Metabolic Signatures of Podocytes under RAAS Overactivation: A Key to better Understand FSGS

health

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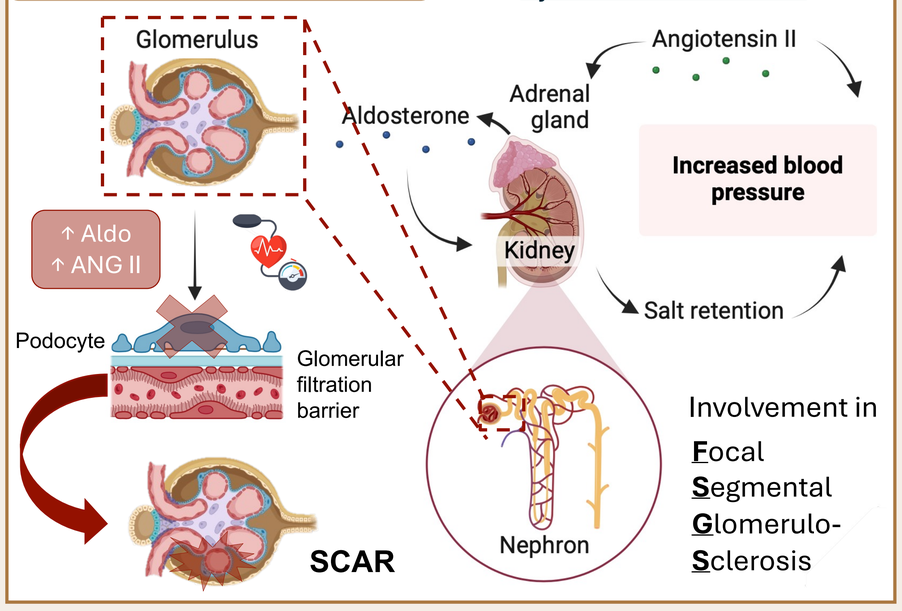
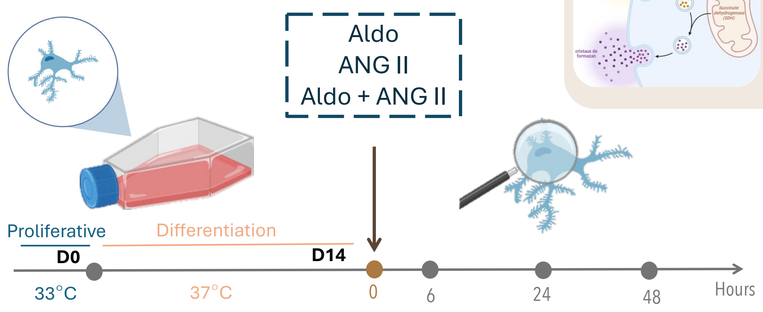
BACKGROUND

Renin Angiotensin Aldosterone System overactivation



METHODS



Experiments were performed on human podocyte immortalized cell line (LY) from the University of Bristol

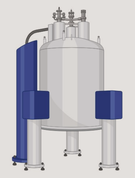
Global metabolic activity

- MTT assay
- Annexin V & Dead cell assay

Mitochondrial impact

- MitoTracker Green: mitochondrial network
- TMRM: Mitochondrial activity

Deep metabolism



600 MHz Spectrometer

^{1}H-NMR based metabonomics study was carried out on cell lysates.

- Aldo 500 nM or DMSO 0,02% (vehicle)
- ANG II 1µM or PBS 0,1% (vehicle).

