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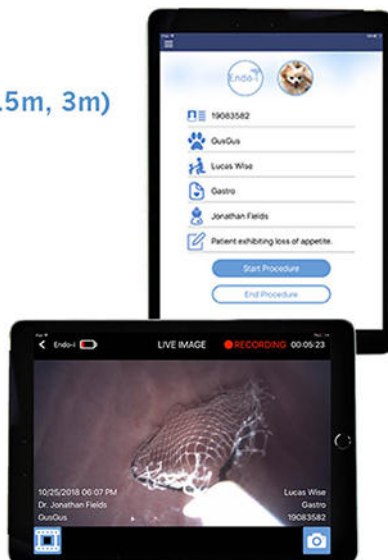
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## STANDARD ARTICLE

# Estimation of glomerular filtration rate by plasma clearance of iohexol in healthy horses of various ages

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**Abstract**

**Background:** Plasma clearance of iohexol is a reliable method to assess the glomerular filtration rate (GFR). The relationship between aging and GFR in horses is unclear.

**Hypothesis/Objectives:** To compare GFR estimated by iohexol clearance in horses of different ages.

**Animals:** Twenty-one clinically healthy horses were enrolled.

**Methods:** Prospective study. Groups: (A) composed by 8 young horses ( $\leq 14$  years old) with serum creatinine  $< 1.5$  mg/dL, (B) by 7 aged horses ( $\geq 20$  years old) with serum creatinine  $< 1.5$  mg/dL, and (C) by 6 aged horses ( $\geq 20$  years old) with serum creatinine  $\geq 1.5$  mg/dL. Iohexol was injected (75.5 mg/kg) through an IV catheter, and plasma samples were collected 5, 30, and 90 minutes later. Plasma clearance of iohexol was obtained by the high-performance liquid chromatography-ultraviolet method.

**Results:** The GFR was 2., 2.1, and 1.45 mL/min/kg (median value) in Groups A, B, and C, respectively. Statistical analysis showed differences between Group A versus C for urea, Group A versus B and A versus C for creatinine, and A versus C for GFR.

**Conclusions and Clinical Importance:** Glomerular filtration rate was significantly reduced in aged horses with serum creatinine  $\geq 1.5$  mg/dL compared to young horses with creatinine  $< 1.5$  mg/dL; no differences were obtained between young and aged horses with creatinine  $< 1.5$  mg/dL. Glomerular filtration rate evaluation should be considered in aged horses even if the plasma creatinine values are normal.

**KEYWORDS**

aging, CKD, equine, GFR, HPLC

**1 | INTRODUCTION**

Age-associated reduction in functional renal mass has been documented in both human and veterinary medicine.<sup>1,2</sup> In humans, the

glomerular filtration rate (GFR) is significantly reduced at birth, and progressively increases over the following 2 years, reaching an average value of 140 mL/min/1.73 m<sup>2</sup>. Although the age-associated loss in renal function may vary considerably,<sup>3</sup> an average decline in GFR of 8 mL/min/1.73 m<sup>2</sup> every 10 years has been documented in humans after the age of 40.<sup>4</sup> Similarly, aged dogs show an increased frequency

**Abbreviations:** AUC, area under the curve; BW, body weight; CKD, chronic kidney disease; Cl<sub>p</sub>, plasma clearance; GFR, glomerular filtration rate; IOX, iohexol; USG, urine specific gravity.

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of glomerular sclerosis and loss of residual renal function.<sup>2</sup> In neonatal Pony foals, GFR did not seem to change significantly over the first 10 days from birth, and was similar to the GFR of adult horses.<sup>5</sup> Plasma clearance (Clp) of iohexol (IOX) is a technically simple and accurate method for GFR evaluation in clinically healthy horses,<sup>6,7</sup> foals,<sup>8</sup> and donkeys.<sup>7</sup>

To the authors' knowledge, there are currently no data concerning possible variations of GFR among clinically healthy horses of various ages. The aim of the present study was to investigate GFR in healthy young and adult horses, using the Clp of IOX.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

