Investigation of podocyte metabolic adaptation to different stresses mimicking focal segmental glomerulosclerosis

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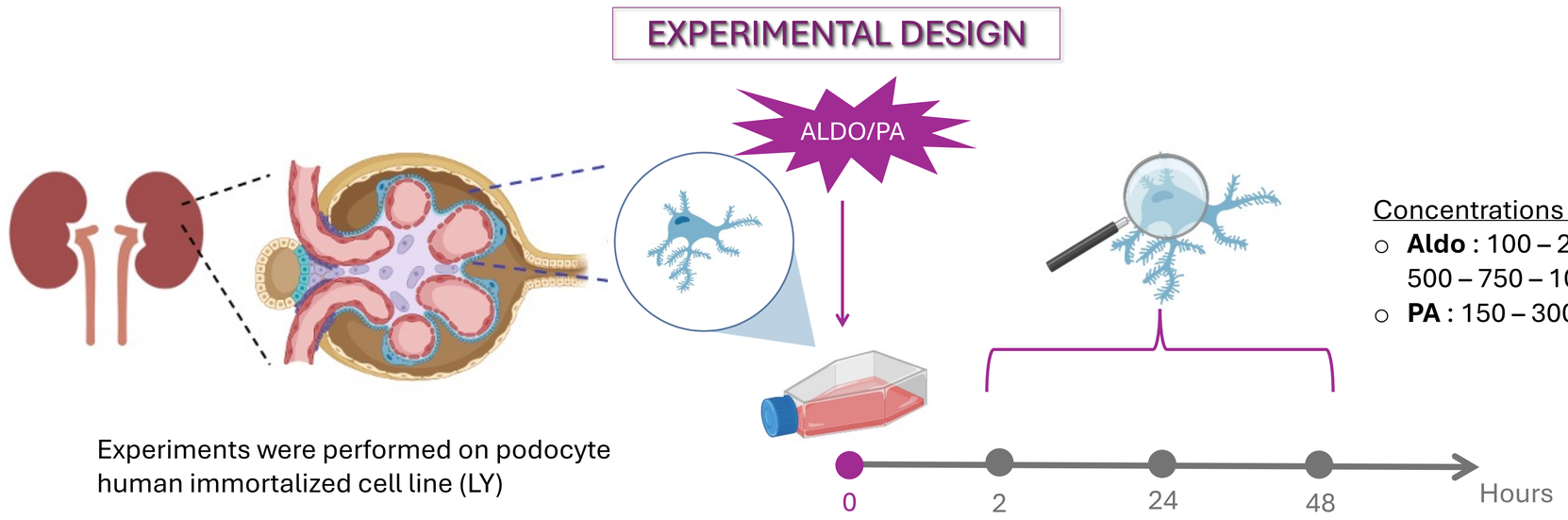
Introduction & Aim

Focal segmental glomerulosclerosis (FSGS) is a complex and diverse subset of renal glomerular disorders[1].
Podocytes, crucial for maintaining the glomerular filtration barrier architecture, and therefore to maintain renal function, are the primary site of the injury in FSGS. 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[2,3].

Therefore, here, we will investigate several stresses related to hypertension through **aldosterone** (aldo) exposure and lipotoxicity through **palmitate** (PA) exposure
Our goal is to identify **metabolic signatures** and **biomarkers** of different FSGS-inducing stresses.

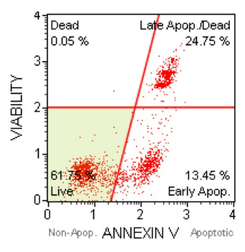
Methods



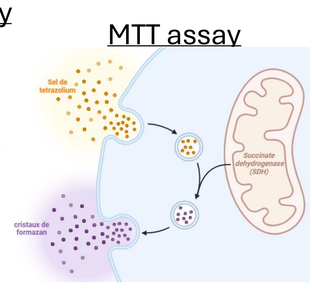
Concentrations tested :
- **Aldo** : 100 – 250 – 500 – 750 – 1000 nM
- **PA** : 150 – 300 µM

1. *In vitro* optimization

Apoptosis assay

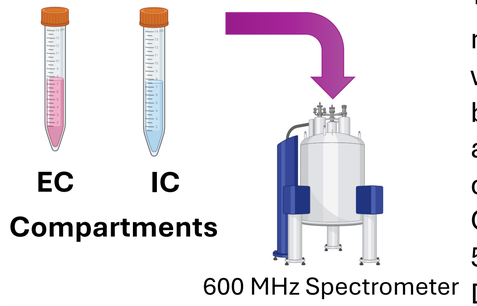


MTT assay



Metabolic activity was evaluated with a MTT assay and confirmed by an Annexin V and cell death assay .