AIM : to identify metabolic signatures and biomarkers of different FSGS-inducing stresses in podocytes, in particular the overactivation of the RAAS

RESULTS

METABOLIC ACTIVITY

ALDOSTERONE

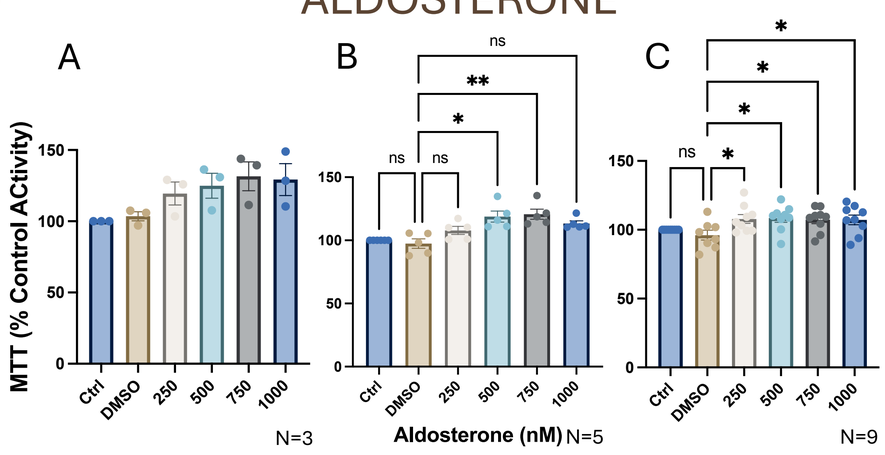


Fig. 1 Effect of Aldo on podocyte metabolic activity after 6 (A), 24 (B) and 48h (C) exposure

Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs DMSO). *: p<0,05, **:p<0,01

ANGIOTENSIN II

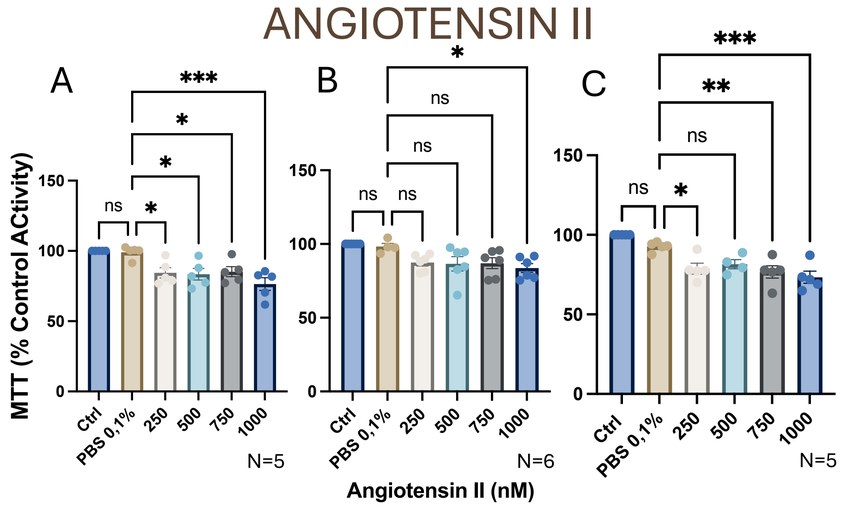


Fig. 2 Effect of ANG II on podocyte metabolic activity after 6 (A), 24 (B) and 48h exposure

Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs DMSO). *: p<0,05, **:p<0,01, ***: p<0,001

