RESEARCH ARTICLE

Morphometric investigations of fresh and fixed rabbit kidney

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Özet

Bolat D, Bahar S, Selçuk ML, Tıpırdamaz S. Taze ve tespit edilmiş tavşan böbreği üzerinde morfometrik araştırmalar. **Eurasian J Vet Sci, 2011, 27, 3, 149-154**

Amaç: Bu çalışmada formaldehit tespitinin böbrek morfometrisine etkisi ile böbreği oluşturan yapıların hacim ölçümlerinin ve ilgili yapıların böbrek içerisindeki hacimsel oranlarının Cavalieri prensibi kullanarak değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çalışmada 9 adet sağlıklı erkek Yeni Zelanda beyaz tavşanına ait böbrekler kullanıldı. Taze böbreklere ait ağırlık verileri elde edildikten sonra craniocaudal uzunluk, dorsoventral ve mediolateral çap ölçümleri dijital kumpas yardımı ile yapıldı. Her bir böbreğe ait hacim ölçümleri Arşimed prensibi ile gerçekleştirildi. Bu işlemlerden sonra taze böbrekler %10 formol içeren kaplarda 15 gün süre ile tespit edildi. Tespit edilen böbrekler üzerinde taze böbreklerde yapılan ölçüm işlemleri tekrarlandı ve böbreği oluşturan yapıların (korteks, medulla ve pelvis renalis) hacimleri Cavalieri prensibi ile hesaplandı.

Bulgular: Taze (15.8±0.26 mm) ve tespit edilmiş (14.8±0.32 mm) sağ böbreklere ait dorsoventral çap değerleri arasında istatistiki olarak önemli fark tespit edildi (p<0.05). Sol ve sağ böbreğe ait rölatif organ ağırlıkları sırası ile %0.38±0.02 ve %0.38±0.01 olarak belirlendi. Formol ile tespit sonrası sol ve sağ böbrek ağırlıklarının sırası ile %7.33 ve %7.56 oranında arttığı görüldü. Tespit sonrası hacim değerlerinde ağırlık artışına paralel olarak sol böbrekte %7.13 (p<0.05), sağ böbrekte ise %3.34 (p>0.05) oranında artış olduğu tespit edildi.

Öneri: Taze ve formol ile tespit edilmiş böbreklere ait elde edilen morfometrik verilerin gelecekte invaziv ve noninvaziv metotların kullanıldığı böbrek hastalıkları üzerinde yapılacak çalışmalara katkı sağlayacağı düşünüldü.

Abstract

Bolat D, Bahar S, Selcuk ML, Tipirdamaz S. Morphometric investigations of fresh and fixed rabbit kidney. **Eurasian J Vet Sci, 2011, 27, 3, 149-154**

Aim: The aim of this study was to investigate the effect of formalin fixation on the morphometry of kidneys, and to assess the volume and fractions of its constituents using the Cavalieri principle.

Materials and Methods: In this study, kidneys of nine healthy male White New Zealand rabbits were used. After weighing the kidneys, craniocaudal length, and mediolateral and dorsoventral diameters of fresh kidneys were measured with digital calipers, and the volume of each kidney was estimated using Archimedes' principle. The fresh kidneys were kept in a container of 10% formalin solution for 15 days. The Cavalieri principle was utilized for the determination of volume and the volume fraction of renal constituents.

Results: The differences in the dorsoventral diameter of fresh (15.8±0.26 mm) and fixed (14.8±0.32 mm) right kidneys were found to be statistically significant (p<0.05). The relative organ weights were determined to be 0.38±0.02% and 0.38±0.01% for the left and right kidneys, respectively. After formalin fixation, the weights of the left and right kidney increased by 7.33% and 7.56% respectively, and an increase in volume was determined between fresh and fixed left (7.13%, p<0.05) and right kidneys (3.34%, p>0.05).

Conclusion: It is believed that obtained morphometric data will contribute to studies involving the investigation of kidney diseases using invasive and non-invasive methods.

