



# NEW INSIGHTS INTO DECLINING KIDNEY FUNCTION IN HYPERTHYROID CATS AFTER TREATMENT WITH $^{131}$I

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Thesis submitted in fulfillment of the requirements for the degree of doctor (Ph.D.) in Veterinary Sciences, Faculty of Veterinary Medicine, Ghent University, 2008

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New insights into declining kidney function in hyperthyroid cats after treatment with $^{131}$I.
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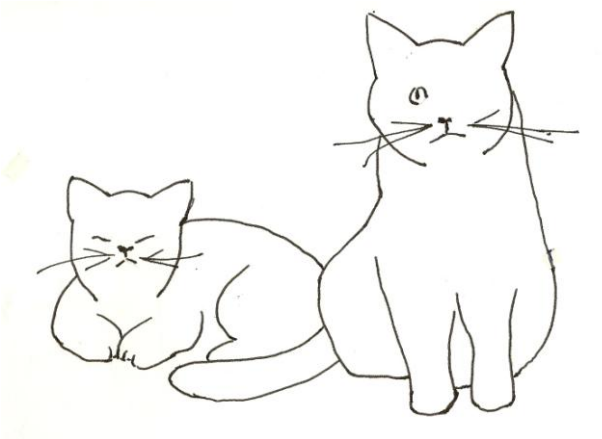
*“Far too noisy, my dear Mozart.  Far too many notes.”*

*Emperor Ferdinand of Austria commenting on "The Marriage of Figaro"*
*1786*



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**CONTENTS**

# LIST OF ABBREVIATIONS

| | |
|---|---|
| Ab | antibody |
| ALARA | as low as reasonably achievable |
| AUC | area under curve |
| BP | blood pressure |
| bTSH | bovine thyroid stimulating hormone |
| BUN | blood ureum nitrogen |
| BW | body weight |
| CKD | chronic kidney disease |
| ClC | chloride channel |
| CO | cardiac output |
| CRF | chronic renal failure |
| C-RBP | cellular retinol binding protein |
| CV | coefficient of variation |
| CysC | cystatin c |
| DIT | di-tyrosine |
| EDTA | ethylene diamine tetraacetic acid |
| ELISA | enzyme linked immunosorbent assay |
| FE | fractional excretion |
| fT4 | free thyroxine |
| GFR | glomerular filtration rate |
| HPLC | high performance liquid chromatography |
| HRP | horse radish peroxidase |
| $^{123}I$ | radio iodine 123 |
| $^{131}I$ | radio iodine 131 |
| IRIS | international renal interest society |
| IV | intravenous |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MBq | megabecquerel |
| MMI | methimazole |
| MD | maximum difference |
| MIT | mono-tyrosine |
| MW | molecular weight |
| NHE | $Na^+/H^+$ exchanger |
| NIS | $Na^+ I^-$ symporter |
| NO | nitric oxide |
| NOS | nitric oxide synthase |
| NTI | non- thyroidal illness |
| PECCT | plasma exogenous creatinine clearance test |
| PEC-ICT | plasma exogenous creatinine-iohexol clearance test |
| PenICT | plasma endo-iohexol clearance test |
| PexICT | plasma exo-iohexol clearance test |
| RAAS | renin-angiotensin-aldosterone system |
| RAIU | radio active iodine uptake |
| RBF | renal blood flow |
| RBP | retinol binding protein |
| RBP/c | retinol binding protein/creatinine ratio |
| rhTSH | recombinant human thyroid stimulating hormone |
| RV | residual variance |
| SC | subcutaneous |
| SD | standard deviation |
| SDS-PAGE | sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| T3 | L-tri-iodothyronine |
| T4 | thyroxine |
| TBS | tris buffered saline |
| TGB | thyroglobulin |
| $^{99m}TcO_4^-$ | technetium |
| TPO | thyroid peroxidase |
| TRH | thyrotropin releasing hormone |
| TSH | thyroid stimulating hormone |
| T/S uptake | thyroid/salivary gland uptake ratio |
| TT4 | total thyroxine |
| TTR | transthyretin |
| UPC | urinary protein/creatinine ratio |
| uRBP | urinary retinol binding protein |
| USG | urinary specific gravity |
| UV | ultraviolet |

# GENERAL INTRODUCTION

# GENERAL INTRODUCTION

There is a complex relationship between hyperthyroidism and kidney function in cats. Feline hyperthyroidism is currently the most diagnosed endocrine disorder in geriatric cats, with a prevalence of 2 % in cats older than 7 years.[1] Chronic kidney disease (CKD) affects almost 8 % of cats over 10 years of age, and this number is doubled in cats over 15 years of age.[2,3] It is therefore not unexpected to find concurrent CKD and hyperthyroidism in a geriatric cat, and indeed CKD is found in up to 40 % of hyperthyroid cats.[4] Further, a decline in kidney function has been reported after treatment of hyperthyroidism in cats.[4-8] This is a serious complication of treatment and early detection of CKD is essential. Indeed, a decrease in glomerular filtration rate (GFR) and an increase in serum creatinine concentration and blood urea nitrogen (BUN) has been reported already 6 days after treatment,[4] although long term effects have not been investigated.

There is a strong need to be able to predict which hyperthyroid cats will develop CKD after treatment, and to detect a declining kidney function early after treatment. An early detection of CKD is essential for an optimal management of these patients. Kidney damage consists of a cascade of events, and each step in the cascade leads to changes in urinary biomarkers of kidney function. These changes can represent disturbances in different functions as well as different locations of the kidney.

Thyroid physiology and feline hyperthyroidism are described in Chapter 1 sections 1.1-1.3. The linkage between thyroid and kidney function in general, in geriatric cats in particular and ways to evaluate kidney function in cats will be described in Chapter 1 sections 1.4 and 1.5.

GFR is an indication of glomerular function. GFR represents the magnitude of ultrafiltration of plasma in the first steps of urine formation and is therefore considered to be the best overall index of kidney function.[9] GFR can be measured directly using clearance of a filtration marker or indirectly and less sensitive by evaluating serum creatinine concentration.[10] Renal clearance of inulin is regarded the gold standard method, but is highly cumbersome, stressful and potentially harmful for the animal. Other methods using plasma clearance of different markers have been evaluated intensively in dogs and cats, although a feasible method suitable for practice has not yet been found. In Chapter 2, we will investigate possible easily applicable clearance measurement methods in cats.

Besides glomerular function, tubular function can also be affected in kidney disease. Moreover, hyperthyroidism has been described to have an influence on several parts of tubular function in humans, rodents and dogs.[11-19] Urinary concentration of the biomarker retinol binding protein (RBP) is a highly sensitive index of tubular damage in humans, because a minor decrease in tubular function may lead to excretion of RBP in urine.[20,21] Evaluation of urinary RBP as a putative marker of kidney dysfunction in cats will be investigated in Chapter 3.

Several studies have investigated follow up of kidney function after treatment of hyperthyroid cats, although these focused on the glomerular part of kidney function, and were performed over a short term period. [4-8] There is a need for long term assessment of glomerular as well as tubular function in hyperthyroid cats after treatment to gain insight into the pathogenesis of declining kidney function in these cats. In Chapter 4, we will perform a long term follow up study of glomerular as well as tubular kidney function in hyperthyroid cats after treatment with radioactive iodine ($^{131}$I). In this study, we will also evaluate differences between cats maintaining a healthy kidney function and cats developing post-treatment renal azotemia, as a first step towards prediction of CKD after treatment.

A diagnostic challenge can occur in cats with hyperthyroidism and CKD, but also in cats developing post-treatment renal azotemia combined with serum total T4 (TT4) below reference range. These diagnostic challenges will be described in Chapter 1 section 1.6. On one hand, iatrogenic hypothyroidism can occur in up to 30 % of cats treated with $^{131}$I [22] and hypothyroidism could contribute to a decline in kidney function. On the other hand, CKD is a non-thyroidal illness (NTI), which can suppress serum TT4 concentrations.[23] Thyroid function can be assessed with serum free T4 (fT4) after equilibrium dialysis, which has a low specificity,[24] or endogenous serum TSH concentration, however feline TSH measurement is not available for cats. Another possibility is stimulation with recombinant human TSH (rhTSH). The application of rhTSH in veterinary medicine will be described in Chapter 1 part 1.6.2. In Chapter 5, we will evaluate stimulation with rhTSH in cats as a tool in the diagnostic challenge represented by cats developing post-treatment renal azotemia and serum TT4 below reference range.

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# SCIENTIFIC AIMS

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The final aim of the thesis was to gain insights into declining kidney function of hyperthyroid cats treated with radioiodine. We evaluated several aspects which could lead to an improved understanding of kidney function in hyperthyroid cats, before as well as after treatment with $^{131}$I. This included evaluation of methods for measuring glomerular as well as tubular kidney function, the follow up of kidney function after treatment in hyperthyroid cats with possible prediction of development of post-treatment renal azotemia, and finally the diagnostic challenge which can occur after treatment in cats developing post-treatment renal azotemia and low serum TT4 concentration.

The scientific aims of the thesis are:

I. To compare the plasma clearance of exogenous creatinine, exo-iohexol and endo-iohexol for GFR measurement
    - in healthy cats (including reproducibility of plasma clearance),
    - in hyperthyroid cats before and after treatment with $^{131}$I to evaluate GFR over a period in which GFR is expected to change
    - in hyperthyroid cats, healthy cats and cats with CKD to evaluate the complete range of GFR values expected in cats

II. To evaluate the potential of urinary retinol binding protein (RBP) measurement as an early marker of tubular damage, by validation of urinary RBP measurements in hyperthyroid cats, healthy cats and cats with CKD

III. To assess the long term effects of $^{131}$I treatment on kidney function, and to investigate whether post-treatment GFR and the development of post-treatment renal azotemia can be predicted from variables measured before treatment

IV. To evaluate thyroid function with rhTSH stimulation followed by thyroid scintigraphy, in cats with post-treatment renal azotemia and serum TT4 below reference range

# SCIENTIFIC AIMS

# CHAPTER 1

# REVIEW OF THE LITERATURE

# CHAPTER 1

## 1.1. Thyroid physiology

The feline thyroid gland consists of two lobes located on the lateral surfaces of the trachea (Figure 1).

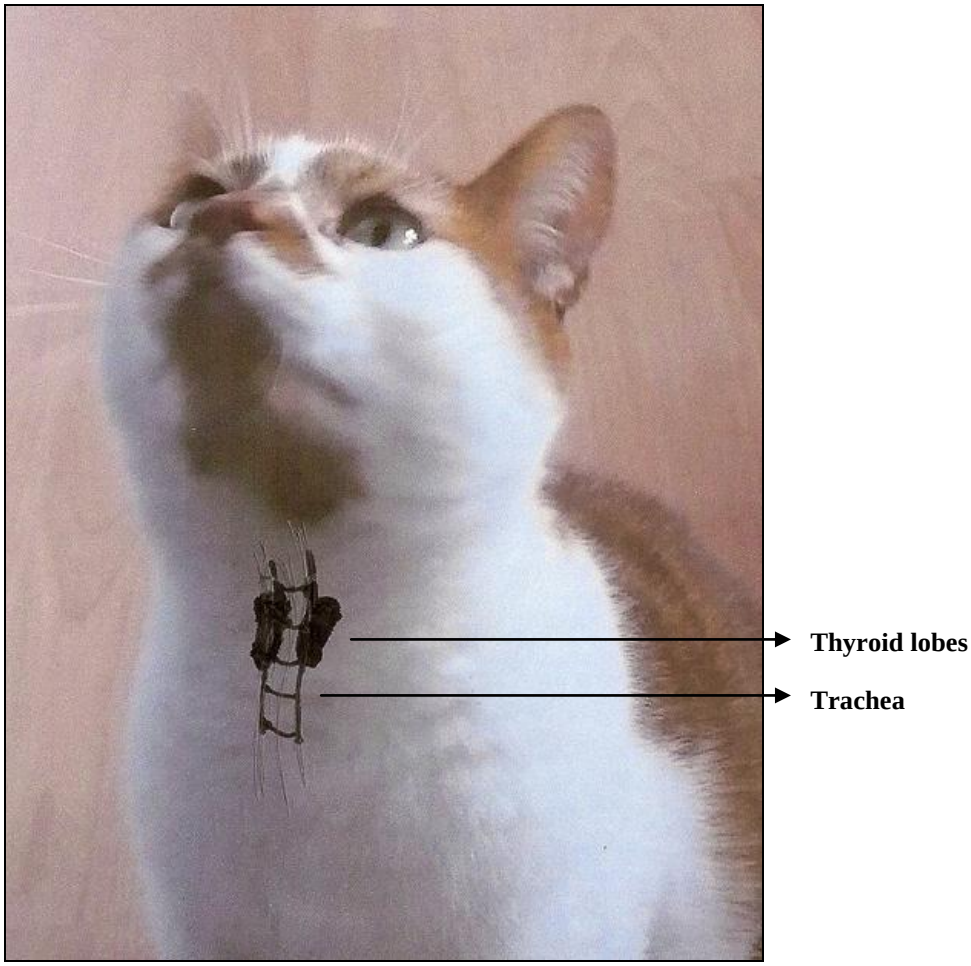


**Figure 1. Thyroid lobes located bilaterally of the trachea in the cat.**
**Picture: I. van Hoek.**

The histological structure of the gland consists of follicles that contain colloid produced by the follicular cells or thyrocytes. Thyroid hormones are synthesized in the thyrocytes and the final assembly occurs extracellular in the lumen of the follicle (Figure 2).

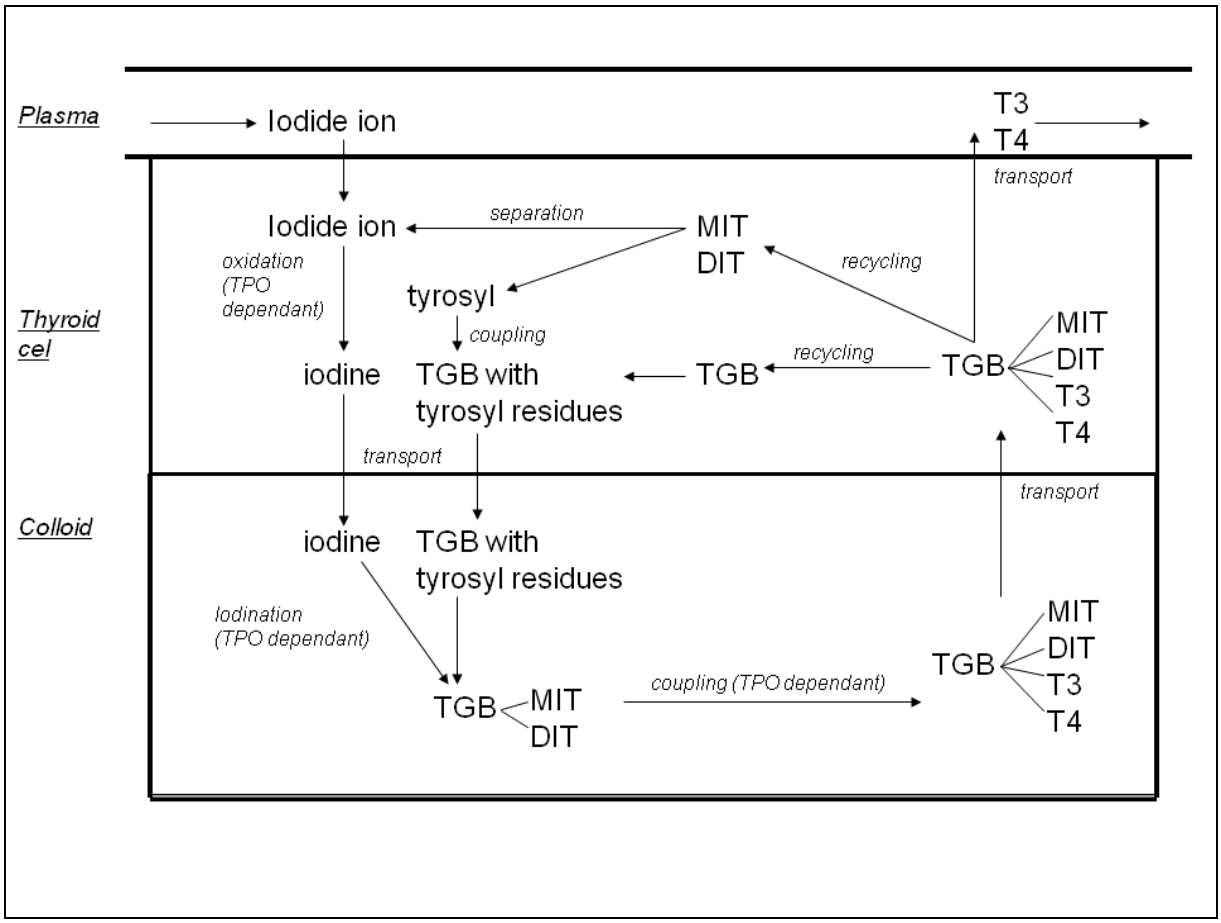


**Figure 2. Synthesis of thyroid hormones.**

**Adapted from: van Hoek et al.[1] TGB: Thyroglobulin, TPO: thyroid peroxidase, MIT: mono-iodotyrosine, DIT: di-iodotyrosine, T3: tri-iodothyronine, T4: thyroxine.**

First, Iodide ($I^-$) ions are actively trapped by follicular cells through the $Na^+/I^-$ Symporter (NIS) from the plasma and transported to the follicular lumen. The follicular lumen generally consists of a pool of glycoproteins called thyroglobulin (TGB) that carry tyrosyl residues. Thyroid peroxidase (TPO) catalyzes iodine to bind to the tyrosyl residues and form mono- and di-iodotyrosines (MIT and DIT respectively).[2] These are coupled together again under the influence of TPO to form T4 and T3, which are then secreted by the thyroid gland into the plasma. Both T4 and T3 are metabolically active, although T3 is much more potent than T4. All T4 is secreted by the thyroid gland, but a considerable amount of T3 is derived from extrathyroidal deiodination of T4. Therefore, T4 has been called a prohormone.[3,4]

**Thyroid stimulating hormone (TSH)**

Thyroid hormone synthesis is regulated by TSH in the thyroidal feedback axis. This axis is shown in Figure 3.

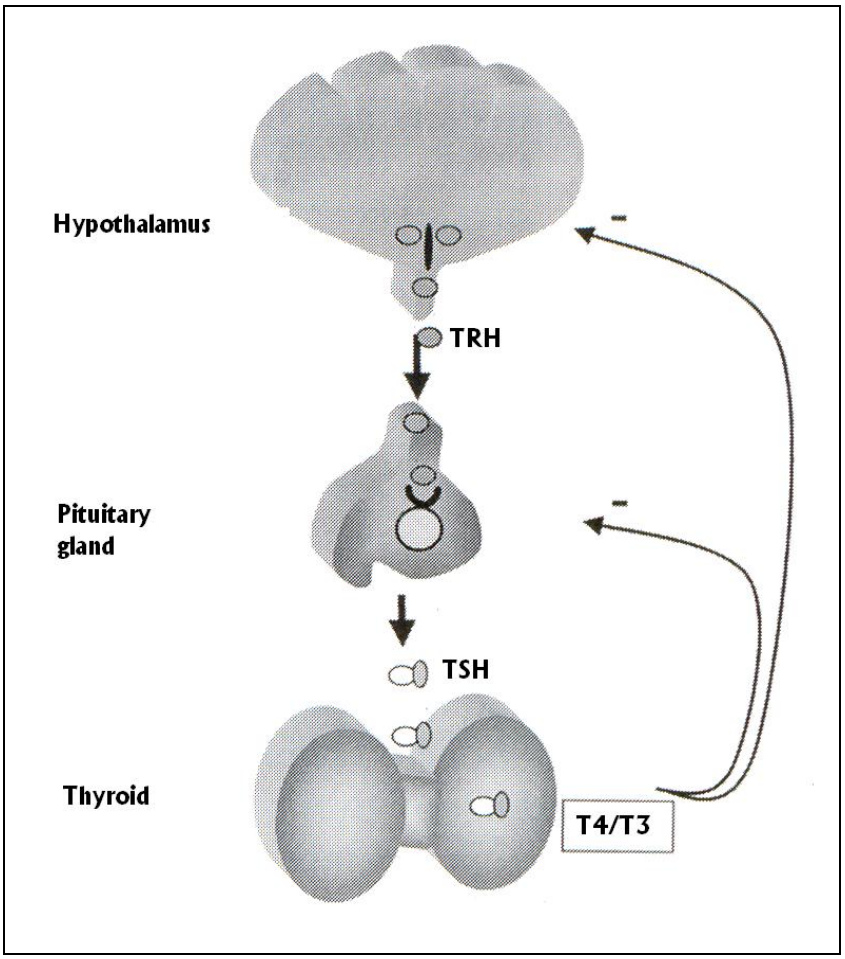


**Figure 3. Thyroidal feedback axis.**

**Adapted from: Cohen and Wondisford.[5] TRH: thyrotropin releasing hormone, TSH: thyroid stimulating hormone, T4: thyroxine, T3: tri-iodothyronine.**

Synthesis and secretion of TSH from the thyrotropes in the pituitary pars distalis is stimulated by thyrotropin releasing hormone (TRH), a small tripeptide released from the hypothalamus into the hypothalamo-hypophyseal capillaries. TSH has a molecular weight of about 30 kDa and consists of an α subunit, identical to the α subunit of other glycoprotein pituitary hormones, and a β subunit, which is specific to the TSH molecule. Despite differences in TSH at the molecular level, TSH of different species share similar biological activity. Response to bovine TSH (bTSH) has been reported in mice,[6] rats,[7,8] dogs,[9-11] cats,[12-14] and humans.[15] TSH binds to a TSH-specific G-protein coupled receptor on the surface of follicular cells, which leads to stimulation of adenylate cyclase and subsequent secretion and

increased production level of thyroid hormones. The thyroid hormones (free and unbound form) are regulated by a homeostatic negative feedback mechanism. The pituitary thyrotrope cell deiodinates T4 derived from the plasma to T3 which inhibits TSH synthesis and secretion by alteration of nuclear receptor binding, mRNA transcription, and protein synthesis.[16,17]

### 1.2. Feline hyperthyroidism

Thyrotoxicosis with excessive production and secretion of thyroid hormones in feline hyperthyroidism is caused in 98 % of the cases by benign adenomatous hyperplasia of the thyroid gland.[18] Single or multiple hyperplastic nodules ranging in size from less than 1 mm to 3 cm (Figure 4) can be found in thyroid glands of hyperthyroid cats in one lobe (unilateral affection) or in both lobes (bilateral affection) in 30 and 70 % of the cases, respectively.[19]

