NMR Metabolomics on Cell Monolayer

Background
The worldwide obesity epidemic and changes in lifestyle to less physical activity have given rise to growing numbers of people suffering from metabolic syndrome. Certain nutritional compounds have been found to have potential health benefits after intake, especially in relation to obesity, diabetes and cardiovascular diseases. A possible mechanism could be that these compounds increase gut barrier integrity and prevent inflammation. However, no clear link has been established.

Caco-2 is a human colon epithelial cancer cell line. By growing a Caco-2 cell monolayer on permeable membranes in-vitro, the cell model can be used to mimic the small intestine epithelium. Intervention studies can be made by adding a component of interest on the apical side of the Caco-2 cell monolayer.

Objective
Using a Caco-2 cell monolayer model, can NMR metabolomics detect:
- differences between the apical and basolateral sides
- an intervention effect of different treatments

Methods
1H NMR spectra were recorded on Bruker Avance III 600 MHz spectrometer with a 5mm BO probe (Exp I) or a Prodigy CryoProbe (Exp II) with 1D NOESY experiments.

Caco-2 cells were seeded onto filter inserts and incubated in 24-well plates using a 21-day differentiation protocol. Interventions were performed for 24-hours with 4 replicates. Treatments were added to the apical side. Cell media from the apical and basolateral sides are collected at the end of the experiment.

Exp I: Test on dose-response sterile fecal water and probiotic supernatant.
Exp II: Intervention of sterile fecal water from 26 subjects. The samples belongs to 5 different groups but are blinded.

Results
- Increased amounts of acetate, EtOH and butyrate as a response of fecal water conc. added to the apical side. These metabolites are typical fermentation products.
- Minor metabolic differences in cell media between the apical and basolateral sides. Two unknown signals seems to be produced by the caco-2 monolayer.
- Grouping of control samples from treatment samples on the basolateral side. This indicates a metabolic effect on the caco-2 monolayer.

Conclusion
- Mechanistic effects can be studied by measuring the Caco-2 cell monolayer integrity and the cell media on the apical and basolateral sides.