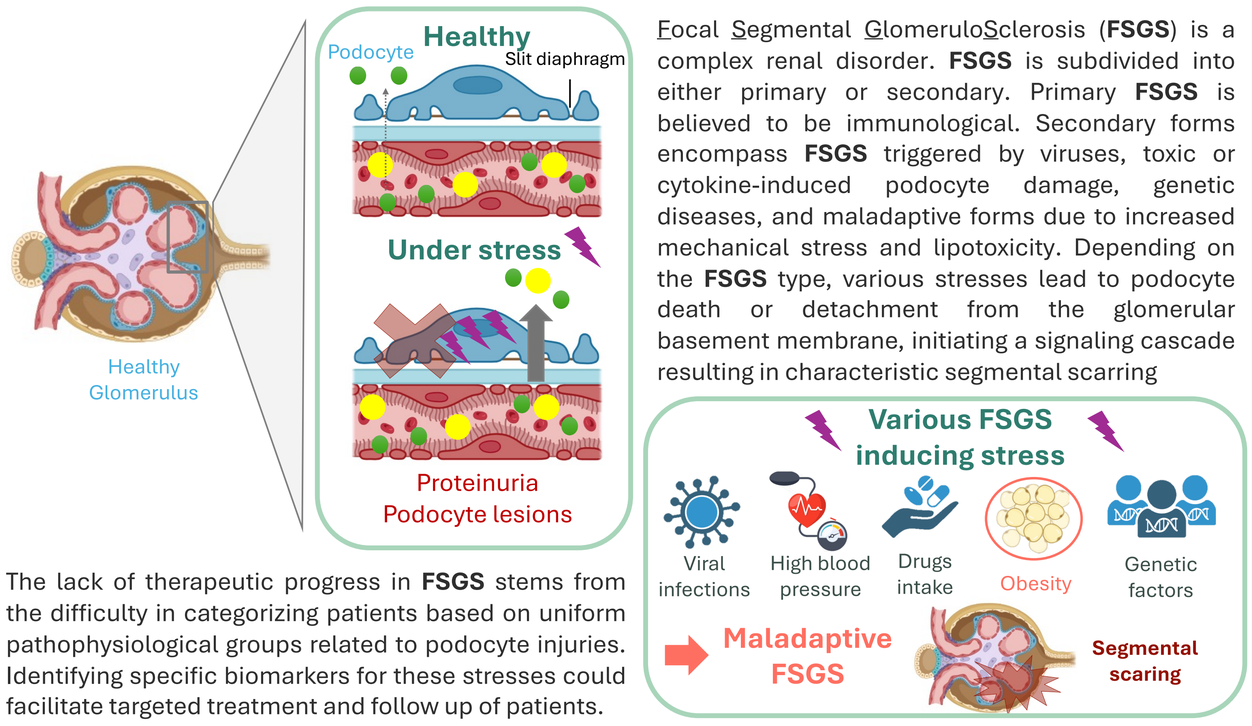
Podocyte metabolic adaptation to lipid overload – application to focal segmental glomerulosclerosis

Marine Thirion[1,2], Aurore Hecq[1], Emmanuel Esteve[1], Vanessa Tagliatti[2], Jean-Marie Colet[2], Anne-Emilie Declèves[1]
[1]Metabolic and Molecular Biochemistry lab, Umons - [2]Human biology and Toxicology lab, Umons

BACKGROUND

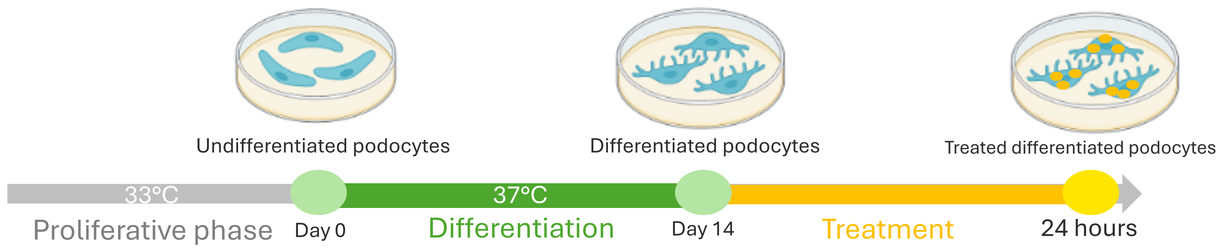


Focal Segmental GlomeruloSclerosis (**FSGS**) is a complex renal disorder. **FSGS** is subdivided into either primary or secondary. Primary **FSGS** is believed to be immunological. Secondary forms encompass **FSGS** triggered by viruses, toxic or cytokine-induced podocyte damage, genetic diseases, and maladaptive forms due to increased mechanical stress and lipotoxicity. Depending on the **FSGS** type, various stresses lead to podocyte death or detachment from the glomerular basement membrane, initiating a signaling cascade resulting in characteristic segmental scarring

