Histomorphometric and biochemical data of rat kidney submitted to warm ischemia associated with resveratrol treatment

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**Abstract**

The data presented here come from the article "Histomorphometric evaluation of the rat kidney submitted to warm ischemia and the protective effect of resveratrol" [1]. Rats of Wistar lineage (n = 39; 9 weeks of age) were obtained and apportioned into 4 groups at random. Both groups Sham (S) and Sham Resveratrol (SR) were submitted to open laparotomy and dissection of the left renal pedicle, the same as groups Ischemia (I) and Ischemia Resveratrol (IR), being the last two also submitted to 1 h left warm renal ischemia. SR and IR were treated with 30 mg/kg of resveratrol intraperitoneally 1 h before the surgical procedure, while S and I received saline injections. Rats were killed a month after surgery by anesthetic overdose. A blood sample was collected by cardiac puncture for determination of serum urea and creatinine serum by biochemical analysis at automated enzymatic method. Kidneys were weighted, Sherleś method was used for measurement of their volume and then both were fixated in buffered formalin for 48 h. Cortex-non-cortex areas ratio (C–NC) was assessed by Cavalieri’s method using a stereoscope. The product of multiplying the renal volume by the C–NC is the cortical volume (CV). Left kidneys were weighted by Sherleś method and Sherleś’s volume was measured by stereoscopy. The product of multiplying the renal volume by Sherleś volume (C–NC × Sherleś’s volume) is the total cortical volume (CV × Sherleś’s volume).
Specific Subject

How Specifications

format data subject were G.F. for Buys-Gonçalves, data data area table collection F.J.B. Biochemistry Renal Surgery The stereoscope During random standardized equipped Brazil), Raw Excel light/dark Softwares: (Carl Scherle’s analyser. volume-weighted light/dark fixation, euthanasia, kidney collection, weighing, volume measurement, cleavage, buffer formalin fixation, Cavalieri method slice analysis for the cortex-non-cortex areas ratio (C–NC), histological processing, histological slide making and stain, drying, random photomicrographs, glomerular volumetric density (Vv [Glom]) and volume-weighted mean glomerular volume (VWGV) analysis, data tabulation and statistical analysis. P-value was always considered significant when <0.05. During each animal’s anesthetic plan, a cardiac puncture was performed to collect blood, which was subsequently centrifuged to obtain the serum and thus, the serum urea and creatinine dosage by a semiautomatic biochemical analyser. The abdominal cavity was then opened to access the kidneys, which were dissected, collected and weighed. The renal volume was measured by the Scherle’s method, which is used to determine the volume of bodies with an

(continued on next page)
irregular surface based on the Archimedes principle, that is, a body totally or partially immersed in a fluid undergoes a thrust that is equal to the weight of the volume of the fluid displaced by the body. To measure it, the weight (W) is recorded to be given by the displacement of an isotonic saline solution by the organ volume. As the density (σ) of the isotonic saline solution is 1.0048 and the volume (V) is obtained by the formula: \( V = \frac{W}{\sigma} \), the volume value is like the weight (\( V \approx W \)). The kidneys were cleaved transversely in the hilar region and fixed in separate flasks containing 3.7% buffered formalin solution. Isotopic, uniform and random fragments of the kidneys were obtained using the vertical cleavage method. The latter were routinely processed for histology in a processor with dehydration by ethyl alcohol baths in increasing concentrations, followed by clarification in xylol and, finally, embedded and embedded in paraffin in the apparatus. After slicing 5 μm thick slices through the microtomy and making slides. After drying, the slides were stained by the haematoxylin and eosin method for histopathological and stereological Vv[Glom] and VWGV. The proportional area of the cortex and non-cortex regions {medulla, capsule and adipose tissue of the renal sinus} was calculated using Cavalieri’s method using the ImageJ software. The kidneys were sectioned into seven to eight transverse slices 2 mm thick and a transversal surface of each slice was photographed in a camera attached to the stereomicroscope, along with a ruler millimeter for further calibration of ImageJ. The images were analysed under 15x magnification. First, the distance occupied by a specified number of pixels of the image in millimeters is calibrated using the ruler. After calibration, the total area of the slice and the area of the non-cortical region in each image were firstly analysed using the “Polygon selections” tool and, through subtraction, it was possible to calculate the area of the cortical region. Multiplying the value obtained by the volume obtained by the Schele method, it was possible to calculate the volume of each region, cortical (CV) and non-cortical. Vv [Glom] is given by Pp/Pr, where Pp is the number of points that overlap the glomeruli and Pr is the total number of points in the grid (42). The Vv is given in percentage and, multiplying this value by the VC and dividing the result by 100, the numerical value of the absolute glomerular density is found in milliliters. The VWGV was estimated using the point interception method. Over the length of the glomerulus intercepted by the line of a grid with lines parallel to each other, a logarithmic ruler of 32 mm in length is placed, composed of a series of 15 classes. This grid is placed over the image at randomly selected angles, with the angle being recalculated, at random, for each image analysed (ranging from 5° to 90°, with 5° intervals). The number of glomeruli per cubic millimeter of renal cortex (\( N_{[\text{Glom}]} \)) was calculated using the formula VCV [Glom]/VWGV.

