Effect of Experimental Hypothyroidism on Glomerular Filtration Rate and Plasma Creatinine Concentration in Dogs

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Background: Hypothyroidism affects renal function in a manner opposite the effects of hyperthyroidism.

Objective: To evaluate the effects of experimentally induced hypothyroidism on glomerular filtration rate (GFR) and basal plasma creatinine concentration in dogs.

Animals: Sixteen anestrous, female dogs.

Methods: Hypothyroidism was induced by administration of $^{131}$I in 8 dogs, and 8 healthy euthyroid dogs acted as controls. Exogenous plasma creatinine clearance (an estimate of GFR) was measured in all dogs before (control period) and 43–50 weeks after induction of hypothyroidism (posttreatment period). Other pharmacokinetic parameters of creatinine were also determined.

Results: No significant difference was observed for basal plasma creatinine concentration and creatinine clearance between control and hypothyroid dogs in the control period. In the posttreatment period, mean ± SD creatinine clearance in the hypothyroid group (2.13 ± 0.48 mL/min/kg) was lower ($P < .001$) than that of the control group (3.20 ± 0.42 mL/kg/min). Nevertheless, basal plasma creatinine concentrations were not significantly different between the hypothyroid and control groups (0.74 ± 0.18 versus 0.70 ± 0.08 mg/dL, respectively) because endogenous production of creatinine was decreased in hypothyroid dogs (22 ± 3 versus 32 ± 5 mg/kg/d, $P = .001$).

Conclusion and Clinical Importance: Hypothyroidism causes a substantial decrease in GFR without altering plasma creatinine concentrations, indicating that GFR evaluation is needed to identify renal dysfunction in such patients.

Key words: Chronic kidney disease; Exogenous creatinine clearance; Thyroid.

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Abnormal thyroid states affect renal function, a phenomenon noted most often in hyperthyroid cats. Glomerular filtration rate (GFR) decreases markedly in hyperthyroid cats after treatment, reflecting in large part decreased renal blood flow after resolution of the hyperdynamic state of the cardiovascular system in this disease.1,2 Hypothyroidism also affects renal function, albeit more subtly than hyperthyroidism. Hypothyroidism in humans is associated with decreased GFR, impaired water excretion, increased urinary sodium loss, and hyponatremia. Serum creatinine concentration frequently is increased in hypothyroid humans, returning to concentrations within the reference range after treatment in most cases.3–5 Dogs with hypothyroidism have been reported to have creatinine concentrations that are significantly higher than euthyroid dogs suspected of hypothyroidism, although an abnormally increased serum creatinine concentration appears to be uncommon.6 Although a relationship between hypothyroidism and chronic kidney disease has not been reported in the dog, a decrease in GFR could affect the diagnosis of intrinsic renal failure, susceptibility of the kidneys to hypotension, or metabolism of drugs.3,7 The purpose of this study was to evaluate the effect of hypothyroidism on plasma creatinine concentration and creatinine clearance in dogs using a model of experimentally induced hypothyroidism.

Materials and Methods

Animals

Sixteen healthy anestrous bitches aged 26–39 months and weighing 8.4–11.6 kg were studied. All bitches were determined to be healthy based on lack of significant abnormalities on physical examination, CBC, serum biochemistry (blood urea nitrogen, creatinine, glucose, phosphorus, calcium, total protein, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, bilirubin, cholesterol, sodium, potassium, chloride), urinalysis, Dirofilaria antigen test, and zinc sulfate fecal floatation. Serum concentrations of total thyroxine (T4), free T4 by equilibrium dialysis, and endogenous canine thyroid stimulating hormone (TSH) were within respective reference ranges. Dogs were housed in indoor runs at 21 °C, with a 12-hour light:dark cycle. Dogs were fed a commercial maintenance diet and offered water ad libitum. During the acclimation period, body weight was measured weekly and the amount of food offered to each dog was adjusted to maintain a

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stable body weight for that individual. This volume of food was fed to each dog for the duration of the study. The study was approved by the Virginia Tech Animal Care and Use Committee.

**Experimental Design**

The dogs were randomly divided into 2 groups of 8 dogs. In the dogs of the first group, hypothyroidism was induced by administration of $^{131}$I. The remaining 8 untreated dogs acted as controls. Exogenous plasma creatinine clearance tests were performed in all dogs before induction of hypothyroidism (pretreatment period) and again 43–50 weeks after $^{131}$I administration (posttreatment period).

