

**Renal denervation does not affect hypertension or the renin-angiotensin system in a rodent model of juvenile-onset polycystic kidney disease: clinical implications.**

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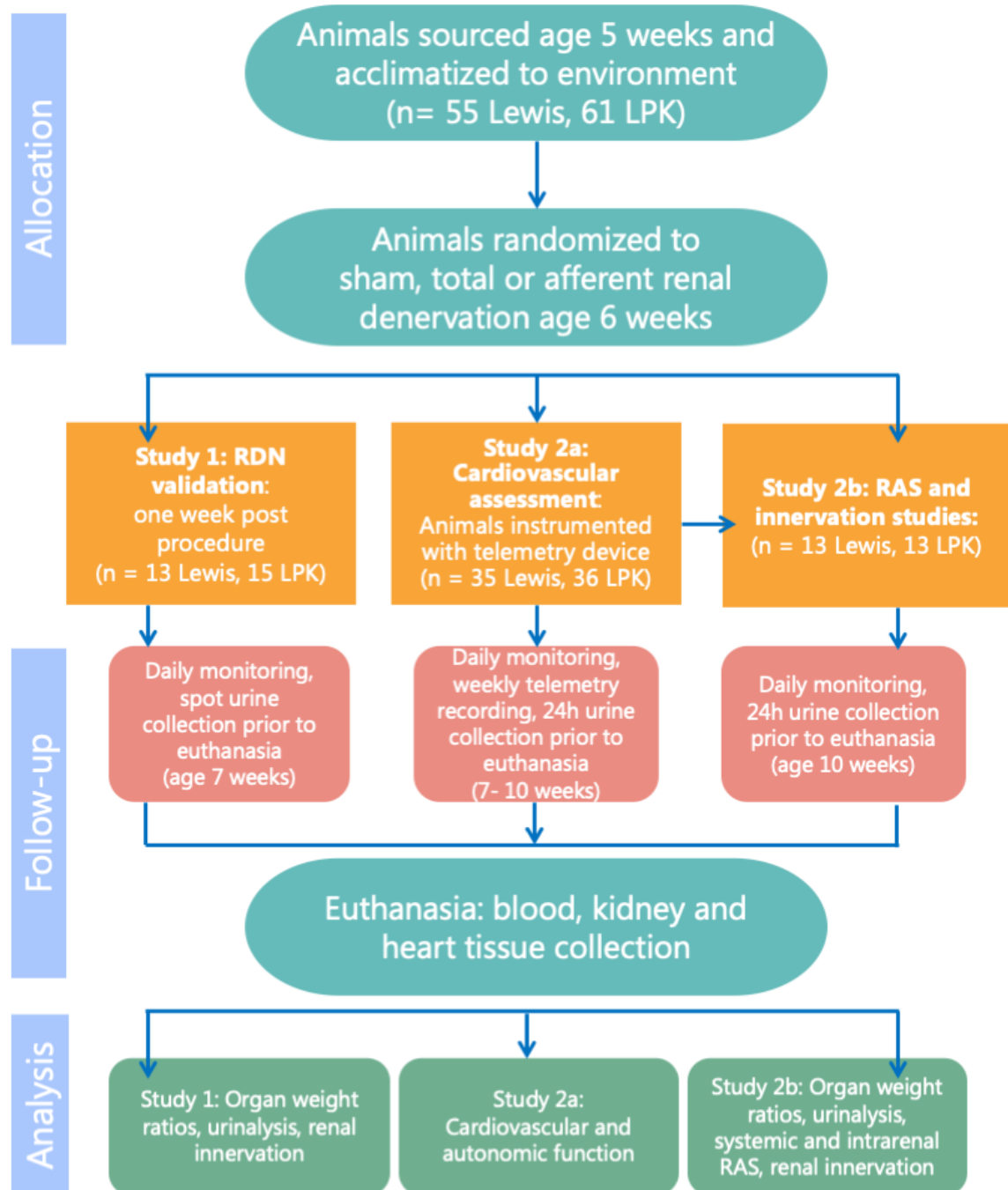
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**Supplementary Figure S1: Flow chart illustrating experimental design**



Animals in each study cohort were allocated to each treatment group as indicated at age 6 weeks, then went through follow-up protocols prior to euthanasia, tissue collection and data analysis. Lewis polycystic kidney (LPK).