COMBINATORY

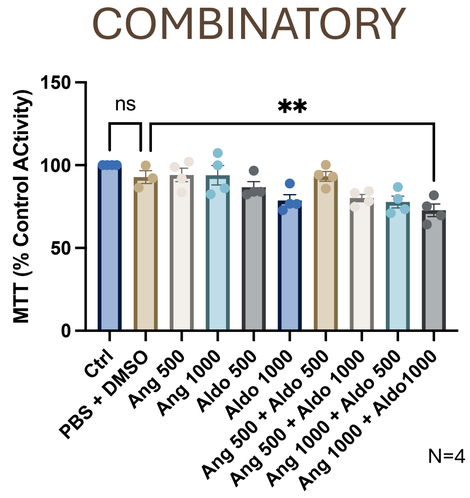


Fig. 3 Combinatory effect on podocyte metabolic activity after 24h exposure

Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs PBS + DMSO). **:p<0,01

MITOCHONDRIAL IMPACT

ALDOSTERONE

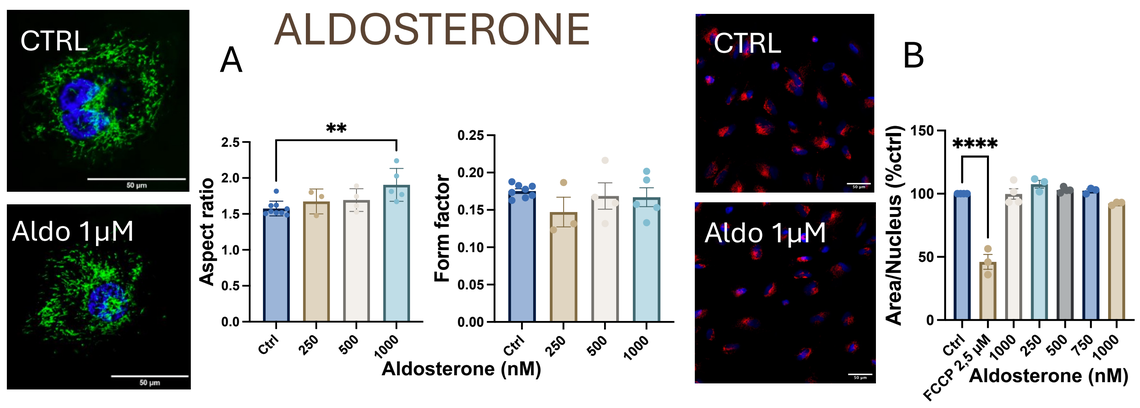


Fig. 4 Mitochondrial network (A) and activity (B) of podocytes exposed 24 hours to Aldo

Blue: Hoescht, Green: MitoTracker green, Red: TMRM. Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs PBS).**:p<0,01, ****: p<0,0001

ANGIOTENSIN II

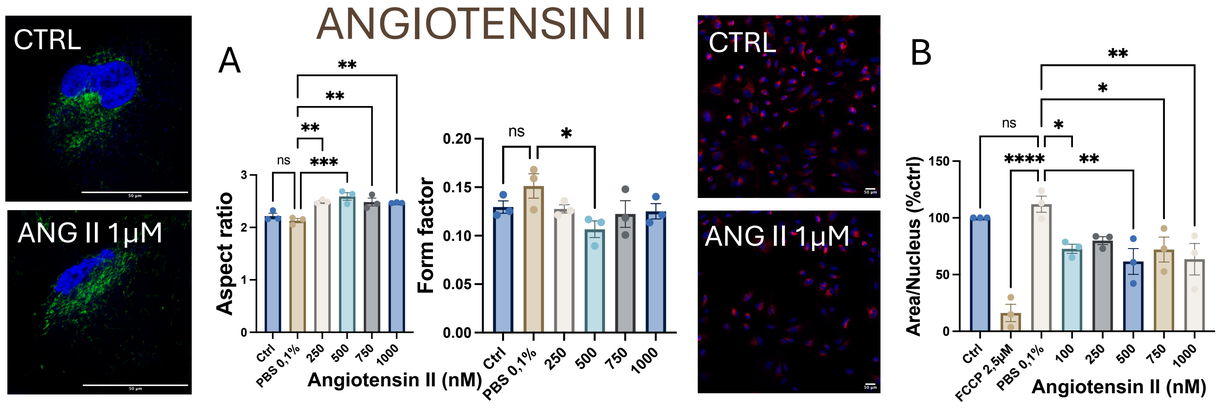
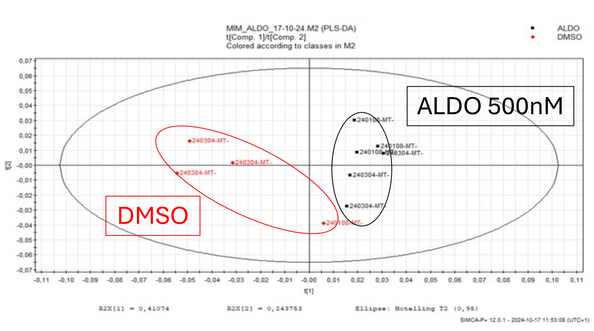