The lack of therapeutic progress in **FSGS** stems from the difficulty in categorizing patients based on uniform pathophysiological groups related to podocyte injuries. Identifying specific biomarkers for these stresses could facilitate targeted treatment and follow up of patients.

AIMS & METHODS

2D Cell Culture Model : human podocyte immortalized cell line (LY)



1. Characterization of podocytes basal métabolism

^{1}H-NMR based metabonomics study was carried out on cell lysates and extracellular media of podocytes differentiated (D14) or not (D0)

2. Global metabolic activity under palmitate exposure

MTT assay was used to determine what impact palmitate (PA) have on metabolic activity

3. Deep lipotoxic metabolic adaptations assessment

^{1}H-NMR based metabonomics study was carried out on cell lysates and extracellular media of podocytes treated to 300µM PA or 0,96% bovine serum albumine (BSA) as control condition.

GOAL Identify metabolic signatures and biomarkers of lipotoxicity as a first FSGS-inducing stress observed in the maladaptive form.

RESULTS

^{1}H–NMR BASED METABONOMICS:MODEL CHARACTERIZATION

Extracellular media

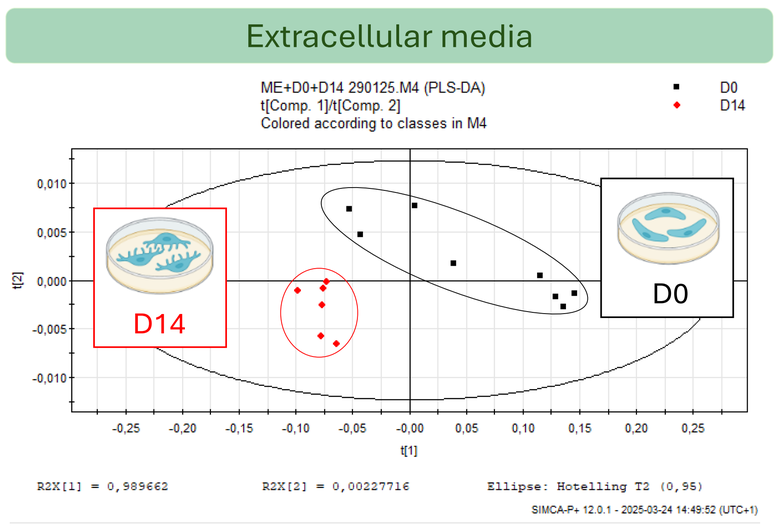


Fig. 1 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from differentiated (D14) or undifferentiated (D0) podocytes extracellular media (p-value = 0,029)