**Supplementary Table S1: Breakdown of animal numbers in each study cohort**

| Cohort                      | Lewis male        | Lewis female     | LPK male         | LPK female | Total             |
|-----------------------------|-------------------|------------------|------------------|------------|-------------------|
| Study 1: 7 wks              |                   |                  |                  |            |                   |
| Sham RDN                    | 4                 | 1                | 3                | 3          | 11                |
| Total RDN                   | 3                 | 1                | 2                | 3          | 9                 |
| Afferent RDN                | 4                 | 0                | 3                | 1          | 8                 |
| Total (M/F)                 | Lewis: 13 (11/2)  |                  | LPK: 15 (8/7)    |            | 28                |
| Study 2a: BP data           |                   |                  |                  |            |                   |
| Sham RDN                    | 6 <sup>(1)</sup>  | 5 <sup>(1)</sup> | 7 <sup>(1)</sup> | 6          | 24 <sup>(3)</sup> |
| Total RDN                   | 8 <sup>(1)</sup>  | 6 <sup>(1)</sup> | 8                | 6          | 28 <sup>(2)</sup> |
| Afferent RDN                | 5 <sup>(1)</sup>  | 5 <sup>(1)</sup> | 5 <sup>(2)</sup> | 4          | 19 <sup>(4)</sup> |
| Total (M/F)                 | Lewis: 35 (19/16) |                  | LPK: 36 (20/16)  |            | 71 <sup>(9)</sup> |
| Study 2b: Tissue collection |                   |                  |                  |            |                   |
| Sham RDN                    | 2                 | 3                | 2                | 2          | 9                 |
| Total RDN                   | 2                 | 2                | 2                | 2          | 8                 |
| Afferent RDN                | 2                 | 2                | 3                | 2          | 9                 |
| Total (M/F)                 | Lewis: 13 (6/7)   |                  | LPK: 13 (7/6)    |            | 26                |

Animals in each study cohort. For Study 2 re BP data: number in superscript indicates number of animals that were also used for tissue collection in Study 2b. Therefore those 9 animals are also counted in Study 2b and overall total number of animals used was 28 + 71 + (26 - 9) = 116.

RDN: renal denervation, BP: blood pressure.

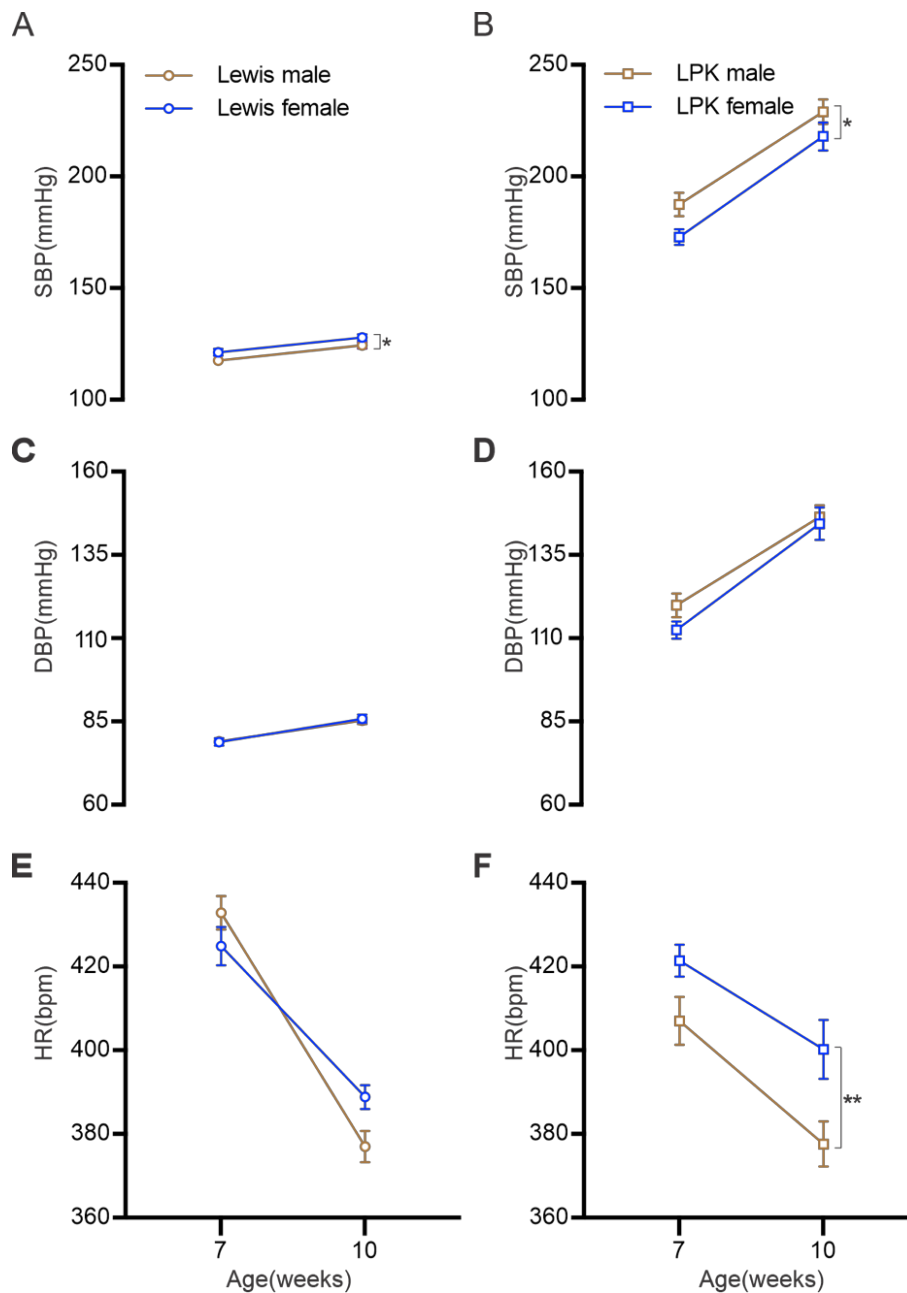
**Supplementary Table S2: List of primer pairs for RT-qPCR.**