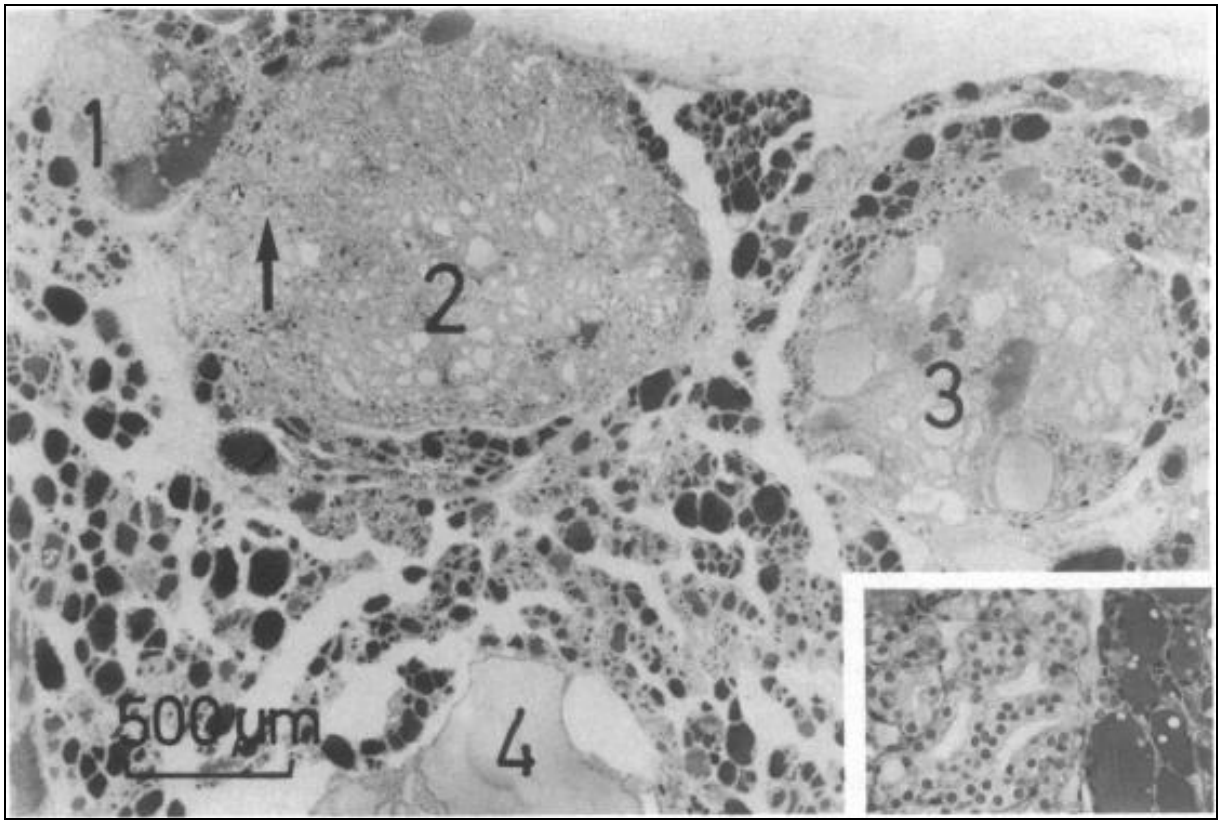


**Figure 4. Histologic section across a nodular goiter of a thyrotoxic cat. Four nodules (1-4) consisting of follicles lined by cuboidal epithelial cells with large nuclei (see inset) and filled with pale, barely stained colloid are shown.**
**Adapted from: Peter et al.[20]**

Malignant tumors are observed occasionally in 2 % of the cases. The etiology of feline hyperthyroidism is likely to be multifactorial. Environmental factors such as feeding of

canned food or the use of cat litter,[21,22] overexpression of oncogenes such as c-*ras*,[23] and altered G protein expression[24] have been associated with the disease. Clinical signs that are commonly observed include polyphagia, polyuria, polydipsia, weight loss, behavioral changes such as hyperactivity, an unkempt haircoat and gastrointestinal signs. Clinical signs can be less pronounced when the disease is diagnosed early. Diagnosis is confirmed by measurement of increased serum TT4 concentration. Tachycardia and a systolic heart murmur are present in 60 and 30 %, respectively, of hyperthyroid cats.[25]

An atypical presentation with the presence of anorexia and lethargy is possible as well, and can often be related to the chronicity and severity of the disease or an underlying disorder complicating the hyperthyroidism.[26,27] Physical examination reveals a palpable cervical nodule(s) in more than 90 % of the cases (Figure 5).[25,28]

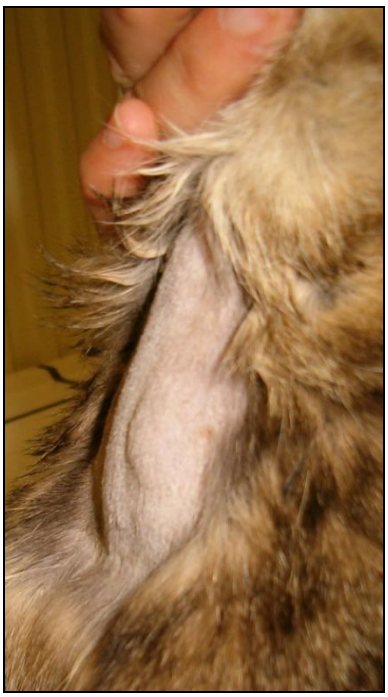

**Figure 5. Enlarged thyroid nodule in a hyperthyroid cat.**
**Picture: I. van Hoek.**

### 1.3. Treatment of feline hyperthyroidism

Feline hyperthyroidism is a debilitating disease and is potentially life-threatening if not treated appropriately. Therapeutic options include administration of radioiodine ($^{131}$I) or antithyroid drugs such as methimazole, and thyroidectomy. The latter treatment method should no longer be recommended routinely because it is invasive and has no advantages to

the $^{131}$I treatment which is available at an increasing level over the world. The administration of $^{131}$I and thyroidectomy are in principle irreversible methods. On the other hand, the use of antithyroid drugs is reversible. Several factors can influence the choice of therapy. Indeed, preference of the owner, availability of $^{131}$I therapy, and the physical condition of the patient are all important considerations. Furthermore, the age of the patient and, more importantly, the presence of concomitant disease such as cardiovascular or renal dysfunction must be taken into account when a therapy is chosen.[29]

Both treatment with antithyroid medication as well as $^{131}$I have specific advantages and disadvantages which are summarized in Table 1.

**Table 1. Advantages and disadvantages of treatment methods currently available for feline hyperthyroidism.**

| Treatment | Advantages | Disadvantages |
|---|---|---|
| Anti-thyroid drugs | ■ inexpensive (at least short term)<br>■ no need for surgery, anesthesia or hospitalization<br>■ reversible | ■ side effects are common<br>■ need for daily medication administration<br>■ close monitoring<br>■ not curative<br>■ life-long treatment |
| Radioiodine treatment | ■ one treatment is sufficient in majority of cases<br>■ rapid cure<br>■ no need for anesthesia<br>■ complications are uncommon | ■ need for sophisticated facilities<br>■ hospitalization time dependent on excretion of radioactivity<br>■ possible risks from radioactivity |

**Adapted from: van Hoek et al.[1]**

### *1.3.1. Anti-thyroid medication*

Thiourylenes are antithyroid drugs derived from a sulfur-containing parent compound called thiouracil. Thiourylenes are actively concentrated in the thyroid, where they exhibit their therapeutic effect by blocking the synthesis of thyroid hormones. More specifically, they block the thyroidperoxidase catalyzed reactions (oxidation of iodide and iodination of tyrosyl residues in thyroglobulin) and the coupling of iodotyrosines to iodothyronines. Thiourylenes also interfere with this coupling by binding to and altering the structure of thyroglobulin.

Thiourylenes have no influence on the iodide uptake mechanism of the thyroid cell (iodide pump) or the release of previously formed thyroid hormones.[30,31]

The most commonly known thiourylenes are methimazole (MMI), a synonym for the pharmaceutical compound thiamazole, and propylthiouracil (PTU). Another agent is carbimazole (CBZ), a carbethoxy derivative of MMI which is not a true thiourylene itself, but an inactive pro-drug. However, CBZ is almost completely bio-activated to an equimolecular amount of MMI after administration. It was developed originally in the search for a drug with a longer duration of activity compared to MMI.[30,32] On a molar basis, MMI and CBZ have the same potential, but CBZ has a greater molecular weight, which necessitates a higher dose in order to obtain an effect equivalent to MMI. A dose of 10 mg CBZ is approximately equivalent to 6.1 mg of MMI.

Adverse reactions occur in approximately 10-15 % of cats treated with a moderate to high dose of MMI (10-15 mg/day) and in 5 % of cats treated with a relatively low dose of MMI.[33,34] The most important clinical side effects during the first weeks of treatment are anorexia, vomiting and lethargy. These are usually transient and may resolve despite continued administration. When gastrointestinal signs persist, hepatic toxicity may be present which requires discontinuation of therapy.[29,33] Another serious side effect is self induced excoriation of the skin of head and neck, which also requires discontinuation of therapy. Hematological side effects can be mild, including lymphopenia, eosinophilia and transient leucopenia, or more serious like severe thrombocytopenia, agranulocytosis or immune mediated hemolytic anemia.[33,35]

In Belgium, only Felimazole® (Thiamazole) tablets of 5 mg are registered for treatment of hyperthyroidism in cats. Methimazole has a bitter taste, but Felimazole® is sugar-coated to simplify administration. Propylthiouracil is very potent in blocking thyroid hormone synthesis but is no longer recommended for use in cats because of severe side effects. These include anorexia, vomiting, lethargy, immune mediated hemolytic anemia and thrombocytopenia.[36]

### *1.3.2. Radioactive iodine ($^{131}$I)*

Administration of $^{131}$I can be intravenous (IV), subcutaneous (SC) or oral.[31,37] Thyroid cells actively take up stable or radioactive iodine and incorporate it into tyrosyl groups during thyroid hormone synthesis. Hyperplastic or tumoral thyrocytes are hyperactive and will take up more $^{131}$I as opposed to healthy cells. Uptake of $^{131}$I by normal cells is suppressed due to

the negative feedback system on the hypothalamic-pituitary-thyroid axis. Radioiodine undergoes β decay and emits β-particles and γ-radiation. The β-particles travel a maximum distance of approximately 2 mm, during which they cause local destruction of the surrounding follicle cells. Surrounding structures, such as the parathyroid and healthy suppressed thyroid cells, are spared.[31,35] The γ-radiation penetrates the tissue, is less radiotoxic than the β-particles, and permits imaging with the γ-camera. Iodine not taken up by the thyroid is excreted in saliva and urine, and through the gastrointestinal system.

Follow up after $^{131}I$ therapy is very important. It is recommended to measure serum TT4, creatinine and BUN after $^{131}I$ therapy. Healthy or atrophied thyrocytes must be activated again after treatment by the increased serum TSH concentration, and this can take several months. When cats have a low serum TT4 concentration 1 month after treatment, it is possible that healthy reactivated cells are not yet producing enough thyroid hormones. This production usually increases hereafter and euthyroidism is achieved 3 to 6 months after $^{131}I$ administration.[37] Optimal timing for assessment of renal function after treatment has not been investigated yet.

Before treatment, a scintigraphic scan of the thyroid using pertechnetate ($^{99m}TcO_4^-$) is often performed to investigate whether there is uni- or bilateral involvement of the thyroid lobes, and whether there is a presence of ectopic thyroid tissue or signs of malignancy. Pertechnetate is trapped by the thyroid gland in the same way as iodide but is not organified by the thyroid gland. It decays with γ-radiation, enabling visualization with a γ-camera.

In rare cases the thyroid pathology can be malignant (adenocarcinoma). Adenocarcinoma gives non-homogenous uptake on the pertechnetate scan, however it can only be confirmed by histology. To treat a suspected adenocarcinoma, a higher dose of $^{131}I$ of 10 to 30 mCi is recommended.[38]

### 1.4. Direct and indirect effects of thyroid hormones on kidney function

The nephron is the functional unit of the kidney. It consists of the renal corpuscle and the tubule. The glomerulus is a dense network of capillaries and filtrates the blood. Filtered substances are re-absorbed from the tubular fluid in segments of the renal tubule. There is also secretion into the tubule of components from plasma like urea, electrolytes such as potassium and components produced by the tubular cell (Figure 6). Alterations of the tubular fluid take place in the formation of urine.[39] The overall physiological effects of thyroid hormone are stimulatory: basal metabolic rate is increased, which demands for increased glycolysis,

gluconeogenesis, protein synthesis and lipid metabolism. The heart rate, cardiac output (CO) and blood flow are stimulated by thyroid hormones as well.

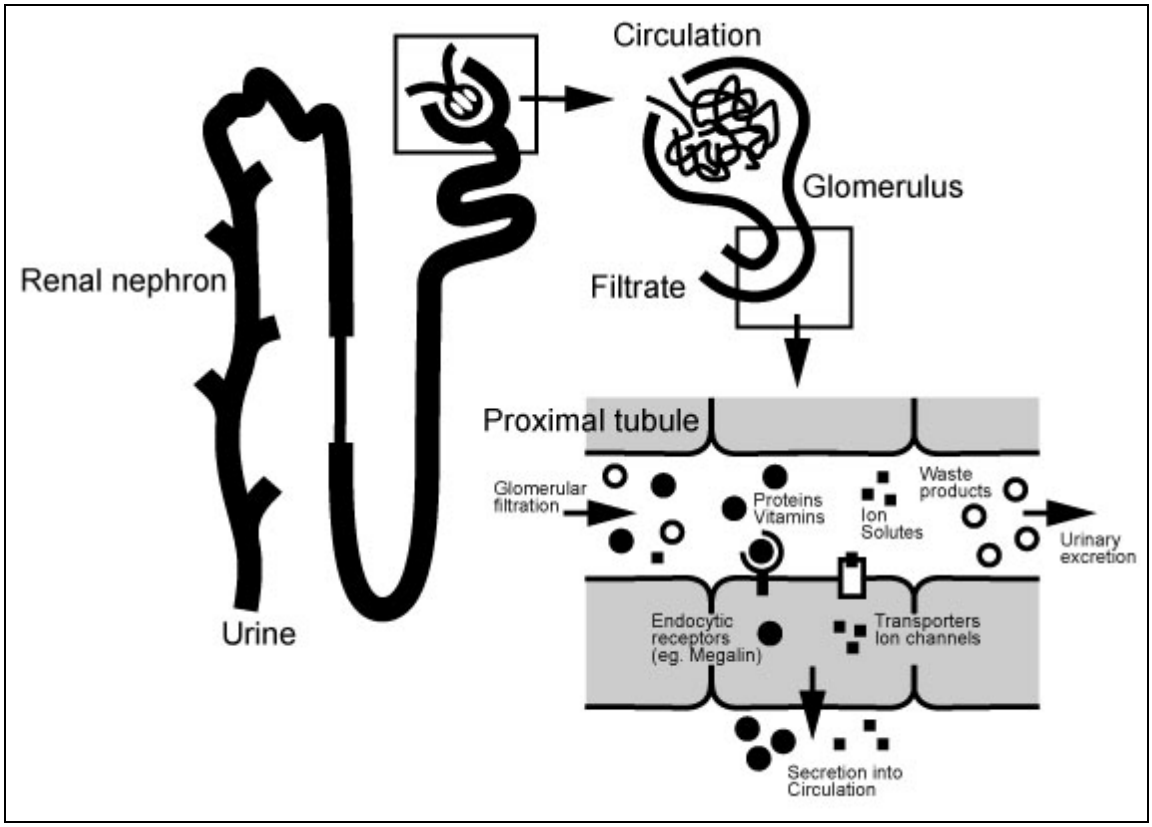


**Figure 6. The nephron in the kidney consists of the renal corpuscle and the tubule. Adapted from: www.recepticon.com/megalinreceptorimage.**

Both hyper- and hypothyroidism cause hemodynamic and vascular changes, which have an influence on kidney function through effects on renal blood flow. These changes are described schematically in Figure 7.

### 1.4.1. Cardiac output

Thyroid hormones have a positive chronotropic effect caused by changes in electrophysiological parameters, shortened atrio-ventricular conduction time and upregulated β-receptors in cardiac tissue[40] which results in tachycardia,[41] and a positive inotropic effect caused by changes in several sodium, potassium and calcium channels and activity of myosin isoenzymes.[42,43] CO is decreased in hypothyroidism[44] which is caused by bradycardia, decreased ventricular filling and cardiac contractility.[45-47]

### 1.4.2. Systemic vascular resistance

Systemic vascular resistance is decreased in the hyperthyroid state. Muscle tissue has a greater number of capillary vessels [48,49] and reduced contractility due to decreased response to norepinephrine and a direct effect of thyroid hormones on vascular smooth muscle cells.[50-52]

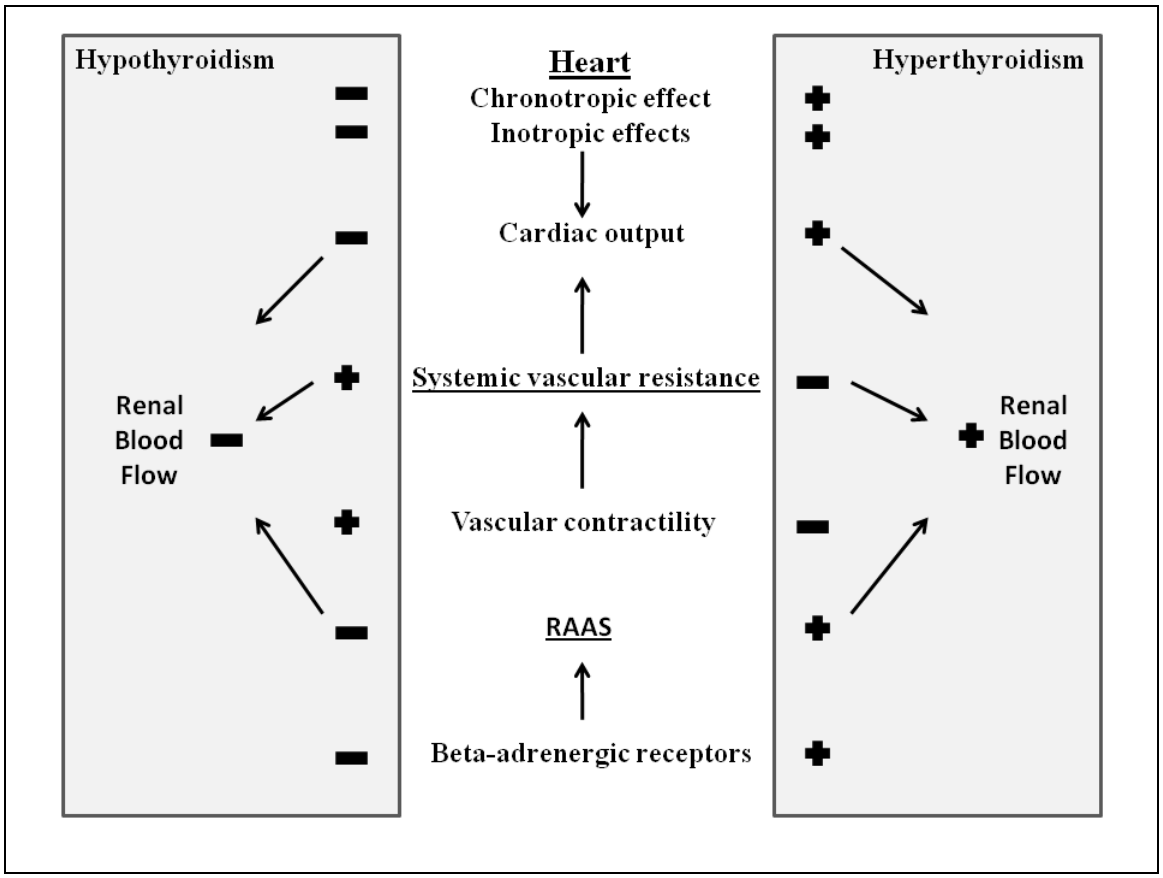


**Figure 7. Hemodynamic and vascular changes that have an effect on renal blood flow in hypothyroidism and hyperthyroidism. RAAS: renin-aldosterone-angiotensin-system.**

A higher number of vascular smooth muscle cells is relaxed due to an increased local release of vasodilators[53] and responsiveness to the endothelium-dependent vasodilator acetylcholine (ACh)[54,55] while activity of the endogenous renal vasoconstrictor endothelin is decreased.[56] Activity of atrial natriuretic factor (ANF) is increased in humans, rats, rabbits and dogs due to either a higher cardiac preload or a direct effect of T4 on gene expression.[57-61] Activity of nitric oxide synthase (NOS) and production of endothelium-derived relaxing factor nitric oxide (NO) is increased in the renal cortex and medulla.[55,62,63] This can be regarded as a protective homeostatic effect in target organs of hypertensive disease and can be due to a direct effect of thyroid hormones on NOS activity. Indirect effects can be a response to high arterial pressure, hyperdynamic circulation with shear stress on the endothelium that

causes expression of NOS,[62,64] or increased release of vaso-active substances.[62] The decreased systemic vascular resistance combined with the changes in β-adrenergic activity caused by an increased number of β-adrenergic receptors in the renal cortex[65-67] causes an increased activity of the renin-angiotensin-aldosterone system (RAAS). A direct effect of T3 on renin gene expression also enhances the RAAS activity. There is increased plasma renin concentration, synthesis of angiotensinogen in the liver, plasma angiotensin II, serum angiotensin converting enzyme (ACE) and angiotensin receptor density and renin release in humans, rats, rabbits an dogs.[58,60,68-73]

### 1.4.3. Renal blood flow

The increased CO caused by positive chronotropic and inotropic effects, decreased vascular resistance and increased blood volume by RAAS activation,[74] leads to an increased renal blood flow (RBF) in hyperthyroid rats, humans and most likely in cats.[56,75,76]

The decreased CO in hypothyroidism leads to a decreased RBF in rats, humans, and most likely in dogs.[77-79] Glomerular lesions seen in hypothyroidism such as thickening of the basement membrane and increased mesangial matrix[44,80] might contribute to the decreased RBF.

Effects of hypo- or hyperthyroidism on RBF and on factors involved in RBF are described in Table 2.

**Table 2. Effects of hypo- and hyperthyroidism on RBF and on factors involved in RBF described in different species (superscript).**

| | **Hypothyroidism** | **Hyperthyroidism** |
|---|---|---|
| RBF | decreased [rat, dog, human] | increased [rat, cat, human] |
| Chronotropic effect | decreased [human] | increased [pig] |
| Inotropic effect | decreased [human] | increased [pig] |
| Systemic vascular resistance | increased | decreased |
| Vascular contractility | increased [rat] | decreased [rat, rabbit, human] |
| ANF | decreased [rat, dog, human] | increased [rat, rabbit, dog, human] |
| NOS | decreased [rat] | increased [rat, human] |
| β adrenergic receptor | decreased [rat, human] | increased [rat, human] |
| RAAS | decreased [rat, rabbit, dog, human] | increased [rat, rabbit, human, dog] |
| CO | decreased [rat] | increased [rat, cat, human] |

**RBF: renal blood flow, ANF: atrial natriuretic factor, NOS: nitric oxide synthase, RAAS: renin-angiotensin-aldosterone system, CO: cardiac output.**

### 1.4.4. Renal tubules

Thyroid hormones have qualitative as well as quantitative effects on renal tubules. Renal tubules are hypertrophic and hyperplastic in hyperthyroidism, which leads to an increased tubular mass, kidney weight, mitotic index, DNA content with a constant protein/DNA ratio,[81] renal expression of renin mRNA,[82] metabolic level and increased tubular secretory and reabsorbtive capacity.[44,82,83]

Hypothyroidism causes a decreased kidney-to-body weight ratio, though there is compensatory renal hypertrophy and increased protein/DNA ratio without changes in DNA content of renal cells.[81] Thyroid supplementation and subsequent doubling of the kidney mass[84] shows reversibility of the decreased kidney mass. Changes in characteristics of tubular function in hypo-and hyperthyroidism are described in Table 3.

