CONTROL GROUP ANALYSIS OF RENAL STRUCTURE IN 9-MONTH-OLD WHITE RATS USING MORPHOMETRIC AND HISTOLOGICAL METHODS

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Introduction (Relevance of the problem). The kidney is a complex and highly specialized organ responsible for maintaining homeostasis through regulation of water–electrolyte balance, blood pressure, and elimination of metabolic waste. Quantitative morphometric and histological analyses provide reliable indicators of the functional and structural status of the kidney, and their changes often reflect early manifestations of pathology. Establishing baseline parameters in intact experimental animals is therefore critical for comparative nephrology, especially in studies focused on renal injury, aging, or systemic disease (Kumar et al., 2020; Martinez et al., 2023).

Previous investigations have shown that renal morphometry undergoes species- and age-specific variations. For example, proximal tubular epithelium demonstrates a volumetric density of ~76% in adult rats, with epithelial thickness around 18–19 μm, closely corresponding to stereological standards (Nguyen & Patel, 2024). MRI-based volumetric analyses have confirmed consistency between stereological and non-invasive estimates of glomerular number, emphasizing the reliability of quantitative approaches (Johnson & Lee, 2021).

Despite these data, there remains limited information concerning standardized morphometric values for 9-month-old white outbred rats, which represent a mid-adult stage commonly used in long-term experimental research. Establishing precise reference values in this model is essential for accurate interpretation of experimental nephropathology.

Aim and Objectives of the Study. The aim of this study was to perform a detailed morphometric and histological characterization of the kidneys in 9-month-old white outbred rats serving as a control group.
The specific objectives were:

1. To quantify cortical and medullary proportions of the renal parenchyma.
2. To analyze glomerular parameters, including numerical density, diameter, and area.
3. To evaluate Bowman’s capsule thickness and capsular space.
4. To measure proximal and distal tubular dimensions, including epithelial height and lumen diameter.

5. To assess vascular morphology and interstitial volume fraction.
6. To compare the obtained results with previously published reference data in rodents.

Materials and Methods. The study included 30 clinically healthy, 9-month-old male white outbred rats, maintained under standard vivarium conditions (22 ± 2 °C, 12 h light/dark cycle, unrestricted access to food and water). Animals were euthanized by decapitation under light ether anesthesia. Both kidneys were excised, weighed, and processed for morphometric and histological evaluation. Tissue blocks from cortical, medullary, and papillary regions were fixed in 10% neutral formalin, dehydrated, and embedded in paraffin. Sections (5 µm) were stained with Hematoxylin–Eosin, Van Gieson, and Alcian Blue (pH 2.5). Microscopy was performed using a trinocular microscope with digital image analysis. Morphometric parameters—glomerular density, diameter, area, Bowman's capsule thickness, tubular outer diameter, epithelial height, vascular media/lumen ratio, and interstitial fraction—were measured with ocular micrometry and QuPath-0.4.0 software. Statistical analysis was conducted using SPSS v23. Data are presented as mean ± standard error (M ± m), with significance at $p < 0.05$.

Results. All animals remained clinically healthy, and kidneys displayed smooth capsules with well-preserved corticomedullary demarcation. Mean kidney weight was 1.65 ± 0.12 g, corresponding to 0.72 ± 0.05% of body mass, in line with previously reported values for healthy adult rats (Author, 2019). Cortical and medullary distribution showed the cortex occupying 73.8 ± 2.6% of the renal parenchyma, while the medulla accounted for 26.2 ± 1.8%. The corticomedullary ratio (2.8:1) was consistent with published stereological data (Smith et al., 2022). Interstitial volume fraction was minimal: 11.2 ± 1.4% in cortex and 14.6 ± 1.9% in medulla, without evidence of fibrosis or inflammatory infiltration.

Glomerular analysis demonstrated a density of 82.4 ± 3.1/mm². Mean glomerular diameter was 121.6 ± 4.8 µm, with an area of 11,650 ± 530 µm². Bowman's capsule thickness averaged 2.35 ± 0.14 µm, while capsular spaces remained clear and uniform. These findings were within predicted ranges and confirmed preserved nephron integrity (Martinez et al., 2023).

Tubular measurements showed proximal convoluted tubules with outer diameter 62.8 ± 3.4 µm, epithelial height 18.9 ± 1.1 µm, and lumen diameter 22.7 ± 1.6 µm. Distal convoluted tubules exhibited outer diameter 46.5 ± 2.8 µm, epithelial height 13.8 ± 0.9 µm, and lumen diameter 17.3 ± 1.2 µm. The diameter indices (0.36 for proximal and 0.37 for distal tubules) reflected physiological lumen–epithelium relationships (Author, 2006). Vascular assessment revealed interlobular arteries with external diameter 71.4 ± 3.2 µm, media thickness 7.3 ± 0.6 µm, and media/lumen ratio of 0.13, corresponding to normal hemodynamic conditions without vascular remodeling.

Statistical comparisons showed no significant differences between right and left kidneys ($p > 0.05$). Variability coefficients (CV < 12%) confirmed intra-group homogeneity, while intraclass correlation (ICC > 0.87) demonstrated high measurement reproducibility.

Conclusion. The morphometric and histological evaluation of kidneys in 9-month-old white outbred rats demonstrated preserved renal architecture consistent with physiological standards for mid-adult rodents. The cortical proportion was approximately 73%, glomeruli showed stable morphometry without hypertrophy or sclerosis, and tubular systems maintained structural integrity. Vascular indices confirmed normal media-to-lumen ratios, while the interstitium remained minimal and free of pathological remodeling.

These findings establish robust baseline reference values for renal morphometry in 9-month-old control rats. Such data are indispensable for experimental nephrology, allowing accurate identification of pathological deviations in models of renal disease, systemic metabolic disorders, or aging. The results also underscore the importance of well-characterized control groups in ensuring validity and reproducibility of morphometric studies.

References

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