Open flow microperfusion (OFM) – a tool for in vivo metabolic profiling of adipose tissue

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Introduction & Aim

One major challenge for healthcare systems is the increasing prevalence of obesity. Several therapeutic approaches are modifying adipose tissue metabolism to induce sustained weight loss but many metabolic pathways that mediate treatment effects remain elusive. The aim of this study was to establish a combined method for the investigation of tissue specific metabolism in brown and white adipose tissue (BAT and WAT). Three rats were implanted with open flow microperfusion (OFM) probes to sample interstitial fluid (ISF) of the respective tissue types (Figure 1 and 2). Samples were analysed by using a Vanquish UHPLC coupled to a QExactive mass spectrometer.

Material & Methods

HPLC-MS[1]

- overnight sample overnight sample extraction
- NH2 Luna HILIC Säule (2 x 150 mm, 3 µm; Phenomenex)
- Vanquish UHPLC
- QExactive mass spectrometer Thermo Fischer Scientific
- positive and negative electro spray ionisation mode
- mass range from 70 to 1050 m/z
- gradient elution 37 minutes

Targeted data analysis

- PeakScout (developed by Joanneum Research)
- reference list (accurate mass + retention time)
- manual signal confirmation
- rigorous data quality controls
- data Log-transformed and median-QC normed (checked for sufficient normality and heteroscedasticity via four different tests)
- Statistics-R [R Core Team] (v3.4.1, packages stats, FactoMineR, missMDA, nlme, lsmeans, readxl, openxlsx)
- TibcoSpotfire for data visualization

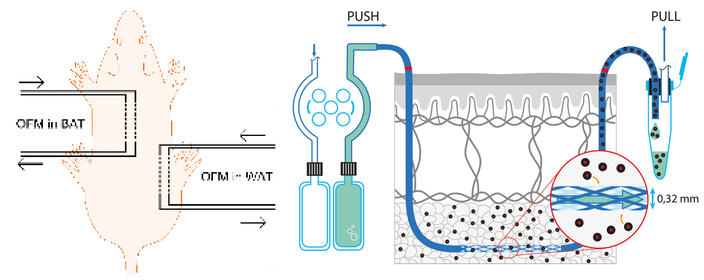

Figure 1*: Experimental set-up for OFM sampling in BAT and WAT. ISF was collected with OFM in 5 sampling intervals (1 hour each) with a total of 119 samples from 12 animals.*

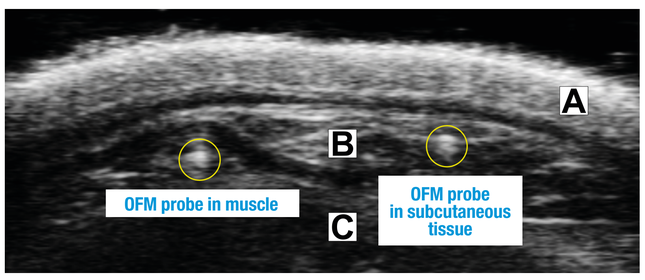

Figure 2*: Ultrasonic image of the interscapular area. A- dermis, B- subcutis, C- muscle. The verification whether subcutaneous BAT or WAT was penetrated is done post mortem via dissection*

Results

Metabolic profiling identified 141 metabolites including lipids, fatty acids and amino acids which were classified into metabolites usable for multivariate (MVA; 95 metabolites) and univariate (UVA; 46 metabolites) data analysis (Figure 4). High quality metabolites for MVA were defined as quality control RSD <30%, blank load <20%, sample data missing <30%. The median standard deviation of peak intensity of the pooled quality control samples was 7.1% with a low ppm deviation (≤10 ppm) and minimal retention-time deviation in comparison to the standards. These criteria made samples usable for subsequent principal component analysis (PCA) and analysis of variance (ANOVA).