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► Introduction

Morphometric features of kidneys and the relative organ weights are important parameters that are used in pharmacological and toxicological studies in addition to the chemical and food industries (Bailey et al 2004, Michael et al 2007). Cortical thickness, area and volume are used in the determination of atherosclerotic renal function and evaluation of the success of treatment (Mounier et al 2007, Padigala et al 2009). Stenosis of the renal artery leads to a decrease in length and thickness of the kidney parenchyma (Prince et al 1997). Creatinine clearance is another important parameter of renal function, which relates directly to lean body mass and kidney volume (Nawaratne et al 1998).

Invasive and non-invasive methods have been used to investigate kidney structures. Magnetic resonance imaging (MRI) and computerized tomography (CT) are used commonly to image anatomical structures during in vivo studies (Heuer et al 2003, Prasad 2006). MRI in conjunction with Archimedes' principle may be used for the calculation of total kidney volume. Cavalieri principle is used to determine the volume of more complicated biological structures such as brain or testis (Cotter et al 1999, Akosman and Ozdemir 2010) in addition to the renal cortex, medulla and pelvis during in vitro studies (Malas et al 2002, Altunkaynak et al 2008, Pazvant et al 2009).

The aim of this study was to investigate the effect of formalin fixation on the morphometry of left and right kidneys, and to assess the volume of renal cortex, renal medulla, renal pelvis and their fractions using the Cavalieri principle.

► Materials and Methods

• *Materials*

Nine healthy White New Zealand male rabbits (1687.8 ± 65.4 g, 4 months old) were used. This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Selcuk University. The animals were anesthetized by administration of xylazine (0.5 mg/kg, IM) plus ketamine (35 mg/kg, IM) (Lipman et al 1990). The abdominal aorta and caudal vena cavae of animals in the supine position were exposed by displacing the intestines, following an incision made along the linea alba of the abdominal wall. Euthanasia was performed by draining blood through cannulae placed in these two vessels. The left and right kidneys were harvested as fresh material and kept in saline solution (0.9%).

• *Morphometric Measurements*

The kidneys were weighed with an assay balance. The mediolateral and dorsoventral diameters were measured at the level of the renal hilus, craniocaudal lengths were evaluated with digital caliper. The volumes of the kidneys were measured in a graded cyl-

inder by application of Archimedes' principle. These measurements were repeated on kidneys that had been kept in a container of 10% neutral formalin solution for 15 days at room temperature, and the results were recorded.

• *Calculation of volume and volume fractions by application of the Cavalieri principle*

The kidneys were sliced for calculation of the volume and volume fractions; all slices were evaluated using the Cavalieri principle. A tissue slicer that was made from microtome knives in the laboratory was used to cut the kidneys. Each kidney was placed with its long axis perpendicular to the blades of the tissue slicer. After the cutting process, and depending on the size of the kidney, 9 to 11 sections were taken from each kidney at 3.290±0.092 mm thickness for the left kidney and at 3.298±0,100 mm thickness for the right kidney (Figure 1). The section faces were photographed as JPEG files, formatted using a Sony DSC-H55 camera, taking into consideration the cut surface of the kidneys. Calculations of area were made on the photographed cut surfaces using an image analysis program, ImageJ (National Institutes of Health). ImageJ was calibrated first for calculation of the area of the images, and the grid function of the software was used to calculate the volume of each kidney and its subcomponents (cortex, medulla, and pelvis). The area per point was set for the kidney at 20 mm^2, for the renal medulla at 5 mm^2 and for the renal pelvis at 3 mm^2 on the grid (Figure 2). Points that fell on each anatomical region were counted and recorded separately. An example of this calculation is given in Table 1. The coefficient of error (CE) was calculated according to previously published methods (Gundersen et al 1999). The following formula was used to calculate the volume of each kidney and its subcomponents by counting the points that fell on the area of interest.

Figure 1. An example of consecutively sectioned kidney with tissue slicer.