Data source location
Institution: State University of Rio de Janeiro
City/Town/Region: Rio de Janeiro
Country: Brazil

Data accessibility
With the article

Related research article
Buys-Goñalves GF, Sampaio FJB, Silva MEM, Pereira-Sampaio MA, De Souza DB. Histomorphometric evaluation of the rat kidney submitted to warm ischemia and the protective effect of resveratrol. Am J Surg. 2020; doi:10.1016/j.amjsurg.2020.02. In press.

Value of the data

- These data bring positive parameters and evidence regarding the nephroprotective effect of resveratrol related to warm ischemia in Wistar rats, an experimental model widely used in preclinical trials
- Such data may be beneficial for researchers who wish to justify studies involving the protective effects of resveratrol against renal or oxidative ischemic damage more generally. Thus, urologists and nephrologists who wish to research and/or use this bioflavonoid as a complementary treatment for their patients who will undergo partial nephrectomy can also benefit
- Scholars with lines of research involving bioflavonoids, or specifically resveratrol, can use this data and methodology to carry out related research, since they can be used as a complement and reference
• The present data demonstrate that resveratrol protects the kidney against damage from warm ischemia in a quantitative, that is, absolute way. This is because the number of glomeruli per kidney is very close to the number of remaining nephrons.

1. Data description

The present dataset describes the levels of serum biochemical markers, as well as morphometric and stereological analysis of rat kidneys submitted to 1-hour arteriovenous ischemia treated previously with resveratrol. Fig. 1 demonstrates the experimental and analytical steps that were conducted to obtain serum biochemical data for urea and creatinine. Fig. 2 demonstrates the experimental and analytical steps that were conducted to obtain the morphometric and stereological data, as well as their analysis. Fig. 3 describes the calculations to obtain specific data so that it was possible to calculate the N[Glom]. Fig. 4 scatter chart representing C–NC raw data from different groups’ animals; transversal black midline represents group’s mean. Fig. 5 grouped column chart representing left renal volume and cortical volume averages of experimental groups; means are shown above the graph’s bars. Fig. 6 scatter chart representing Vv[Glom] raw data from different groups’ animals; transversal black midline represents group’s mean. Fig. 7 scatter chart representing N[Glom] raw data from different groups’ animals; transversal black midline represents group’s mean. Table 1 shows raw data of serum urea and creatinine of rats submitted to sham surgery or to left renal warm ischemia with or without resveratrol treatment. Table 2 contains raw data of animals’ body weight, renal weight and volume of experimental groups. Table 3 includes raw data of kidney morphological data of rats submitted to sham surgery or to left renal warm ischemia with or without resveratrol treatment.

2. Experimental design, materials, and methods

All experiments were performed according to the national and international laws for scientific use of animals, and this project was formally approved by the local Ethics Committee for animal experimentation.

