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Single-injection inulin clearance for routine measurement of glomerular filtration rate in cats

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Glomerular filtration rate (GFR) was determined in 53 cats using an inulin single-injection method. Thirty healthy young adult cats were used to establish normal values. The procedure was also used in 23 cats that were either older than 10 years or had borderline serum creatinine levels. The total clearance was calculated from the decay of the serum inulin concentration after injection of 3000 mg/m^2 body surface area using a two-compartment model. Concomitant inulin and iohexol clearance in nine cats showed excellent correlation between the two methods. Calculated normal values for GFR in 30 healthy cats were 35.9–58.5 (median 46.0) ml/min/m^2 or 2.07–3.69 (median 2.72) ml/min/kg. A few cats with normal creatinine or blood urea nitrogen levels were detected as having reduced GFR and therefore being in a state of early renal dysfunction. The study indicates that single-injection inulin clearance is a valuable tool for routine GFR measurement in cats. An 'inulin excretion test' using only one blood sample 3 h after the administration of 3000 mg/m^2 body surface area could prove an attractive alternative for the assessment of renal function in daily practice.

Introduction

Renal disease is a very common disorder in cats, especially in older cats. Glomerular filtration rate (GFR) is considered to be the best single parameter for assessing overall renal function, because it is directly related to functional renal mass. The commonly used screening tests for estimating GFR are blood urea nitrogen (BUN) and creatinine levels. Unfortunately, they provide only a crude estimate of GFR as their levels only start to rise when 75% of the nephrons are not functioning anymore (DiBartola 2000). To detect early renal dysfunction, a more sensitive test is required. Several methods for the determination of GFR in cats have been investigated (Krawiec 1994). Most of them require quantitative urine sampling (by means of a metabolic cage or repetitive bladder catheterization) or the use of radioactive labelled markers, both markedly limiting their use in routine practice. Determination of inulin disappearance from serum or plasma after a single injection is an attractive alternative as there is no need for quantitative urine sampling or the use of radioactive compounds. The method has been described for the measurement of GFR in humans (Florijn et al 1994), for routine measurement of GFR in dogs (Haller et al 1998) and in experimental settings in cats (Brown et al 1996b, Miyamoto 1998, Miyamoto, 2001). The first goal of this study was the evaluation of single-injection inulin clearance for the routine measurement of GFR in healthy cats and in cats with naturally occurring reduced renal function. The second goal was to determine the usefulness of the serum inulin concentration 3 h after the administration of 3000 mg/m^2 body surface area as an indicator of renal function in a sort of 'inulin excretion test'.

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In the literature, there is some discussion about the point, in progressing renal disease, at which cats lose their ability to concentrate urine (Elliott and Barber 1998, Ross and Finco 1981). As we expected to see cats in a state of very early renal disease, we also evaluated urine concentration ability in those cats.

Material and methods

Cats

On 30 healthy young adult cats (group A) we performed the single-injection inulin clearance to establish normal values for this method. Cats were included in this group according to the following criteria: age between 1 and 6 years and no signs of illness based on physical examination, serum biochemistry and urinalysis. Eighteen cats of this group were males and 12 females. Bodyweight ranged from 2.6 to 6.5 kg (mean 4.63 kg).

Group B consisted of 23 cats of which at least some of the cats were expected to be in early renal dysfunction. In this group, 11 cats were older than 10 years but had normal serum creatinine levels (reference range for our laboratory <158 µmol/l), and 12 cats had elevated serum creatinine levels. Fourteen of these cats were males and nine females. All these cats were clinically healthy and were presented for routine blood checks and/or dental procedures. Cats with clinical or laboratory signs of hyperthyroidism were not included in this group.

In a total of nine cats (three healthy cats from group A and six cats from group B) concomitant single-injection inulin clearance and iohexol clearance were performed to compare our method with an established method for measuring GFR in cats.

Inulin clearance

Prior to the clearance determination, food was withheld for at least 12 h but free access to water was given. None of the cats showed any sign of dehydration, and none was receiving any fluids prior to the procedure. A urine sample was obtained by cystocenthesis to evaluate urine specific gravity using a hand refractometer. A single bolus of 3000 mg/m^2 body surface area of a 25% solution of inulin (Inutest®, Fresenius-Kabi, Linz, Austria) was administered in the left antebrachial vein using an indwelling catheter, and the catheter was flushed with sterile saline. Blood samples (volume 1.5 ml) were obtained before the injection and 3, 10, 20, 40, 80, 120 and 180 min after injection from the right antebrachial vein or a jugular vein. Samples were allowed to clot, centrifuged, and the serum samples (volume at least 0.5 ml) were sent by normal mail to the laboratory where they were frozen until analysis.