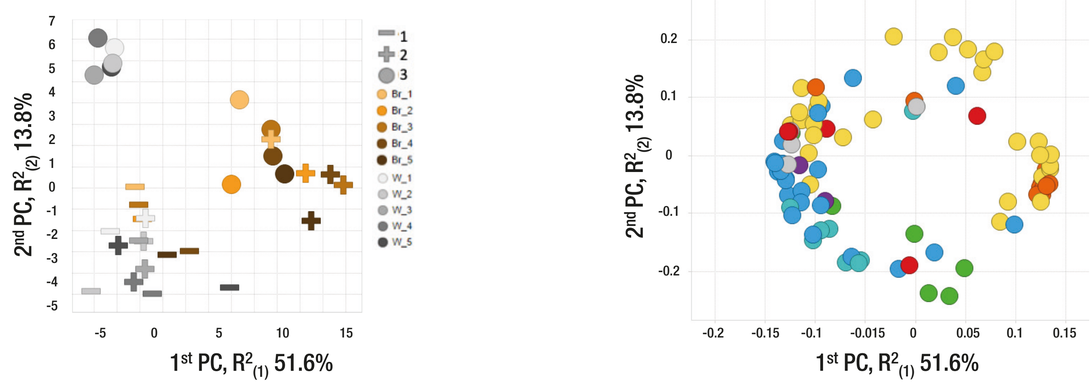

Figure 3: *PCA Scores Plot (left) and PCA Loading Plot (right). BAT (Br) samples coloured brown, WAT (W) samples coloured white; Metabolite colouration in the loading plot according to Figure 4.*

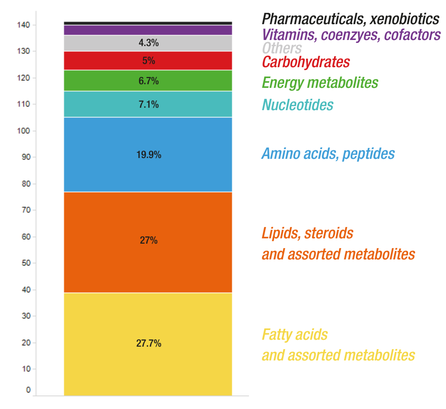

Figure 4: *Metabolite classess sorted according to percentages.*

The metabolite pattern in the loading plot showed clear differences among the 94 MVA metabolites (Figure 3), especially for „fatty acids“ and „amino acids“ allowing a distinction of BAT and WAT. In animals #2 and #3 strong metabolic differences were particularly driven by these metabolic groups but also in part by „energy metabolites“. This indicates that BAT metabolizes more energy metabolites, leading to an increase in energy consumption. Notably, rat #3 was nearly double the weight of the other animals and exhibited a distinct separation regarding WAT.ANOVA results were in good agreement with PCA. In BAT, 42 metabolites were significantly higher than in WAT and 36 were lower ($p < 0.01$; Benjamini-Hochberg corrected for multiple testing).

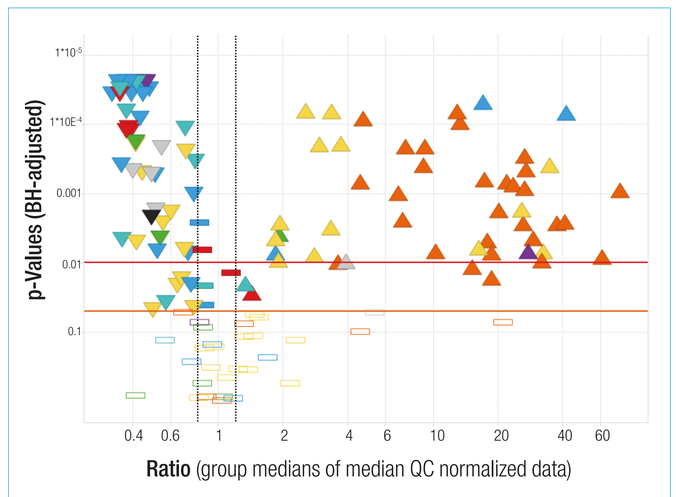

Figure 5*: Volcano plot for metabolites showing significant changes from the ANOVA model. Benjamini-Hochberg adjusted p-values (inverse log scale) versus metabolite ratios (median QC normalized data; mixed Tissue, random rat ID). Left side metabolites with increase in WAT; right side with increase in BAT. Metabolites are coloured according to their respective classes.*

Conclusion

Our results are a first proof-of-concept for a **direct in-vivo investigation of adipose tissue metabolism**. Stable measurements were possible even for samples with small volumes of < 15 µl. A combination of OFM and metabolomics allows first insights into **metabolic differences between brown and white adipose tissue**.

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Disclosure

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References

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