Male rats of Wistar lineage (n = 39; 9 weeks of age) were used, being allocated into 4 groups at random: Sham (S) – group submitted to open laparotomy and dissection of the renal pedicle; Sham Resveratrol (SR) – group previously treated with resveratrol and submitted to the same procedures of group Sham; Ischemia (I) - group submitted to 1-hour renal warm ischemia; Is-
How was the morphometric data obtained?

- Kidney dissection and collection

- Experimental groups euthanasia

- Kidney weight using precision scale

- Kidney volume by Scherle’s method

- Kidney fixation on buffered formalin

- Measurement of the C-NC areas by Cavalieri method - Software Image J

- Photography of the pieces in a digital camera attached to a stereoscope

- Kidney vertical cleavage in 7-8 pieces

- Data obtained tabulated and analyzed statistically

- Photograph of 25 random fields per kidney in a digital camera coupled to a light microscope

- Random vertical cleavage - histological processing - slide making - HE staining

- Quantification of Vv[Glom] in M42 grid - Software Image J

- VWGV quantification using the point interception method and 15-class logarithmic ruler

- Data obtained tabulated and analyzed statistically

Fig. 2. How was the morphometric data obtained?


Calculations used:

**Cortical area** = kidney total area – Non-cortical area

C-NC = cortical area / kidney total area

CV = kidney volume x C-NC

Total glomerular volume (mL) = CV x (Vv [Glom] / 100)

Total glomerular volume (μm³) = Total glomerular volume (mL) x 10¹¹

N[Glom] = Total glomerular volume (μm³) / VWGV

Fig. 3. Calculations Used.

**Cortex-non-cortex areas ratio**

Fig. 4. Cortex-non-cortex areas ration.

Chemia Resveratrol (IR) - group previously treated with resveratrol and submitted to 1-hour renal warm ischemia. Groups SR and IR received 30 mg/kg of resveratrol (Resveratrol, Terraternal, Santa Clara, USA) intraperitoneally 1 h before surgery, while untreated groups (S and I) received saline injections.

The animals were anesthetized via intramuscular ketamine (Cetamin, Syntec, Santana de Parnaiba, Brazil, 100 mg/kg) and xylazine (Xilazin, Syntec, 20 mg/kg). Once the surgical field was aseptic, a ventral midline incision was used to expose the abdominal viscera, which were displaced to expose the retroperitoneal area and the left kidney. The left renal artery and vein were isolated by blunt dissection. In rats of groups I and IR the renal vessels were clamped for 1 h, while in groups S and SR the pedicle was only dissected, and no ischemia was induced. All animals remained under anesthesia for 1 h, when the abdominal viscera were replaced, and the surgical wound was covered with moistened gauze. At the end of this period, vascular clamps
were removed, and left kidney reperfusion was observed in groups I and IR. For all groups abdominal cavity was closed routinely.

The animals were killed a month after surgery by anesthetic overdose (Isoflurane, BioChimico, Rio de Janeiro, Brazil). A blood sample was collected by cardiac puncture during rats' anesthetic
**Number of Glomeruli per Kidney**

![Number of Glomeruli per Kidney graph](image)

**Table 1**
Raw data: serum urea and creatinine of rats submitted to sham surgery or to left renal warm ischemia with or without resveratrol treatment.