A total of 21 stallion horses were enrolled in this prospective study. All the subjects were submitted to clinical examination, CBC, total proteins, serum creatinine, and urea concentrations. The horses were divided in 3 groups based on age<sup>9,10</sup> and serum creatinine concentration. For serum creatinine, a cutoff value of 1.5 mg/dL was considered. We decided to use the cutoff of 1.5 mg/dL because it represented the upper third of the reference range for horses in our laboratory (0.9-1.7 mg/dL). Aged horses were divided into 2 groups according to a serum creatinine less or greater than 1.5 mg/dL. In particular, Group A was composed by 8 young horses ( $\leq 15$  years old)<sup>9</sup> with serum creatinine concentration  $< 1.5$  mg/dL, Group B was composed by 7 aged horses ( $\geq 20$  years old)<sup>10</sup> with serum creatinine concentration  $< 1.5$  mg/dL, and Group C was composed by 6 aged horses ( $\geq 20$  years old)<sup>10</sup> with serum creatinine  $\geq 1.5$  mg/dL. Glomerular filtration rate was assessed by plasma IOX clearance. Horses were provided with hay and water ad libitum during all the study period. During the entire time of the procedure, horses were checked for vital parameters (body temperature, respiratory rate, heart rate, and mental status), and possible anaphylactic reactions every 30 minutes. Approval to conduct this study was obtained by the Ethic Committee on Animal Experimentation of the University of Pisa (D.L. 26/2014). An owner written consent has been obtained.

### 2.2 | Plasma IOX clearance

A sterile 14-gauge IV catheter (Surflash, Terumo Italia S.r.l., Rome, Italy) was aseptically placed in both left and right jugular veins and locked with a sterile cap. The right IV catheter was used only for IOX administration. The left catheter was used for blood sampling, in order to minimize venipuncture. Before and after each blood sampling, the catheter was flushed with 2.5 mL of sterile 0.9% saline solution. The first 5 mL of blood collected were discarded, then 2.5 mL of blood was collected in lithium heparin tubes.

A commercially available IOX formulation (Omnipaque 350, Nycomed Imaging AS, Oslo, Norway) was administered as an IV bolus (within 1 minute) at a dose of 75.5 mg/kg, through the right jugular catheter. To determine the exact dose administered, the syringe and the needle used for IOX infusion were weighed before and after

injection. Blood samples were collected through the left jugular catheter before IOX bolus (T0), and at 5, 30, and 90 minutes from completion of IOX inoculation. Blood samples were centrifuged at 3000 rpm for 10 minutes. Plasma was stored in aliquots at  $-20^{\circ}\text{C}$  until high-performance liquid chromatography analysis.<sup>7</sup>

### 2.3 | Pharmacokinetic and statistical analysis

Pharmacokinetic analyses were performed by PKSolver.<sup>11</sup> Plasma data were subjected to noncompartmental analysis with a statistical moment approach. The area under the curve (AUC) was calculated by trapezoidal rule with extrapolation to infinity; the Clp was calculated as follows:  $\text{Clp} = \text{Dose}/\text{AUC}$ . Glomerular filtration rate was determined by dividing Clp, by body weight (BW) (mL/min/kg). The administered dose was established by assuming that the 85% of IOX was *eso*-IOX.<sup>12</sup>

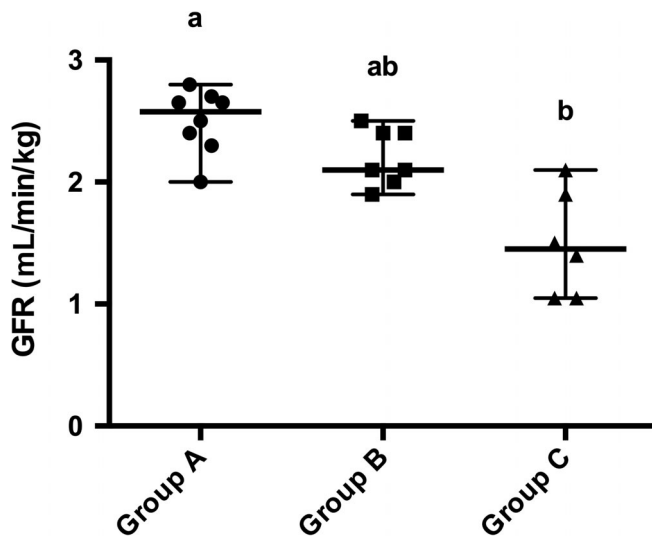
Data were analyzed using GraphPad Prism (GraphPad Software Inc, La Jolla, California). Data distribution was assessed through the Shapiro-Wilk normality test. Because of only few data showed a Gaussian distribution, we decided to express all our results as median minimum and maximum values. Glomerular filtration rate, urea, and creatinine values of the 3 groups of horses were compared by Kruskal-Wallis test and Dunn's test as post hoc. Results were considered statistically significant for  $P < .05$ .