$V = t \times a(p) \times \sum P$ (Mayhew and Gundersen 1996). In this formula, V refers to the volume of the region of interest (V_K= volume of kidney, V_M= volume of renal medulla, V_P= volume of renal pelvis), t refers to the mean section thickness, a(p) expresses the area per point and $\sum P$ is equal to the total number of points

hitting the region of interest. The volume of the renal cortex was calculated by subtracting the volume of the renal pelvis and renal medulla from the total kidney volume.
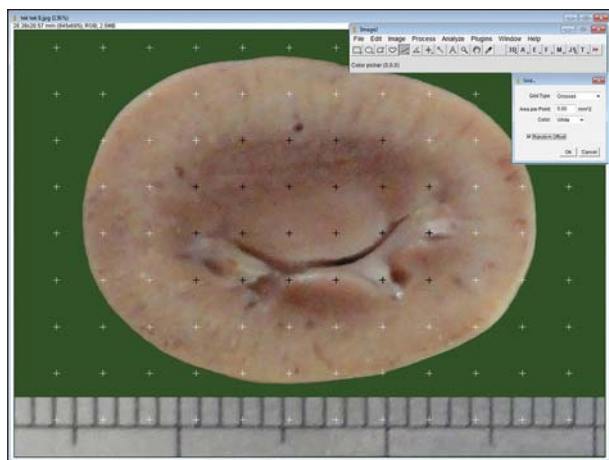

Figure 2. Superimposed point counting frame on cross-section of kidney using ImageJ (area per point = 5 mm²).

Table 1. Calculation of volume and volume fractions of the samples. a(p)= 20 mm² for total kidney, a(p)= 5 mm² for renal medulla, a(p)= 3 mm² for renal pelvis, t=3.339 mm.

Consecutive Section no:	Number of points in region		
	Kidney	Renal Medulla	Renal Pelvis
1	4	0	0
2	8	12	0
3	12	20	1
4	14	25	4
5	14	23	9
6	13	22	5
7	12	20	1
8	10	15	0
9	7	4	1
Total number of points ($\sum$P)	94	141	21

• *Volume fractions of kidney subcomponents*

Volume fraction refers to the ratio of the volume of any structure to the volume of the entire structure. The determination of the ratio in this study followed the formula used by (Turgut et al 2007).

• V_{VA}= *(Volume of A located within B)/(Volume of B) = V_A/V_B*

Volume fractions were expressed in the following forms: V_{VC} (renal cortex, kidney), V_{VM} (renal medulla, kidney), V_{VP} (renal pelvis, kidney). The volume fractions mentioned above were calculated using the following formulas: $V_{VC} = V_C / V_K$, $V_{VM} = V_M / V_K$, $V_{VP} = V_P / V_K$ (V_K=Volume of the kidney).

The density of each kidney was calculated using the following formula: D (density) = weight (W) / volume (V) (Malas et al 2002). Measurements of weight, in grams, were converted to milligrams for the calculation of kidney densities.

• *Statistical Analysis*

The two-sample t-test was used in the statistical evaluation of the measurements made on fresh and fixed kidneys, and also on the volume parameters of fresh and fixed kidneys (Archimedes' principle), and estimations made using the Cavalieri principle on fixed kidneys only. The paired t-test was used to compare the parameters between fresh and fixed left kidneys and fresh and fixed right kidneys (SPSS 17.0). The results are presented as mean±SEM and p <0.05 was considered to be statistically significant.

► **Results**

The morphometric measurements performed on fresh and fixed kidneys are given in Table 2 and the volume results are presented in Table 3. The relative weights of the left and right kidneys before formalin fixation were calculated to be 0.38 ± 0.022%, 0.38 ± 0.019% respectively. After formalin fixation, the relative weights of the left and right kidneys were 0.41 ± 0.025%, 0.42 ± 0.022% respectively. There was no statistically significant difference between the values for the left and right kidneys (p>0.05). After fixation, the relative weights were observed the left kidney 7.4% and the right kidney 7.6% to be increased (p<0.01).

An increase in weight was observed after fixation for the left 7.33% and for the right 7.56% kidney (p<0.05). The dorsoventral diameter of the right kidney was decreased (p<0.05) compared with the measurements made before fixation (Table 2).