Laboratory analysis

All laboratory analyses were performed in the laboratory ALOMED, D-78315 Radolfzell-Böhringen, Germany, in two steps. Firstly, inulin was split into its monomer d-fructose by acid hydrolysis. Secondly, d-fructose was determined by means of a modified enzymatic assay (Beutler 1984) using a commercially available testkit (Testkit d-glucose/d-fructose, Nr. 139106, Boehringer Mannheim, Germany). With this method a minimum of 100 µl serum or plasma is required for each determination. As inulin is not a homogenous substance, its concentration is expressed in micrograms per millilitre.

Clearance determination

GFR was calculated from the decay of serum inulin concentration with the aid of the computer program Inusoft® (Fresenius-Kabi, Linz, Austria) based on a previous study by Estelberger et al (1995). This program is especially designed for GFR determinations. All calculations were based on a two-compartment model as this model yielded an excellent description of the concentration–time curve of inulin.

Concomitant iohexol clearance

In a total of nine cats (three healthy cats and six cats from group B), concomitant iohexol clearance and inulin clearance were performed. Iohexol (Omnipaque®300, Schering, Germany) was administered at a dosage of 300 mg/kg intravenously immediately after the inulin injection. Iohexol concentrations were determined from the same blood samples as those used for inulin determination.

Determination of iohexol was performed by the Department of Pharmacokinetics and Drug Metabolism, Byk Gulden Konstanz. For the determination of iohexol serum concentrations, serum samples were diluted 1:1000 with an ammonium acetate buffer (50 mM, pH=6.7). For reversed phase HPLC separation, a phenomenex Aqua C18

(50 mm×2 mm, 5 µm particles) column was used and the following gradient was applied:

0 min: 0% Solvent B;
1 min: 0% Solvent B;
3.20 min: 70% Solvent B;
3.50 min: 100% Solvent B;
4.50 min: 100% Solvent B.

Solvent A was 5 mM ammonium acetate in water, pH=5, solvent B was 5 mM ammonium acetate in a mixture of 10% water and 90% acetonitril. The mass spectrometer (Applied Biosystems, Sciex API 3000) was coupled online to the HPLC system (Agilent, 1100) by using the heated nebulizer source. For iohexol, the SRM transition (SRM, single reaction monitoring) from the parent mass of iohexol ($[M+H]^+$=803.1 m/z) to the dominant production at a mass to charge ratio of 622.0 was detected.

As no appropriate internal standard was available for iohexol, quantitation was performed without internal standardization. Two calibration curves in the concentration range from 1 to 10 µg/ml as well as three sets of quality control samples at different concentration levels (1, 5 and 10 µg/ml) were prepared by spiking known amounts of analyte in ammonium acetate buffer (50 mM ammonium acetate in water, pH=6.7). One set of calibration standards were analysed at the beginning of the sample sequence, the second were analysed at the end. Quality control samples (in duplicate) were analysed at the beginning, the middle and the end of the sample sequence. Assay performance was demonstrated by the back-calculated concentrations for the calibration standards and by the concentrations determined for the quality control samples. The accuracy of the back-calculated concentrations found for the calibration standard ranged between −9.3 and +12.9%, the accuracy of the quality control samples ranged between −8.0 and +9.2%. A linear relationship between the concentration and the signal for the analyte was found; the calibration curve was described by the equation $y=19x-1.11\,e^4$ and the correlation coefficient (r) was 0.9977.

Clearance of iohexol was calculated as described above for inulin clearance.

Statistical analysis

Normal values of clearance and inulin concentration 3 h after the administration of 3000 mg/m^2 body surface area (results of group A) are expressed as 5 and 95 percentiles. The linear relationship between inulin clearance and iohexol clearance was evaluated using Pearson product-moment correlation. Sensitivity, specificity and diagnostic safety of the inulin excretion test and of serum creatinine levels for the detection of reduced renal function are calculated from the results of group B according to the following definitions:

$$\text{sensitivity}=\frac{n_{\text{true positive}}}{n_{\text{true positive}}+n_{\text{false negative}}}$$

$$\text{specificity}=\frac{n_{\text{true negative}}}{n_{\text{true negative}}+n_{\text{false positive}}}$$

$$\text{diagnostic safety}=\frac{n_{\text{true positive}}+n_{\text{true negative}}}{n_{\text{total}}}$$

Break points were defined as 5th and 95th percentiles of the control group (group A) for GFR values and as 5th and 95th percentiles of the control group (group A) for inulin concentration at 180 min.