## 3 | RESULTS

All the enrolled animals did not show a history of clinical signs suggestive of chronic kidney disease (CKD; such as polyuria-polydipsia, dehydration, weight loss, poor appetite, or anorexia). Hydration status was evaluated on the basis of a combination of physical exam (skin turgor, mucous membrane, and BW), and laboratory parameters (hematocrit and total solutes). Each horse showed a normal physical exam, and CBC, plasmatic urea and creatinine concentrations within the normal reference range for our laboratory (0.9-1.7 mg/dL). None of the horses showed clinically relevant adverse reactions (such as anaphylactic reactions) to the inoculation of IOX assessed by physical exam.

Group A consisted of 8 young horses (5 Warmbloods and 3 Italian Draft horses), with a median age of 7 years (1-14 years old) and a median BW of 580 kg (522-613 kg). Group B consisted of 7 aged horses (5 Warmbloods and 2 Italian Draft horses), with a median age of 23 years (21-23) and median BW of 600 kg (553-650 kg). Group C consisted of 6 aged horses (6 Warmbloods), with a median age of 21 years old (20-26) and a median BW of 500 kg (450-600 kg).

In Group A, urea, creatine, and GFR were 11 mg/dL (10-13 mg/dL), 1 mg/dL (0.9-1.2 mg/dL), and 2.60 mL/min/kg (2.00-2.80 mL/min/kg) (median, minimum, and maximum), respectively. In Group B the urea, creatine, and GFR were 20 mg/dL (12-32 mg/dL), 1.3 mg/dL (1.0-1.4 mg/dL), and 2.10 mL/min/kg (1.90-2.50 mL/min/kg), respectively. In Group C, urea, creatin and GFR were 21 mg/dL



**FIGURE 1** Dot plots displaying the actual values of glomerular filtration rate (GFR) for horses in Groups A (young horses with creatinine <1.5 mg/dL), B (aged horses with creatinine <1.5 mg/dL), and C (aged horses with creatinine ≥1.5 mg/dL). The different lowercase letters above the dot plots denote a significant difference ( $a \neq ab \neq b$ ;  $P < .05$ ). The horizontal lines represent the median, minimum, and maximum values

(15-40 mg/dL), 1.5 mg/dL (1.5-1.7 mg/dL), and 1.45 mL/min/kg (1.05-2.10 mL/min/kg), respectively.

The Kruskal-Wallis test showed statistically differences for urea ( $P = .0001$ ), creatinine ( $P = .0001$ ), and GFR ( $P = .0003$ ) among groups. Dunn's post hoc test showed a significance difference between Group A versus C for urea, between Group A versus B and Group A versus C for creatinine, and between Group A versus C for GFR (Figure 1).

#### 4 | DISCUSSION

Plasma clearance of IOX showed a clinically suitable and safe procedure for GFR determination in both young and aged horses. During the entire time of the procedure, horses were checked for vital parameters (body temperature, respiratory rate, heart rate, and mental status) and possible anaphylactic reactions. None of the horses showed clinically relevant adverse reactions to the inoculation of IOX. Glomerular filtration rate was not significantly different between Groups A and B. In addition, the GFR values of both young horses (2.50 mL/min/kg) and aged horses with serum creatinine <1.5 mg/dL (2.20 mL/min/kg) were close to previously findings for plasma IOX clearance (2.15 mL/min/kg in foals, and 2.38 mL/min/kg and 2.36 mL/min/kg in adult horses, respectively).<sup>6-8</sup> In our study, aged horses with serum creatinine ≥1.5 mg/dL (Group C) showed a significantly lower GFR (1.50 mL/min/kg) than young horses (Group A).

Our initial explanation for the reduction of GFR in aged horses was secondary to the physiological aging process of kidneys. This finding seems to be in agreement with current data in both human and veterinary medicine, in which the aging kidney is characterized by structural and physiological changes that are responsible for a

reduction in GFR.<sup>1,2,13</sup> In humans, the kidney aging process starts around 50 years of age, and it is characterized by both macroscopic and microscopic structural changes. Macroscopic alterations are represented by an increase in medullary volume, number of renal cysts, surface roughness, and a reduction in cortical volume. Microscopic alterations are characterized by a reduction in nephron number, and increased glomerular and interstitial fibrosis.<sup>13</sup> People <40 years old are expected to have <10% of globally sclerotic glomeruli, whereas this percentage increases significantly with aging, particularly after 50 years of age.<sup>14</sup> Aging-related glomerulosclerosis has a multifactorial pathogenesis.<sup>15</sup>

Similar findings have been reported in veterinary species, where a review of histopathology kidney changes in aged dogs, showed an increased frequency in glomerulosclerosis and obsolescent glomeruli.<sup>2</sup> Aged dogs are more commonly characterized by increased thickness of the glomerular basement membrane.<sup>16</sup> In dogs, tubulointerstitial lesions seem to be a less frequent consequence of renal aging. Occasionally, signs of interstitial fibrosis and tubular atrophy are reported.<sup>2</sup> Tubulointerstitial lesions seem to be more frequent in aged humans, where they are generally associated with glomerulosclerosis.<sup>17</sup> Beside structural changes in kidney architecture, aging is also responsible for a reduction in overall renal function.

