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Sexually dimorphic proteinuria in Wistar rats: Relevance to clinical models

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ABSTRACT

The study investigated the relationship between physiological proteinuria and the histomorphometry of the renal corpuscles in apparently healthy Wistar rats of both sexes, belonging to the same age group. This was with a view to appraise any possible connection between potential expression of sexual dimorphism and the histomorphometry of some integral parts of the glomerular filtration barrier. Twenty Wistar rats of both sexes between ages 9 and 10 weeks were used for this study. This comprised 10 male and 10 female rats weighing 110–200 g which were housed in separate metabolic cages for the collection of urine samples.They were sacrificed 24 h and 7 days after 2 weeks of acclimatization, respectively. The rats were fasted for 24 h during the collection of urine samples. The results showed 74.75% significantly higher urine total protein ($p < 0.0001$), 187.29% significantly higher mg protein/100 g body weight ($p < 0.0001$), 32.34% significantly higher Bowman's capsular thickness ($p < 0.0001$), 30.64% significantly higher glomerular thickness ($p = 0.0002$), 59.47% significantly higher Bowman's capsular space ($p = 0.003$), 5.30% insignificantly lower creatinine clearance ($p = 0.24$) and 28.05% significantly higher level of urine protein to creatinine ratio ($p < 0.0001$) in the male when compared with their female counterpart. In conclusion, Wistar rats express sexually dimorphic proteinuria which is structural in origin.

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1. Introduction

The excessive presence of serum protein in urine (proteinuria) is of clinical significance to man because it represents a symptom of renal disease. Rats, on the other hand, demonstrate a striking physiological proteinuria [1,2]. The term "physiological proteinuria" could mean one of two things, depending on the species in description. In humans, it is defined as the presence of protein in urine which disappears after a short time following some biological conditions such as fever, gestation, hypoxia, posture and spasm of renal blood vessels [3]. Also, the consumption of a diet rich in protein or intravenous infusion of protein compounds can cause transient proteinuria in humans [3]. In rats, however, the term is used to describe the incidence of excessive presence of serum protein in the urine of apparently healthy, normal rats without the aforementioned conditions [1]. In humans, proteinuria can be pathological. This is associated with defects of the glomerular filtration barrier [4,5] and, therefore, persists with or without the biological conditions.

The study of physiological proteinuria in rats had been of scientific interest until the late 1980s. Afterwards, it seems unfounded that this area of scientific exploration was abruptly suspended; hence, references in this regard are often stranded in antiquity [1,2,6–8]. A proper understanding of the maintenance of homeostasis, regardless of proteinuria (physiological proteinuria) in apparently healthy rats, may point toward how best to treat or manage an imbalance in homeostatic conditions that are associated with proteinuria (pathological proteinuria) in humans.

The integrity of the glomerular filtration barrier (GFB) is a factor that determines the appearance of varied levels of protein in the urine [5,9]. Although literature exists on the incidence of proteinuria and the amount excreted by male and female rats of various strains [2,6–8], there is a dearth of literature on the relationship between the potential expression of physiological proteinuria and the histomorphometry of some integral features of the renal corpuscles; which constitute parts of the glomerular filtration barrier. Therefore, this current investigation seeks to bridge this gap in knowledge.

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2. Materials and methods

2.1. Metabolic cage

Metabolic cages used for this study were fabricated by Central Technological Laboratory and Workshops (CTLW), OAU, Ile-Ife, Osun State, Nigeria. Standard laboratory kit for assay of plasma and urine levels of creatinine was purchased from Randox Laboratories Limited, United Kingdom.

2.2. Animal management and experimental design

Twenty apparently healthy Wistar rats of both sexes between ages 9 and 10 weeks were used for this study. These comprised 10 male and 10 female Wistar rats and were certified healthy by the animal scientist in the animal holdings of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The male rats weighed 150–200 g while the female rats weighed 110–150 g. At about 7 weeks of age, the rats were transferred into separate metabolic cages and allowed to adapt to life there for two weeks before the collection of urine samples and commencement of the study. During this period, they were allowed access to food and water *ad libitum*. Before sacrifice, the rats were deprived of food for twenty four hours but allowed access to water *ad libitum*. They were divided into four groups of five rats each as follows; group 1 (male 1), group 2 (female 1), group 3 (male 2) and group 4 (female 2). While groups 1 and 2 were sacrificed twenty four hours after acclimatization, groups 3 and 4 were sacrificed 7 days thereafter (Fig. 1). The gap in the period of sacrifice was intended to increase the sample size for the determination of the total mean of the parameters of study and or appraise possible variations in the urine total protein level that could occur within this period. Blood samples were collected by cardiac puncture into separate EDTA bottles and thereafter centrifuged at −4 °C using a cold centrifuge (Centurium Scientific, Model 8881) that was set at 4000 revolutions per minute for 15 min. The plasma obtained was decanted with a sterile syringe into separate plain bottles for biochemical assays.