| Genes  | NCBI Reference              | Primer Sequence (5'→3') |                        | Primer concentration | Size (bp) |
|--------|-----------------------------|-------------------------|------------------------|----------------------|-----------|
|        |                             | Forward                 | Reverse                |                      |           |
| Renin  | NM_012642.4 <sup>1</sup>    | CACTCTTGTTGCTCTGGACCT   | GGGGTACCAATGCCGATCTC   | 600nM                | 250       |
| AGT    | NM_134432.2 <sup>2</sup>    | CACGGACAGCACCTATTTT     | GCTGTTGTCCACCCAGAACT   | 600nM                | 103       |
| ACE1   | NM_012544.1 <sup>3</sup>    | CACCGGCAAGGTCTGCT       | CTTGGCATAGTTTCGTGAGGAA | 300nM                | 97        |
| ACE2   | NM_001012006.1 <sup>4</sup> | CCCAGAGAACAGTGGACCAAAA  | GCTCCACCACACCAACGAT    | 300nM                | 64        |
| Agtr1a | NM_030985.4 <sup>5</sup>    | CACAGTGTGCGCGTTTCAT     | GTAAGGCCCAGCCCTATGG    | 300nM                | 63        |
| β-ACT  | NM_031144.3 <sup>6</sup>    | GGTCCACACCCGCCACCAG     | CGATGGAGGGGAAGACGGC    | 300nM                | 128       |
| CYC-1  | NM_001277194.1              | GCATGGCTCCTCCCATCTAC    | CCCATGCGTTTTTCGATGGTC  | 300nM                | 139       |

AGT, angiotensinogen; ACE, angiotensin-converting enzyme; Agtr1α, angiotensin type 1α receptor; β-ACT (β-actin); CYC1, cytochrome c1

### Supplementary Figure S2

The effect of sex on systolic (SBP; A,B) diastolic blood pressure (DBP; C, D) and heart rate (HR; E, F) in Lewis (A, C and E) and LPK (B, D and F) rats between 7-10 weeks of age.