**Induction of Hypothyroidism**

Hypothyroidism was induced in 8 randomly selected bitches after 12–18 weeks of acclimation and data collection by IV administration of 1 mCi/kg of $^{131}$I. Hypothyroidism was confirmed 9 weeks and 38–45 weeks after $^{131}$I administration by finding serum T4 concentrations of <10 nmol/L both before and 4 hours after administration of 50 μg human recombinant TSH. This model of experimental hypothyroidism has been used previously to successfully induce hypothyroidism. Serum T4 concentrations were measured by a previously validated assay.

**Plasma Exogenous Creatinine Clearance Test**

Dogs were fasted for 12 hours before obtaining blood samples, and fed 10 hours after sample collection. Water was not restricted before the study, and was offered every 2 hours during the study until 10 hours, and then given free choice. A catheter was placed in a jugular vein. Blood samples were obtained by first withdrawing 2 mL of blood into a syringe containing 1 mL of heparinized saline (5 U/mL), withdrawing 2 mL blood for analysis, and then injecting the initial blood and saline into catheter followed by 1 mL of heparinized saline. Blood samples were placed in lithium heparin tubes and submitted for analysis of creatinine or centrifuged within 10 minutes of collection at 3500 $g$ for 10 minutes and stored at 4°C until analyzed within 16 hours of collection. Plasma creatinine concentration was measured using a kinetic modification of the Jaffe procedure on an automated chemistry analyzer. Creatinine was added to sterile water to make a solution of 80 mg/mL. The creatinine solution was passed through a 0.2-μm filter before administration. After obtaining the baseline blood sample, creatinine was administered IV into the right cephalic vein at a dosage of 40 mg/kg. Blood samples then were collected at 0, 2, 5, 10, 20, and 30 minutes and 1, 1.5, 2, 3.5, 6, 10, and 24 hours after creatinine administration.

Pharmacokinetic analysis was performed using WinNonlin, a specific pharmacokinetic software by a noncompartmental approach as previously validated in dogs. Briefly, the area under the plasma creatinine concentration versus time curve (AUC) was determined by the trapezoidal rule with extrapolation to infinity. The plasma clearance of creatinine was determined by dividing the IV dose of creatinine by the AUC. Steady-state volume of distribution ($V_{ss}$) and mean residence time (MRT) were calculated by standard pharmacokinetic equations. The endogenous daily production rate of creatinine ($Q$) was estimated by multiplying the area under the basal creatinine over 24 hours by the plasma clearance of exogenous creatinine, as published previously. The area under the basal creatinine over 24 hours was calculated by multiplying the basal plasma creatinine concentration by 24 hours.

**Statistical Analysis**

All data sets were found to be normally distributed by the Shapiro-Wilk test. All measurements were compared between the control and hypothyroid groups as well as within groups between times by use of analysis of variance using the general linear model procedure. All data are expressed as mean ± standard deviation. A $P$ value <0.05 was considered to be significant.

**Results**

All hypothyroid dogs showed clinical signs of hypothyroidism including weight gain, thin hair coat or alopecia, and lethargy. Hypothyroid dogs gained weight (pretreatment 9.8 ± 0.77 versus posttreatment 11.5 ± 0.36 kg; $P < 0.001$) whereas control dogs did not (9.7 ± 1.18 versus 9.8 ± 1.24 kg; $P = 0.46$). Serum T4 concentrations before and after TSH administration were <5 nmol/L in all hypothyroid dogs. In control dogs, all post-TSH serum T4 concentrations were >35 nmol/L. The mean ± SD serum T4 concentration before and after TSH in control dogs was 26 ± 7 and 57 ± 18 nmol/L, respectively.

The plasma exogenous creatinine clearance test was well tolerated in all dogs at both time periods. The plasma profiles of creatinine are shown in Figure 1. The extrapolated part of the AUC did not represent >21% of the total AUC in all dogs and was <11% in
28/32 kinetic studies. Because the plasma creatinine concentration had returned to baseline at 24 hours in most dogs, calculations were made using only data between 0 and 10 hours. The pharmacokinetic parameters of creatinine for control and hypothyroid dogs in the pre- and posttreatment periods are given in Table 1. No effect of the group was observed for basal plasma creatinine concentration and $V_{ss}$ regardless of the period. The plasma clearance of creatinine (mL/min/kg) was not significantly different between the 2 groups in the pretreatment period but was less ($P < .0001$) in the hypothyroid group in the posttreatment period compared with the posttreatment results in the control group. No dog in either group before radiiodine treatment or any dog in the control group demonstrated a change in creatinine clearance within the same period compared with the control group at the same time. The endogenous daily production of creatinine was decreased ($P = .0004$) and the MRT was increased ($P < .0001$) in hypothyroid dogs in the posttreatment period when compared with control dogs.