**Table 3. Changes in characteristics of tubular function in hypo- and hyperthyroidism, described in different species (superscript).**

| **Tubular characteristics** | **Hypothyroidism** | **Hyperthyroidism** |
|---|---|---|
| All-over characteristics | atrophic [rat] | hypertrophic and hyperplastic [rat] |
| DNA content | unchanged [rat] | increased [rat] |
| Protein/DNA ratio | increased [rat] | unchanged [rat] |
| Kidney weight | decreased [rat] | increased [rat] |
| $Na^+$-$K^+$-ATPase activity across basolateral membrane | decreased [rat, rabbit] | activated [rat] |
| $Na^+$ reabsorbtion through $Na^+/H^+$ exchanger (NHE) | decreased [rat] | increased [rat] |
| Urine concentrating ability | impaired [rat]<br>due to:<br>• decreased osmotic driving force [rat]<br>• decreased response of vasopressin to osmotic stimuli [rat]<br>• impaired water handling [human] | decreased [human]<br>due to:<br>• disturbed metabolisation or sensitivity of distal tubuli to vasopressin<br>• reduced sodium concentration [human]<br>• osmotic diuresis [human] |

***Tubular transport processes***

Thyroid hormones stimulate active, carrier-mediated tubular transport processes by an increased gene expression, synthesis and activity of carrier proteins,[85,86] such as $Na^+$-$K^+$-ATPase across the basolateral membrane, and $Na^+/H^+$ exchanger (NHE) activity in brush border membrane vesicles which leads to an increased uptake of $Na^+$ in exchange for $H^+$.[87] More specifically, NHE-2 and NHE-3 isoforms mRNA levels increase after transition from the hypothyroid to the hyperthyroid state.[88] The increased tubular reabsorption of sodium combined with a decreased load of filtered sodium causes a decreased pressure-diuresis-natriuresis response[89,90] and enhanced $Na^+Ca^{2+}$ exchange activity and $Ca^{2+}$ reabsorbtion[91] in the basolateral membrane through modulation of the uptake of $Ca^{2+}$ in brush border membrane vesicles.

The influence of short term hypothyroidism on tubular function is only modest,[92] however tubular transport capacity is below normal[77] and phosphate reabsorption is reduced in the proximal tubule.[93] Urinary acidification is impaired with increased sodium and bicarbonate excretion rates.[94]

***Ability to concentrate urine***

Human patients with thyrotoxicosis can have a decreased ability to concentrate urine[95] though without clinical importance.[44] A low urine specific gravity (USG) has also been described in hyperthyroid cats compared to healthy cats.[96,97] Suggested reasons for the impairment of urine concentration are disturbances in the metabolism or sensitivity to vasopressin in the distal nephrons,[77] reduced sodium concentration secondary to increased RBF[95] or osmotic diuresis caused by an increased filtered solute.[98]

Ability to concentrate urine is impaired in hypothyroidism.[99,100] This is reversible with thyroid hormone replacement and is not associated with a decreased GFR, serum urea, solute excretion or plasma arginine-vasopressine (AVP) concentration.

### 1.4.5. Glomerular filtration rate

The ultrafiltrate is formed by the glomerulus into the capsule of Bowman by filtration through the glomerular capillary wall (Figure 8).

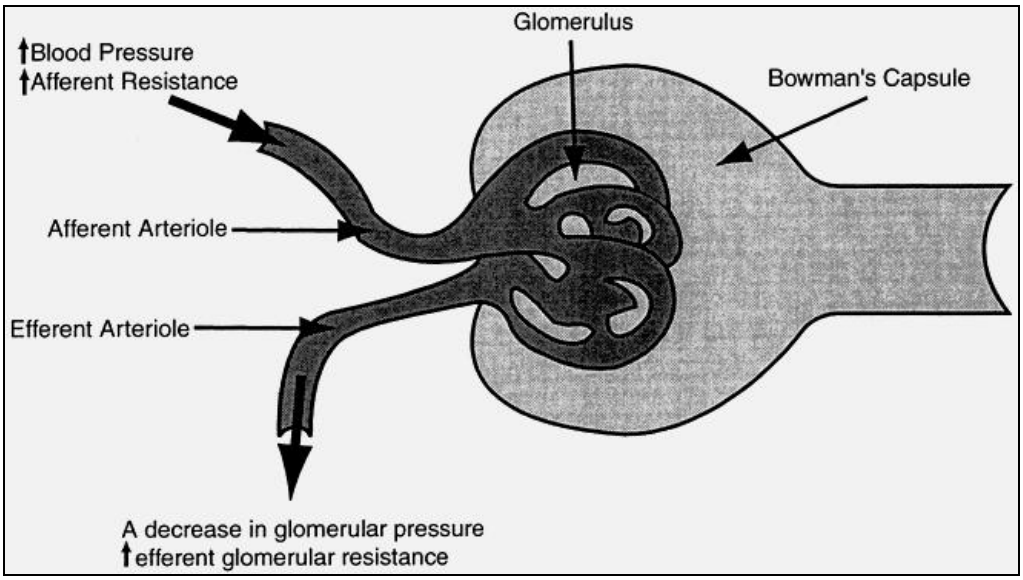


**Figure 8. Schematic presentation of the afferent and efferent arterioles forming the glomerulus in the Bowman's Capsule of a nephron.**
**Adapted from: Weir.[101]**

The rate of this filtration, the glomerular filtration rate (GFR), is the result of the mean net filtration pressure, the permeability of the filtration barrier and the surface available for filtration. The permeability is dependent on the structural and chemical characteristics of the glomerular capillary wall.[39] GFR represents the magnitude of ultrafiltration of plasma in the first steps of urine formation. It is therefore regarded to be the best overall index of kidney function.[102] Hyperthyroidism and hypothyroidism have a respectively increasing and decreasing effect on GFR through several mechanisms, which are described in Figure 9 and summarized in Table 4.

**Table 4. Effects of hypo- and hyperthyroidism on GFR and on aspects of kidney function with a direct or indirect effect on GFR described in different species (superscript).**

| | **Hypothyroidism** | **Hyperthyroidism** |
|---|---|---|
| GFR | decreased [rat, dog, human] | increased [rat, cat, human] |
| Glomerular vasoconstriction | increased [efferent arterioles dog] | decreased [afferent arterioles rat] |
| Chloride channels | decreased [rat] | increased [rat, human] |
| Tubulo-glomerular feedback | decreased [rat] | increased [cat] |

**GFR: glomerular filtration rate.**

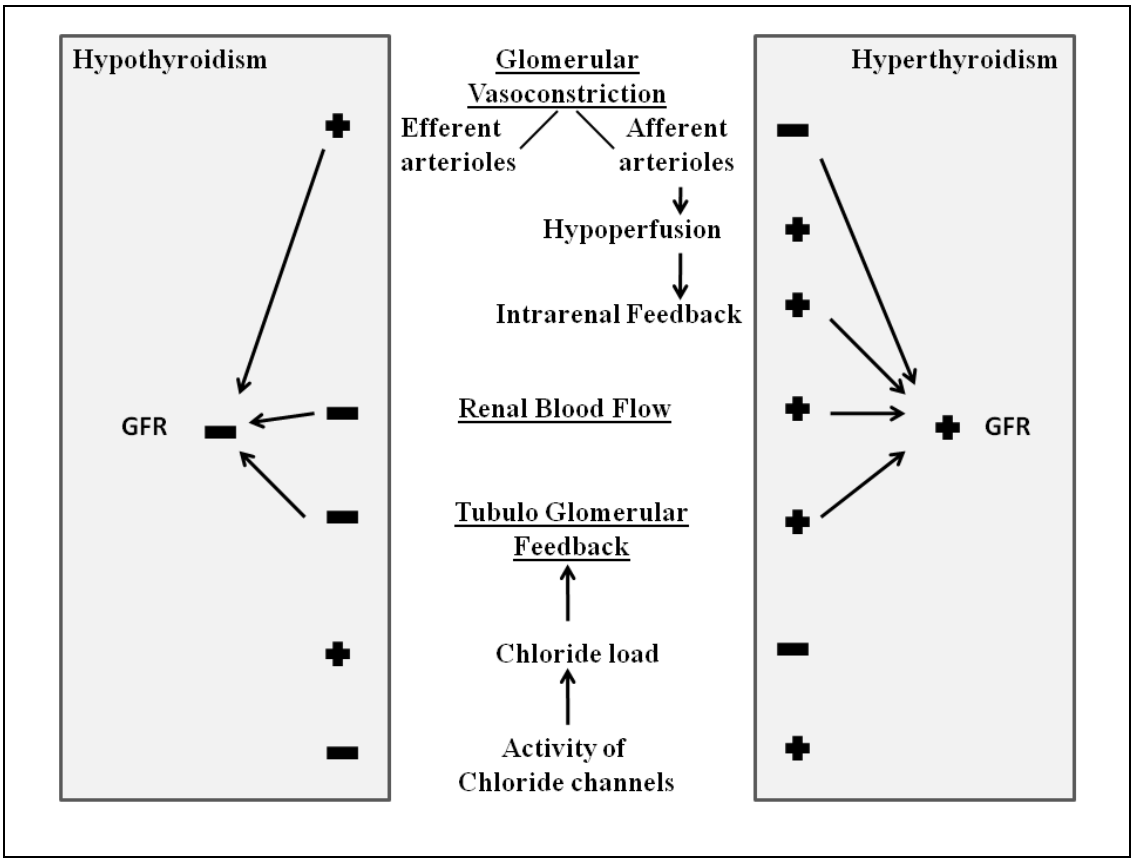


**Figure 9. Schematic overview of the major changes in the kidney leading to an effect on GFR in hyperthyroidism and hypothyroidism. GFR: Glomerular filtration rate.**

***Glomerular filtration rate in hyperthyroidism***

Hyperthyroidism increases GFR up to 18 % in hyperthyroid rats[103,104] and humans[105] by several mechanisms. It leads to a decreased resistance of afferent arterioles in the kidney which increases the glomerular hydrostatic pressure and subsequently GFR[106] though it also increases the risk of hypoperfusion of the proximal tubule. The intrarenal feedback mechanism increases GFR to cope with the threatening hypoperfusion and the escape of urine entering the distal tubule which has to be replaced by delivery of proximal tubule fluid.[107] Thyroid hormones increase mRNA expression of chloride channels (ClC) in a dose-dependent way[108,109] and increase activity of ClC and Cl absorption in the proximal tubule and Henle's loop. Tubulo-glomerular feedback adjusts GFR after a decreased chloride load is sensed within the distal tubule by the macula densa.[96] The increased GFR is reversed after treatment of hyperthyroidism in humans and cats.[96,97,105]

Glomerular filtration rate can be reduced up to 40 % in hypothyroid humans[92,104,105,110] and 30 % in hypothyroid rats.[103] GFR has been reported to be decreased in dogs after thyroidectomy,[77] and is significantly decreased in dogs diagnosed with thyroid

deficiency.[77,111] The decreased GFR is corrected after treatment with thyroid hormone in humans with a normal renal function[79,92,105,112] which is suggestive of only functional renal changes that do not cause permanent cellular damage.[112]

***Serum creatinine concentration***

Serum creatinine concentration is an indirect estimate of GFR and is often significantly decreased in humans with hyperthyroidism due to the increased GFR, increased clearance and tubular secretion of creatinine but also to decreased muscle mass.[113-116] Serum creatinine concentration increases after therapy of hyperthyroidism.[105,113] Serum concentration of cystatin C (CysC) is another estimator of GFR[117] because it is freely filtered by the glomerulus, and catabolised by the tubuli.[118] Serum CysC is independent of age, sex, malignancy or inflammation.[119] However, intra-individual variability is high[120] and thyroid hormones influence the general metabolism thereby increasing the production rate of CysC in humans[115,121,122] and rats.[123]

Serum creatinine is increased in hypothyroid humans caused by the reduced glomerular function and creatinine generation from possible myopathy and rhabdomyolysis.[80,124] The increased serum creatinine is not caused by an impaired creatinine metabolism.[92] It is reversible after treatment with thyroid hormone supplementation.[105,125]

### 1.4.6. Proteinuria

Proteinuria with an increased total urinary protein/creatinine ratio (UPC) and urinary albumin/creatinine ratio is often present in hyperthyroid humans and rats.[106,110,113] It is not related to activity of the RAAS, blood pressure or oxidative stress[126,127] though it can be a reflection of glomerular hypertension and hyperfiltration, changes in tubular protein handling, or a change in the structure of the glomerular barrier.[90] Proteinuria resolves quickly after treatment of hyperthyroidism in humans.[113]

Hypothyroid humans and rats can have an increased transcapillary leaking of the plasma proteins such as albumin which leads to mild proteinuria and albuminuria.[79,128,129] The albuminuria is considered to be present before the decrease in GFR in hypothyroid patients.[110]

## 1.5. Evaluation of kidney function in hyperthyroid cats

### 1.5.1. Measurement of glomerular filtration rate

Routinely, kidney function is assessed through evaluation of BUN and creatinine measurements in serum. However, these parameters only give a rough estimate of kidney function. Indeed, 75 % of the kidney mass needs to be lost before these blood values increase. Measurement of GFR is a more efficient and accurate method for assessment of kidney function because it allows detection of a decreased glomerular function in an early stage of renal disease, before renal azotemia develops.[130]

Clearance is defined as the volume of plasma cleared of a substance during a given interval of time (mL/min).[131] GFR can be measured directly by measuring clearance of a filtration marker or indirectly and less sensitive with serum creatinine concentration. GFR can be measured with urine clearance of a substance which is not metabolized by the body, completely filtrated and not secreted nor reabsorbed by the tubules after filtration. When GFR is measured with plasma clearance, the substance can only be filtrated by the kidney.

Renal clearance is calculated from the classical formula $CL_{renal} = (U \times C_u)/C_p$, where U = urine flow (mL/min), $C_u$ = marker concentration in urine (mg/mL), and $C_p$ = marker concentration in plasma (mg/mL).[131] Renal clearance of inulin is regarded the gold standard method, however only a few studies on urinary clearance of inulin in cats have been performed.[132-134] Indeed, urinary clearance is highly cumbersome, is difficult to propose for client-owned cats, is tedious and time consuming for the staff, is stressful (e.g., anesthesia may be required) and potentially harmful (e.g., urinary tract infection) for the animal, and an accurate measurement of urine volume is often difficult. Another marker accepted for measuring renal clearance in cats is urinary clearance of exogenous creatinine.[132,135-138] Other methods using different markers have been evaluated intensively in dogs and cats over the recent years, although an easy to apply method suitable for practice has not yet been found.

Plasma clearance of an intravenous administered marker reflects total body clearance and is calculated by the formula $CL_{plasma} = D/AUC$ where D = dose of marker and AUC = area under plasma concentration versus time curve. The AUC is calculated with specific formulas according the appropriate pharmacokinetic model.[131] To correct for the wide variety in body composition, plasma clearance must be standardized. In cats, normalization by body surface area, BW or extracellular fluid volume (ECFV) are all considered satisfactory.[139] It is important to respect the number of samples and sampling time when plasma clearance is

measured. If renal function is normal or slightly reduced, elimination of the marker is generally achieved within 4-6 hours. However, most of the marker will still be in the body after 6 hours if renal function is severely reduced and it may be necessary to prolong the sampling period. Also, frequent sampling during the first half hour is necessary to avoid overestimation of clearance due to an erroneously small AUC. Markers for plasma clearance investigated for use in cats are inulin,[132,134,137,140-142] iohexol,[136,138,139,141,143-145] exogenous creatinine,[140,142,146] and radiolabelled substances like $^{99m}$Tc-DTPA (diethylene triamine penta-acetic acid), $^{99m}$Tc-mercaptoacetyl triglycine, $^{131}$I-IOH (orthoiodohippuric acid) and $^{51}$Cr-EDTA (ethylene diamine tetraacetic acid).[134,147-150] Studies comparing different methods of GFR measurement with regard to reproducibility and age in cats are lacking. Moreover, no studies investigating different GFR techniques over a period in which GFR is expected to change, nor studies investigating different GFR techniques over the complete range of GFR values expected in cats, have been performed.

### 1.5.2. Retinol binding protein as an urinary marker

The nephrons in the kidney consist of different regions with structural and functional specialization. Damage to a specific region would result in characteristic changes in the profile of biomarkers in the urine (urinary markers). Urinary biomarkers for diagnosis of acute renal failure in humans were recently reported.[151] Further, urinary biomarkers prove to be an important tool for early assessment of therapeutic efficacy in clinical settings in human medicine.[152] Damage to the kidney consists of a cascade of events, which will lead to kidney failure if not stopped. It is important to detect changes already early in the cascade. Depending on their origin in the kidney, biomarkers can be site-specific or reflect regional function.[153] An overview of urinary tests for initial screening for nephrotoxicity is described in Table 5. There is a need in veterinary medicine for a simple urinary marker to detect early renal damage in hyperthyroid cats. New promising urinary markers are urinary N-acetyl-β-glucosaminidase (NAG) and urinary retinol binding protein (RBP). Hyperthyroid cats had an increased urinary NAG concentration compared to healthy controls, though this did not change after treatment.[154]

**Table 5. Overview of urinary markers with their corresponding unit of the nephron.**

| Test | Functional unit tested |
|---|---|
| Albumin | Glomerulus |
| N-acetyl-β-glucosaminidase (NAG) | Proximal tubule |
| Retinol binding protein | Proximal tubule (lysosomes) |
| Alanine aminopeptidase | Proximal tubule (brush border) |

**Adapted from: Price.[153]**

Urinary NAG was higher before treatment in cats developing post-treatment renal azotemia compared to cats maintaining a healthy kidney function. Nevertheless, the usefulness of NAG in hyperthyroid cats for detection of tubular damage remains unclear from these preliminary data, moreover because hyperthyroid cats have an increased prevalence of urinary tract infection.[155] Active infection of the urinary system also increases urinary NAG.[156]

Urinary RBP is a tubular type of proteinuria. It is a highly sensitive index of renal tubular damage in humans because a minor decrease in tubular function may lead to RBP excretion in urine.[157,158] RBP is a low molecular weight (MW) carrier protein of 21 kilodalton (kDa). RBP is a specific carrier for the lipophilic vitamin A (retinol) in blood, transporting the retinol ligand as a holo-RBP complex. Holo-RBP binds physiologically to transthyretin (TTR), the thyroid hormone transport protein in plasma, and this prevents the loss of both RBP and its bound retinol through glomerular filtration.[159] Upon release of its ligand, the uncomplexed apo-RBP no longer has affinity for TTR and can be freely filtered in the glomerular ultrafiltrate and is normally reabsorbed through a megalin-receptor dependent endocytosis mechanism in the proximal tubules, where RBP is degraded.[160] However, when tubular function fails, elimination of RBP shifts from intra-tubular catabolisation to urinary excretion.[161] Although previous studies have investigated RBP in veterinary medicine, it was not yet detected in cat urine and its potential use as an early renal marker of renal damage needs to be investigated.[162-164]

### 1.5.3. Hyperthyroidism and kidney failure

As mentioned earlier, hyperthyroidism is the most diagnosed endocrine disorder in geriatric cats with a median age at diagnosis of 13 years[165] and is reported to affect 0.3 % of

all cats with no apparent sex or breed predilection.[166] CKD affects 7.7 % of cats over 10 years of age and 15.3 % of cats over 15 years of age.[167,168] Therefore it is not surprising that the prevalence of pre-existing CKD in hyperthyroid cats in different studies has been reported to be 14 % (n=167)[169], 23 % (n=202)[26], 27 % (n=22)[27] and 40 % (n=22).[170] It is important to mention that pre-existing renal disease is associated with shorter survival after treatment of hyperthyroidism.[169] A summary of the literature concerning kidney function after treatment of hyperthyroid cats is outlined in Table 6.

**Table 6. Effect of treatment of hyperthyroid cats on kidney function.**

| Ref. | Number of cats per treatment method | Period (days) | USG pre | USG post | GFR pre | GFR post | Creatinine (μmol/L) pre | Creatinine (μmol/L) post | Azotemia Number (%) post |
|---|---|---|---|---|---|---|---|---|---|
| 96 | Tx: n = 13 | 30 | 1.038 | 1.030 | 2.51 ±0.69 | 1.4 ±0.41 | 111 ± 3 | 181 ± 53 | n = 5 (39) |
| | Controls: n = 11 | - | 1.058 | 1.058 | 2.02 ±0.27 | 2.18 ±0.5 | 128 ± 18 | 136 ± 24 | - |
| 171 | $^{131}$I: n = 27 | 90 | 1.046 | 1.043 | - | - | 115 ± 35 | 177 ± 53 | - |
| | MMI: n = 9 | 90 | 1.042 | 1.037 | - | - | 150 ± 80 | 239 ± 221 | - |
| | Tx: n = 22 | 90 | 1.033 | 1.033 | - | - | 150 ± 53 | 212 ± 71 | - |
| 170 | $^{131}$I: n = 22 | 6<br>30 | 1.032 | 1.031<br>1.028 | 2.25 | 2.1<br>- | 115 ± 53 | 106 ± 44<br>168 ± 62 | - |
| 97 | MMI: n = 12 | 42 | 1.041 | 1.033 | 3.83 ±1.82 | 2.02 ±0.81 | 117 ± 18 | 159 ± 85 | n = 2 (17) |
| | Controls: n = 10 | - | 1.057 | 1.056 | 1.83 ±0.56 | 2.05 ±0.3 | 144 ± 11 | 146 ± 14 | - |

**Adapted from: Daminet.[172] MMI: Methimazole, Tx: thyroidectomy, USG: urine specific gravity, GFR: glomerular filtration rate mL/min/kg, pre: before treatment, post: after treatment.**

Evaluation of kidney function in a cat with thyroid dysfunction is important but difficult at the same time, because clinical signs of hyperthyroidism and CKD overlap, and hyperthyroidism can mask and might even worsen CKD. A geriatric cat presented with weight loss, vomiting and polyuria and polydipsia can present clinical signs of both hyperthyroidism and co-existing CKD.[173]

Systemic hypertension was previously assumed to be highly common in hyperthyroid cats.[174] Systemic hypertension can be transmitted onto the glomeruli when there is failure of autoregulation.[171] However, due to the more accurate and precise methods that are used for measuring blood pressure nowadays, systemic hypertension appears to be less common in

hyperthyroid cats than previously presumed and is not expected to be the most important mediator of progressive renal injury.[175,176] Proteinuria is frequently present in hyperthyroid cats either developing azotemia or maintaining a healthy kidney function after treatment,[177] therefore a change in glomerular barrier is less likely.[178]

USG is lower in cats with hyperthyroidism compared to healthy cats[96,97] and decreases[96,170] or remains equal[97,171] after treatment, hence USG after treatment stays lower compared to USG in healthy cats.[96,97]

### 1.5.4. Hyperthyroidism masking co-existing CKD

CKD can become apparent after treatment of hyperthyroidism. GFR and plasma creatinine have an inverse relationship in hyperthyroid cats before and after treatment. Plasma creatinine concentration is decreased in hyperthyroid cats and healthy cats supplemented with thyroxine,[75,96] but values increase after treatment of hyperthyroidism.[96,170,171] Glomerular filtration rate is increased in cats with hyperthyroidism, and decreases after treatment.[96,97,170] A proposed cause for the increased GFR is renal hypertrophy caused by hyperthyroidism. The hypertrophy reverses after treatment and might cause normalization of GFR per gram of renal tissue.[178] This is however less likely because GFR is already decreased 6 days after treatment.[170] Unmasking of renal failure with development of post-treatment renal azotemia is present in 39 % (n = 13)[96], 17 % (n = 12)[97], 37 % (n = 67)[169] and ± 30 % of cats.[173]

Cats with hyperthyroidism and pre-existing renal disease should first preferably undergo a reversible treatment of hyperthyroidism. This will allow to assess renal changes caused by the treatment of hyperthyroidism.[27] The effects of methimazole treatment on renal function are reversible because discontinuation of methimazole in hyperthyroid cats developing post-treatment renal azotemia increases GFR and decreases serum creatinine concentration.[97]

It would be ideal if pre-existing, though masked renal failure, could be detected in hyperthyroid cats, in order to predict post-treatment renal azotemia. An early detection of CKD before the onset of CKD and azotemia is crucial for good management of these patients.[179] Several studies have showed that only pre-treatment assessment of GFR was predictive for development of CKD.[97,170] Further, pre-treatment baseline values of serum creatinine, BUN and urinary protein or USG were not proven to be predictive for the development of CKD.[170,177,180]

## 1.6. Diagnostic challenges with non-thyroidal illness

### 1.6.1. Concurrent hyperthyroidism and CKD

Diagnosing mild hyperthyroidism in cats with CKD can pose difficulties, because CKD can lower serum TT4 concentration into reference range values.[181,182] CKD can act as a non-thyroidal illness (NTI) causing the euthyroid sick syndrome. The decrease in thyroid hormones is caused by changes in peripheral hormone metabolism, thyroid hormone binding proteins and central effects.[183-185] Extrathyroidal conversion of T4 to T3 is decreased due to decreased delivery of T4 to intracellular deiodinases and activity of these deiodinases. At tissue level there is decreased uptake of T4 and T3, impaired activity of nuclear receptors to T3 and post-receptor actions of T3. Production of thyroid hormone-binding proteins (thyroxine binding globulin, transthyretin and albumin) and their affinity for thyroid hormones is decreased. Thyrotropin (TSH) secretion is decreased which causes a decreased thyroidal secretion of T3 and decreased availability of T4 for peripheral conversion to T3. The hypothalamic-pituitary axis is intact in human patients with CKD, because TSH can elevate in patients with CKD and primary hypothyroidism, and TSH is suppressed in patients with CKD and hyperthyroidism.[183-185] The decreased TSH secretion despite the low level of circulating thyroid hormone explains the euthyroid sick syndrome as a host's mechanism of defense against protein wasting and therefore treatment with thyroid hormone supplementation remains debatable in human medicine.[186,187]

Other methods with less diagnostic value are analysis of fT4 which in these cats is only of limited value due to false positive concentrations in systemic illness,[188] T3 suppression test which has not yet been evaluated in cats with concurrent hyperthyroidism and CKD,[189] or the TRH stimulation test which could not confirm hyperthyroidism in sick cats that were believed to be hyperthyroid.[190] A recent study described TSH measurement in hyperthyroid cats with CKD.[182] Results of the study seemed promising because there was a significant difference between cats with hyperthyroidism and concurrent CKD and cats with CKD or healthy cats. However, the assay used was a canine TSH assay and it was therefore not species specific. Moreover, it is a 1$^{st}$ generation assay with low sensitivity. In human medicine, 3$^{rd}$ and 4$^{th}$ generation assays with higher sensitivity are used nowadays.[191] Also, it was not validated in the higher range of TSH concentration expected in hypothyroid cats. Combined measurement of serum TSH with fT4 might be of merit in diagnosing hyperthyroidism in any cat with mild or previously diagnosed CKD.[182] The diagnostic value

of TSH stimulation and/or thyroid scintigraphy in cats with CKD and suspected hyperthyroidism has not yet been investigated in cats.