2. ^{1}H-NMR based metabonomics



^{1}H-NMR based metabonomics study was carried out on both intracellular (IC) and extracellular(EC) compartments.
Cells were exposed to 500 nM of Aldo or DMSO 0,02% (vehicle).

Results

Metabolic activity : differences between aldosterone and palmitate exposure

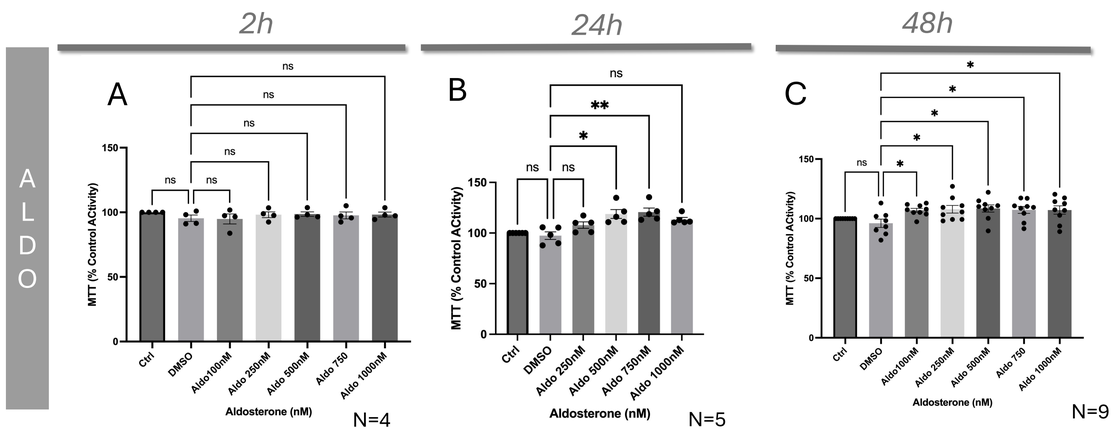


Fig. 1 Effect of aldosterone on podocyte metabolic activity after 2 (A), 24 (B) and 48h exposure
Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs DMSO). *: p<0,05, **:p<0,01

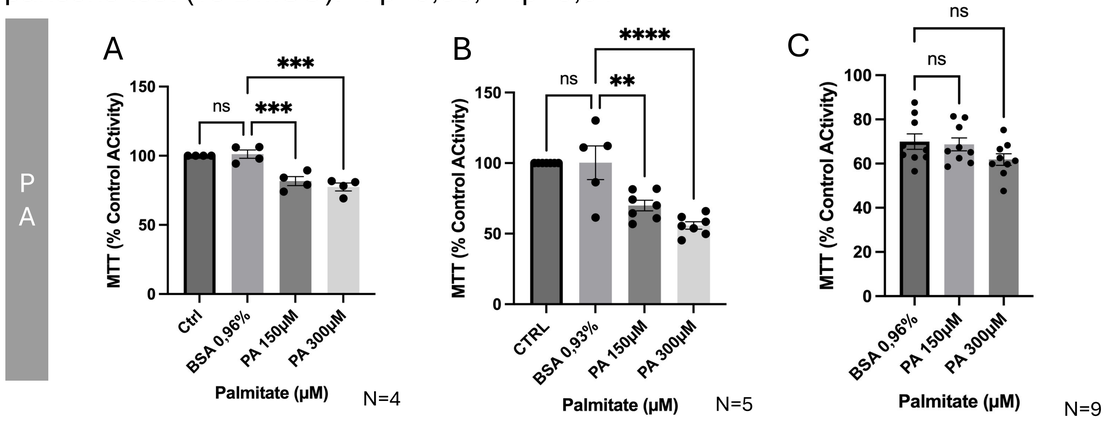


Fig. 2 Effect of palmitate on podocyte metabolic activity after 2 (A), 24 (B) and 48h exposure
Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs DMSO). **: p<0,01, ***:p<0,001, ****:p<0,0001

