition cows (Rico et al., JDS, 2015) and promotes apoptosis and endothelial dysfunction in humans (Maceyka and Spiegel 2014)
- Ceramide-1-phosphate: modulates inflammation and immune response in humans (Maceyka and Spiegel 2014)
- Sphingomyelin dihydroceramide as precursors



(Maceyka and Spiegel, Nature, 2014)



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Thank you for your attention!