### 1.6.2. Post-treatment renal azotemia and low serum TT4 concentration

Besides in cats with mild hyperthyroidism and CKD, a diagnostic challenge can also occur in cats developing a serum TT4 concentration below reference range and azotemia after irreversible treatment of hyperthyroidism. These cats could have iatrogenic hypothyroidism which occurs in 6 to 30 % of the cats treated with $^{131}$I and which could contribute to azotemia.[92,192-194] However, these cats could also have CKD suppressing serum TT4 below reference ranges,[181,182] or both. The differentiation between these two pathological conditions has not yet been investigated in cats, in contrast to dogs. Historically, most investigators regard the TSH response test as the best single test for evaluating canine thyroid function. This dynamic test has the advantage of better differentiating between a hypothyroid dog and one suffering from NTI.[195,196] A recent study showed that quantitative measurement of thyroidal $^{99m}TcO^-$ uptake had the highest discriminatory power in one study with regard to the differentiation between primary hypothyroidism and non-thyroidal illness.[11] This diagnostic challenge has not yet received much attention in cats, as spontaneous hypothyroidism is extremely rare.

#### *Application of recombinant human thyrotropin (rhTSH)*

Recombinant human thyrotropin or thyrotropin $\alpha$ is a heterodimeric glycoprotein produced by recombinant DNA technology in a Chinese hamster ovary cell line.[197] The principal clinical utility of rhTSH which has been approved is the diagnostic monitoring in human patients with differentiated thyroid cancer. Recent investigations have also proven the use of rhTSH to enhance uptake of $^{131}$I (RAIU) in treatment of differentiated thyroid cancer with thyroid ablation by $^{131}$I [198] or in treatment of toxic and nontoxic nodular goiter.[199,200] This results in therapeutic doses which are lower and therefore irradiation to extra-thyroidal tissue is decreased.[201-204] Because reliable TSH assays have been developed in human medicine, there is no need for dynamic function testing with TSH stimulation in hypothyroid humans.[191]

Limiting factors for the use of rhTSH in veterinary practice are the cost and the limited storage time after reconstitution of a vial. However, there was no proof of loss in biological activity in euthyroid dogs after storage of rhTSH at 4 °C for 4 weeks or at -20 °C for 8 weeks, which makes the clinical application of rhTSH in veterinary practice realistic.[205]

Administration of rhTSH in dogs causes significant elevation of serum thyroid hormones and rhTSH has comparable biological activity as bTSH in dogs.[206-210] Also in cats, administration of rhTSH causes increased thyroid hormones in the serum.[211] The α and β subunits of feline TSH show 68 % and 88 % homology with human TSH respectively.[212] This however does not seem to be of significant importance, because homologues glycohormones do not necessarily have the highest affinity for the receptor in the same species. For example, bovine and porcine TSH have higher affinities for the human TSH receptor than human TSH itself.[213]

Hypothyroidism is considered one of the most difficult to diagnose canine endocrine disorders. Historically, the bovine TSH (and now rhTSH) response test was regarded as the best single test for evaluating canine thyroid function because it had the advantage of better differentiating between a hypothyroid dog and one receiving certain medications or suffering from a NTI.[209,210] This has not yet been investigated in cats.

Another area in which rhTSH could proof valuable, is administration of rhTSH previous to therapeutic $^{131}I$ for treatment of hyperthyroidism. A recent study showed that 25 μg of rhTSH caused an increase in RAIU of 7 %.[214]

### 1.7. Conclusion

The influence of hyperthyroidism on kidney function is extensive. There is evidence that besides the glomerular changes described in hyperthyroid cats, tubular changes also occur. Radioactive iodine is the treatment of choice for feline hyperthyroidism, however an important aspect that must be considered is the declining kidney function after treatment. Kidney function could be evaluated in hyperthyroid cats by measurement of GFR or evaluation of urinary markers which appear when there is tubular damage. These could be an aid in the early detection of kidney dysfunction. The declining kidney function can also lead to challenges in the accurate diagnosis of iatrogenic hypothyroidism.

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# CHAPTER 2

# EVALUATION OF GFR TECHNIQUES

# CHAPTER 2

Introduction to Chapter 2

Glomerular filtration rate (GFR) is regarded as the best overall index of kidney function. GFR can be measured directly by measuring clearance of a filtration marker. Renal clearance of inulin is considered the gold standard method, however it is cumbersome and rarely performed. In order to evaluate GFR in a feasible and reliable way in our further studies, we first had to compare and validate different techniques to measure GFR.

In the first section (§ 2.1) we compared the plasma exogenous creatinine clearance test (PECCT), plasma exo-iohexol clearance test (PexICT) and plasma endo-iohexol clearance test (PenICT) in healthy cats, and investigated reproducibility and the ability to distinguish between GFR values caused by age differences.

Another important point for GFR measurement is the ability to distinguish between GFR values measured over a time period in which the GFR is expected to change. A decrease in GFR is expected in hyperthyroid cats after treatment. In the following section (§ 2.2) we compared PECCT, PexICT and PenICT in hyperthyroid cats after treatment with radioiodine, as well as the distinguishment between GFR values measured at different time points after treatment.

In the final section (§ 2.3), we evaluated the ability of these methods to detect differences in GFR between groups that represent the whole range of GFR which can be expected in cats: low in cats with CKD, normal in healthy cats and high in untreated hyperthyroid cats.

# CHAPTER 2

# COMPARISON AND REPRODUCIBILITY OF GFR MEASUREMENTS IN YOUNG ADULT AND AGED HEALTHY CATS

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# CHAPTER 2


**Summary**

Important characteristics determining the usefulness of a method for GFR measurement are convenience, availability, and reproducibility. The use of different plasma clearance methods could lead to different results and differences in reproducibility.

Twelve healthy cats: 6 young adult cats (age 7-12 months) and 6 aged cats (age 9-12 years) were included in this study. A cross-over design was used to compare the plasma clearance of exogenous creatinine (PECCT), exo-iohexol (PexICT), endo-iohexol (PenICT) and Chromium-51 ethylenediaminetetraacetic acid ($^{51}$Cr-EDTA), and to investigate reproducibility of these methods. Cats of different ages were included to determine if differences in GFR in young adult versus aged cats would be detected with these methods. The PECCT, PexICT and PenICT were performed in a combined manner. Plasma data were subjected to non-compartmental (creatinine, exo-iohexol and endo-iohexol) or bicompartmental ($^{51}$Cr-EDTA) analysis with a statistical moment approach. Area under the concentration-time curve was calculated using the trapezoidal rule with extrapolation to infinity. Statistical analyses were carried out using a random effects model.

Globally, the 4 methods differed significantly in GFR assessment. Clearance of exo-iohexol and $^{51}$Cr-EDTA showed the highest and lowest reproducibility, respectively. Only plasma clearance of creatinine differed significantly between young adult and aged cats. These findings should be taken into account not only in practice but also in future studies involving GFR measurement.

## Introduction

Kidney function can be crudely estimated by assessing blood urea nitrogen (BUN) and creatinine concentrations in serum, or more precisely evaluated by estimating glomerular filtration rate (GFR). Indeed, measurement of GFR allows detection of decreased kidney function in an early stage of kidney disease (International Renal Interest Society [IRIS] stage I), before insufficiency develops (IRIS stage II or higher).[1,2] Important characteristics determining the usefulness of a method for GFR measurement are not only convenience and availability but also accuracy and reproducibility. Many methods have several disadvantages including labor intense nature, risks caused by anesthesia, cost of the test substance, assay of the substance used, or need for specialized licensing and equipment.

The traditional gold standard for GFR measurement is urinary clearance of inulin. Urinary clearance of creatinine during exogenous creatinine administration is comparable with urinary clearance of inulin in cats.[3] However, urinary clearance techniques have the disadvantage of being laborious and difficult to apply in a clinical setting.

Studies have shown that GFR measurement using plasma clearance of iohexol (plasma iohexol clearance test, PICT) is comparable with urinary clearance of exogenous creatinine in healthy cats and dogs,[4,5] and is useful for detection of renal dysfunction in cats.[6] The PICT has good reproducibility in humans with normal or compromised renal function,[7-10] as well as in dogs and in cats with normal renal function.[11,12] Iohexol is best analysed by high performance liquid chromatography (HPLC) which has good specificity, sensitivity, accuracy, and reproducibility, and measures both stereoisomers exo- and endo-iohexol.[13] Thus, PICT can be specified to plasma clearance of exo-iohexol and endo-iohexol (PexICT and PenICT, respectively). However, this method is expensive and not readily available in practice.

In human medicine, radiolabelled markers that use chelating agents such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) also have been introduced as alternatives to inulin clearance.[14] Clearance of chromium-51 EDTA ($^{51}$Cr-EDTA) shows excellent correlation to iohexol clearance in humans and is reproducible in humans with normal or compromised renal function and in dogs with normal renal

function.[15-19] This method has not yet been investigated in cats. Disadvantages are the need for specialized licensing and equipment.

A promising alternative for GFR measurement in cats is the plasma exogenous creatinine clearance test (PECCT)[20] as it does not require specialized equipment, use of radionuclides, or anesthesia.[21] PECCT could be an alternative to more complicated methods to assess GFR in a research setting and could even be used in referral practice. It is important however to assess accuracy and reproducibility of any clearance method before it can be used routinely in monitoring renal function in cats that are at risk for developing renal disease, in follow-up of treatment of kidney failure, or in a research setting. Combined use of creatinine and iohexol in a plasma exogenous creatinine-iohexol clearance test (PEC-ICT) has been described in cats[20] and allows determination of 2 GFR methods with minimal stress for the animals and minimal variation between the 2 methods.

Aging has a degenerative effect on the kidney, thereby decreasing glomerular function and decreasing GFR with increasing age. This inverse relationship has been described in humans and rats.[22,23] Chronic kidney failure in cats increases in prevalence with increasing age. When kidney function is monitored in an aging cat, it is interesting to know whether GFR can be decreased due to physiological circumstances.

Studies comparing different methods of GFR measurement with regard to reproducibility and age in cats are lacking. The objectives of this study were to compare and investigate the reproducibility of clearance of exogenous creatinine, exo-iohexol and endo-iohexol, and $^{51}$Cr-EDTA. Cats of different ages were included to investigate whether these methods are capable of detecting differences in GFR between young adult and aged cats.

## Materials and Methods

### Cats

This study was conducted according to guidelines for animal care, with consent of the Ethical Committee of the Faculty of Veterinary Medicine from Ghent University, Belgium.
Twelve healthy domestic shorthair cats were obtained from the population of laboratory animals of Ghent University. They were divided according to age in group 1 (n = 6; age range, 7-12 months; body weight (BW) range, 4.3-5.5 kg [mean ± standard deviation (SD), 4.7 ± 0.5 kg]), and in group 2 (n = 6; age range, 9-12 years; BW range, 2.2-5.9 kg [mean ± SD, 4.9 ± 0.7 kg]). To assess the health of the cats, initial screening was performed, which included physical and routine laboratory examinations (CBC, biochemistry and measurement of total T4 [TT4], evaluation of feline immunodeficiency virus [FIV] and feline leukemia virus [FeLV] status), and systolic blood pressure measurement (Doppler method). Cats underwent abdominal ultrasonography and cystocentesis. Urinalysis (dip-strip tests, microscopic analysis, protein/creatinine ratio, urine specific gravity, and bacteriologic culture) also was performed. Cats were included in the study if these examinations showed no abnormalities. Cats were placed on a commercial diet (Hill's Science Diet Adult Original Cat Food, Etten-Leur, The Netherlands) throughout the study. The cats were acclimated to the experimental conditions (food and investigator) for 2 weeks before the start of the study. Cats were fasted for at least 10 hours before the start of the clearance test and fed immediately after the end of the sampling period. Water was offered ad libitum.

### Experimental design

A cross-over design was used to compare clearance and to investigate reproducibility for the PECCT, PexICT and PenICT. The study comprised a period of 8 weeks. The study design is shown in Table 1. To limit the number of cats undergoing the complete protocol of iohexol-creatinine clearance and $^{51}$Cr-EDTA clearance, and because the target population was aged cats, the complete protocol was only performed in 3 aged cats.
Twelve healthy cats were divided into group I (n = 6) with young adult cats and group II (n = 6) with aged cats. Cats in groups I were randomly assigned to subgroup I-A (n = 3) or I-B (n = 3); cats in group II were randomly assigned to subgroup II-A (n = 3) or II-B (n = 3). All cats in groups I and II underwent PEC-ICT on day 1 or 2 of week 1. All cats in subgroup II-A underwent $^{51}$Cr-EDTA clearance on day 1 of week 2. In week 3, tests performed during week

1 were repeated in all cats from group I and II. After a resting period of 3 weeks, the $^{51}$Cr-EDTA clearance (as in week 2) was repeated in week 7 in all cats from subgroup II-A. Finally, in week 8, the same 3 cats from subgroup II-A underwent PEC-ICT on day 1 and $^{51}$Cr-EDTA clearance on day 2.

**Plasma exogenous creatinine-iohexol clearance test**

The PEC-ICT protocol was slightly modified from a method previously reported.[20] Animals received 40 mg/kg creatinine and 64.7 mg/kg iohexol (Omnipaque 300 (300 mg I/mL), Nycomed Imaging AS, Oslo, Norway). Creatinine was dissolved in 0.9 % sodium chloride (NaCl) (Natrii Chloridum 0.9 %, B.Braun Melsungen AG, Deutschland). First iohexol, then creatinine was administered IV in the cephalic vein. The dead space in the catheter was rinsed with 2 mL of 0.9 % NaCl and the timer was started. Blood samples (2 mL) were taken by jugular venipuncture before iohexol-creatinine administration, after 5, 15 and 30 minutes, and after 1, 2, 3, 6, 8 and 10 hours, and then placed in EDTA tubes and centrifuged. Aliquots of plasma were stored at -20 ºC until assayed.

**$^{51}$Cr-EDTA clearance test**

For every new day of $^{51}$Cr-EDTA clearance testing, a new stock of $^{51}$Cr-EDTA was made and aliquoted into parts for injection and parts to serve as standard for that particular day. A standard dose of approximately 1 g $^{51}$Cr-EDTA (equivalent to approximately 3.7 mCi) was injected IV. The catheter was flushed with 2 mL 0.9 % NaCl and the timer started. Blood samples (1 mL) were taken by jugular venipuncture after 5, 15 and 30 minutes, and after 1, 2, 3, and 4 hours, placed in EDTA tubes and centrifuged. Plasma was stored at 4 ºC until assayed. The precise dose of injected $^{51}$Cr-EDTA was calculated by weighing the syringe before and after administration with comparison to the previously prepared standard.

**Assays**

Plasma creatinine was assayed by an enzymatic method (Vettest analyzer, Idexx Laboratories Europe B.V., Amsterdam, The Netherlands). This technique was validated in-house by measuring samples with increasing creatinine concentration 4 times per day, on each of 3 consecutive days. Samples from the same animal of 2 separate GFR measurements were assayed on the same day. Quality control samples were measured on each day that a run of assays was performed. The limit of quantification was 13.6 mg/dL. Within- and between-day

coefficients of variation were < 3 % in the lower, upper-middle and higher range of concentration (1.6, 9.0 and 12.4 mg/dL, respectively), and there was linear correlation between theoretical and measured concentration within quantification limits. Accuracy varied from -0.7 % to 8.8 % for the different concentrations. The basal plasma creatinine concentration measured on the day of PECCT testing was subtracted from the creatinine concentrations measured in the samples from that cat. The basal plasma creatinine concentrations in groups 1 and 2 were 1.6 ± 0.2 mg/dL and 1.7 ± 0.2 mg/dL.

Plasma concentrations of iohexol stereo-isomers exo-iohexol and endo-iohexol were determined by an HPLC method with ultraviolet (UV) detection, slightly modified from a method previously reported.[24] A Varian Product (Varian, Walnut Creek, CA, USA) HPLC system equipped with a ternary gradient pump type 9012, an autosampler type Prostar 410 and an UV-photo diode array (PDA) detector type Prostar set at 254 nm were used for the quantitation of both exo- and endo-iohexol. Chromatographic separation was achieved on a 250 x 4.6 mm inner diameter (ID) polymer laboratories reversed phase-S polymeric column (Polymer Laboratories Ltd., Shropshire, UK) (8 μm), attached to a guard column of the same type (5 x 3 mm ID). The mobile phase consisted of HPLC grade water and acetonitrile, run with a gradient solvent program. The flow rate was 1.0 mL/min. Samples were prepared by pipetting 100 μL of plasma into a 1-mL Eppendorf tube (Novolab, Geraardsbergen, Belgium), followed by the addition of 100 μL of HPLC grade water, 25 μL of an internal standard solution of 5 mg/mL of iohexol impurity J: 5-amino-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-tri-iodobenzene-1,3-dicarboxamide (chemical reference substance [CRS] European Pharmacopoeia, Strasbourg, France) and 15 μL of trifluoroacetic acid. After vortex mixing briefly and centrifugation (13.000 x *g* for 10 min at room temperature), the upper layer was transferred to a HPLC-vial and 50 μL was injected on the HPLC instrument. For the preparation of the calibrators, the same procedure was followed with the exception that 100 μL of appropriate solutions of iohexol (CRS) were added to 100 μl of blank cat plasma instead of 100 μl of HPLC grade water. The method was validated for exo-iohexol and endo-iohexol before the start of the analysis. Calibration parameters were determined in accordance with the recommendations as defined by the European Union and with criteria based on the literature.[25-28] Linear calibration curves ($r \geq 0.9965$) were obtained at a low concentration range (1.2-11.9 μg/mL for endo-iohexol and 8.8-88.1 μg/mL for exo-iohexol) and a high concentration range (11.9-595.0 μg/mL for endo-iohexol and 88.1-6607.5 μg/mL for exo-iohexol). The goodness-of-fit coefficient was < 10 % for both calibration curves. The within-

and between-day precision fell within the ranges specified by Heitzman at 4 different concentrations (1.2-11.9-59.5-595.0 µg/mL for endo-iohexol and 8.8-88.1-440.5-4405 µg/mL for exo-iohexol); i.e. coefficient of variation (CV) ≤ 5 %.[27] The trueness fell within ranges of -20 % to +10 % at the same concentrations and varied between -9.5 % and + 6.5 %. The limit of detection (LOD) was defined as the lowest concentration of iohexol that could be recognized by the detector with a signal-to-noise ratio of ≥ 3 and was calculated to be 0.97 and 4.7 µg/mL for endo-iohexol and exo-iohexol, respectively. The limit of quantification (LOQ) was defined as the lowest concentration for which the method was validated with a trueness and precision that fall within the recommended ranges and was set at 1.2 and 8.8 µg/mL for endo-iohexol and exo-iohexol, respectively. Retention times for both isomers were 6.23 and 6.73 min for endo-iohexol and exo-iohexol, respectively. The ratios of exo- and endo-iohexol stereo-isomers were quantified in the analytical standard (88.1 % exo-iohexol and 11.9 % endo-iohexol) and in the Omnipaque solution administered (81.9 % exo-iohexol and 18.1 % endo-iohexol).

The counts from radioactivity of $^{51}$Cr-EDTA in each plasma sample were measured for 3 minutes in a gamma counter (COBRA II Auto Gamma Counter Packard, model 05003 S/N 41110, Canberra Group, Zellik, Belgium). Each time a run of measurements was performed, the method was validated by measuring the counts of the standard made from the same stock as the solution injected, and each time a zero sample was measured to correct for background signal.

**Pharmacokinetic analysis**

All analyses were performed using WinNonlin (WinNonlin Version 4.0.1, Scientific Consulting Inc. Apex, NC). Plasma data were subjected to non-compartmental analysis (creatinine, exo-iohexol and endo-iohexol) or bicompartmental analysis ($^{51}$Cr-EDTA) with a statistical moment approach. The area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity, as described by Watson et al.[21] Plasma clearance of creatinine, exo-iohexol and endo-iohexol and $^{51}$Cr-EDTA was determined by dividing dose administered by AUC, and indexed to BW (mL/min/kg).

**Statistical analysis**

A mixed model with cat as random effect was fitted to the GFR data (SAS Version 9.1, SAS Institute. Inc., Cary, IN). First, the different techniques were compared with one

other by a mixed model with cat as random effect and technique, week and age (young adult or aged) as categorical fixed effects at a global significance level of 0.05. The 4 techniques were compared pairwise at a Bonferroni-adjusted comparison-wise significance level of 0.008 (= 0.05/6). Both a global analysis and separate analyses for young and aged cats were performed. The Bland-Altman approach was used to measure bias over the range of values. The graph generated from this approach displays a scatter diagram of the differences between creatinine and exo-iohexol clearance and endo-iohexol clearance, and between exo-iohexol and endo-iohexol clearance on the y-axis plotted against the averages of the 2 measured clearances on repeated occasions on the x-axis. The correlation among exo-iohexol, endo-iohexol and creatinine was quantified by the Spearman rank correlation coefficient. Secondly, reproducibility was investigated. The residual variance (RV) describes the variability between the repeated measures of the same cat with the same technique, and its estimate therefore is a measure of test reproducibility. Reproducibility was expressed as the between-day CV and given by the square root of the residual error divided by the overall mean. The maximum difference (MD) was the absolute difference between the highest and lowest GFR measurement. GFR measurements were compared between young adult and aged cats to assess whether there was an age effect when a specific method was used. All results are expressed as mean ± SD.