When within-group comparisons were made, the clearance of creatinine (mL/min/kg) was lower at the posttreatment period than in the pretreatment period in hypothyroid dogs ($P < .0001$), but not in the control group ($P = .54$). When creatinine clearance was expressed as mL/min, creatinine clearance was less in the posttreatment period in the hypothyroid group ($P = .007$), but not in the control group ($P = .53$). MRT increased in the hypothyroid group after induction of hypothyroidism ($P < .0001$) but not in control dogs ($P = .85$). $Q$ decreased in hypothyroid dogs ($P < .0001$) but not control dogs ($P = .244$). No significant effect was detected for $V_{ss}$ and basal plasma creatinine concentration.

### Table 1. Pharmacokinetic parameters of creatinine determined after IV bolus of exogenous creatinine before (pretreatment period) and after (posttreatment period) induction of hypothyroidism in 8 dogs and in 8 euthyroid control dogs.

<table>
<thead>
<tr>
<th>Study period</th>
<th>Group</th>
<th>Basal Creatinine (mg/dL)</th>
<th>Cl (mL/min/kg)</th>
<th>Cl (mL/min)</th>
<th>$V_{ss}$ (mL/kg)</th>
<th>MRT (min)</th>
<th>$Q$ (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>Control</td>
<td>0.76 ± 0.15</td>
<td>3.33 ± 0.31</td>
<td>32.3 ± 4.3</td>
<td>615 ± 66</td>
<td>187 ± 33</td>
<td>37 ± 9</td>
</tr>
<tr>
<td></td>
<td>Hypothyroid</td>
<td>0.71 ± 0.08</td>
<td>3.20 ± 0.40a</td>
<td>31 ± 3.5a</td>
<td>625 ± 63</td>
<td>198 ± 26a</td>
<td>33 ± 4a</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>Control</td>
<td>0.70 ± 0.08</td>
<td>3.20 ± 0.42b</td>
<td>31.3 ± 3.3b</td>
<td>606 ± 32</td>
<td>191 ± 24b</td>
<td>32 ± 5b</td>
</tr>
<tr>
<td></td>
<td>Hypothyroid</td>
<td>0.74 ± 0.18</td>
<td>2.13 ± 0.48a,b</td>
<td>24.4 ± 5.1a,b</td>
<td>576 ± 52</td>
<td>284 ± 68a,b</td>
<td>22 ± 3a,b</td>
</tr>
</tbody>
</table>

See the text for $P$ values.

Measurements derived from the pharmacokinetic analysis of the plasma exogenous creatinine clearance test. The control group consisted of 8 euthyroid dogs while the hypothyroid group consisted of 8 initially euthyroid dogs made hypothyroid by administration of $^{131}$I. Time period 1 was before $^{131}$I administration while time period 2 was 43–50 weeks after $^{131}$I administration.

$^a$Significant difference within a group.

$^b$Significant difference between groups.

Basal creatinine, basal plasma creatinine; Cl, plasma exogenous creatinine clearance; $V_{ss}$, steady-state volume of distribution; MRT, mean residence time; $Q$, daily endogenous creatinine production.

### Discussion

The major findings of this study are that hypothyroidism induced a decrease in plasma exogenous creatinine clearance and endogenous creatinine production without a significant change in plasma creatinine concentration. The decrease in GFR by 33% in hypothyroid dogs of the present study was substantial, and 3/8 dogs had a GFR $< 2$ mL/min/kg, a value previously proposed as the lower limit of normal. A similar decrease in GFR has been found in hypothyroid humans and rats. Alteration of cardiovascular hemodynamics and myocardial function are responsible for much of the alteration in renal function in hypothyroidism. Decreased cardiac output occurs as a result of decreases in heart rate, blood volume, and myocardial contractility. Decreased myocardial contractility and cardiac output have been documented in hypothyroid dogs.