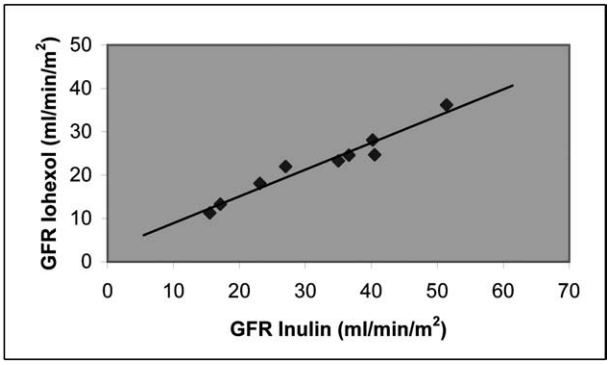

Fig 1. Relationship between single-injection inulin clearance and iohexol clearance for simultaneous measurements in nine cats (r=0.9745).

Results

Side effects were not seen in any of the cats receiving inulin alone, but three cats undergoing concomitant inulin and iohexol clearance vomited once immediately after the iohexol administration. Calculated normal values for inulin clearance in 30 healthy cats were 35.9–58.5 (median 46.0) ml/min/m^2 or 2.07–3.69 (median 2.72) ml/min/kg. An excellent correlation (r=0.9745) was found in the nine cats undergoing concomitant inulin and iohexol clearance using the two different methods (Fig 1). Eleven of the 23

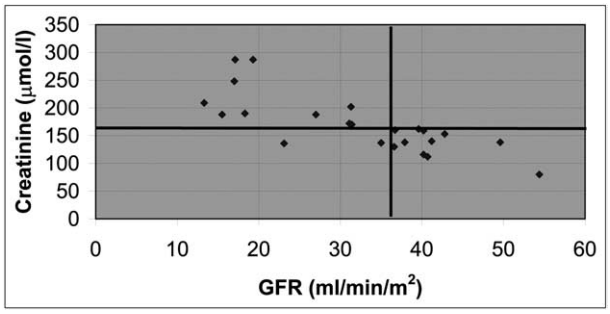

Fig 2. Relationship between serum creatinine level and GFR in 23 cats of group B. Vertical line indicates lower limit of GFR normal values. Horizontal line indicates upper limit of serum creatinine normal values.

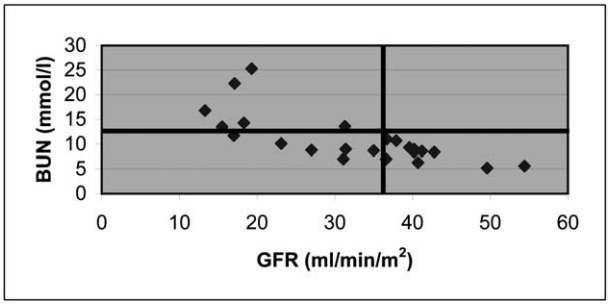

Fig 3. Relationship between serum BUN level and GFR in 23 cats of group B. Vertical line indicates lower limit of GFR normal values. Horizontal line indicates upper limit of serum BUN normal values.

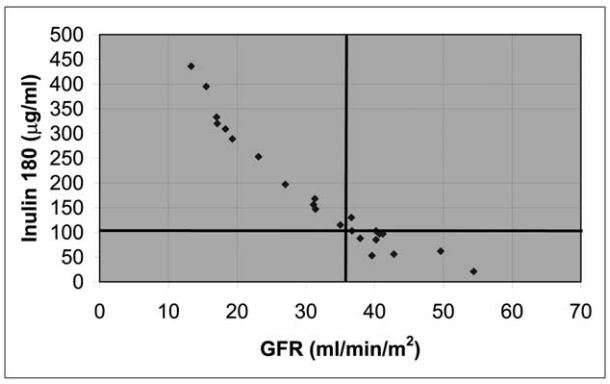

Fig 4. Relationship between serum inulin level after 180 min and GFR in 23 cats of group B. Vertical line indicates lower limit of GFR normal values. Horizontal line indicates upper limit of serum inulin normal values after 180 min.