**Table 1. Study design.**

| Week | 1 | | 2 | 3 | | 4-5-6 | 7 | 8 | |
|---|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 1 | 1 | 2 | | 1 | 1 | 2 |
| Clearance method | PECCT<br>PenICT<br>PexICT | PECCT<br>PenICT<br>PexICT | $^{51}$Cr-EDTA | PECCT<br>PenICT<br>PexICT | PECCT<br>PenICT<br>PexICT | Resting period | $^{51}$Cr-EDTA | PECCT<br>PenICT<br>PexICT | $^{51}$Cr-EDTA |
| Group[a] | I-A<br>II-A | I-B<br>II-B | II-A | I-A<br>II-A | I-B<br>II-B | | II-A | II-A | II-A |

**PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test; $^{51}$Cr-EDTA, chromium-51 ethylenediaminetetraacetic acid.**

**[a] Group I comprised young adult cats (n = 6), divided into 2 subgroups I-A (n = 3) and I-B (n = 3). Group II comprised aged cats (n = 6), divided into 2 subgroups II-A (n = 3) and II-B (n = 3).**

## Results

### Pharmacokinetic analysis

Figure 1 represents the plasma concentration versus time curves of plasma creatinine clearance, exo-iohexol and endo-iohexol clearance in the repeated weeks. The proportions of the AUC extrapolated to infinity for creatinine, exo- and endo-iohexol in the total group and the young adult and aged cats separately are presented in Table 2. The AUC was not calculated for $^{51}$Cr-EDTA. The plasma curves for exo-iohexol have the largest AUC thereby expressing the lower GFR.

### GFR measurements

The mean, standard deviation, and range for the plasma clearance of creatinine, exo-iohexol and endo-iohexol, and $^{51}$Cr-EDTA are presented in Table 3. The correlation among creatinine, exo-iohexol and endo-iohexol was good. The correlation between creatinine and exo-iohexol was highest, where Spearman rank was 0.866 ($P < 0.001$). Globally, the 4 methods differed significantly ($P < 0.001$) in GFR assessment. The largest difference was between endo-iohexol and exo-iohexol (1.2 mL/min/kg, $P < 0.001$) and the smallest difference was between $^{51}$Cr-EDTA and exo-iohexol (-0.2 mL/min/kg, $P < 0.001$). Also, in young adult cats there were significant ($P < 0.001$) differences among creatinine, exo-iohexol and endo-iohexol clearance. In aged cats, there were significant ($P < 0.0001$) differences among the 4 methods, except when exo-iohexol was compared with $^{51}$Cr-EDTA ($P = 0.012$) or with creatinine ($P = 0.074$). Figure 2 describes the Bland-Altman approach. A clear bias is visible for comparison between creatinine and exo-iohexol (graph A) and between exo-iohexol and endo-iohexol (graph C), as with increasing average of GFR, the difference between GFR measurements is increasing.

### Reproducibility

Residual variance, between-day CV and maximum difference are given in Table 4. Reproducibility was the highest for exo-iohexol clearance, followed by creatinine clearance, endo-iohexol clearance and $^{51}$Cr-EDTA clearance. Reproducibility is demonstrated in Figure 1, where curves of different methods agree among different weeks. RV differs significantly between creatinine and exo-iohexol clearance ($P = 0.021$) and between exo- and endo-iohexol

clearance (P = 0.003). When different methods were compared within young adult or aged cats, reproducibility of endo-iohexol in aged cats was the lowest.

**Age effect**

There was a significant difference between young adult and aged cats for creatinine clearance (P = 0.039), but no significant age effect for clearance of exo-iohexol (P = 0.41) or endo-iohexol (P = 0.75).

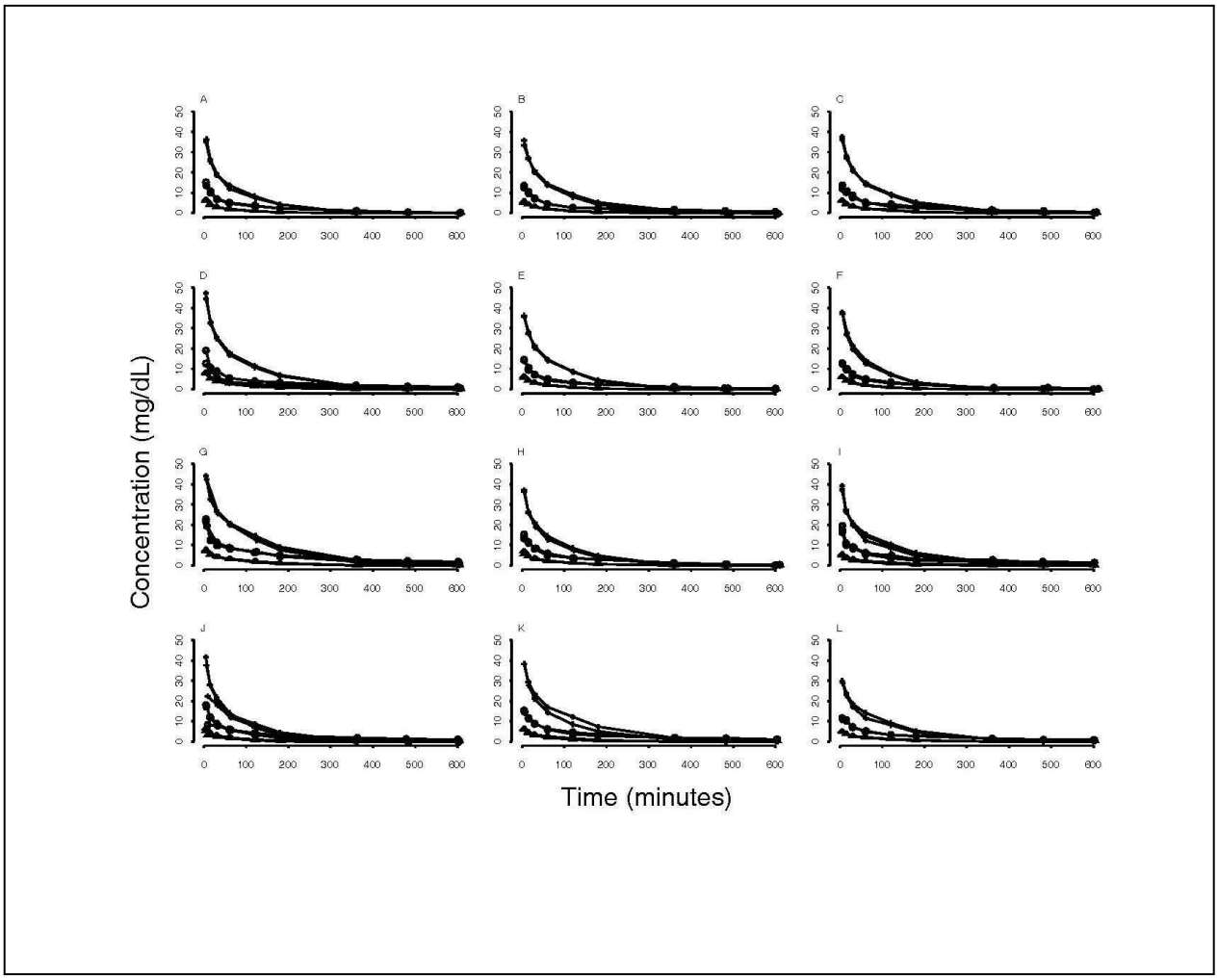


**Figure 1. Plasma concentration versus time curves from repeated measurements for plasma creatinine clearance (o), exo-iohexol (+) and endo-iohexol (∆) of 12 healthy young adult (A-F) and aged (G-L) cats.**

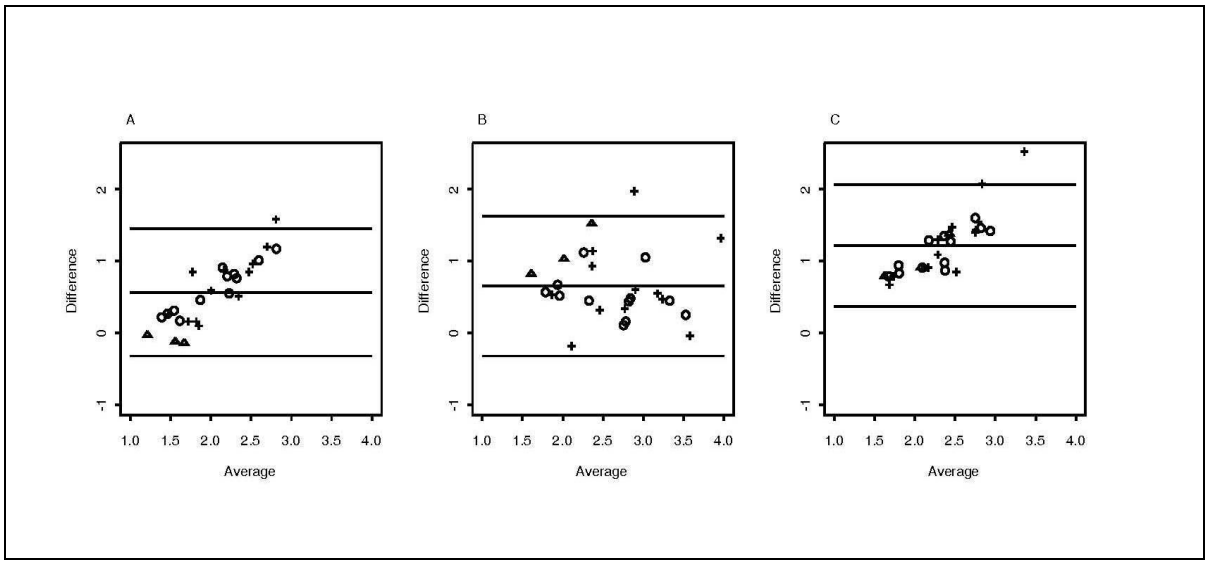


**Figure 2. Bland-Altmann plots of plasma creatinine and exo-iohexol clearance (A), creatinine and endo-iohexol clearance (B), and exo-iohexol and endo-iohexol clearance (C). The middle horizontal line corresponds to the average difference; the upper and lower horizontal lines correspond to the 95% limits of agreement. All cats (n = 12) were assessed at week 1 (+), week 3 (o) and week 8 (Δ).**

**Table 2. Proportion of the AUC extrapolated to infinity in plasma concentration versus time curves of creatinine, exo-iohexol and endo-iohexol in 12 healthy cats.**

| | **Complete group (n = 12)** | **Young adult cats (n = 6)** | **Aged cats (n = 6)** |
|---|---|---|---|
| PECCT | 9 % | 6 % | 12 % |
| PexICT | 9 % | 11 % | 8 % |
| PenICT | 11 % | 10 % | 12% |

**AUC, area under the curve; PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test.**

**Table 3. Mean ± standard deviation (range) of GFR measurements (mL/min/kg) with creatinine, exo-iohexol and endo-iohexol, and $^{51}$Cr-EDTA clearance in healthy cats.**

| | | Complete group (n = 12) | Young adult cats (n = 6) | Aged cats (n = 6) |
|---|---|---|---|---|
| PECCT | Total period | 2.3 ± 0.66 (1.2 - 3.6) | 2.7 ± 0.52 (1.7 - 3.6) | 2.0 ± 0.57 (1.2 - 3.3) |
| | week 1 | 2.5 ± 0.63 (1.6 - 3.6) | 2.8 ± 0.52 (2.2 - 3.6) | 2.2 ± 0.64 (1.6 - 3.3) |
| | week 3 | 2.4 ± 0.62 (1.5 - 3.4) | 2.7 ± 0.58 (1.7 - 3.4) | 2.0 ± 0.50 (1.5 - 2.7) |
| | week 8 | | | 1.4 ± 0.21 (1.2 - 1.6) |
| PexICT | Total period | 1.7 ± 0.29 (1.2 - 2.1) | 1.8 ± 0.27 (1.4 - 2.2) | 1.7 ± 0.28 (1.2 - 2.1) |
| | week 1 | 1.8 ± 0.27 (1.3 - 2.1) | 1.8 ± 0.28 (1.4 - 2.1) | 1.8 ± 0.29 (1.3 - 2.1) |
| | week 3 | 1.7 ± 0.31 (1.3 - 2.2) | 1.8 ± 0.30 (1.4 - 2.2) | 1.6 ± 0.29 (1.3 - 2.0) |
| | week 8 | | | 1.5 ± 0.26 (1.2 - 1.7) |
| PenICT | Total period | 3.0 ± 0.64 (2.0-4.6) | 3.1 ± 0.54 (2.0 - 3.7) | 2.9 ± 0.71 (2.1 - 4.6) |
| | week 1 | 3.1 ± 0.73 (2.0-4.6) | 3.1 ± 0.61 (2.0 - 3.6) | 3.2 ± 0.88 (2.1 - 4.6) |
| | week 3 | 2.9 ± 0.53 (2.1 - 3.7) | 3.1 ± 0.52 (2.2 - 3.7) | 2.7 ± 0.52 (2.1 - 3.6) |
| | week 8 | | | 2.5 ± 0.55 (2.0 - 3.1) |
| $^{51}$Cr-EDTA[a] | Total period | | | 1.34 ± 0.59 (0.8 - 2.5) |
| | week 2 | | | 0.87 ± 0.07 (0.8 - 0.9) |
| | week 7 | | | 1.1 ± 0.27 (0.9 - 1.4) |
| | week 8 | | | 2.0 ± 0.43 (1.7 - 2.5) |

**GFR, glomerular filtration rate; PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test; $^{51}$Cr-EDTA, chromium-51 ethylenediaminetetraacetic acid.**

**[a] Only three cats underwent $^{51}$Cr-EDTA test.**

**Table 4. Reproducibility of GFR measurements using creatinine, exo-iohexol and endo-iohexol, and $^{51}$Cr-EDTA clearance in 12 healthy cats globally and young adult and aged cats separately.**

| | | Complete group (n = 12) | Young adult cats (n = 6) | Aged cats (n = 6) |
|---|---|---|---|---|
| PECCT | RV | 0.165 | 0.139 | 0.183 |
| | CV | 17.31 % | 13.69 % | 21.58 % |
| | MD | 1.0 | 1.0 | 0.7 |
| PexICT | RV | 0.0153 | 0.009 | 0.019 |
| | CV | 7.05 % | 5.25 % | 8.28 % |
| | MD | 0.4 | 0.2 | 0.4 |
| PenICT | RV | 0.175 | 0.033 | 0.266 |
| | CV | 14.16 % | 5.97 % | 19.07 % |
| | MD | 1.4 | 0.5 | 1.4 |
| $^{51}$Cr-EDTA[a] | RV | | | 0.344 |
| | CV | | | 43.72 % |
| | MD | | | 1.60 |

**GFR, glomerular filtration rate; PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test; $^{51}$Cr-EDTA, chromium-51 ethylenediaminetetraacetic acid.**

**RV, residual variance; CV, between-day coefficient of variation; MD, maximum difference.**

**[a] Only three cats underwent $^{51}$Cr-EDTA test.**

## Discussion

In the present study, we investigated GFR measurements, reproducibility and age effect for clearance of exogenous creatinine, exo-iohexol and endo-iohexol, and $^{51}$Cr-EDTA in healthy young adult and aged cats. Clearance of $^{51}$Cr-EDTA has never been studied in the cat, and plasma clearance of exogenous creatinine has been investigated only minimally in cats.[20] Therefore, this study is the first to report GFR results using these methods. Plasma clearance with exo-iohexol reported in this study was lower compared to values described in the literature,[5,6,12,29,30] but in agreement with studies using the same analysis method.[20] Plasma clearance using iohexol has been investigated thoroughly in cats, but frequently colorimetric methods,[5,6] atomic emission spectroscopy,[12] or most commonly x-ray fluorescence methods[29,30] are used. The latter has a high detection limit and requires a higher dose of iohexol to be administered with increased risk for toxicity.[31] The colorimetric method is nonlinear above 120 mg/L.[32] These 3 methods measure the amount of iodine in the sample, which agrees with the total amount of iohexol instead of exo-iohexol or endo-iohexol specifically. Conversely, HPLC measures the amount of exo-iohexol and endo-iohexol in the sample. When HPLC is used for iohexol analysis in plasma clearance of dogs, exo-iohexol frequently is selected as a GFR marker.[11,13] However, discrepancies have been described between GFR calculation using exo- and endo-iohexol in dogs[13] and cats,[20] although not in humans.[15] In this study, there was a significant difference in the clearance of exo-iohexol and endo-iohexol. The ratio between exo-iohexol and endo-iohexol is constant, so the reason for this difference is unclear.

Correlation among clearances of creatinine, exo-iohexol and endo-iohexol was high. However, the correlation coefficient does not measure agreement among methods but only the strength of relationship among the methods. Agreement can be shown graphically with the use of Bland-Altman plots (Figure 2), which visualize between-method differences in GFR as a function of the average GFR over the 2 methods.[33] The difference between exo-iohexol clearance and creatinine clearance increased, as did the difference between exo-iohexol and endo-iohexol clearance with increasing mean GFR. This finding can be the result of an overestimation of creatinine and endo-iohexol clearance, an underestimation of exo-iohexol clearance, or both. Mean difference was the highest between exo-iohexol and endo-iohexol. The limits of agreement were wide for all between-method differences. Whether the observed

differences among the different methods in healthy cats are acceptable in clinical practice must be addressed by evaluating clearance of creatinine, exo-iohexol and endo-iohexol and $^{51}$Cr-EDTA in a clinical setting with healthy cats and cats expected to have either decreased or increased GFR.

Differences can be due to systematic methodological errors or to biological differences in the renal handling of the substances.[34] Methodological errors can result from laboratory variation, calculation of the dose of the test substance, or the degree of binding of the filtered substance to its tracer (iodine and $^{51}$chromium). GFR measurement by means of clearance of $^{51}$Cr-EDTA was assessed in only 3 of the aged cats, on different occasions as compared with the combined PEC-ICT. The small number of cases and time-related difference of $^{51}$Cr-EDTA clearance can result in methodological errors. In our study, all of the 3 substances used were assayed in different laboratories. The enzymatic method used for creatinine assay is not considered the gold standard method but is the most frequently used in routine clinical laboratories. However, the creatinine as well as the iohexol assay were validated previously and the $^{51}$chromium assay was validated with every run of assays. Storage time and temperature of samples were comparable for both iohexol and creatinine. Also, a non-compartmental pharmacokinetic model was used to assess GFR using creatinine, exo-iohexol and endo-iohexol, as described in dogs.[13,21] This method is different from the bicompartmental approach for GFR calculation with $^{51}$Cr-EDTA in this study, also described in dogs.[19] Furthermore, because a combined PEC-ICT was used, factors related to the cats themselves cannot completely explain the difference in plasma clearance by means of creatinine, exo-iohexol and endo-iohexol.

A method that is used to measure GFR must have high reproducibility in research settings. The CVs for reproducibility were < 22 % for all markers except $^{51}$Cr-EDTA. This variation is not excessively high and means that a change in GFR in 1 cat from 2.5 to 2 mL/min/kg (ie a decrease of 0.5 mL/min/kg) can be due to between-day variability and not to a biological change. At least two thirds to three quarters of renal function must be impaired to induce changes in routine indirect parameters of renal function such as serum creatinine concentration. In the present study, a change greater than 7 to 17% for the whole tested population could be considered clinically relevant. Therefore, the reproducibility, except for $^{51}$Cr-EDTA, is sufficient for screening patients for early renal dysfunction (e.g. a decrease by

50% in GFR, from 2.5 to 1.25 mL/min/kg). Nevertheless, increased precision in GFR measurements could allow detection of subtle changes in renal function in different physiologic and disease states.

We found good reproducibility for the clearance of exo-iohexol. This finding is in accordance with other studies. Becker et al. repeated plasma iohexol clearance in healthy cats and found there was no significant difference between the mean GFR on initial and repeat evaluation.[12] Miyamoto found a CV of 5 ± 3% for 2 consecutive iohexol clearance studies in 4 healthy cats.[5] In this study, the clearance of creatinine had lower reproducibility compared to exo-iohexol and endo-iohexol, because variability among repeated measures of the same cat was larger. In dogs, several factors such as state of hydration, food intake, and adrenergic stimulation may cause fluctuation in GFR.[35] However, clearances of creatinine, exo-iohexol and endo-iohexol were measured simultaneously, therefore if 1 or more of these factors were present, it would have influenced the reproducibility of all markers. Factors related to individual cats have more influence on the clearances of creatinine and endo-iohexol, because variance between repeated measures in the same cat is larger for these clearances compared to exo-iohexol clearance.

This study suggests that the PECCT may be good as an alternative method for GFR measurement. Calculation of GFR from clearance of creatinine is in agreement with values of GFR described in the literature.[20,36-38] There was no significant difference between clearance of creatinine and exo-iohexol in the aged cats, which is the major target population for GFR measurement. Reproducibility was acceptable and, more importantly, this study reported a significant difference in GFR measurement using creatinine clearance between young adult and aged cats. Possibly, clearance of creatinine has a higher sensitivity to detect changes in GFR, or the results are an artifact due to the lower reproducibility of creatinine clearance. Weaknesses of this study were the use of clearance of exo-iohexol and endo-iohexol for comparison instead of the gold standard method being urinary clearance of inulin. Additional studies are required to determine which technique is most accurate for assessment of GFR in young and aged cats. In these studies, the differences in reproducibility must be considered. However, only a few studies on urinary clearance of inulin in cats have been performed. These give comparable values for GFR (mL/min/kg): 2.71 ± 0.12 (mean ± SD),[36] 3.51 ± 0.6 (mean ± SD),[37] and 3.01 (median; range, 1.91-4.67).[39] Nevertheless, differences in the

protocols, and differences in the number and age of animals could account for differences among these GFR values measured by inulin clearance and the results in the present study.

The lower creatinine clearance with increasing age observed in the present study also has been described in humans and rats.[22,23] Possible explanations for this lower value in cats could be the same as for humans and rats, being either structural or hemodynamic changes in the kidney or both. Histological studies in humans and rats describe focal and segmental glomerulosclerosis causing a decrease in functional glomeruli which decreases creatinine clearance and increases the prevalence of microalbuminuria.[23,40-42] Hemodynamic causes are increased renal vasoconstriction, as well as loss of hydraulic permeability and reduction of filtration surface leading to decreased renal blood flow and GFR.[22,43] To the authors' knowledge this is the first study that included cats of different ages to investigate whether differences in GFR in young adult versus aged cats would be detected using these methods. Our study suggests the possibility of lower GFR with increased age when creatinine clearance is used. Nevertheless, additional studies in larger populations still are needed to determine the potential effect of aging on GFR and concomitant histopathological changes in cats, and the best way to detect a true age effect is to perform longitudinal follow-up in the same individuals.

**Conclusion**

The considerable differences in reproducibility found in this study should be taken into account in practice and in future studies to determine which technique is most accurate for detecting the possible difference in GFR between young adult and aged cats.