Another factor contributing to the decrease in renal blood flow in hypothyroidism is an increase in systemic vascular resistance that accompanies the disease. Increased vascular resistance in glomerular afferent and efferent arterioles in hypothyroid rats, associated with a decrease in single nephron and whole kidney GFR, is thought to occur in a large part as a result of increased sensitivity to angiotensin II and decreased response to nitric oxide (NO). That angiotensin II plays an important role in renal function is evidenced by the finding that administration of an angiotensin II antagonist reversed the hypothyroidism-induced arteriolar vasoconstriction and decrease in single nephron GFR in hypothyroid rat kidneys. However, other investigators have found no difference in the renal vasomotor response to angiotensin II in hypothyroidism. Plasma renin activity typically is not increased in hypothyroid humans or rats, therefore increased sensitivity to, rather than increased secretion of, angiotensin II appears central to the pathogenesis in these species. Because increased renin activity has been found in hypothyroid dogs, other factors also may play a role in the increased systemic resistance.
vascular resistance in dogs.\textsuperscript{20} Generation of NO from the endothelium and the vascular response to this important vasodilator are decreased in many tissues by the hypothyroid state.\textsuperscript{19} The decreased response to NO and acetylcholine in kidneys from hypothyroid animals appears to play a role in the decreased GFR of hypothyroidism.\textsuperscript{18,21} The ultimate effect of these factors on glomerular hemodynamics is a decrease in glomerular blood flow due to affenter and efferent arteriolar vasoconstriction and decreased glomerular filtration pressure.

The decrease in GFR in hypothyroid dogs in the current study was not associated with a concomitant increase in plasma creatinine concentration. A higher serum creatinine concentration, albeit slight, was found in dogs with naturally occurring hypothyroidism (1.17 mg/dL) compared with euthyroid dogs suspected of hypothyroidism (0.99 mg/dL).\textsuperscript{5} Mild increases in serum creatinine concentration are common in hypothyroid humans, occurring in 26–54% of affected individuals.\textsuperscript{3–5}

Discrepancies between inverse changes in GFR and plasma creatinine concentration result from the fact that the relationship between the 2 variables is curvilinear, and consequently in early renal dysfunction, a large change in GFR is associated with little or no change in plasma creatinine concentration.\textsuperscript{22} Plasma creatinine concentration is a hybrid parameter that depends on GFR as well as on the distribution of creatinine in the body and its endogenous production.\textsuperscript{10} In the present study, the values observed in the control conditions for $V_{ss}$, MRT, and $Q$ were similar to those published previously.\textsuperscript{10} $V_{ss}$ was not affected by hypothyroidism and MRT was increased because of the decrease in plasma creatinine clearance. More interestingly, $Q$ was decreased in hypothyroid dogs by approximately 33%. Consequently, a decrease in $Q$ may mitigate the effect of decreased GFR on plasma creatinine concentration, as observed previously in dogs with subclinical renal impairment.\textsuperscript{10} The effect of $Q$ on discrepancies between plasma creatinine concentration and GFR was also recently demonstrated in cats where, for example, a cat with a plasma creatinine of 2.5 mg/dL was shown to have a GFR similar to that of a cat with a plasma creatinine of 1.6 mg/dL.\textsuperscript{23} The cause of decreased $Q$ in hypothyroid dogs in the present study is unknown. However, because plasma creatinine is derived primarily from the skeletal muscle, it may result from a decrease in the muscle mass of hypothyroid dogs. The practical implication of this observation is that measurement of plasma creatinine concentration is not a sensitive approach for assessing renal function in dogs with hypothyroidism, and measurement of GFR is necessary to accurately determine renal function.

With the possible exception of immune-complex glomerulonephritis occurring rarely in dogs with lymphocytic thyroiditis,\textsuperscript{24} chronic kidney disease has not been reported in retrospective studies of canine hypothyroidism.\textsuperscript{6,25,26} However, the clinical relevance of the 30% decrease in GFR of hypothyroid dogs observed in the present study is unclear because GFR was not evaluated after reestablishing a euthyroid state with levothyroxine administration and the kidneys were not evaluated histologically. Adequate thyroid hormone replacement therapy would be expected to normalize GFR if the cause of renal impairment were functional, as has been recently reported in dogs with spontaneous hypothyroidism where GFR increased approximately 25% after levothyroxine administration.\textsuperscript{8} Whatever the mechanism of renal impairment, the detrimental effects of hypothyroidism on renal function would be particularly important in dogs with concurrent renal disease or abnormal cardiac function. Recently, GFR was shown to be <2 mL/min/kg in 7/9 dogs with NYHA class III or IV valvular heart disease and hypothyroidism would be expected to compound this abnormality.\textsuperscript{27} In addition, hypothyroid dogs might be more susceptible to the effects of anesthetic agents on renal perfusion and also to those of potentially nephrotoxic drugs. Impaired renal metabolism and excretion of some drugs (including digoxin, warfarin, phenytoin, and propranolol) may enhance the toxicity of these agents as has been documented in hypothyroid humans.\textsuperscript{28} However, unlike in humans, digoxin pharmacokinetics are not affected by hypothyroidism in dogs, so it is not possible to extrapolate studies of drug metabolism in humans to dogs.\textsuperscript{7}