Cell lysates

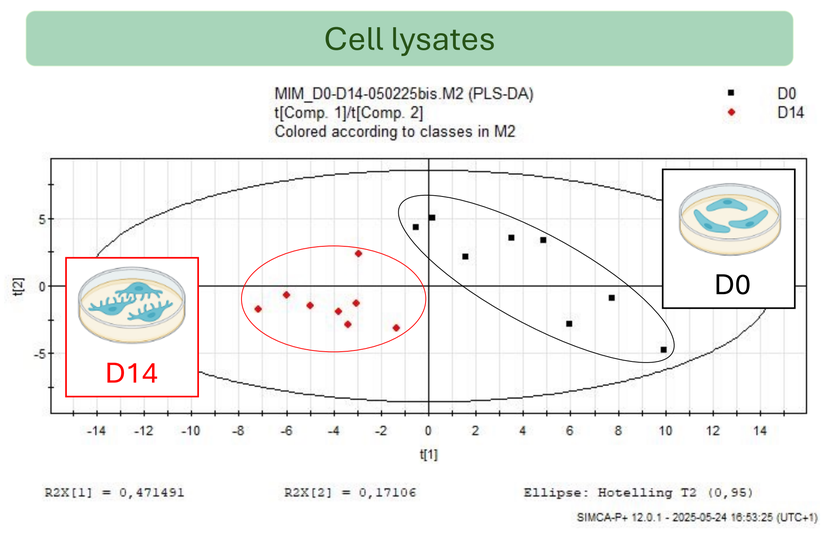
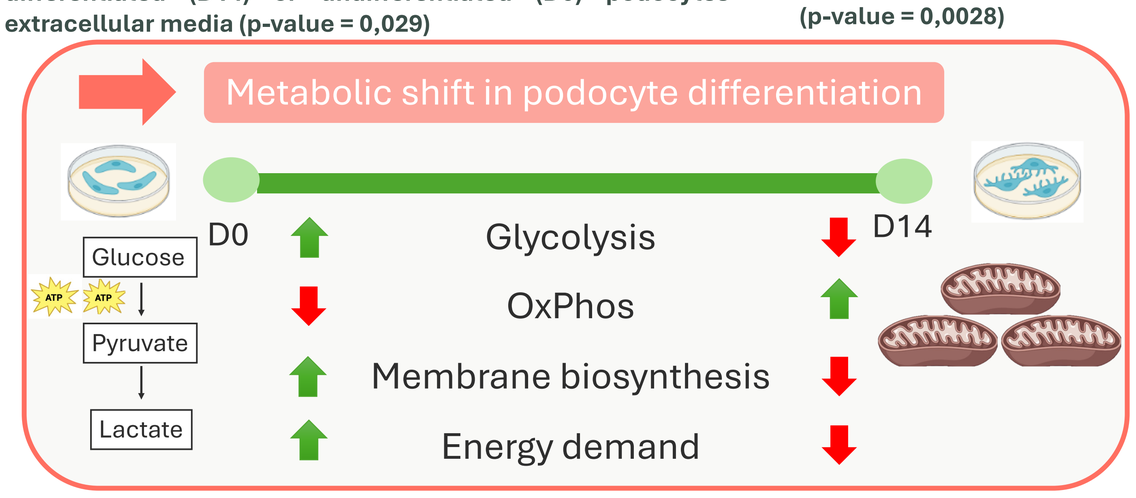


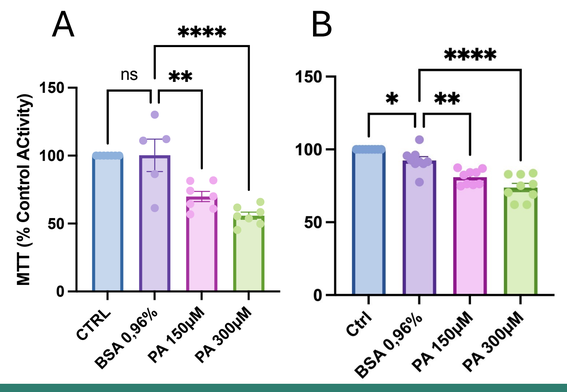
Fig. 2 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from differentiated (D14) or undifferentiated (D0) podocytes lysates (p-value = 0,0028)

Metabolite	Chemical shift (ppm) and multiplicity	Changes in EC D14	Changes in IC D14
Lactate	1.32 (d), 4.12 (q)	↓	↓
Glucose	3.88 (dd), 5.2 (d)	↓	↑
Myo inositol	3.29 (t), 3.63 (t), 4.07 (t)	-	↑
Glutamate	2.06 (q), 2.34 (m), 3.76 (q)	↑	↑
Glutamine	2.12 (m), 2.44 (t), 3.77 (t)	↑	-
Pyroglutamate	2.42 (ABX), 4.18 (q)	↓	-
Valine	0.99 (d), 1.05 (d), 2.28 (m)	↑	-
Leucine	0.96 (t), 1.71 (m)	↓	-
Isoleucine	0.94 (t), 1.01 (d), 1.26 (m)	↓	-
Pyruvate	2.38 (s)	↑	-
Creatine	3.04 (s), 3.94 (s)	-	↓
Choline	3.21 (s)	-	↑
O-p-ethanolamine	3.96 (m)	-	↑

Tab. 1 Discriminant metabolites in PLS-DA of extracellular (EC) and intracellular (IC) compartments of D0 and D14 podocytes and their variation