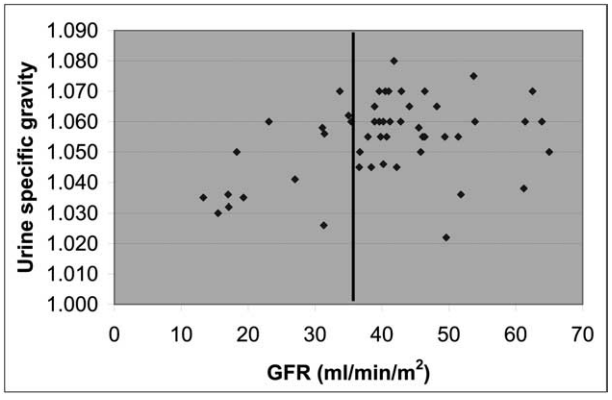

Fig 5. Relationship between urine specific gravity and GFR in all cats. Vertical line indicates lower limit of GFR normal values.

cats of group B showed GFR in the normal range. Figure 2 shows the relationship between creatinine and GFR. From the 12 cats with reduced GFR, two would have been classified as normal based on creatinine values alone. Cats with creatinine levels from 188 to 202 µmol/l showed calculated GFR values from 15.5 to 31.3 ml/min/m^2 (0.92–1.86 ml/min/kg). The relationship between BUN and GFR is shown in Fig 3. Six of the 12 cats with reduced GFR would have been classified as normal based on BUN values alone. Inulin concentrations after 180 min in healthy cats were 26–103 (median 59) µg/ml (5th to 95th percentile). The relationship between GFR and inulin concentration after 180 min is shown in Fig 4. Comparison of calculated GFR and inulin levels after 180 min showed agreement in 22 of 23 cats classified as having normal or reduced renal function. One cat had a calculated GFR in the low normal range and an inulin concentration after 180 min slightly above the normal range and was therefore classified as false positive. Calculated sensitivity, specificity and diagnostic safety for detecting reduced renal function with this inulin excretion test were 100, 91 and 96%, respectively. For serum creatinine levels, sensitivity was 83%, specificity 100% and diagnostic safety 91% for detecting reduced renal function.

The relationship between urine specific gravity and GFR in all cats is shown in Fig 5, and that between urine specific gravity and creatinine in Fig 6. Several cats with markedly reduced GFR produced urine with a specific gravity of 1.050–1.060, but cats with a GFR lower than 18 ml/min/m^2 or a creatinine higher than 200 µmol/l did not produce urine with a specific gravity higher than 1.035.

Discussion

Assessment of renal function in clinical practice is based on the determination of BUN and

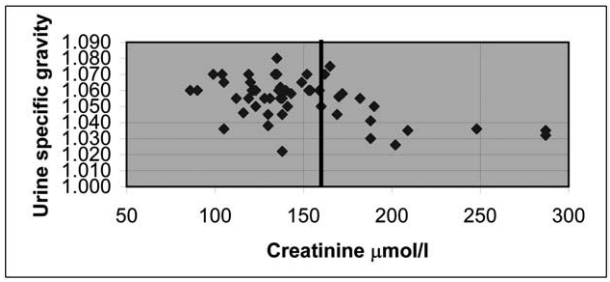

Fig 6. Relationship between urine specific gravity and serum creatinine level in all cats. Vertical line indicates upper limit of serum creatinine normal values.

creatinine levels. Unfortunately, they are not sensitive in early renal dysfunction as they are not able to detect renal disease until 75% of renal function is lost (DiBartola 2000). Furthermore, both are influenced by extrarenal factors. More precise methods to evaluate GFR are needed for the detection of early renal disease. This question will gain importance with the introduction of new therapeutic regimens in renal disease such as treatment with the angiotensin converting enzyme inhibitor benazepril. Most of the methods for GFR determination described in the literature require quantitative urine sampling (by means of a metabolic cage or by repetitive bladder catheterization) or the use of radioactive compounds (Krawiec 1994). Neither of these procedures can be used on a routine basis in clinical practice due to their expense in time and cost, their risk of bladder infection or their need for special devices. These were also the reasons for choosing iohexol clearance as the reference procedure in our study with clinically healthy, client owned cats. Iohexol clearance is also a single-injection procedure, which provides a reliable estimate of GFR in cats (Brown et al 1996a). An excellent correlation was found in our study between single-injection inulin clearance and single-injection iohexol clearance. Single-injection inulin clearance was compared with other procedures in a previous study and was found to provide a reliable estimate of GFR in cats (Brown et al 1996b), but the validity of this test was questioned in another study due to poor correlation to exogenous creatinine clearance (Rogers et al 1991). One explanation of this discrepancy could be the fact that the study by Brown et al (1996b) as well as our study included healthy and renal impaired cats with a broad range of GFR, whereas Rogers et al (1991) only determined GFR in healthy cats.