ALDO increases metabolic activity >< PA decreases metabolic activity

^{1}H – NMR based metabonomics analysis

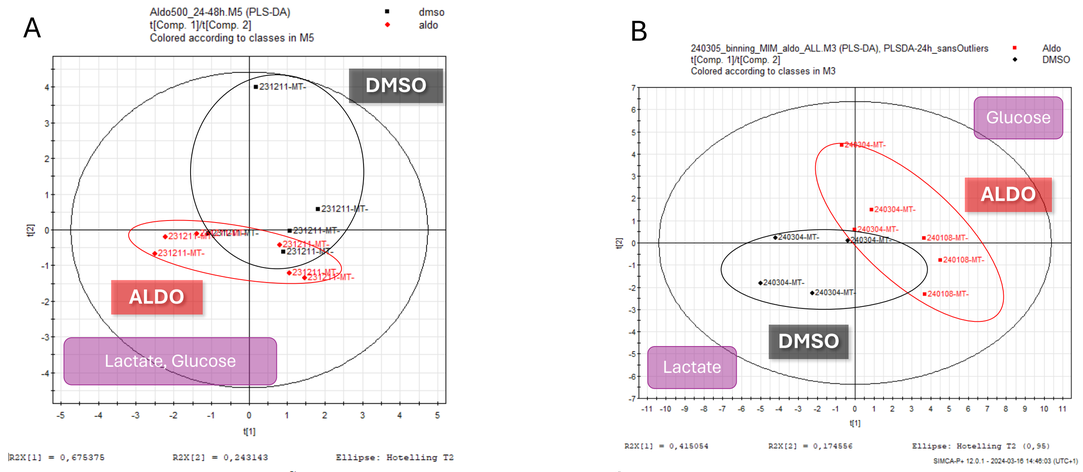


Fig. 4 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from extracellular media (A) and intracellular media (B, polar phase) of podocytes exposed for 24h either to aldosterone 500 nM or DMSO 0,02%

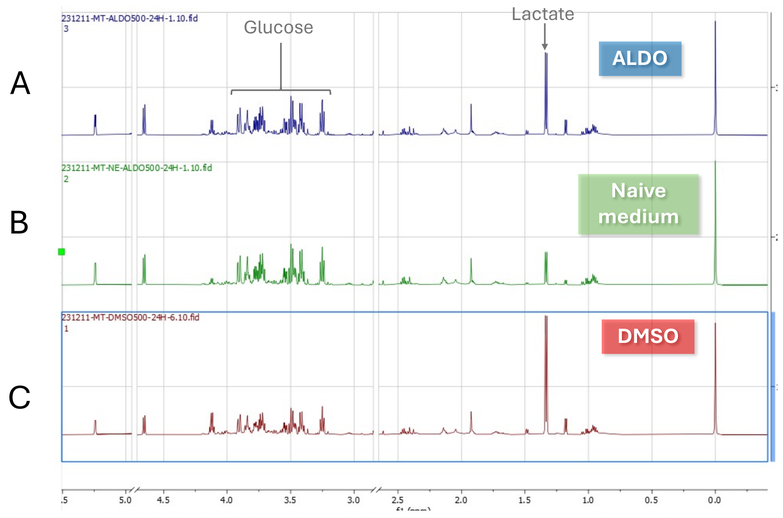


Fig. 5 Typical 600 MHz 1H-NMR spectra of naive medium (B) and extracellular media after 24h exposure of 700000 podocytes to aldosterone 500 nM (A) or DMSO (C)

Conclusion & Perspectives

- Future experiments include metabonomics analysis with palmitic acid exposure
- To better understand our results, respirometric analysis (seahorse) are planned to see oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of our podocytes

Following these comprehensive analyses will contribute to a more thorough comprehension of podocyte responses and metabolic alterations in the context of FSGS-inducing stresses.

References

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2. Fogo, A. B. Causes and pathogenesis of focal segmental glomerulosclerosis. Nat Rev Nephrol 11, 76–87 (2015).
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