GLOBAL METABOLIC ACTIVITY



PA decreases metabolic activity in podocytes after 24 and 48 hours as demonstrated by MTT assay

Fig. 3 Effect of PA on podocyte metabolic activity after 24 (A) and 48h (B) exposure
Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs DMSO). *: p<0.05, **:p<0.01, ****: p<0.001

^{1}H–NMR BASED METABONOMICS:PALMITATE EXPOSURE

Extracellular media

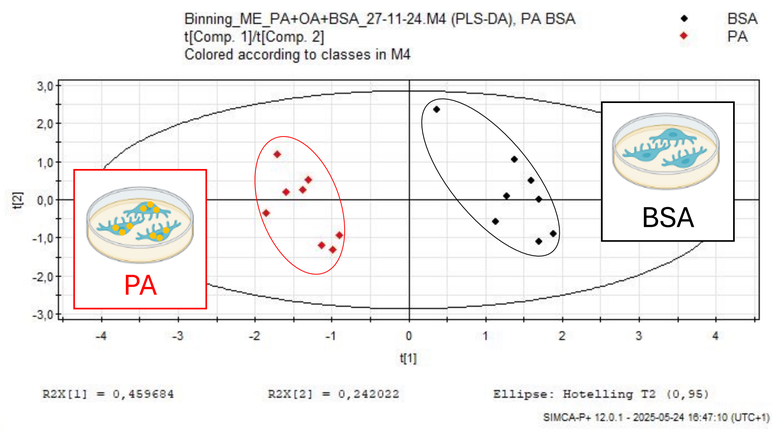


Fig. 4 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from podocytes extracellular media exposed for 24h either to PA 300µM or BSA 0.96% (p-value = 1.3e^{-5})

Cell lysates

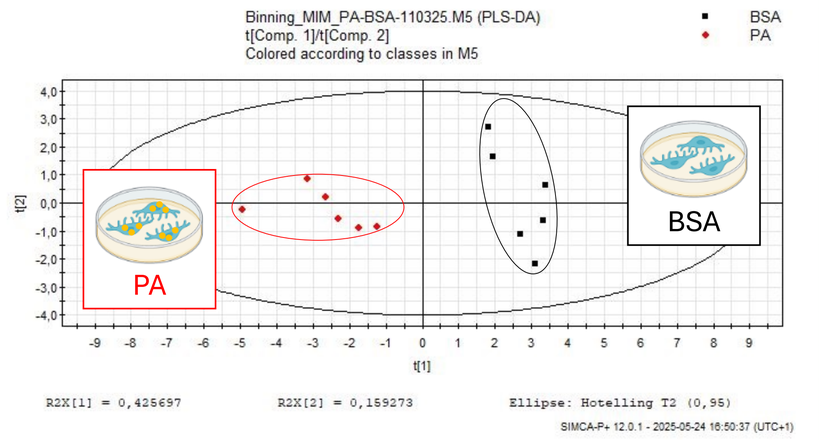


Fig. 5 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from podocytes lysates exposed for 24h either to PA 300µM or BSA 0.96% (p-value = 0,029)

Metabolite	Changes in EC PA	Changes in IC PA
Lactate	↑	↓
Glucose	↑	↑
Myo inositol	-	↑
Glutamate	-	↑
Glutamine	↑	↓
Pyroglutamate	-	↓
Valine	↑	-
Leucine	↑	-
Isoleucine	↑	-
Acetate	↑	-
Alanine	↑	-
Choline	↑	↓
Lysine	↑	-

Tab. 2 Discriminant metabolites in PLS-DA of extracellular (EC) and intracellular (IC) compartments of podocytes exposed to PA or BSA and their variation

PA induces deep metabolic changes in podocytes after 24 hours as demonstrated by ^{1}H-NMR

TAKE-HOME MESSAGE

This study enabled the characterization of basal podocyte metabolism using ^{1}H-NMR based- metabolomics and demonstrated that PA induces distinct metabolic signals, making it a potential source of biomarkers. These comprehensive analyses contribute to a deeper understanding of podocyte responses and metabolic alterations in the context of FSGS-related stresses.

REFERENCES

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Contacts : Marine Thirion (marine.thirion@umons.ac.be), Anne-Emilie Declèves (anne-emilie.decleves@umons.ac.be), Vanessa Tagliatti (vanessa.tagliatti@umons.ac.be)