In human beings, GFR declines after 30 years of age by approximately 1 mL per year.<sup>18</sup> However, the exact influence of age on renal function and GFR in veterinary medicine is still debated.

For dogs, there are contrasting results. In one study,<sup>19</sup> 2-month-old puppies had a significantly higher mean GFR than mature dogs. On the other hand, Bexfield and Colleagues<sup>20</sup> showed that GFR was affected by age only in the group of dogs with a BW of between 1.8 and 12.4 kg. In this group of dogs, age alone was responsible for 12.5% of GFR variation. In dogs with BW > 12.4 kg, no effects of age on GFR were reported. Bexfield and Colleagues<sup>20</sup> hypothesized that the discrepancy between what is found in human medicine and their results might be affected by the lower life span of dogs, compared to humans.<sup>20</sup> In aged dogs, the reduction in GFR might not be as evident as in humans, despite the presence of similar histopathological lesions.<sup>20</sup> Companion horses have a higher average life span than dogs, which may predispose horses to develop a level of glomerulosclerosis that is more similar to geriatric human patients.<sup>21</sup> Therefore, it is possible that the discrepancy in GFR between young and aged horses may be secondary to a physiological increase in glomerulosclerosis, because of the aging process. Unfortunately, no histopathological exam was performed at the time GFR was evaluated, so we are unable to confirm our hypothesis.

On the other hand, it is possible that the significantly lower GFR of old horses of Group C, compared to Group A, was determined by the presence in Group C of horses with early stages of CKD. This hypothesis may be supported by the lack of significant difference in GFR between young horses and aged horses with serum creatinine <1.5 mg/dL of Group B. Horses of Group C had a serum creatinine ≥1.5 mg/dL, which was at the upper third of the reference range of our laboratory (0.9-1.7 mg/dL). Therefore, it is possible that these horses had early stage CKD, despite no clinical signs and normal

creatinine values. The reference range for serum creatinine of our laboratory may be too wide, thus classifying horses with low grade CKD as normal. A reduction in GFR despite normal serum creatinine is a typical finding of early stage CKD. Glomerular filtration rate is, in fact, considered the best indicator of overall renal function, with a higher sensitivity than serum creatinine in detecting small changes in renal function. As the kidney has a high reserve capacity, increase in serum creatinine is not evident until GFR reduces over 75%.<sup>22</sup>

The present study has some limitations. First, GFR evaluation was not accompanied by kidney biopsy. As a consequence, we had no opportunity to evaluate the degree of glomerulosclerosis, or other pathological findings, in the Group C kidneys. Second, a limited number of horses were included in the study. Another limitation was the lack of urinalysis for all the horses of the study. Urinalysis would have been a useful tool to rule out early signs of CKD (such as reduction in urine specific gravity [USG]), particularly for horses of Group C. However, as all horses of Group C were stallions, which did not urinate spontaneously during the procedure, we opted not to catheterize them. Urinary catheterization in stallions is usually performed under sedation, and vasoactive drugs might affect glomerular hemodynamics, and GFR. To support our choice, the absence of clinical signs of renal failure, the normal hydration status, and serum creatinine within the normal reference range made prerenal azotemia unlikely.

Another limitation of the present study was represented by the lack of a group of young horses with serum creatinine  $\geq 1.5$  mg/dL. As a consequence, the role of a serum creatinine  $\geq 1.5$  mg/dL in affecting GFR could not be investigated. The relatively low number of patients, together with a significant variability in terms of breed and BW, might affect the homogeneity of the sample. For this reason, a study with a larger number of patients, and with horses of various ages, breeds, and BWs would be recommended.

In conclusion, GFR was significantly reduced in aged horses with serum creatinine  $\geq 1.5$  mg/dL compared to aged horses with creatinine  $< 1.5$  mg/dL and to young horses. Although serum creatinine, serum urea, and USG remain the most commonly used parameters for the diagnosis of CKD in equine medicine, GFR evaluation should be encouraged, particularly in aged horses.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The in vivo trial was approved by the Ethic Committee on Animal Experimentation of the University of Pisa (D.L. 26/2014). An owner written consent has been obtained.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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