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# CHAPTER 2

# EVALUATION OF GFR MEASUREMENTS IN HYPERTHYROID CATS BEFORE AND AFTER TREATMENT WITH RADIOIODINE

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# CHAPTER 2


**Summary**

Glomerular filtration rate (GFR) can be measured by clearance methods of different markers showing discrepancies and different reproducibility in healthy cats. Studies comparing different methods of GFR measurement in hyperthyroid cats have not yet been performed. Plasma clearance of exogenous creatinine (PECCT), exo-iohexol (PexICT) and endo-iohexol (PenICT) could lead to differences in GFR measurement and the need to use the same clearance method when comparing GFR before and after radioiodine treatment in hyperthyroid cats.

Fifteen client-owned hyperthyroid cats were included. GFR was measured 1 day before and 1, 4, 12 and 24 weeks after treatment. Intravenous injection of iohexol was followed immediately by IV injection of creatinine. Plasma creatinine was measured by an enzymatic method. Plasma endo- and exo-iohexol were measured using high performance liquid chromatography coupled to ultraviolet (UV) detection.

Globally, the 3 GFR methods resulted in significantly different GFR results. GFR results among the different methods were the same at all time points. All 3 techniques showed decreasing GFR after $^{131}I$ treatment. For each GFR technique, a significant decrease in GFR was observed between time point 0 and all other time points. This decrease stabilized 4 weeks after treatment, with very little decline afterwards. It is mandatory to use the same GFR technique in follow-up studies. GFR testing at 4 weeks post treatment allowed assessment of the final renal functional loss after treatment in hyperthyroid cats.

## Introduction

Glomerular function can be crudely estimated by assessing circulating blood urea nitrogen (BUN) and creatinine concentrations or more precisely evaluated by estimating glomerular filtration rate (GFR). Measurement of GFR allows detection of decreased kidney function at an early stage of kidney disease (International Renal Interest Society [IRIS] stage I), before insufficiency develops (IRIS stage II or higher).[1,2] The traditional gold standard for GFR measurement is urinary clearance of inulin. However, only a few studies on urinary clearance of inulin in cats have been performed.[3-5] In cats, the urinary clearance of exogenous creatinine is comparable to the urinary clearance of inulin.[6]

Plasma clearance techniques have the advantage of being less laborious and easier to apply in a clinical setting than urinary clearance techniques. GFR measurement using plasma clearance of iohexol (plasma iohexol clearance test, PICT) provides comparable results as urinary clearance of exogenous creatinine in healthy cats and dogs[7,8] and is useful for detection of renal dysfunction in cats.[9] With high performance liquid chromatography (HPLC), both stereoisomers of iohexol (i.e., exo-iohexol and endo-iohexol) can be measured. For this reason, use of HPLC allows determination of the plasma clearance of both exo-iohexol and endo-iohexol (PexICT and PenICT, respectively), thereby providing 2 measures of GFR after iohexol administration.[10] However, HPLC is expensive and not readily available in veterinary practice.

The plasma clearance of exogenous creatinine (plasma exogenous creatinine clearance test, PECCT) seems to be a promising alternative for GFR measurement in cats. The PECCT is less complicated and does not require specialized equipment, use of radionuclides, or anesthesia,[11] and therefore can be used in clinical practice.[12] Combined use of creatinine and iohexol in a plasma exogenous creatinine-iohexol clearance test (PEC-ICT) also has been described in cats,[12,13] and allows determination of different measures of GFR with minimal stress for the animals and minimal time- and space-related variation among the methods.

Cardiac output (CO) is increased in thyrotoxicosis due to positive chronotropic and inotropic effects, decreased systemic vascular resistance, and activation of the renin-angiotensin-aldoseterone-system (RAAS).[14] Autoregulatory mechanisms in the kidney of

healthy animals would counteract these changes thereby maintaining normal GFR. In thyrotoxicosis, however, intrarenal vasodilatation occurs, which, combined with increased CO causes increased renal blood flow (RBF), glomerular hydrostatic pressure, and GFR.[15] Autoregulatory mechanisms in the kidney that respond to the increased sodium and chloride reabsorption in the tubules caused by the thyrotoxicosis lead to an additional increase in GFR.[16-18]

GFR will decrease after restoring euthyroidism in hyperthyroid cats, regardless of the treatment chosen.[18-20] Increased pre-treatment GFR can mask underlying decreased kidney function that then is identified after treatment.[18,21] The objective of this study was to evaluate 3 different GFR assessment methods (PECCT, PexICT and PenICT) for follow-up of renal function in hyperthyroid cats before and after treatment with $^{131}I$.

## Materials and Methods

### Cats

Fifteen client-owned hyperthyroid cats were included in the study. Age at time of inclusion in the study was 12.7 ± 2.2 years. Cats were studied when diagnosed with hyperthyroidism, presented for treatment with radioiodine at the faculty of veterinary medicine of Ghent University (Belgium) and 24 weeks after treatment (i.e., decrease in serum total thyroxine [TT4] concentration and amelioration of clinical signs). Diagnosis of hyperthyroidism was based on clinical signs compatible with hyperthyroidism, increased TT4 serum concentration and increased thyroidal uptake of $^{99m}TcO_4^-$. To assess the clinical condition of the cats, initial screening included physical and routine laboratory examinations (e.g., CBC, biochemistry). GFR was measured 1 day before and 1, 4, 12 and 24 weeks after treatment with $^{131}I$. Biochemistry and measurement of TT4 were repeated 1, 4, 12 and 24 weeks after treatment. Cats maintained their original diet throughout the study period.

### Plasma Exogenous Creatinine-Iohexol Clearance Test

Cats were fasted for at least 10 hours before the start of the clearance test and fed immediately after the end of the sampling period. Water was offered *ad libitum*. GFR was measured by the combined clearance of exogenous creatinine, exo- and endo- iohexol (PEC-ICT), as previously described.[12,13] Briefly, animals received 40 mg/kg creatinine and 64.7 mg/kg iohexol (Omnipaque 300 [300 mg I/mL], Nycomed Imaging AS, Oslo, Norway). Creatinine was dissolved in 0.9 % sodium chloride (NaCl) (Natrii Chloridum 0.9 %, B.Braun Melsungen AG, Deutschland). First, iohexol then creatinine was administered IV via the cephalic vein. The dead space in the catheter was rinsed with 2 mL of 0.9 % NaCl and the timer was started. Blood samples (2 mL) were taken by jugular venipuncture immediately before iohexol-creatinine administration, and at 5, 15 and 30 minutes, and 1, 2, 3, 6, 8 and 10 hours after administration, placed in EDTA tubes and centrifuged. Aliquots of plasma were stored at -20 ºC until assayed. Plasma creatinine concentration was measured using a validated enzymatic method (Vettest analyzer, Idexx Laboratories Europe B.V., Amsterdam, The Netherlands). The upper limit of quantification was 13.6 mg/dL, within- and between-day coefficients of variation (CV) were < 3 %, and there was linear correlation between theoretical and measured concentration within quantification limits. Plasma exo-iohexol and endo-iohexol concentration was measured using a validated high performance liquid

chromatographic (HPLC) method with ultraviolet (UV) detection.[13] The lower limit of quantification was 1.2 and 8.8 μg/ml for endo-iohexol and exo-iohexol, respectively. CV was < 5 % and calibration curves were linear at low and high concentration ranges. The ratios of exo-iohexol and endo-iohexol stereo-isomers in the Omnipaque solution were 81.9 and 18.1 % respectively.

**Pharmocokinetic Analysis**

Pharmacokinetic analyses were performed using WinNonlin (WinNonlin Version 4.0.1, Scientific Consulting Inc. Apex, NC). Plasma data were subjected to non-compartmental analysis with a statistical moment approach. The area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity.[11] Plasma clearance of creatinine, exo- and endo-iohexol was determined by dividing dose administered by AUC and indexed to bodyweight (BW) (mL/min/kg).

**Statistical Analysis**

A general linear model (Systat version 8.0, SPSS Inc. Chicago IL) was used to test for differences between GFR techniques before and 1, 4, 12 and 24 weeks after treatment at a global significance level of 0.05 and the 3 techniques were compared pairwise at a Bonferroni-adjusted comparison-wise significance level of 0.017 (= 0.05/3). The same model was used to test for differences in GFR techniques among the time-points (i.e. before and 1, 4, 12 and 24 weeks after treatment) and for interactions between time-point and method at a global significance level of 0.05. Moreover, for each GFR marker, time points before (0) and 1, 4, 12 and 24 weeks after treatment were compared pairwise at a significance level of 0.05. The correlation between GFR values calculated by PexICT and PECCT, PenICT and PECCT and PexICT and PenICT was expressed in scatter plots. The 95 % confidence intervals of the slopes and intercepts of these scatter plots were calculated to evaluate respective relative and absolute systematic errors in 1 or both of the clearance methods compared in the scatter plot. These errors lead to between-method differences which can be evaluated with a Bland-Altman plot. A Bland-Altman plot was used to measure bias over the range of measured GFR values by comparison of PexICT and PECCT, PenICT and PECCT and PexICT and PenICT. The difference between 2 GFR values by 2 methods in a cat at a specific time point before or 1, 4, 12, or 24 weeks after $^{131}$I treatment, was plotted on the *y*-axis. The average of these

same GFR values of the 2 methods was plotted on the *x*-axis, which generates a scatter diagram.[22]

Results are expressed as mean ± standard deviation (SD).

## Results

### Animals

BW before treatment was 4.1 ± 1.3 kg, and increased to 5.3 ± 1.5 kg at 24 weeks after treatment. One cat was lost for follow-up 12 weeks after treatment owing to euthanasia because of malignant neoplasia of the pleura. Two cats failed to receive follow-up because of aggressive behavior, 12 and 24 weeks after treatment, respectively. The basal plasma creatinine concentration increased from 85 ± 34 μmol/L (1.0 ± 0.4 mg/dL) before treatment to 144 ± 49 μmol/L (1.6 ± 0.6 mg/dL) 24 weeks after treatment (reference values, 9-133 μmol/L [0.1-1.5 mg/dL]). Before and 1 week after treatment, 2 cats were azotemic. At 4, 12 and 24 weeks after treatment, the number of azotemic cats was 4, 8 and 9 respectively.

The serum TT4 concentration decreased from 104 ± 56 nmol/L before treatment to 20 ± 20 nmol/L 24 weeks after treatment (reference values, 14-45 nmol/L).

### Comparison of GFR methods

Seventy-two GFR assessments (each of them including the 3 markers) were performed. The mean ± SD and range for the PECCT, PexICT and PenICT and the mean ± SD serum TT4 concentration at the different time points are presented in Table 1. The ratio between exo-iohexol and endo-iohexol concentration in the analysed samples was 3.5 ± 0.9. The part of the AUC extrapolated to infinity expressed as percent of the total AUC was higher than 25 % in 14/72 kinetics of creatinine clearance (range, 0.3-48 %), but was below 25 % in all kinetics of exo-iohexol and endo-iohexol clearance (range, 1-20 and 1-22 %, respectively). Globally, the 3 GFR methods resulted in significantly ($P < 0.001$) different GFR values. A statistically significant difference between mean values of PECCT and PexICT (-0.254 mL/min/kg, $P < 0.05$), PECCT and PenICT (-0.716 mL/min/kg, $P < 0.001$), and PexICT and PenICT (0.463 mL/min/kg, $P < 0.001$), was observed before and 1, 4, 12 and 24 weeks after treatment. These differences in GFR among different methods were the same ($P = 0.999$) at all time points. The scatter plots of GFR values calculated by either PexICT or PenICT versus PECCT and of GFR values calculated by PenICT versus PexICT are shown in Figures 1A, 2A and 3A. A good correlation among the 3 methods is visible in Figures 1A, 2A and 3A. The 95 % confidence intervals for the slope and intercept respectively of these correlation plots are [0.825; 0.954] and [-0.158; 0.187] for 1A, [0.656; 0.799] and [-0.251; 0.132] for 2A and [0.742; 0.867] and [-0.194; 0.109] for 3A. All correlation plots had no evidence of systematic

errors (value of 0 was included in all confidence intervals), however all 3 correlation plots showed a relative error (value of 1 not included in confidence intervals). This relative systematic error is indicated by bias in Bland-Altman plots. Bland-Altman comparisons of PECCT, PexICT and PenICT are shown in Figures 1B, 2B and 3B. Bias among clearance methods is clearly visible for comparison among PenICT and PECCT (2B) and PexICT (3B), with average GFR (along the *x*-axis) increasing difference among GFR measurements (along the *y*-axis). The bias is less clearly visible in comparison of PECCT and PexICT. Nonetheless, in all 3 plots the majority of the measurements are spread in the area of the *y*-axis above 0, proving that PECCT structurally generates higher GFR values than do PexICT (1B) and PenICT (2B), and PexICT generates higher GFR values than does PenICT (3B). The highest difference was between PECCT and PenICT, which is visible in the highest limits of agreement (mean difference ± 2*SD) and mean difference (Figure 2B).

**Evaluation of GFR after treatment**

All 3 techniques indicated decreased GFR after $^{131}$I treatment in all cats (Table 1). For each of the 3 techniques separately, there were significant differences ($P < 0.001$) in GFR value for all time points.

There was a significant decrease in PexICT between timepoint 0 and 1 (-23%), 4 (-39 %), 12 (-41 %) and 24 weeks (-47 %) after treatment ($P < 0.01$). There also was a significant -23 % decrease in PexICT between 1 and 12 weeks ($P = 0.041$) and a significant -33 % decrease between 1 and 24 weeks ($P = 0.002$). At 1, 4, 12 and 24 weeks after treatment, respectively, 12, 14, 14 and 13 cats showed a decrease in GFR higher than the between-day variability of 8.3 %, which has been described in aged healthy cats.[13] Changes in PexICT are shown in Figure 4A.

Similarly, PenICT decreased significantly between time point 0 and 1 (-28 %), 4 (-42 %), 12 (-44 %) and 24 (-50 %) weeks after treatment ($P < 0.01$). It also decreased by 26 % between 1 and 12 weeks after treatment ($P = 0.020$), and by 31 % between 1 and 24 weeks after treatment ($P = 0.001$). At 1, 4, 12 and 24 weeks after treatment, respectively, 10, 13, 13 and 12 cats showed a decrease in GFR higher than the between-day variability of 19.1 % which has been described in aged healthy cats.[13] Changes in PenICT are shown in Figure 4B.

There was also a significant decrease in PECCT between time point 0 and 1 (-22 %) ($P = 0.002$), 4 (-34 %), 12 (-34 %), and 24 (-40 %) weeks ($P < 0.001$) after $^{131}$I treatment. No other statistically significant differences however, were observed among other time points. At

1, 4, 12 and 24 weeks after treatment, respectively 8, 12, 9 and 11 cats indicated a decrease in GFR higher than the between-day variability of 21.6 %, which has been described in aged healthy cats.[13] Changes in PECCT are shown in Figure 4C.

**Table 1. Mean ± standard deviation (range) of GFR measurements (mL/min/kg) with exo-iohexol (PexICT), endo-iohexol (PenICT) and creatinine (PECCT) in 15 hyperthyroid cats before (time point 0) and 1, 4, 12 and 24 weeks after treatment with radioiodine.**

| Time point | Cats (n) | Serum TT4 concentration (nmol/L) | PexICT | PenICT | PECCT |
|---|---|---|---|---|---|
| 0 | 15 | 104 ± 56 | 3.1 ± 1.33 [a] (1.3 - 5.6) | 2.6 ± 1.20 [a] (0.9 - 4.5) | 3.4 ± 1.50 [a] (1.2 - 6.0) |
| 1 week | 15 | 37 ± 30 | 2.3 ± 0.95 [b] (1.1 - 3.8) | 1.9 ± 0.83 [b] (0.7 - 3.7) | 2.5 ± 0.96 [b] (1.1 - 4.0) |
| 4 weeks | 15 | 15 ± 26 | 1.9 ± 0.89 [b,c] (0.9 - 3.5) | 1.4 ± 0.61 [b,c] (0.6 - 2.5) | 2.1 ± 0.98 [b] (1.1 - 4.2) |
| 12 weeks | 14 | 19 ± 25 | 1.7 ± 0.75 [c] (0.7 - 3.2) | 1.3 ± 0.52 [c] (0.5 - 2.2) | 1.9 ± 0.89 [b] (0.8 - 3.8) |
| 24 weeks | 13 | 20 ± 20 | 1.7 ± 0.71 [c] (0.8 - 2.9) | 1.2 ± 0.49 [c] (0.5 - 2.1) | 2.0 ± 0.83 [b] (0.9 - 3.7) |

**When the superscripts ([a, b, c]) are different between time points for a specific marker, a statistically significant difference is observed between the values. P values are provided in "Results".**

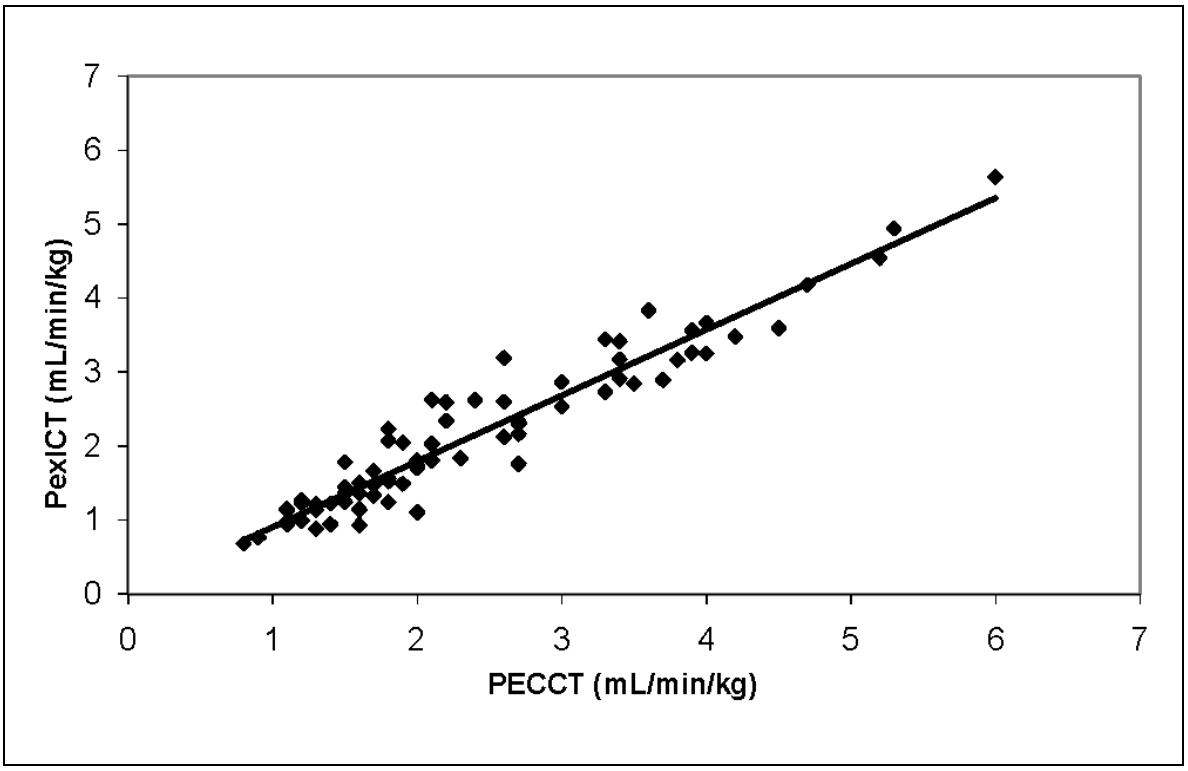


**Figure 1A. Glomerular filtration rate (GFR) calculated by PexICT plotted against GFR values calculated by PECCT. The linear regression equation was $y = 0.8894x + 0.0145$ ($r^2 = 0.9152$).**

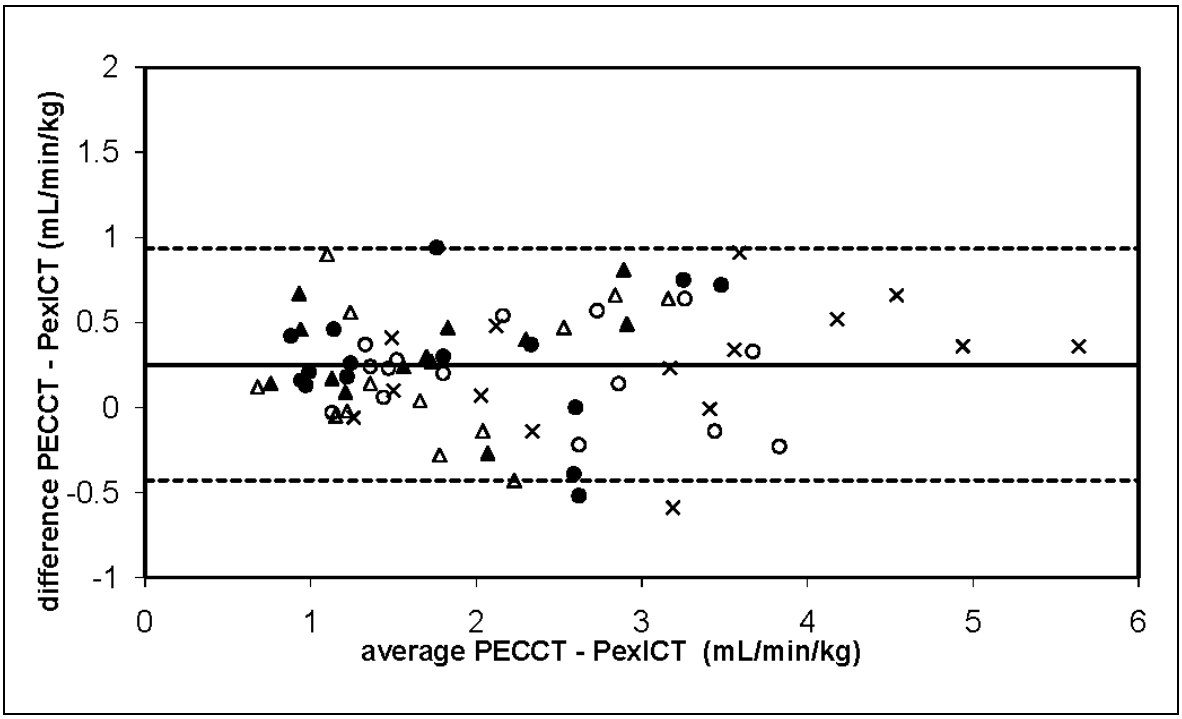


**Figure 1B. Bland-Altman plot of the differences between PECCT and PexICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean difference ± 2SD. x time point 0; ○1 week after treatment; ● 4 weeks after treatment; Δ 12 weeks after treatment; ▲24 weeks after treatment.**

**PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test.**

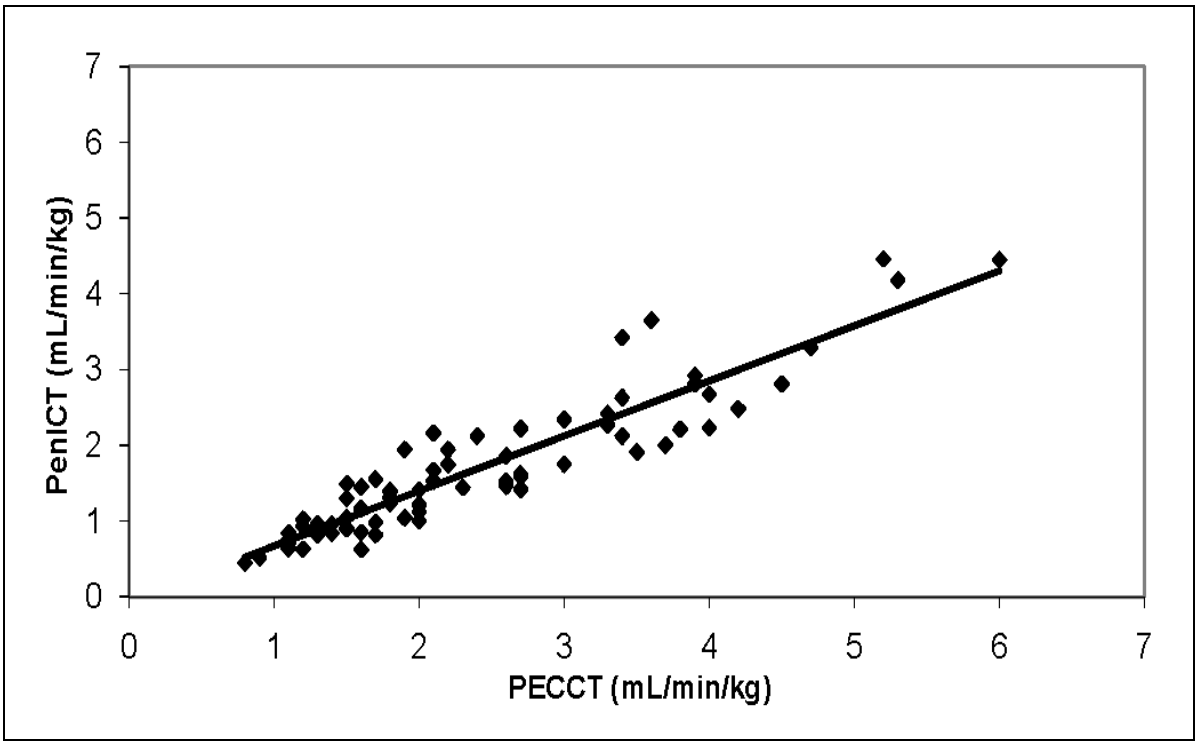


**Figure 2A. Glomerular filtration rate (GFR) calculated by PenICT plotted against GFR values calculated by PECCT. The linear regression equation was $y = 0.7274x - 0.0593$ ($r^2 = 0.8540$).**

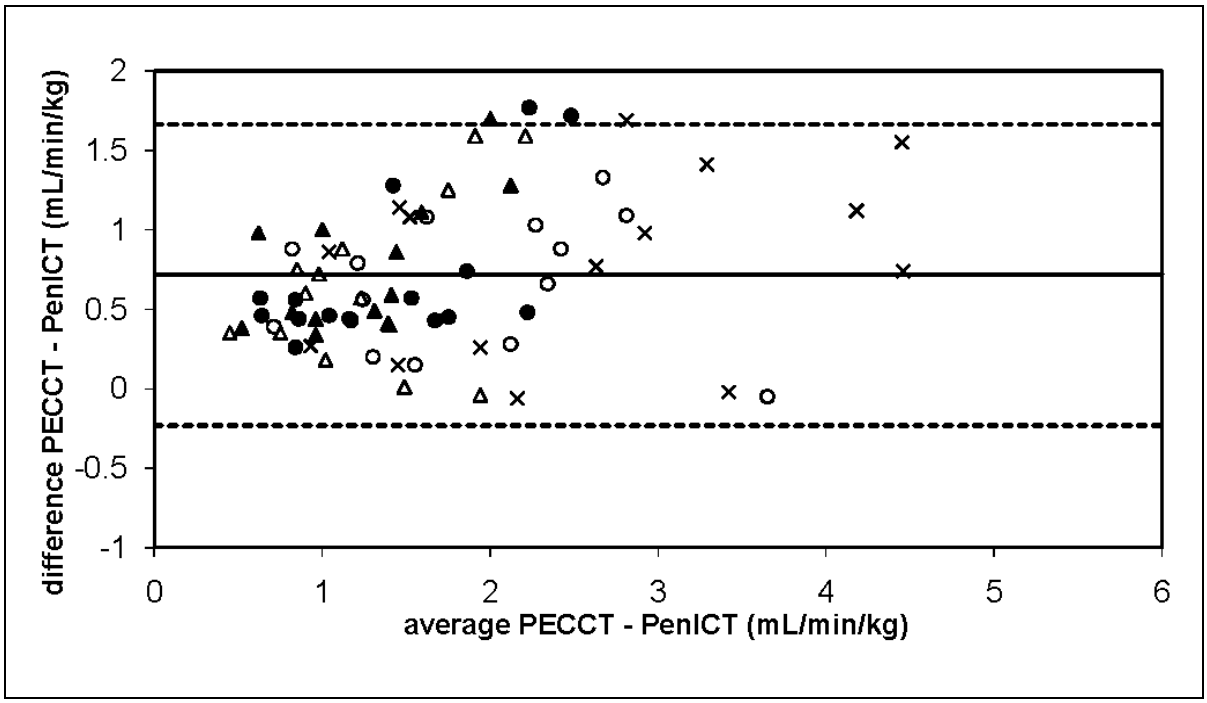


**Figure 2B. Bland-Altman plot of the differences between PECCT and PenICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean difference ± 2SD. x time point 0; ○1 week after treatment; ● 4 weeks after treatment; Δ 12 weeks after treatment; ▲24 weeks after treatment.**

**PECCT, plasma exogenous creatinine clearance test; PenICT, plasma endo-iohexol clearance test.**

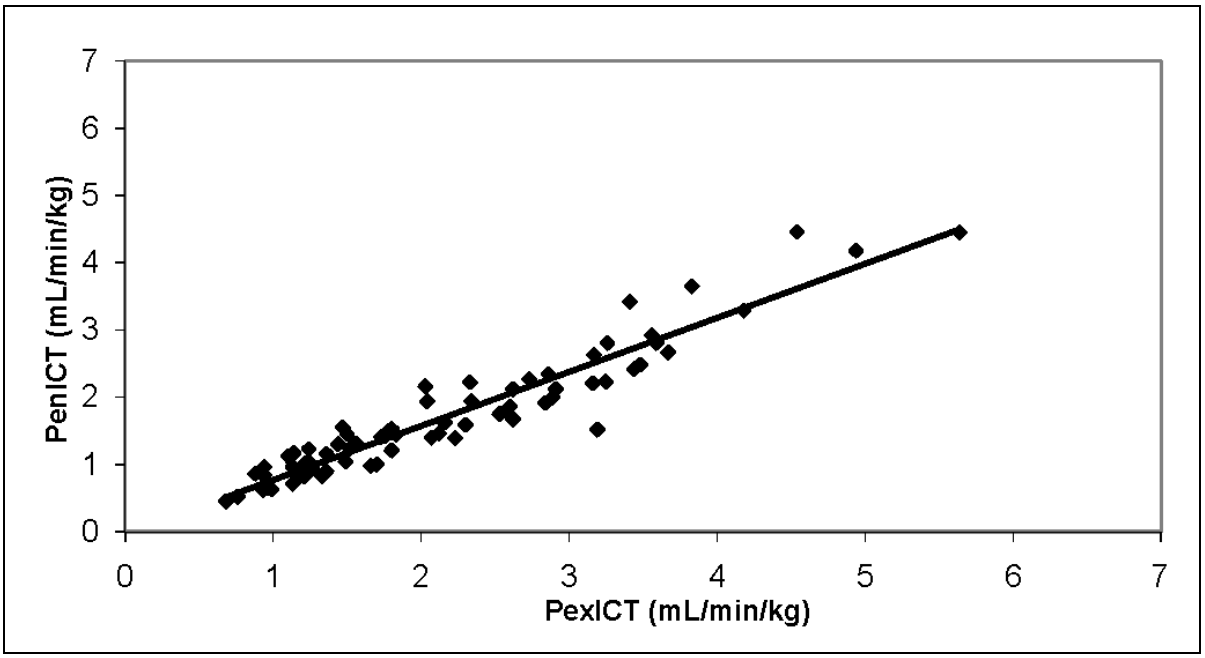


**Figure 3A. Glomerular filtration rate (GFR) calculated by PenICT plotted against GFR values calculated by PexICT. The linear regression equation was $y = 0.8045x - 0.0424$ ($r^2 = 0.9029$).**

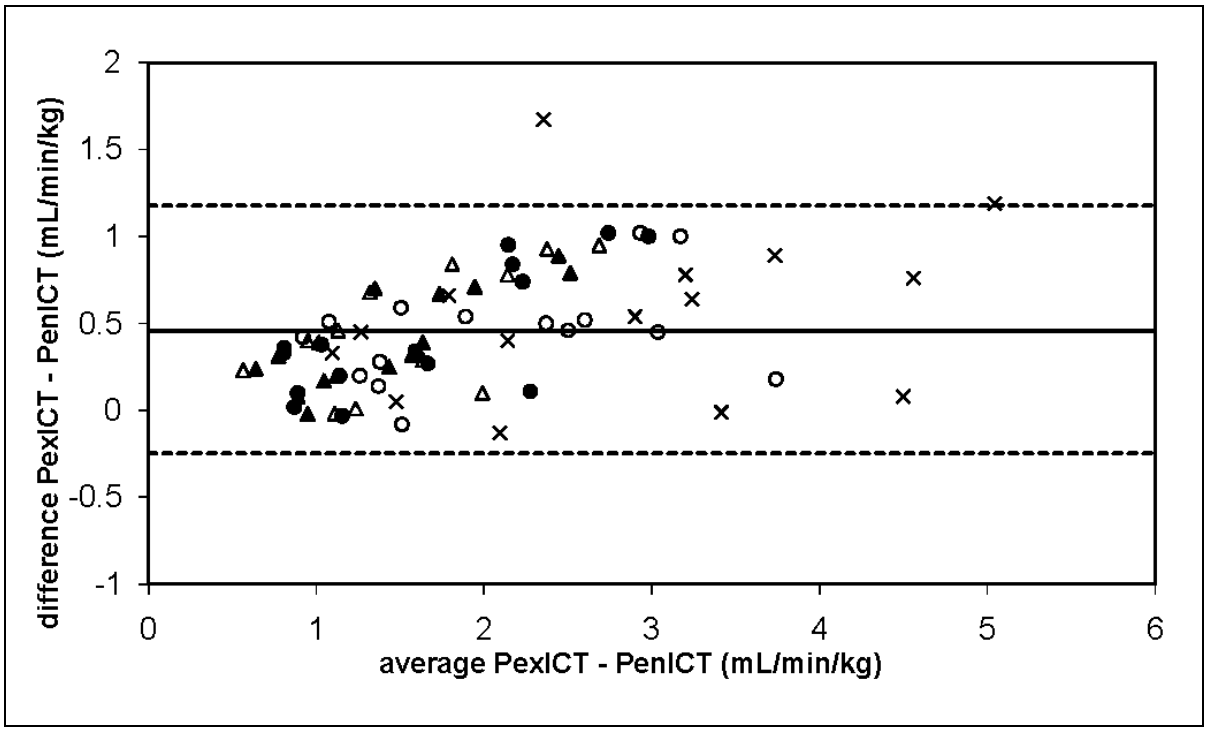


**Figure 3B. Bland-Altman plot of the differences between PenICT and PexICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean difference ± 2SD. x time point 0; ○1 week after treatment; ● 4 weeks after treatment; Δ 12 weeks after treatment; ▲24 weeks after treatment.**

**PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test.**

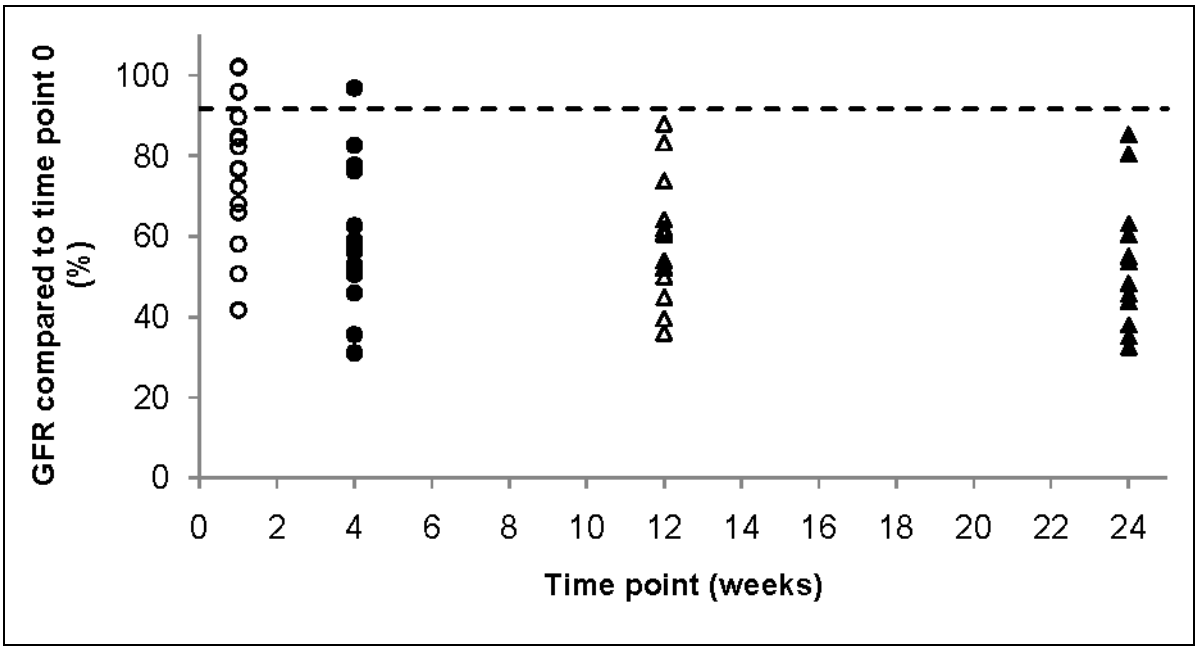


**Figure 4A. Changes in glomerular filtration rate (GFR) value, expressed as percent of GFR at time point 0, at 1 (○), 4 (●), 12 (Δ) and 24 (▲) weeks after treatment measured with PexICT. Dotted lines represent between-day variability.**

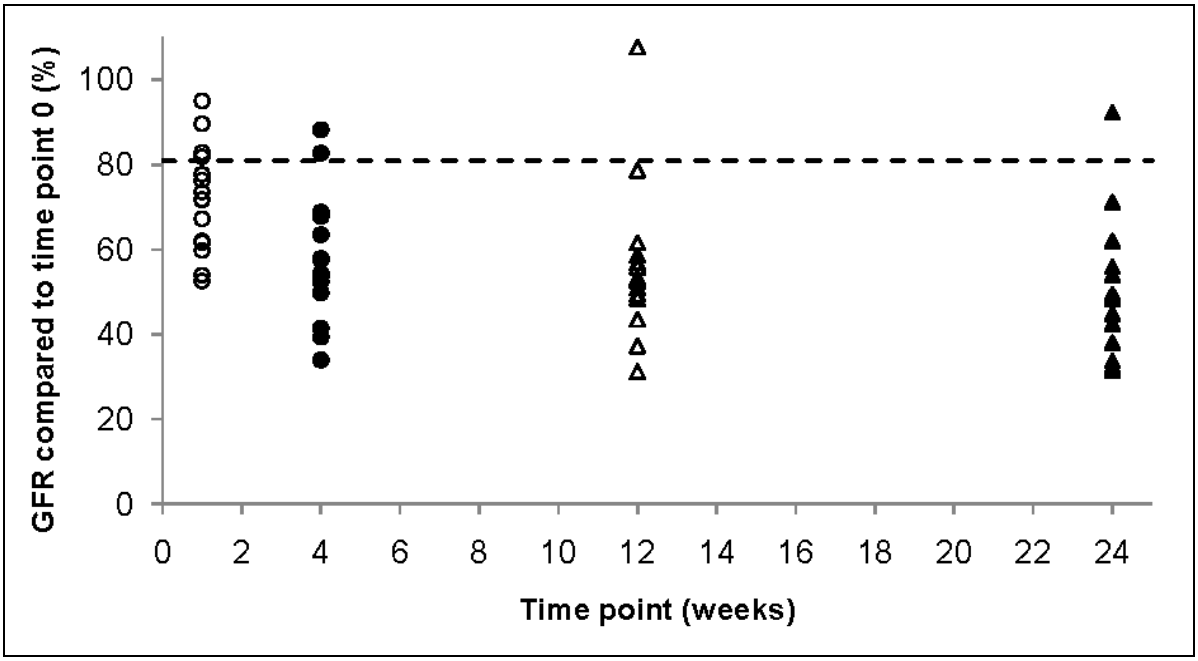


**Figure 4B. Changes in glomerular filtration rate (GFR) value, expressed as percent of GFR at time point 0, at 1 (○), 4 (●), 12 (Δ) and 24 (▲) weeks after treatment measured with PenICT. Dotted lines represent between-day variability.**

**PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test.**

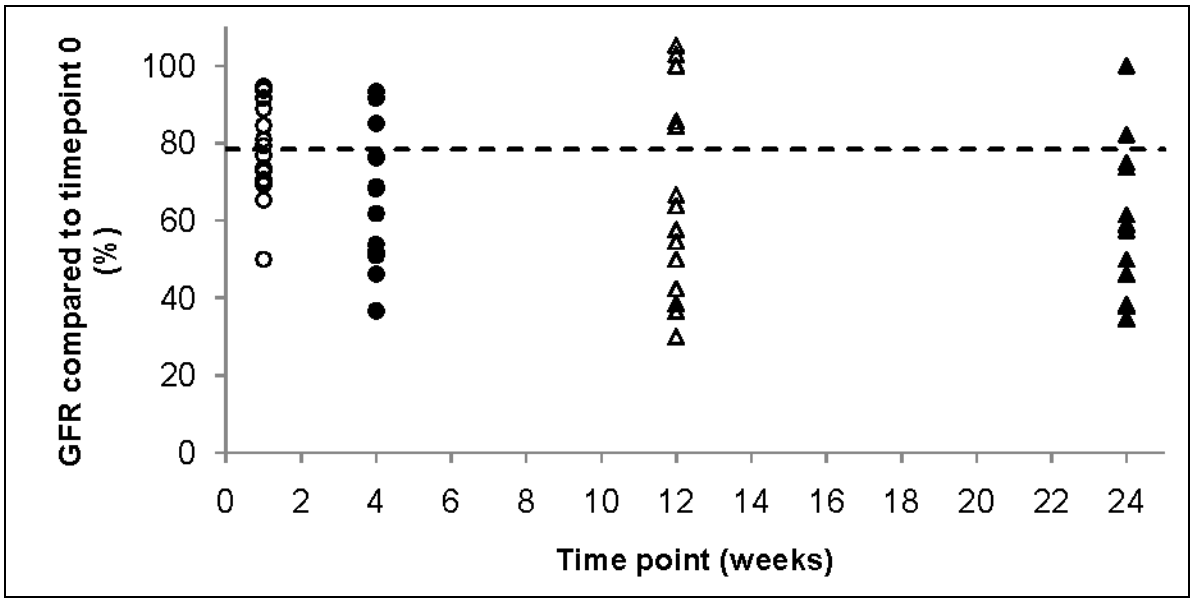


**Figure 4C. Changes in glomerular filtration rate (GFR) value, expressed as percent of GFR at time point 0, at 1 (○), 4 (●), 12 (∆) and 24 (▲) weeks after treatment measured with PECCT. Dotted lines represent between-day variability.**

**PECCT, plasma exogenous creatinine clearance test.**

## Discussion

One of the major finding of this study is that a statistically significant difference ($P < 0.001$) in GFR value was observed among the 3 GFR assessment techniques, whatever the time of GFR testing (i.e., before and after treatment). The difference in GFR assessment among the techniques is noted in the deviation of the slope from value 1 but the correlation among the 3 different clearance techniques, however, seems to be acceptable (Figure 1A, 2A and 3A). A more important factor when comparing different techniques is between-method differences as a measurement of agreement with each other. This can be shown graphically in Bland-Altman plots (Figure 1B, 2B and 3B). The difference among PenICT and PECCT and PexICT respectively increased with increasing mean GFR, thereby producing a bias. This can be caused by an underestimation of PenICT, an overestimation of PECCT and PexICT respectively, or both. PECCT generated systematically higher values of GFR compared to PexICT in hyperthyroid cats before and after treatment, which is comparable to findings in healthy cats.[13] However, GFR values measured with PenICT are higher compared to PECCT and PexICT in healthy cats, in contrast to the lower PenICT compared to PECCT and PexICT in the hyperthyroid cats described in this study. There is no major bias visible in the Bland-Altman graph showing the comparison between the PECCT and PexICT (Figure 1B). The mean difference between these techniques is low. In combination with the good reproducibility that is described for PECCT,[13] the results of the present study suggest that the PECCT is a reasonable alternative for PexICT in hyperthyroid cats before and after treatment.

The differences in GFR values according to the technique used can first be explained by laboratory variations. Creatinine and iohexol were measured in different laboratories using different assays. Nevertheless, creatinine as well as iohexol assays used in the present study had been validated previously.[13] The gold standard assay method for creatinine in plasma is HPLC. However, the enzymatic method used here is most frequently used in routine clinical laboratories and is proven to be a reasonable alternative to the HPLC method.[12] Other conditions (e.g., storage time and temperature of plasma samples) were similar for both iohexol and creatinine. Interestingly, a significant difference between GFR values obtained by PexICT and PenICT was observed in this study whereas both markers were assayed using the same HPLC method in the same laboratory. Colorimetric methods,[8,9] atomic emission spectroscopy,[20] or x-ray fluorescence methods,[23-25] measuring the amount of iodine and

consequently indirectly iohexol, have been proposed also for plasma iohexol assay. Iohexol assay by HPLC has good specificity, sensitivity, accuracy and reproducibility, and moreover allows separate measurement of the 2 stereoisomers: endo-iohexol and exo-iohexol.[26] In dogs, this latter compound, which represents the major stereoisomer, is frequently selected as a GFR marker.[10,26] Discrepancies have been described between GFR calculation using exo- and endo-iohexol in dogs[10] and cats,[12,27] but not in humans.[28] The reason for this difference remains unclear, but these results emphasize the fact that disposition of exo-iohexol and endo-iohexol are not the same and consequently assay of total iohexol could lead to misinterpretation because it is an hybrid concentration reflecting the sum of 2 stereoisomers with different clearances.

The combined use here of creatinine, exo-iohexol and endo-iohexol, as performed previously in dogs and cats,[7,8,12,13] also raises the issue of a potential interference among the analytes. Such an interference however is unlikely. Creatinine is an endogenous compound and the peak concentration observed here (up to 1795 μmol/L [20.3 mg/dL]) could be observed in severely azotemic patients. Disposition of exo-iohexol and endo-iohexol moreover does not seem to be affected by mild to moderate azotemia in cats.[12]

In the present study, pharmacokinetic analysis also cannot explain the differences observed because a non-compartmental approach was used similarly for PECCT, PenICT and PexICT, as described in dogs.[10,11] The non-compartmental and compartmental approaches moreover are comparable for clearance of iohexol[29,30] and creatinine[11] in dogs. Despite the wide ranges of the AUC parts extrapolated to infinity, 80 % of the pharmacokinetic analyses showed an AUC part extrapolated to infinity lower than 25 % of the complete AUC. Consequently, the sampling strategy (i.e. number of blood samples and time of last sampling) can be considered appropriate in healthy, hyperthyroid and moderately azotemic cats. In more severely azotemic cats, the last blood sample should be delayed, especially for PECCT.