Volume of the left kidney increased at a rate of 7.13% (p<0.05) and the right kidney 3.34% (p>0.05) after formalin fixation, as calculated according to Archimedes' principle. A statistically significant difference was found between the volumes of fresh and fixed left kidneys with the use of Archimedes' prin-

Table 2. Morphometric measurements made on fresh and fixed kidneys (n=9, mean±SEM).

	Weight (g)		Craniocaudal length (mm)		Dorsoventral diameter (mm)		Mediolateral diameter (mm)	
	Left	Right	Left	Right	Left	Right	Left	Right
Fresh	6.19±0.24 [B]	6.23±0.26 [B]	31.9±0.53	32.2±0.65	16.4±0.29	15.8±0.26 [A]	21.3±0.36	21.2±0.36
Fixed	6.68±0.29 [A]	6.74±0.31 [A]	31.9±0.50	32.1±0.60	16.1±0.36 [a]	14.8±0.32 [b,B]	21.1±0.41	21.5±0.33

Different letters in the same row ([a,b]) and in the same column ([A,B]) represent statistically significant differences (p<0.05).

ciple and the Cavalieri principle (p<0.05). It was observed that left kidneys were more affected by formalin fixation than right kidneys. Neither the results obtained using Archimedes' principle nor those from the Cavalieri principle showed a statistically significant difference between the left and right kidneys. Differences in the volumes of the left and right renal cortex, renal medulla and renal pelvis estimated using the Cavalieri principle were not found to be statistically significant (p>0.05) (Table 3). Volume fractions of the left and right renal cortex, renal medulla and renal pelvis were estimated to be 59.8%, 36.4%, 3.8% and 61.8%, 34.7%, 3.4% respectively. The coefficient of error (CE) values were calculated for the kidney, renal cortex, renal medulla and renal pelvis and were found to be 3.5%, 3.7%, 3.3% and 11% respectively. It was found that the density of the left kidney was 0.97 and of the right kidney was 1.00 (p>0.05).

was calculated as 0.76±0.040 and observed that this value was similar to the results of previously studies conducted by Hall and Macgregor (1937) and Taylor et al (1923).

Relative weights of kidneys showed that use of the fresh kidney weight is the most accurate approach to the calculation of relative organ weight when comparing fixed organ weight. There was no difference in the relative weight between the left and right kidneys. After 10% neutral formalin solution an increase in the relative weights was observed for both kidneys 7.5% and this value can be used to calculate fresh kidney weight and relative weight.

Calculation of kidney density has been developed to investigate the possible relationship between weight and volume of the kidney. It is assumed that 1 g kidney weight is equal to 1 ml volume of kidney when

Table 3. Volume measurements of fresh and fixed kidneys (n=9, mean±SEM).

	Fresh (mm^3)		Fixed (mm^3)		Cavalieri principle (mm^3)	
	Left	Right	Left	Right	Left	Right
Kidney	6222 ±260 [b,B]	6444±280	6700±290 [a]	6667±270	6880±272 [A]	6726±254
Renal Cortex	N/A	N/A	N/A	N/A	4111±165	4160±172
Renal Medulla	N/A	N/A	N/A	N/A	2505±140	2336±100
Renal Pelvis	N/A	N/A	N/A	N/A	259±31.0	230±34.0

[a, b, A, B]; different letters in the same row represent statistically significant differences (p<0.05). N/A = Not available.

► Discussion

Tissue shrinkage or swelling can be seen in biological tissues after fixation using chemical agents such as formaldehyde and alcohol. In the present study it was observed that the weight and volume of the left and right kidneys were increased after formalin fixation. However the right kidney was less affected compared the left one. It is thought that these differences may have been caused by the decrease in the dorsoventral diameter of the right kidney. Kidneys are composed of cortex and medulla, which show different histological features, and these subcomponents can be affected to different degrees by fixative solutions. It was reported that the renal cortex of the rabbit kidney swelled by 29% as a result of fixation in 10% neutral formalin solution (Stowell 1941). Considering the current data, it was thought that the positive effect of formalin fixation on the renal cortex or renal medulla can be seen more clearly when they are fixed separately in formalin.