Fig. 5 Mitochondrial network (A) and activity (B) of podocytes exposed 24 hours to ANG II

Blue: Hoescht, Green: MitoTracker green, Red: TMRM. Bars: Mean ± SEM. Statistical test: One way ANOVA followed by Dunnett's multiple comparisons test (vs PBS). *: p<0,05, **:p<0,01, ***: p<0,001, ****:p<0,0001

^{1}H–NMR BASED METABONOMICS

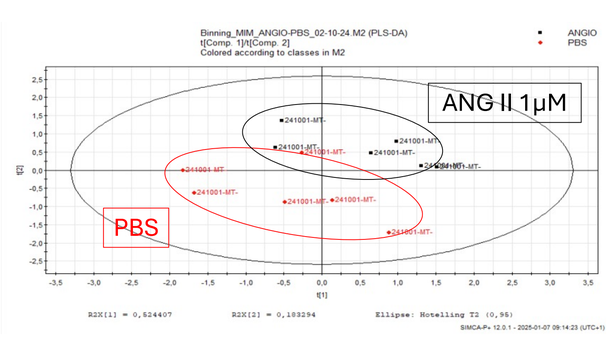
ALDOSTERONE



Metabolite	Chemical shifts (ppm)	VIP score	P-value	Metabolism
Dimethylamine	2.72 (s)	11,6873	/	DMSO metabolite
Glucose	3.24 (m) – 3.28 (m) – 3.38 (m) – 3.42 (m) – 3.88 (dd) – 4.64 (d) – 5.2 (d)	5,95612	0,3339	Cell energy
Myo-inositol	3,28 (t)– 3,64 (t)	3,49844	0.0043 **	Cell signaling
Glutamate	2.12 (m)	1,25134	0.4519	Energy metabolism
Pyroglutamate	2.04 (m)	1,19005	0.5722	Energy metabolism

Fig. 6 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from podocytes lysates exposed for 24h either to Aldo 500 nM or DMSO 0,02%

ANGIOTENSIN II



Metabolite	Chemical shifts(ppm)	VIP score	P-value	Metabolism
Glucose	3.24 (m) – 3.28 (m) – 3.38 (m) – 3.42 (m) – 3.88 (dd) – 4.64 (d) – 5.2 (d)	3.71975	0.0129 *	Cell energy
Lactate	1.32 (d) – 4.12 (q)	3.52502	0.3939	Glycolysis
Choline	3.2 (s)	4.42908	0.4848	Cell membranes
Sarcosine	2.72 (s)	3.04146	0.0022 **	Cell signaling
Myo-inositol	3,28 (t)– 3,64 (t)	2.32202	0.2173	Cell signaling
Proline	3.32 (m)	1.82886	0.0317*	Amino acid
Glutamate	2.32 (m)	1.89207	0.0724	Energy metabolism

Fig. 7 PLS-DA, Scores plot of ^{1}H -NMR spectra acquired from podocytes lysates exposed for 24h either to ANG II 1µM or PBS 0,1%

TAKE-HOME MESSAGES

High levels of angiotensin II and aldosterone, recognized as contributors to podocyte dysfunction in FSGS, yield distinct metabolic and mitochondrial signals, making them promising sources of biomarkers.
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

Shabaka, A., Ribera, A. T. & Fernández-Juárez, G. Focal Segmental Glomerulosclerosis: State-of-the-Art and Clinical Perspective. NEF 144, 413–427 (2020).
Lichtnekert, J., Kaverina, N. V., Eng, D. G., Gross, K. W., Kutz, J. N., Pippin, J. W., & Shankland, S. J. (2016). Renin-angiotensin-aldosterone system inhibition increases podocyte derivation from cells of renin lineage. Journal of the American Society of Nephrology, 27(12), 3611-3627.

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