

# Dynamics of renal electrolyte excretion in growing mice

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## Supplementary Information

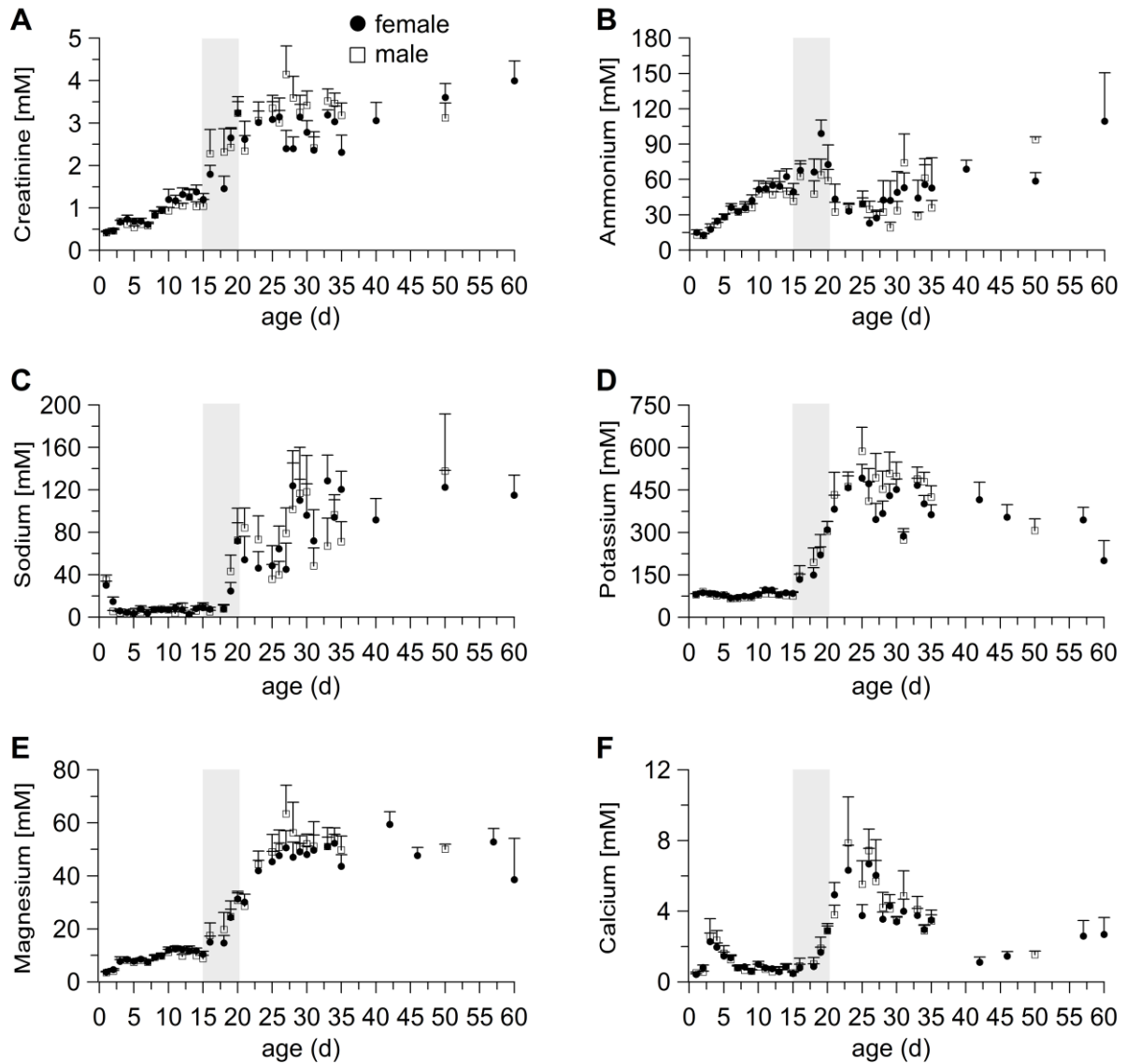
### Supplementary material and methods:

#### Quantification of inorganic ions and creatinine in urine samples

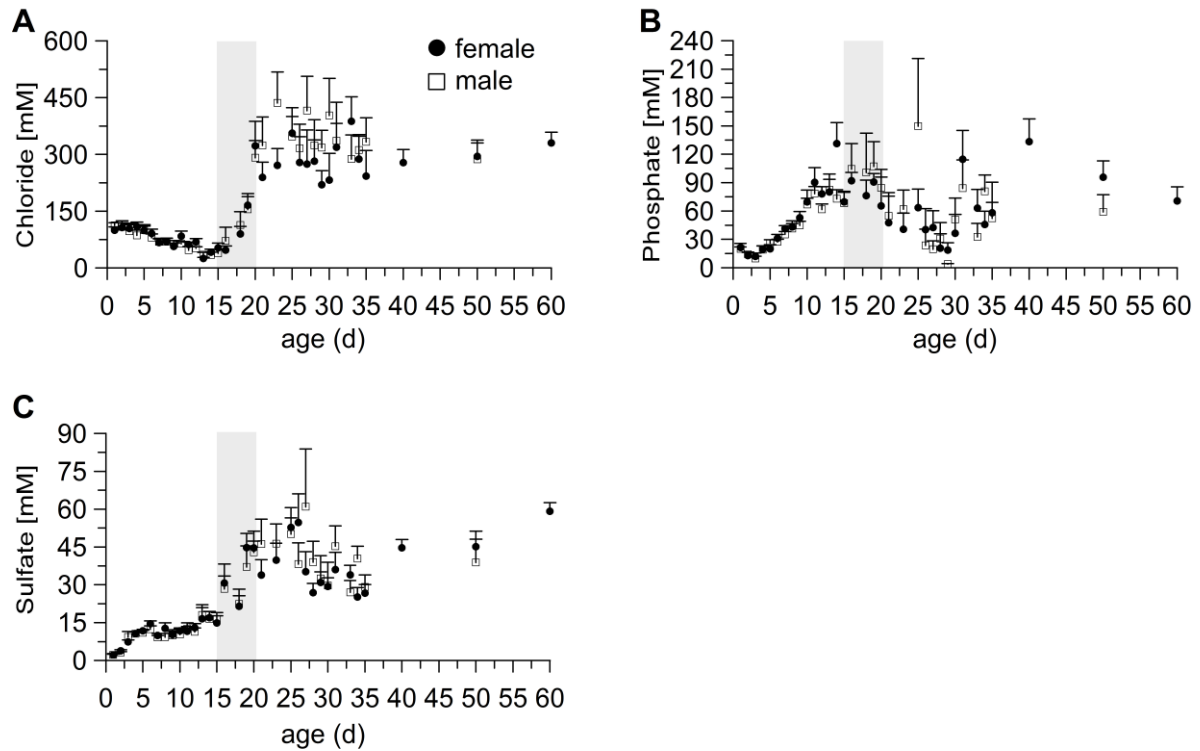
Mouse urine was centrifuged 15 min at 10000 g, and the supernatant was diluted with deionized water as required (1:100 or 1:400). Dilution series of appropriate standards for inorganic anions, cations, or creatinine were used for the quantification of the ion-specific peak areas in diluted samples.

Anions ( $\text{Cl}^-$ , phosphate, sulfate), cations ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ), and creatinine were quantified in urine samples using an automated ion chromatography system (Isocratic dual ICS-1600-System, ICS-3000 autosampler; hardware and columns were purchased from Dionex/Thermo Fisher Scientific, Idstein, Germany). The following reagents and standards were used: sodium carbonate (Dionex/Thermo Fisher Scientific); sulfuric acid; standards for inorganic ions, and for creatinine (Sigma-Aldrich GmbH, Taufkirchen, Germany); deionized water (18.8 MOhm\*cm). All reagents and inorganic ion standards were IC-certified stock solutions. The conditions for anion measurements were the following: columns: IonPac AS9-HC analytical column, IonPac NG1 and AG9-HC guard columns; eluent: 9 mM sodium carbonate; detection: suppressed conductivity (ASRS, AutoSuppression™ recycle mode, 15 mA current); background conductivity: <1  $\mu\text{S}$ ; system operating backpressure: 1360 psi; flow rate: 0,25 mL/min; injection volume: 25  $\mu\text{L}$ .