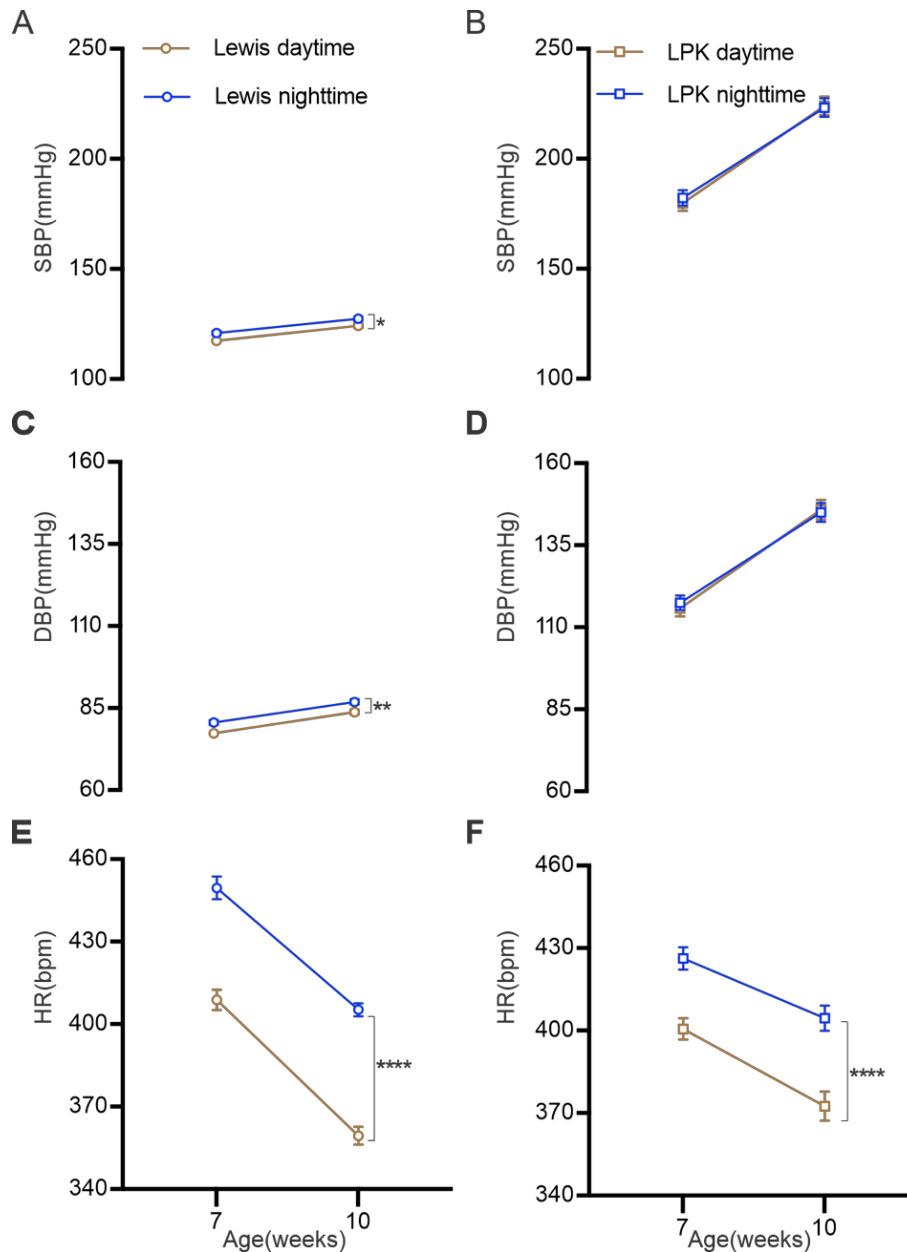


N values at each week for Lewis male = (18, 18); Lewis female = (15, 16), LPK male = (20, 16) , LPK female = (16, 15) respectively. Data is expressed as mean  $\pm$  SEM and analysed with two-way ANOVA for sex and age effect.

Female Lewis had a significantly higher level of SBP but comparable level of DBP and HR relative to male Lewis. Male LPK had a significantly higher level of SBP and lower level of HR compared to female LPK. \* indicates  $P < 0.05$  and \*\* indicates  $P < 0.01$  difference between male and females, analysed using two-way ANOVA and Bonferroni's post hoc analysis.

**Supplementary Figure S3**

The effect of day and night period on systolic (SBP; A, B), diastolic blood pressure (DBP; C, D) and heart rate (HR; E, F) in all Lewis (A, C and E) and LPK (B, D and F) rats between 7-10 weeks of age.