In previous research, 17 male rabbits (weight; 453-3690 g) were used and reported that mean relative weight was 0.68 (range; 0.52-1.27) (Taylor et al 1923). It was showed that the relative organ weights of animals up to 1750 g of body weight decrease rapidly but this value increases in the heavier male animals while females don't show any increment (Hall and Macgregor 1937). In the present study, relative organ weight

calculating kidney density and there is no statistically significant difference between the weight and volume of rat kidneys (Christiansen et al 1997). In the current study this value was found to be 0.97 for the left kidney and 1.00 for the right kidney (p>0.05). While a search of the literature found no information about the renal density of rabbits, a study conducted on the kidneys of male rats that applied the Cavalieri principle showed that kidney density was 1.19 for the left kidney and 0.997 for the right kidney (Malas et al 2002).

In the present study, use of the Cavalieri principle did not show any statistically significant difference between the volumes of left kidneys (6880±272 mm^3) and right kidneys (6726±254 mm^3) (p>0.05) (Table 3). While the volumes and volume fractions of renal cortex, renal medulla and renal pelvis are calculated using MRI during in vivo studies, MRI is also used to calculate such parameters, in addition to the Cavalieri principle and histological techniques, during in vitro studies. It has been reported that the volumes of pig kidneys that were calculated using MRI were greater than the volumes of autopsy material calculated using Archimedes' principle (Coulam et al 2002). In another study of pig kidneys comparing MRI and the Cavalieri principle, there was found to be no statistically significant difference in the volumes obtained by the two different methods (Karstoft et al 2007). It was

stressed that the reason for the higher volume calculated by MRI in comparison with the Archimedes' principle can be caused by the amount of blood in the kidney, low-Tesla imaging and the fat tissue surrounding the kidney structures which may result in failure to differentiate kidney subcomponents (Coulam et al 2002). However, Karstoft et al (2007) reported no statistically significant difference between the two methods. Considering the results of two studies, there may be a methodological problem in MRI studies previously done.

In the present study, it was found that the left kidney consisted of 59.7% renal cortex, 36.4% renal medulla and 3.8% renal pelvis, whereas right kidney consisted of 61.8% renal cortex, 34.7% renal medulla and 3.4% renal pelvis (Table 3). The ratio of the constituent parts of biological structures is of great importance (Cibulskyte et al 2007, Bas et al 2009, Akdogan et al 2010). It has been reported in previous studies that the ram kidney consists of 70.3% renal cortex, 26.4% renal medulla and 3.1% renal pelvis (Pazvant et al 2009). In pigs the ratio of renal medulla was found to be 10–20 % (Coulam et al 2002), and the rat kidney consisted of 48.7% renal cortex, 47.9% renal medulla and 2–3.3% renal pelvis (Christiansen et al 1997). When the current results are evaluated, the ram, rat and White New Zealand rabbit have the same ratio of renal pelvis, but there were no similarities among their ratios of renal cortex and renal medulla.

The coefficient of error (CE) value was determined to be less than 5% for renal cortex and renal medulla but greater than 10% for renal pelvis. The CE value shows the quality of the sampling procedure (Slomianka and West 2005), and it should be less than 10% in biological studies (Gundersen et al 1999). In the present study, the CE in the estimated volumes of renal cortex and renal pelvis was acceptable, but the value was higher for the renal pelvis. This problem could be solved by increasing the number of sections obtained from the renal pelvis or by decreasing the distance between two test points in the grid (Mouton 2002).

► **Conclusions**

Formalin fixation is usually believed that causes tissue shrinkage not to swelling. In the light of current study, it can be mentioned that formalin fixation causes tissue swelling in the kidney. This important finding can be useful for anatomists, histologists and pathologists. The volume of the kidney and fractions of its subcomponents (cortex and medulla) are important in chronic, atherosclerotic renal diseases and diabetes mellitus. It is thought that the obtained results from the kidneys of White New Zealand rabbits, which are used as a model animal for many diseases, including renal pathologies, in experimental studies, can be used as reference values in future studies.

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