2.3. Determination of urine total protein

The total protein in the urine of the rats was determined according to the method of Lowry et al. [10] and as described by Holme and Peck [11]. The procedure was as follows; 0.2 mL of the test sample (urine) was added to 2.1 mL of alkaline copper reagent which was freshly prepared by mixing 2% Na_2CO_3 in 0.1 M NaOH, 1% $CuSO_4{\cdot}5H_2O$ and 1% Na, K tartrate·$4H_2O$ (98:1:1, by volume). The mixture was vortexed and allowed to stand for 10 min after which 0.2 mL of Folin–Ciocalteau color reagent was added. The resulting reaction mixture was vortexed and allowed to stand at room temperature in a dark locker for about an hour after which the absorbance of the mixture was read at 550 nm against a reagent blank. The blank was made up of 0.2 mL of distilled water and appropriate volume of the diluents and color reagent.

The protein concentrations of the test samples were estimated from a standard curve obtained using bovine serum albumin (BSA). To 0.2 mL of five different concentrations (50, 100. 150, 200 and 250 μg/mL) of BSA was added to appropriate volumes of diluents and color reagent as given above. The values obtained were then used to plot a curve of absorbance against BSA concentration using linear regression:

$$\text{mg protein/mL} = A_{\text{sample}} \times \frac{\text{Dilution factor}}{E}$$

where E is the slope of the best-fit linear regression line obtained from the graph of the standard curve for BSA.

2.4. Measurement of Bowman's capsular and glomerular circumferences

After the representative photomicrographs (at ×100 magnification) were imported into Motic Image Plus (MIP) 2.0 ML software (Motic China Groups Co., Ltd.), a free hand tracing of the circumferences of both the glomerulus and Bowman's capsule was carried out using "free-line tool". The values obtained were directly recorded in micrometer (μm) for either the glomerulus or Bowman's capsule, depending on the selected structure.

2.5. Measurement of Bowman's capsular space

The difference between the Bowman's capsular circumference and the glomerular circumference, as generated by the MIP, was taken to be the measurement for the Bowman's capsular space. This is the actual distance between the Bowman's capsule and the beginning of the glomerulus embedded in it, as revealed by the representative photomicrographs. The values obtained were directly recorded and expressed in micrometer (μm).

2.6. Measurement of Bowman's capsular and glomerular diameters

The diameter was taken as a measure of the thickness of the feature of interest. This involved the following three steps:

1. using the "rectangle tool", a rectangle was traced around the feature of interest such that the edges of the feature under examination were within and in contact with the edges of the rectangle.
2. a vertical and a horizontal line were drawn within the rectangle of step 1 above using the "line tool" of MIP. These lines were made such that each originated and terminated on one of the four sides of the rectangle so that a cross was formed within each rectangle.
3. the values of both lines (forming the cross within the rectangle) were recorded as expressed in micrometer (μm) and the average was taken as the diameter/thickness of each feature under examination.

2.7. Measurement of relative kidney weight (RKW)

First, the weight of both kidneys (left and right) of each rat was weighed using Camry sensitive weighing balance. The summation of both kidney weights was divided by the final body weight (at the point of sacrifice) and multiplied by 100% as depicted by the formula below:

$$\text{RKW}(\%) = \frac{\text{Weight of left kidney} + \text{Weight of right kidney}}{\text{Final Body Weight}} \times 100\%$$

2.8. Measurement of glomeruli per 5×10^{-3} cm^2

Each slide that was imported to MIP was about 3.2×10^{-3} cm^2. Using the "rectangle tool", six boxes each with an area of 5×10^{-3} cm^2 were traced on each slide and the number of glomeruli per unit area was counted.

2.9. Measurement of creatinine levels and creatinine clearance

Plasma and urine levels of creatinine were estimated using standard laboratory protocols, as provided by Randox Laboratory kit

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