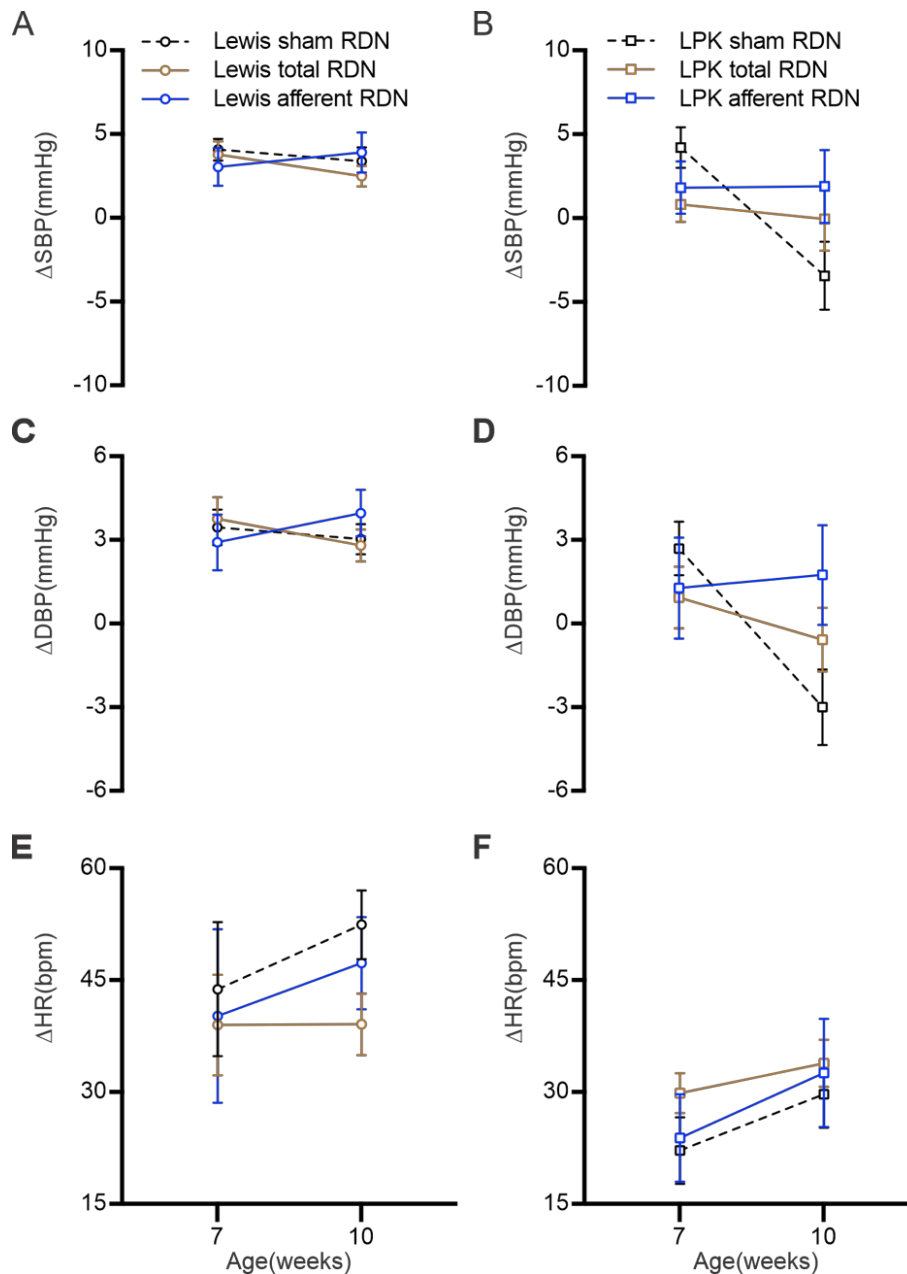


N values at each week for Lewis = (33, 34); LPK = (36, 31) for both day and night values, respectively. Data is expressed as mean  $\pm$  SEM and analysed with two-way ANOVA for day period and age effect.

Lewis rats had a significantly higher level of SBP, DBP and HR during night-time compared with daytime. LPK rats had a significantly higher level of HR during night-time vs. daytime, but the level of SBP and DBP were comparable during both periods, suggesting a non-dipping status. \*indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$  \*\*\*\* indicates  $P < 0.0001$  difference between daytime and night-time values, analysed using two-way ANOVA and Bonferroni's post hoc analysis.

**Supplementary Figure S4**

The effect of renal denervation (RDN) on the differences between night-time and daytime SBP ( $\Delta$ SBP, A and B), DBP ( $\Delta$ DBP, C and D) and HR ( $\Delta$ HR, E and F) in Lewis (A, C and E) and LPK (B, D and F) rats between 7-10 week of age after sham, total and afferent RDN procedures.



Data is expressed as mean  $\pm$  SEM, analysed using Two-way ANOVA. N values at each week for Lewis sham = (10, 11); Lewis total = (14, 13); Lewis afferent = (9, 10) respectively and for LPK sham = (13, 11), LPK total = (14, 12) and LPK afferent = (9, 8), respectively.

No overall treatment effect was present for any of these parameters in either strain.

## References

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