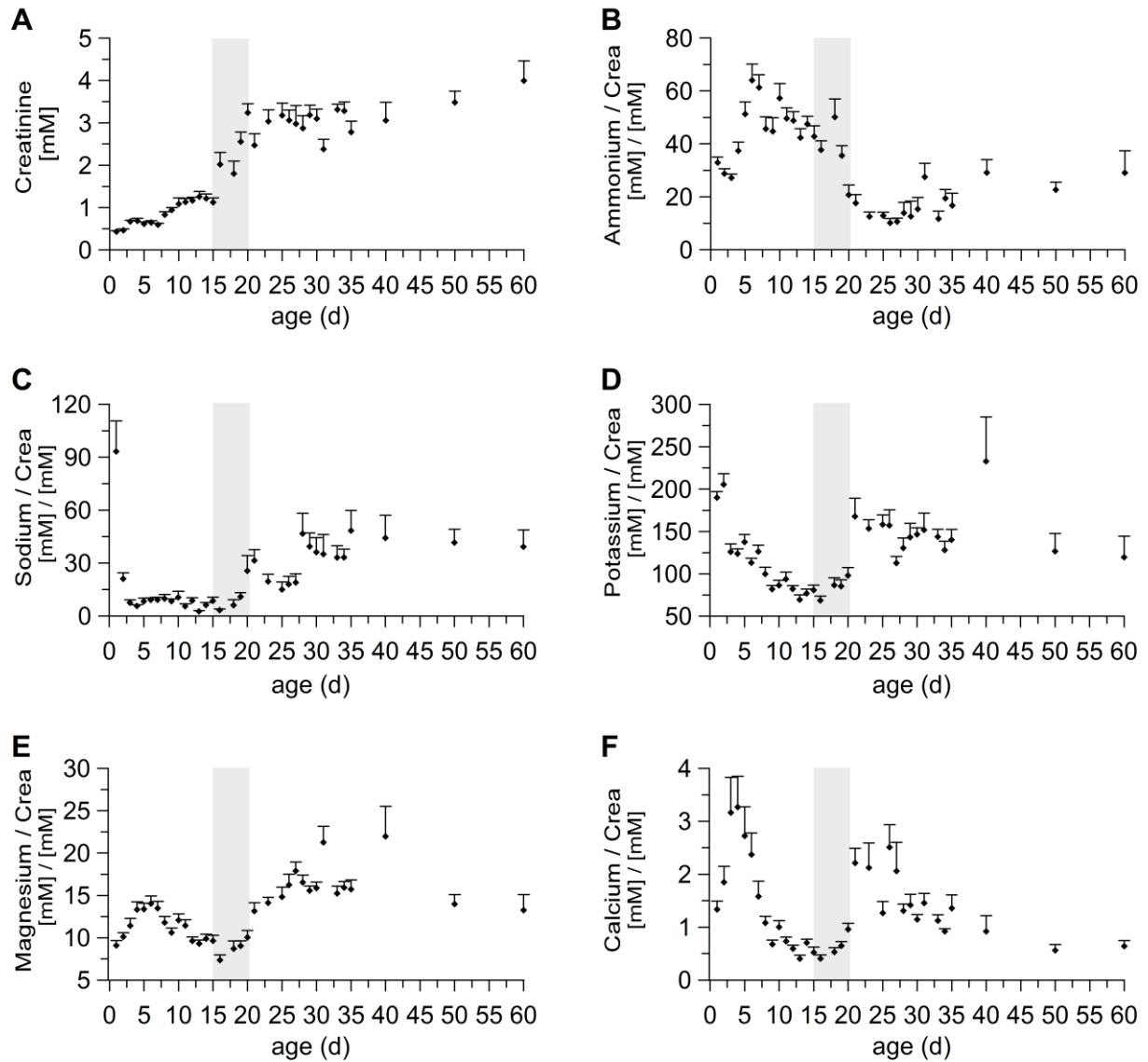
The conditions for cation measurements were the following: columns: IonPac CS12A analytical column, IonPac NG1 and CG12A guard columns; eluent: 9 mM sulfuric acid; detection: suppressed conductivity (CSRS, AutoSuppression™ recycle mode, 16 mA current) and UV-absorbance at 210 nm (for creatinine); background conductivity: <26  $\mu\text{S}$ ; system operation backpressure: 960 psi; flow rate: 0,25 mL/min; injection volume: 25  $\mu\text{L}$ .