The experimental model used in this study was chosen because it has been used successfully previously and allowed for a consistent degree of hypothyroidism and control of any factors that might influence thyroid or kidney function.\textsuperscript{8} Hypothyroidism was present for 10–11.5 months in this study, and all hypothyroid dogs had clinical signs at the time of testing. The group of dogs studied here likely would represent dogs severely affected with hypothyroidism because of the duration of the hormone deficiency and overt clinical signs. Moreover, this study was controlled by the use of a healthy control group under the same conditions as the hypothyroid group.

Plasma exogenous creatinine clearance, used here as an estimate of GFR, has been previously validated in healthy dogs and in those with impaired renal function.\textsuperscript{10} One of its distinct advantages compared with other GFR testing methods is that it also allows calculation of other pharmacokinetic parameters of creatinine. These measurements are relevant to understanding relationships between GFR, the best indicator of the overall renal function, and basal plasma creatinine concentration, the most frequently measured indirect marker of renal function in routine practice. Estimation of endogenous creatinine production may be useful for exploring this relationship, as noted in the present study where creatinine production rate was decreased in hypothyroid dogs. The rationale for the calculation of creatinine production is derived from the equation used for determination of the plasma clearance (Cl) (Cl= Dose/AUC [equation 1]). A given endogenous amount of creatinine enters the bloodstream daily and in part determines the basal plasma creatinine concentration. By equation 1, the daily endogenous amount or production is equal to the clearance multiplied by the area under the basal creatinine over 24 hours. The area under the basal creatinine over 24 hours is calculated by multiplying the basal plasma concentration by 24 hours because the basal plasma creatinine concentration is stable in dogs in fasted conditions.\textsuperscript{10} Creatinine clearance and production are 2
independent variables that control basal plasma creatinine. For example, in cats the endogenous production of creatinine can vary considerably despite similar values for creatinine clearance.23 The estimation of daily production of endogenous creatinine from plasma clearance and the plasma concentration of creatinine initially was proposed by Watson et al., but this pharmacokinetic approach also has been applied to estimation of the endogenous production rate of cortisol in horses during rest and exercise,29 and that of cortisol and ACTH in ewes in healthy and diseased conditions.30,31 It also has been used to quantify the amount of creatine kinase released from skeletal muscle as an indicator of muscle damage after exercise32 or IM administration of drugs.33,34 Further evaluation of this method for estimating endogenous production of various compounds will help define its clinical utility.

In conclusion, appropriate assessment of early renal dysfunction in hypothyroid dogs requires GFR testing because serum creatinine concentration alone is an insensitive measure. Endogenous production of creatinine needs to be considered when interpreting plasma creatinine concentration in dogs with hypothyroidism. Measurement of GFR should be considered in dogs with hypothyroidism and concurrent renal or cardiac dysfunction.

Footnotes

1 Hill’s Science Diet Adult dry Kibble, Topeka, KS
2 1-131 sodium, Cardinal Health, Charlottesville, VA
3 Thyrogen, Genzyme Corp, Framingham, MA
4 Coat-A-Count Canine T4 radioimmunoassay, Diagnostic Products Corporation, Los Angeles, CA
5 18 ga Venocath; Abbot Ireland, Sligo, Republic of Ireland
6 Monoject, Tyco Healthcare, Mansfield, MA
7 Olympus AU400, Olympus America Inc, Center Valley, PA
8 Anhydrus creatinine; Sigma-Aldrich Inc, St Louis, MO
9 WinNonLin Version 4.0.1, Pharsight, Mountain View, CA
10 SAS 9.1.3, SAS Institute, Cary, NC

Acknowledgment

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References