| Sham group (Resveratrol group) | Serum urea (mg/dL) | Serum creatinine (mg/dL) | Ischemia group | Serum urea (mg/dL) | Serum creatinine (mg/dL) |
|-------------------------------|--------------------|--------------------------|----------------|--------------------|--------------------------|
| Animal S1                    | 42                 | 0.46                     | Animal I1      | 43                 | 0.42                     |
| Animal S2                    | 41                 | 0.44                     | Animal I2      | 50                 | 0.44                     |
| Animal S3                    | 40                 | 0.44                     | Animal I3      | 44                 | 0.45                     |
| Animal S4                    | 42                 | 0.45                     | Animal I4      | 40                 | 0.51                     |
| Animal S5                    | 41                 | 0.42                     | Animal I5      | 43                 | 0.50                     |
| Animal S6                    | 43                 | 0.45                     | Animal I6      | 50                 | 0.51                     |
| Animal S7                    | 39                 | 0.45                     | Animal I7      | 51                 | 0.46                     |
| Animal S8                    | 41                 | 0.42                     | Animal I8      | 45                 | 0.50                     |
| Animal S9                    | 45                 | 0.44                     | Animal I9      | 44                 | 0.45                     |
| Animal S10                   | 41                 | 0.45                     | Animal I10     | 48                 | 0.50                     |
| Animal SR1                   | 46                 | 0.46                     | Animal IR1     | 49                 | 0.46                     |
| Animal SR2                   | 45                 | 0.45                     | Animal IR2     | 42                 | 0.53                     |
| Animal SR3                   | 47                 | 0.50                     | Animal IR3     | 43                 | 0.49                     |
| Animal SR4                   | 46                 | 0.50                     | Animal IR4     | 43                 | 0.51                     |
| Animal SR5                   | 48                 | 0.43                     | Animal IR5     | 45                 | 0.43                     |
| Animal SR6                   | 39                 | 0.44                     | Animal IR6     | 42                 | 0.50                     |
| Animal SR7                   | 43                 | 0.49                     | Animal IR7     | 44                 | 0.49                     |
| Animal SR8                   | 44                 | 0.43                     | Animal IR8     | 42                 | 0.44                     |
| Animal SR9                   | 45                 | 0.45                     | Animal IR9     | 40                 | 0.39                     |
|                              |                    |                          | Animal IR10    | 43                 | 0.40                     |

*Fig. 7. Number of Glomeruli per Kidney.*
| Animals from Sham group | Body weight (g) | Left kidney weight (g) | Left kidney volume (ml) | Right kidney weight (g) | Right kidney volume (ml) |
|------------------------|----------------|------------------------|-------------------------|-------------------------|-------------------------|
| Animal S1              | 298.0          | 0.93                   | 0.91                    | 1.08                    | 1.04                    |
| Animal S2              | 301.0          | 1.05                   | 1.01                    | 1.11                    | 1.08                    |
| Animal S3              | 278.5          | 1.00                   | 0.98                    | 1.09                    | 1.05                    |
| Animal S4              | 291.5          | 1.01                   | 1.00                    | 1.01                    | 0.99                    |
| Animal S5              | 368.5          | 1.11                   | 1.07                    | 1.13                    | 1.10                    |
| Animal S6              | 299.0          | 0.98                   | 0.88                    | 0.95                    | 0.93                    |
| Animal S7              | 329.5          | 1.04                   | 1.00                    | 0.97                    | 0.93                    |
| Animal S8              | 357.5          | 1.20                   | 1.17                    | 1.13                    | 1.11                    |
| Animal S9              | 369.0          | 1.17                   | 1.19                    | 1.28                    | 1.29                    |
| Animal S10             | 346.5          | 1.41                   | 1.37                    | 1.32                    | 1.31                    |

| Animals from Ischemia group | Body weight (g) | Left kidney weight (g) | Left kidney volume (ml) | Right kidney weight (g) | Right kidney volume (ml) |
|-----------------------------|----------------|------------------------|-------------------------|-------------------------|-------------------------|
| Animal I1                   | 300.0          | 1.05                   | 1.01                    | 1.01                    | 1.00                    |
| Animal I2                   | 361.0          | 1.37                   | 1.34                    | 1.24                    | 1.22                    |
| Animal I3                   | 295.0          | 0.85                   | 0.90                    | 0.95                    | 0.94                    |
| Animal I4                   | 362.5          | 1.55                   | 1.31                    | 1.36                    | 1.37                    |
| Animal I5                   | 339.0          | 1.32                   | 1.25                    | 1.36                    | 1.32                    |
| Animal I6                   | 292.0          | 1.00                   | 1.05                    | 0.94                    | 0.96                    |
| Animal I7                   | 295.0          | 1.18                   | 1.20                    | 0.99                    | 1.02                    |
| Animal I8                   | 342.0          | 1.37                   | 1.31                    | 1.29                    | 1.29                    |
| Animal I9                   | 328.0          | 1.35                   | 1.26                    | 1.03                    | 1.31                    |
| Animal I10                  | 356.0          | 1.24                   | 1.18                    | 1.40                    | 1.38                    |