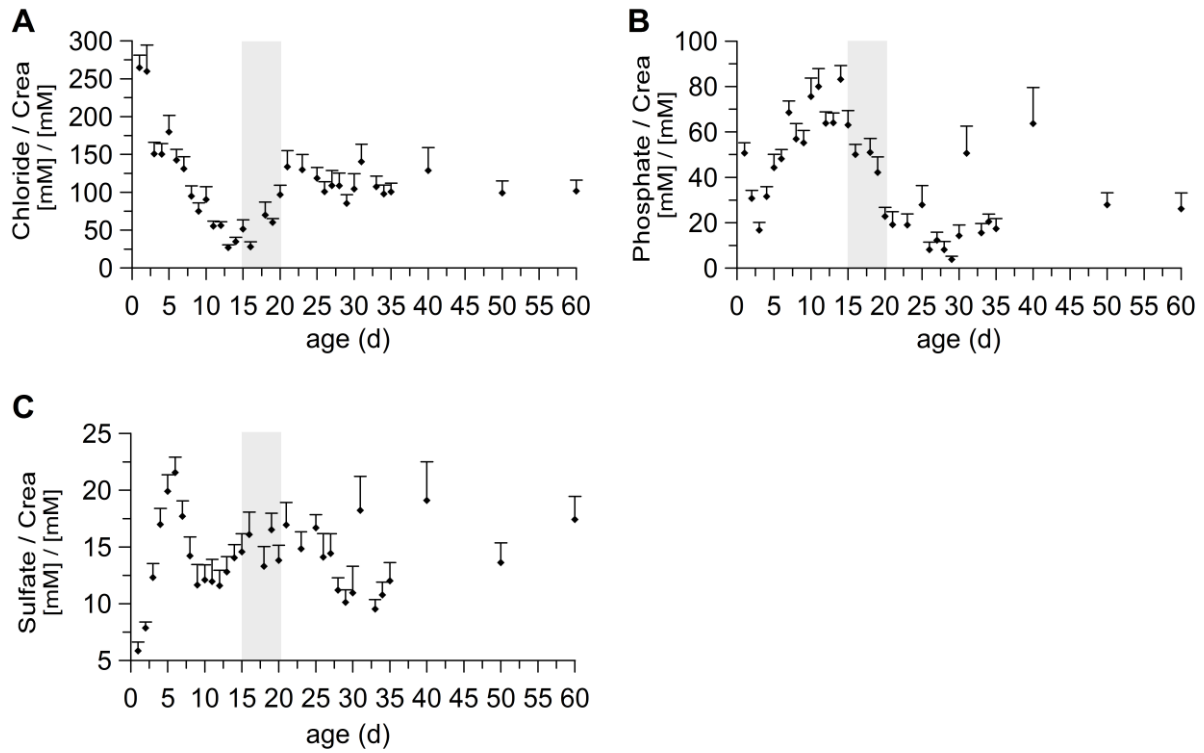
**Supplemental Figure 1: Urinary concentration of creatinine (A) and different cations ( $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , B-F) during postnatal development of mice.** Individual profiles are shown for male and female mice. Concentrations were measured from day 1 up to day 60 after birth using an ion chromatography system. The grey background marks the weaning phase. Profiles were quite similar comparing both sexes before weaning, but show a high scattering after weaning without significant differences between sexes.



**Supplemental Figure 2: Urinary concentration of different anions (Cl<sup>-</sup>, phosphate and sulfate, A-C) during postnatal development of mice.** Individual profiles are shown for male and female mice. Concentrations were measured from day 1 up to day 60 after birth using an ion chromatography system. The grey background marks the weaning phase. Profiles were quite similar comparing both sexes before weaning, but show a high scattering after weaning without significant differences between sexes.



**Supplemental Figure 3: Urinary concentration of and different cations normalized to creatinine excretion during postnatal development of mice.** For comparison, excretion of creatinine as shown in Fig. 2 is also depicted in this figure (A). Concentrations were measured from day 1 up to day 60 after birth using an ion chromatography system. The grey background marks the weaning phase. Values of male and female animals were pooled.



**Supplemental Figure 4: Urinary concentration of and different anions normalized to creatinine excretion during postnatal development of mice.** Concentrations were measured from day 1 up to day 60 after birth using an ion chromatography system. The grey background marks the weaning phase. Values of male and female animals were pooled.