Normal values established in our study are considerably lower than the values obtained for dogs using the same method (Haller et al 1998). They compare nicely with previously reported values for GFR determination in cats (Krawiec 1994) although some precautions must be taken when comparing values obtained by different methods. Furthermore, most of the previous studies established their normal values using a considerably smaller number of cats than those used in our study.

GFR determination in old cats and in cats with elevated creatinine concentration revealed, as expected, some cats with slightly reduced renal function. Comparison with creatinine values revealed two old cats of the 12 cats with reduced GFR which would have been classified as normal based on creatinine values alone, thus emphasizing the need for more sensitive methods than the determination of creatinine levels to detect early renal dysfunction (Fig 2). Determination of BUN levels appears to be an even less sensitive method in early renal dysfunction as six of 12 cats with reduced GFR had BUN levels in the normal range (Fig 3). Comparison of GFR and creatinine levels also shows that large differences in GFR can be found in cats with small differences in creatinine levels early in the course of renal disease (Fig 2). This phenomenon is well known in the veterinary literature where the relationship of serum creatinine to GFR is described as a rectangular hyperbola (DiBartola 2000).

Although single-injection inulin clearance is an easy, safe and simple procedure, obtaining eight blood samples at fixed time points might be too time consuming to be used in daily routine practice. Furthermore, the determination of inulin levels in seven samples is more expensive than in just one sample. Therefore, we also evaluated the use of a single determination of inulin levels 3 h after the administration of 3000 mg/m^2 body surface area in a kind of inulin excretion test to detect early renal dysfunction. For this reason, we also based the inulin dosage on body surface area to obtain as accurate a dose as possible in each animal. This would not have been essential for GFR determination alone as long as the exact dosage in every single cat was recorded. As only one cat had to be classified as false positive, this inulin excretion test showed excellent sensitivity and good specificity in detecting early renal dysfunction (Fig 4). The two cats with normal creatinine and reduced GFR were also detected

with early renal dysfunction using this test. This results in a higher sensitivity of 100% compared with a sensitivity of 83% for creatinine. We conclude that determination of serum inulin level 180 min after the intravenous administration of a single bolus of 3000 mg/m^2 body surface area of inulin (Inutest®, Fresenius-Kabi, Linz, Austria) is a simple, easy and cheap method of obtaining more information about renal function. It is especially indicated in cases with suspected early renal dysfunction, borderline creatinine levels and/or discrepancy between BUN and creatinine levels. In spite of the fact that creatinine is a breakdown product of phosphocreatine in muscle, and daily production is determined by the muscle mass of the individual (DiBartola 2000), most commercial laboratories do not provide normal values for creatinine corrected for muscle mass. Creatinine levels in small cats might therefore be in the normal range in spite of a reduction in GFR. This was found to be the case in two cats in our study. On the other hand, the test can also be used to verify elevated creatinine levels in large cats.

Based on the studies on partially nephrectomized cats it has been concluded that cats might become azotemic before they lose their ability to concentrate urine (Ross and Finco 1981). This conclusion was questioned in naturally occurring renal disease where a study with a large number of cats suffering from renal insufficiency revealed that most of the cats were not able to concentrate urine to a specific gravity of more than 1.035 (Elliott and Barber 1998). Some cats in our study showed normally concentrated urine despite considerably reduced GFR, but none of them had a creatinine level higher than 200 µmol/l. Based on our data from this small number of cats, we conclude that urine concentration ability in cats is maintained at a higher degree of renal damage than in dogs, as has been shown in a study with partially nephrectomized cats (Ross and Finco 1981). But as indicated by comparison of urine specific gravity and creatinine levels, cats with naturally occurring renal disease lose their urine concentration ability soon after azotemia has started. Elliott and Barber (1998) used in their study a creatinine level above 180 µmol/l as inclusion criteria. Based on our data, this creatinine level reflects an already considerable reduction in GFR and therefore the finding that cats had lost their ability to concentrate urine is not surprising.

Conclusions

Single-injection inulin clearance is a simple method for detecting early renal dysfunction. It is useful in the approach to determine borderline serum creatinine levels in a cat. Determination of serum creatinine and/or BUN levels correlate poorly with GFR in early renal dysfunction. An inulin excretion test using a single blood sample 3 h after the administration of 3000 mg/m^2 body surface area could prove to be a simple and easy test to evaluate early renal dysfunction. Cats with naturally reduced renal function may retain substantial urine concentrating ability despite substantial reductions in GFR.

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