| Animals from Sham Resveratrol group | Body weight (g) | Left kidney weight (g) | Left kidney volume (ml) | Right kidney weight (g) | Right kidney volume (ml) |
|-------------------------------------|----------------|------------------------|-------------------------|-------------------------|-------------------------|
| Animal SR1                          | 384.5          | 1.12                   | 1.14                    | 1.16                    | 1.17                    |
| Animal SR2                          | 376.0          | 1.14                   | 1.15                    | 1.25                    | 1.23                    |
| Animal SR3                          | 348.0          | 1.14                   | 1.08                    | 1.28                    | 1.28                    |
| Animal SR4                          | 353.0          | 1.12                   | 1.05                    | 1.11                    | 1.09                    |
| Animal SR5                          | 363.0          | 1.23                   | 1.25                    | 1.34                    | 1.33                    |
| Animal SR6                          | 298.0          | 1.03                   | 1.04                    | 1.12                    | 1.11                    |
| Animal SR7                          | 334.0          | 1.21                   | 1.21                    | 1.19                    | 1.20                    |
| Animal SR8                          | 348.5          | 1.50                   | 1.50                    | 1.37                    | 1.37                    |
| Animal SR9                          | 368.0          | 1.54                   | 1.50                    | 1.52                    | 1.46                    |

| Animals from Ischemia Resveratrol group | Body weight (g) | Left kidney weight (g) | Left kidney volume (ml) | Right kidney weight (g) | Right kidney volume (ml) |
|----------------------------------------|----------------|------------------------|-------------------------|-------------------------|-------------------------|
| Animal IR1                              | 276.5          | 0.89                   | 0.89                    | 0.80                    | 0.81                    |
| Animal IR2                              | 369.5          | 1.12                   | 1.12                    | 1.11                    | 1.22                    |
| Animal IR3                              | 337.0          | 1.19                   | 1.31                    | 1.10                    | 1.10                    |
| Animal IR4                              | 302.5          | 1.04                   | 1.04                    | 0.98                    | 0.98                    |
| Animal IR5                              | 278.0          | 0.92                   | 0.93                    | 0.75                    | 0.74                    |
| Animal IR6                              | 323.5          | 1.31                   | 1.31                    | 1.16                    | 1.17                    |
| Animal IR7                              | 350.5          | 1.15                   | 1.15                    | 1.34                    | 1.39                    |
| Animal IR8                              | 331.0          | 1.27                   | 1.23                    | 1.17                    | 1.13                    |
| Animal IR9                              | 324.5          | 1.25                   | 1.20                    | 1.25                    | 1.23                    |
| Animal IR10                             | 290.5          | 1.05                   | 0.99                    | 1.05                    | 0.98                    |

Both kidneys were dissected, collected and weighed. The renal volume was measured by the Scherle’s method [1,2]. Left kidneys fixed in 4% buffered formaldehyde. The cortex-non-cortex areas ratio (C–NC) was achieved by morphometrical analysis of 2 mm transversal slices of left kidneys and calculated by the Cavalieri method [3–5]. The cortical volume (CV) was calculated by multiplying the renal volume by the C–NC [5].
Table 3
Raw data: Kidney morphological data of rats submitted to sham surgery or to left renal warm ischemia with or without resveratrol treatment.

| Group | Sham | Resveratrol |
|-------|------|-------------|
|       | C-NC Ratio | Cortical Volume (ml) | Vv[Glom] (%) | Total glomerular volume (μm³) | VWGV (μm³) | N[Glom] (millions per kidney) |
| Animal S1 | 0.76912 | 0.69990 | 5.81428 | 40,694,579,756 | 1,887,222,214 | 21,563.21574 |
| Animal S2 | 0.71523 | 0.72238 | 6.09523 | 44,031,304,147 | 1,388,244,613 | 31,717.25194 |
| Animal S3 | 0.77467 | 0.75918 | 5.95238 | 45,189,604,684 | 1,477,171,314 | 30,591.98636 |
| Animal S4 | 0.78191 | 0.78191 | 5.72380 | 44,755,106,953 | 1,635,263,227 | 27,368.74786 |
| Animal S5 | 0.71621 | 0.76635 | 6.49947 | 49,808,765,114 | 1,659,965,089 | 30,005.91124 |
| Animal S6 | 0.76534 | 0.67350 | 7.17428 | 51,955,834,587 | 1,284,496,795 | 40,448.39566 |
| Animal S7 | 0.75988 | 0.75988 | 6.38095 | 48,468,087,942 | 1,422,827,219 | 34,078.60916 |
| Animal S8 | 0.74567 | 0.87244 | 4.98842 | 43,521,230,900 | 1,353,662,007 | 32,150.73680 |
| Animal S9 | 0.72388 | 0.87213 | 5.61904 | 49,005,799,925 | 1,556,217,271 | 31,490.33291 |
| Animal S10 | 0.71888 | 0.98487 | 5.14285 | 50,650,821,126 | 1,422,827,219 | 35,598.71533 |

| Group | Ischemia | C-NC Ratio | Cortical Volume (ml) | Vv[Glom] (%) | Total glomerular volume (μm³) | VWGV (μm³) | N[Glom] (millions per kidney) |
|-------|----------|-------------|---------------------|-------------|-------------------------------|---------|-----------------------------|
| Animal I1 | 0.69766 | 0.70463 | 4.56190 | 32,144,958,118 | 1,783,474,396 | 18,023.78447 |
| Animal I2 | 0.69092 | 0.92583 | 4.00000 | 37,033,390,707 | 1,452,456,453 | 25,496.84652 |
| Animal I3 | 0.71733 | 0.64560 | 4.99084 | 25,824,003,346 | 1,393,184,985 | 18,535.94722 |
| Animal I4 | 0.67768 | 1.02330 | 3.90476 | 39,957,625,409 | 1,452,456,453 | 27,510.13134 |
| Animal I5 | 0.65233 | 0.81541 | 4.57142 | 37,276,064,573 | 1,758,772,534 | 21,194.36359 |
| Animal I6 | 0.71521 | 0.75097 | 3.71428 | 27,893,249,915 | 1,195,570,094 | 23,330.50154 |
| Animal I7 | 0.70938 | 0.85126 | 4.00000 | 34,050,483,234 | 1,496,932,802 | 22,746.83484 |
| Animal I8 | 0.66900 | 0.87639 | 3.80952 | 33,386,406,368 | 1,146,166,371 | 29,128.76108 |
| Animal I9 | 0.64778 | 0.81620 | 3.14285 | 25,652,688,840 | 1,343,781,262 | 19,089.66414 |
| Animal I10 | 0.68102 | 0.80361 | 4.66666 | 37,502,018,120 | 1,338,840,890 | 28,010.81025 |

Random samples from all 39 left kidneys were processed for paraffin embedding, sectioned at 5 μm thickness, and resulting histological blades were stained with haematoxylin and eosin. From each kidney, 25 histological fields, obtained from five different sections of the renal cortex, were photographed with a camera in a light microscope to be examined. Glomerular volumetric density (Vv[Glom]), which indicates the proportional volume occupied by the glomeruli in the cortex, was estimated by the point-counting method [3–5]. The volume-weighted mean
glomerular volume (VWGV) was estimated by using the point-sampled intercepts method \cite{3–5}. The estimation of the total number of glomeruli per kidney (N[Glom]) was achieved through the formula CVxVv[Glom]/VWGV \cite{5}.

Analyses were performed using GraphPad Prism 8.3.1 (GraphPad Software, San Diego, USA). The quantitative results were compared by one-way ANOVA with Tukey’s post-test and all results were considered significant when the value of \( p<0.05 \).

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105545.

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