Discrepancies in GFR results also can be caused by physiologic differences in renal handling of the substances.[23] Because a combined PEC-ICT was used, factors related to the cats themselves cannot explain the difference in PECCT, PexICT and PenICT. Moreover, creatinine[7] and iohexol[8] appear to be reliable GFR markers for cats. Use of urinary clearance of inulin, considered the gold standard method, would have been helpful to compare the

accuracy of each GFR marker in the follow-up of the animals. Urinary clearance testing, however, is difficult to propose to owners in our experience, because it is tedious and time-consuming for the staff, it is stressful (e.g. anesthesia may be required) and potentially harmful (e.g., urinary tract infection) for the animal, and accurate measurement of urine volume often is difficult. Clearances of both creatinine and iohexol already have been proposed as alternatives for GFR measurements in cats.[7-9,12]

Whatever the cause of the differences among PexICT, PenICT and PECCT, all 3 techniques nevertheless indicated the same trend with decreasing GFR after $^{131}$I treatment. This finding indicates that if the same marker is used for repeated GFR testing, it will provide clinically relevant information similar to what would have been provided by the use of the 2 other GFR markers. For each of the 3 techniques, a significant decrease in GFR value was observed between time point 0 and all other time points. This decrease stabilized 4 weeks after treatment, with very little decline afterwards, although GFR values determined by PECCT at time point 1 week were not statistically significant from those observed at time points 4 and 24 weeks. These results show that PECCT is a promising alternative for GFR measurement, although it might be less sensitive to small changes in GFR compared to PexICT and PenICT. The decrease in GFR from 0 to 24 weeks after treatment was relatively consistent whatever the marker used (47 %, 50 % and 40 % for PexICT, PenICT, and PECCT, respectively). This is much higher than the between-day CV of each method (8.3, 19.1, and 21.6 %, respectively) in aged healthy cats[13] and proves that the decrease in GFR is caused by a change in glomerular function, and not by intrinsic between-day variability. Several studies in the literature have investigated GFR in hyperthyroid cats before and after treatment and describe a decrease in GFR after treatment as shown in this study. However, the follow-up in these studies was measured only before and at 1 time point (6 days, 30 days or 6 weeks) after treatment. Hence, these follow up studies are shorter and less extensive compared with the study described here.[18-20]

## Conclusion

In conclusion, PexICT, PenICT, and PECCT, although providing different GFR values, can be used for follow-up of the decrease in GFR observed in hyperthyroid cats after treatment. Nevertheless, the same GFR marker should be used throughout the follow-up period. GFR testing at 4 weeks post-treatment could also be reasonably recommended to estimate the final loss in renal function in cats after $^{131}$I treatment. Nevertheless, further investigations in a larger population are needed.

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# CHAPTER 2

# EVALUATION OF GFR MEASUREMENTS IN HEALTHY CATS, CATS WITH HYPERTHYROIDISM AND CATS WITH CHRONIC KIDNEY DISEASE

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# CHAPTER 2

**Summary**

The study investigated plasma clearance of exogenous creatinine (PECCT), exo-iohexol (PexICT) and endo-iohexol (PenICT) in 6 healthy (H) cats, 4 cats with chronic kidney disease (CKD) and 6 hyperthyroid (HT) cats to assess potential differences in GFR measurement over the low, normal and high range of GFR values possible in cats.

The PECCT, PexICT and PenICT were performed in a combined manner. All pairwise comparisons between PexICT and PenICT and PECCT were significantly different in H cats. GFR values differed significantly only between CKD cats and HT cats for PexICT, PenICT and PECCT, between H and HT cats for PexICT, PECCT, and between CKD and H cats for PenICT and PECCT.

The PexICT and PenICT observed larger differences between CKD and HT cats, although PECCT observed smaller differences between H, HT and CKD cats. Differences between clearance techniques seem to be correlated to range in GFR.

## Introduction

Glomerular filtration rate measurement is a precise and direct evaluation of glomerular function, in contrast to the indirectly and crudely estimating assay of circulating blood urea nitrogen (BUN) and serum creatinine concentration. Moreover, measurement of GFR is more sensitive in detecting a decreased kidney function before insufficiency or chronic kidney disease (CKD) develops.[1,2] Plasma clearance methods are less laborious and easier to apply in a clinical setting compared to urinary clearance techniques. When applying plasma clearance of iohexol, plasma iohexol concentration can be assayed with high-performance liquid chromatography (HPLC), which measures both stereo-isomers exo- and endo-iohexol. This way, two measures of GFR are provided after iohexol administration: plasma clearance of exo-iohexol (PexICT) and of endo-iohexol (PenICT).[3-5] The plasma clearance of exogenous creatinine test (PECCT) has been suggested to be a promising alternative for GFR measurement in cats.[4,5] Combined use of creatinine and iohexol in a plasma exogenous creatinine-iohexol clearance test (PEC-ICT) has been described in healthy cats, moderately azotemic cats and hyperthyroid cats before and after treatment with radioiodine ($^{131}$I).[4-6] The combined use of different markers allows minimal time- and space related variation between the methods.

Discrepancies within H cats, HT cats or cats with CKD exist when GFR is measured using 2 or 3 different clearance techniques due to external and internal factors.[4-10] We hypothesized that discrepancies can arise due to the range of GFR in which the clearance method is applied. To date there is no study comparing different techniques in separate groups expected to have GFR values spread over the whole range possible in cats: low in cats with CKD, normal in H cats and high in HT cats. The objectives of this study were to compare PexICT, PenICT and PECCT within as well as between groups of H cats, HT cats and cats with CKD.

## Materials and Methods

### Cats

The study was conducted according to guidelines for animal care, with consent of the Ethical committee of the Faculty of Veterinary Medicine from Ghent University, Belgium and informed consent by the owners of the cats with CKD and HT cats. The study included 16 cats divided into 3 groups: cats with CKD (n = 4), H cats (n = 6) and HT cats (n = 6). Healthy cats were obtained from the population of laboratory animals of Ghent university and included if there were no clinically significant abnormalities on initial screening (physical and routine laboratory examinations [CBC, biochemistry and measurement of total T4 (TT4)], evaluation of feline immunodeficiency virus [FIV] and feline leukemia virus [FIV] status), abdominal ultrasonography and cystocentesis followed by urinalysis (dip-strip tests, microscopic analysis, protein/creatinine ratio, urine specific gravity and bacteriologic culture). To assess the clinical condition of the HT cats and cats with CKD, initial screening included physical and routine laboratory examinations (CBC, biochemistry) and urinalysis after cystocentesis. HT cats were included when clinical signs compatible with hyperthyroidism were present, increased serum total thyroxine (TT4) concentration and increased thyroidal uptake of $^{99m}TcO_4^-$ on a scintigraphic scan were observed. Antithyroid drugs had to be discontinued at least 3 weeks prior to inclusion. Cats with CKD were included based on compatible clinical signs and azotemia compatible with International Renal Interest Society [IRIS] stage II or III (www.IRIS-kidney.com).

### Plasma Exogenous Creatinine-Iohexol Clearance test

The combined clearance of exogenous creatinine, exo- and endo-iohexol was performed as previously described.[4-6] Pharmacokinetic analyses were performed using WinNonlin (Version 4.0.1, Scientific Consulting Inc. Apex, NC). Plasma data were subjected to non-compartmental analysis with a statistical moment approach. The area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity.[11] Plasma clearance of creatinine, exo- and endo-iohexol was determined by dividing dose administered by AUC, and indexed to BW (mL/min/kg).

**Statistical Analysis**

A mixed model with cat as random effect (Systat version 8.0, SPSS Inc. Chicago IL) was used to test for differences between GFR techniques in cats with CKD, HT cats and H cats and for differences between health status (cats with CKD, H and HT cats) within a GFR technique, at a global significance level of 0.05. The 3 techniques in cats with CKD, H or HT cats and the 3 different health statuses (CKD, H , HT) within a technique were compared pair wise at a Bonferroni-adjusted comparison-wise significance level of 0.017 (= 0.05/3) with ANOVA.

The correlation between GFR values calculated by PexICT, PenICT and PECCT in cats with CKD, H and HT cats was expressed in scatter plots. The 95 % confidence intervals of the slopes and intercepts of these scatter plots were calculated, to evaluate respective relative and absolute errors in one or both of the clearance methods compared in the scatter plot. These errors lead to between-method differences which can be evaluated with a Bland-Altman plot. A Bland-Altman plot was used to measure bias over the range of measured GFR values by comparison of PECCT and PexICT, PECCT and PenICT, and PexICT and PenICT in cats with CKD, H and HT cats. The difference between two GFR values using two methods in a cat with CKD, a H or HT cat was plotted on the *y*-axis. The average of these same GFR values of the two methods was plotted on the *x*-axis, which generates a scatter diagram.[12] Limits of agreement are expressed by the mean difference ± 2 * standard deviation (SD).
Results are expressed as range and mean ± SD.

## Results

### Cats

The study included 6 H cats with an age of 7-12 months and BW range of 4.3 - 5.6 kg (4.7 ± 0.5 kg), 6 HT cats with an age of 8-16 years and BW range of 2.6 - 6.2 kg (4.0 ± 1.2 kg), and 4 cats with CKD (IRIS stage II, n = 3; IRIS stage III, n = 1) with an age of 10-13 years and BW range of 4.5 - 6.6 kg (5.6 ± 0.9 kg).

### Comparison between GFR methods

Sixteen GFR assessments (each of them including the 3 markers) were performed. The mean ± SD and range for the PECCT, PexICT and PenICT are presented in Table 1. The ratio between plasma exo- and endo-iohexol concentration in the analyzed samples was 5.8 ± 2.4. The part of the AUC extrapolated to infinity expressed as % of the total AUC, was higher than 25 % in 1/16 (1 cat with CKD) kinetics of creatinine clearance (range 1-57 %), but was below 25 % in all kinetics of exo-iohexol and endo-iohexol (range 1.5-20 % and 2.8-19 % respectively). The part of the AUC extrapolated to infinity for the three markers in cats with CKD, H and HT cats is described in Table 2. Plasma creatinine concentration did not return to pre-dosing level before the end of the sampling period in 7/16 cats (CKD n = 4, H n = 1, HT n = 2). Plasma exo- and endo-iohexol returned to pre-dosing level in 13/16 cats (CKD n = 1, H n = 6, HT n = 6) before the end of the sampling period.

The GFR methods globally resulted in significant different GFR values ($P < 0.001$) but there was a statistically significant interaction between GFR method and the different groups ($P = 0.004$). A statistically significant difference between mean values of PECCT and PexICT (average difference 0.95 mL/min/kg, $P < 0.001$) and PexICT and PenICT (average difference 1.3 mL/min/kg, $P < 0.001$), though not between PECCT and PenICT (average difference 0.3 mL/min/kg, $P = 0.21$) was observed in H cats. There was no statistically significant difference between GFR values obtained with PexICT, PenICT or PECCT in cats with CKD ($P = 0.386$) or HT ($P = 0.185$) cats.

The scatter plots of GFR values calculated by either PexICT or PenICT versus PECCT and of GFR values calculated by PenICT versus PexICT are shown in Figures 1A, 2A and 3A respectively. A good correlation is visible in figure 1A, but correlation is less good in figures 2A and 3A. The 95% confidence intervals for the slope and intercept respectively of these correlation plots are [0.776;1.042] and [-0.783;0.133] for 1A, [0.687;1.063] and [-

0.333;0.962] for 2A and [0.564;1.149] and [0.050;1.727] for 3A. Correlation plots of PECCT, PexICT and PenICT clearance had no evidence of absolute systematic errors except for correlation between PexICT and PenICT (value of 0 not included in confidence interval of the intercept).

A small bias is visible in the Bland-Altman plots for comparison between PenICT and PECCT (2B) and PexICT (3B), as with increasing average of GFR (along the *x*-axis), the difference between GFR measurements is increasing (along the *y*-axis). The Bland-Altman plots also show the interaction between GFR method and group (P = 0.004), because the majority of measurements of cats with CKD are spread in the area below (Figure 1B) or above (Figures 2B and 3B) the mean difference, the majority of measurements of H cats are spread in the area above (1B) or below (2B and 3B) the mean difference and the majority of measurements of HT cats are spread around the mean difference (1B, 2B and 3B).

The statistical significant difference between PexICT and PECCT clearance (1B) and PenICT (3B) in H cats is visible in the wide spread of differences between GFR measurements along the *y*-axis, and these differences clearly positively (1B) or negatively (3B) differing from 0. The systematic error between PexICT and PenICT (3B) is visible in these majority of values spread below 0, proving that PenICT generates higher clearance values than PexICT, though this is only significant in H cats. The highest difference is between PexICT and PenICT which is visible in the highest limits of agreement (mean ± 2SD) combined with the high mean difference (3B). The mean difference is smallest in 2B, which is in accordance with the absence of significant difference between PECCT and PenICT in cats with CKD, H and HT cats. Only 1 mean value is located outside the limits of agreement in all three Bland-Altman comparisons.

**Comparison of GFR values between cats with CKD, H and HT cats**

There was a significant difference in GFR between cats with CKD, H cats and HT cats for PECCT, PexICT and PenICT (P < 0.001).

There was a significant difference in PexICT between cats with CKD and HT cats (average difference 3.183 mL/min/kg, P < 0.001), and H cats and HT cats (average difference 2.3 mL/min/kg, P < 0.001) but not between cats with CKD and H cats (average difference 0.9 mL/min/kg, P = 0.280). PenICT also significantly differed between cats with CKD and HT cats (average difference 3.4 mL/min/kg, P < 0.001), and differed significantly between cats with CKD and H cats (average difference 2.2 mL/min/kg, P = 0.004) but not between H cats

and HT cats (average difference 1.2 mL/min/kg, P = 0.067). There was a significant difference between all groups for PECCT with a significant difference between cats with CKD and HT cats (average difference 3.6 mL/min/kg, P < 0.001), cats with CKD and H cats (average difference 1.7 mL/min/kg, P = 0.018) and H cats and HT cats (average difference 1.9 mL/min/kg, P = 0.005). These results cannot be seen in the correlation plots, which show clear delineation between cats with CKD and H and HT cats without overlap between groups for PexICT (1A and 3A). There is however a small overlap between H and HT cats for PECCT and PenICT (2A and 3A).

**Table 1. Mean ± SD (range) of plasma clearance (mL/min/kg) of exo-iohexol (PexICT), endo-iohexol (PenICT) and exogenous creatinine (PECCT) in cats with chronic kidney disease (CKD), healthy cats (H) and cats with hyperthyroidism (HT).**

| Health status | Cats (n) | PexICT | PenICT | PECCT |
|---|---|---|---|---|
| CKD | 4 | $0.9 \pm 0.2^{a}$ (0.7 - 1.1) | $0.9 \pm 0.1^{a}$ (0.8 - 1.1) | $1.0 \pm 0.1^{a}$ (0.9 - 1.1) |
| H | 6 | $1.8 \pm 0.3^{a}$ (1.4 - 2.1) | $3.1 \pm 0.6^{b}$ (2.0 - 3.6) | $2.8 \pm 0.5^{b}$ (2.2 - 3.6) |
| HT | 6 | $4.1 \pm 1.2^{b}$ (2.6 - 5.6) | $4.3 \pm 1.2^{b}$ (2.6 - 6.2) | $4.7 \pm 1.2^{c}$ (3.4 - 6.0) |

**When the superscipts (a, b, c) are different between groups for a specific marker, a statistically significant difference is observed between the values. P values are provided in the results.**

**Table 2. Mean ± SD (range) of AUC extrapolated to infinity expressed as % of the total AUC for plasma clearance (mL/min/kg) of exo-iohexol (PexICT), endo-iohexol (PenICT) and exogenous creatinine (PECCT) in cats with chronic kidney disease (CKD), healthy cats (H) and cats with hyperthyroidism (HT).**

| Health status | Cats (n) | PexICT | PenICT | PECCT |
|---|---|---|---|---|
| CKD | 4 | 8.0 ± 8.1 (3.8 - 20.1) | 8.4 ± 7.3 (2.8 - 19.0) | 26.9 ± 20.8 (12.4 - 57.1) |
| H | 6 | 10.7 ± 5.9 (3.1 - 16.4) | 10.3 ± 5.0 (5.6 - 18.2) | 5.0 ± 4.1 (0.9 - 11.8) |
| HT | 6 | 3.9 ± 2.2 (1.5 - 6.8) | 5.7 ± 2.9 (2.9 - 9.5) | 3.1 ± 2.9 (1.0 - 8.7) |

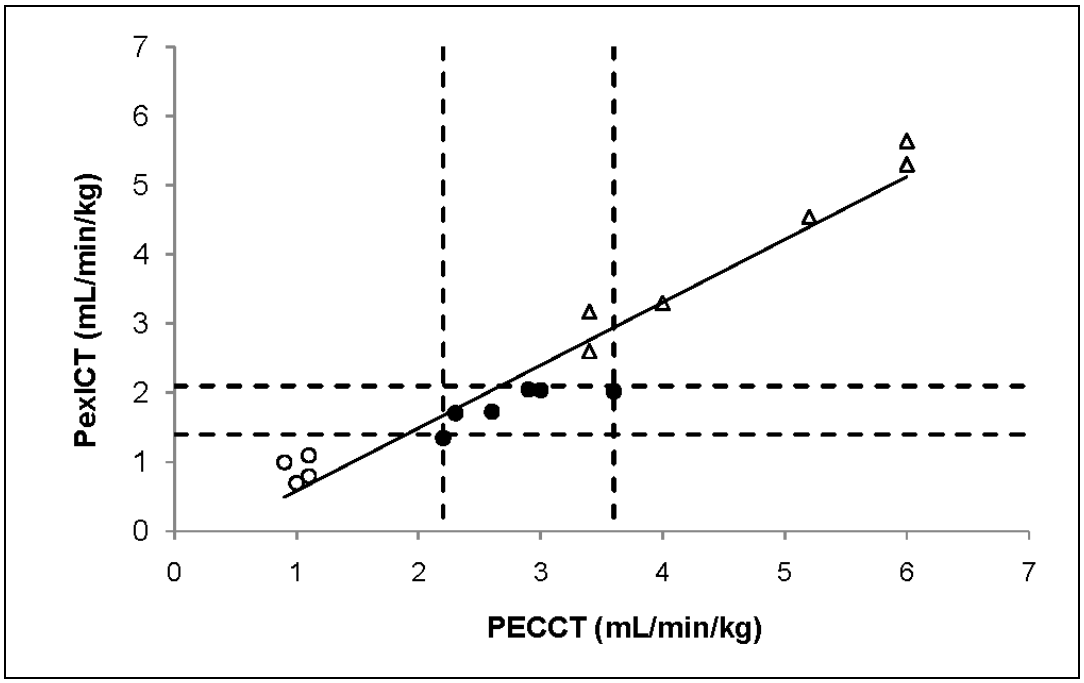


**Figure 1A. GFR calculated by PexICT plotted against GFR values calculated by PECCT. The linear regression equation was $y = 0.908x - 0.325$ ($r^2 = 0.938$). Ranges of H cats for PECCT and PexICT are represented by vertical and horizontal dotted lines respectively.**

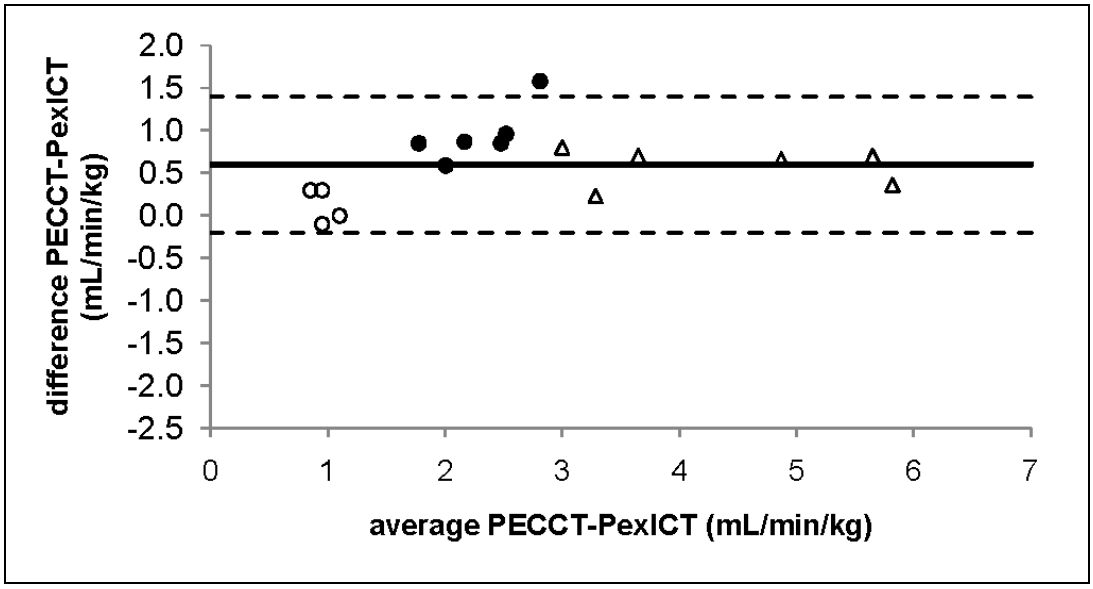


**Figure 1B. Bland-Altman plot of the differences between PECCT and PexICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean differences ± 2*SD.**

**○ cats with CKD, ● H cats, Δ HT cats.**

**PECCT, plasma exogenous creatinine clearance test; PexICT, plasma exo-iohexol clearance test.**

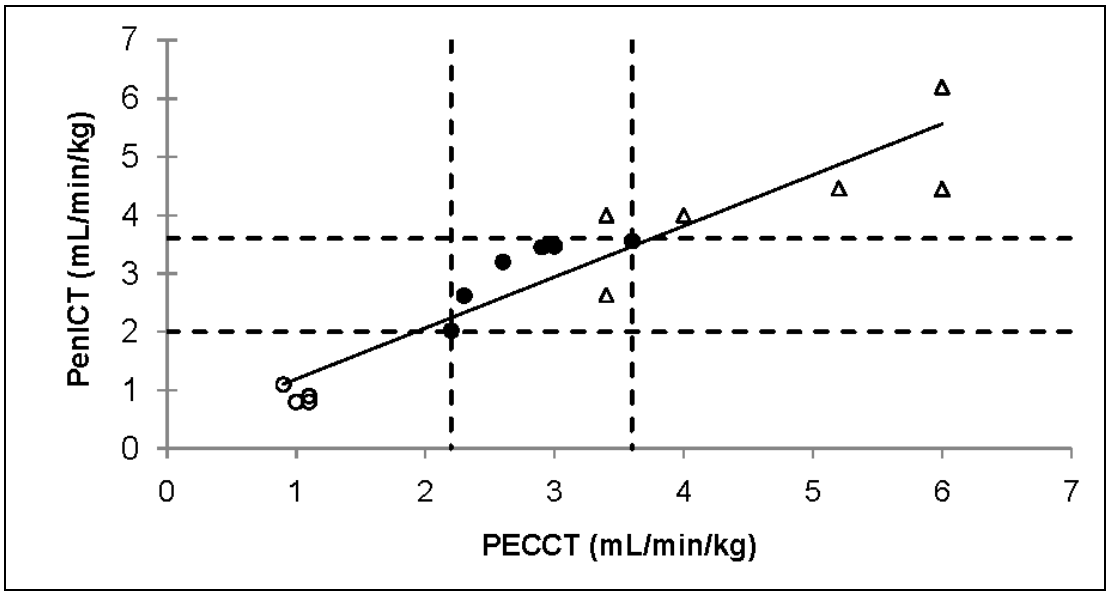


**Figure 2A. GFR calculated by PenICT plotted against GFR values calculated by PECCT. The linear regression equation was $y = 0.875x + 0.314$ ($r^2 = 0.876$). Ranges of H cats for PECCT and PenICT are represented by vertical and horizontal dotted lines respectively.**

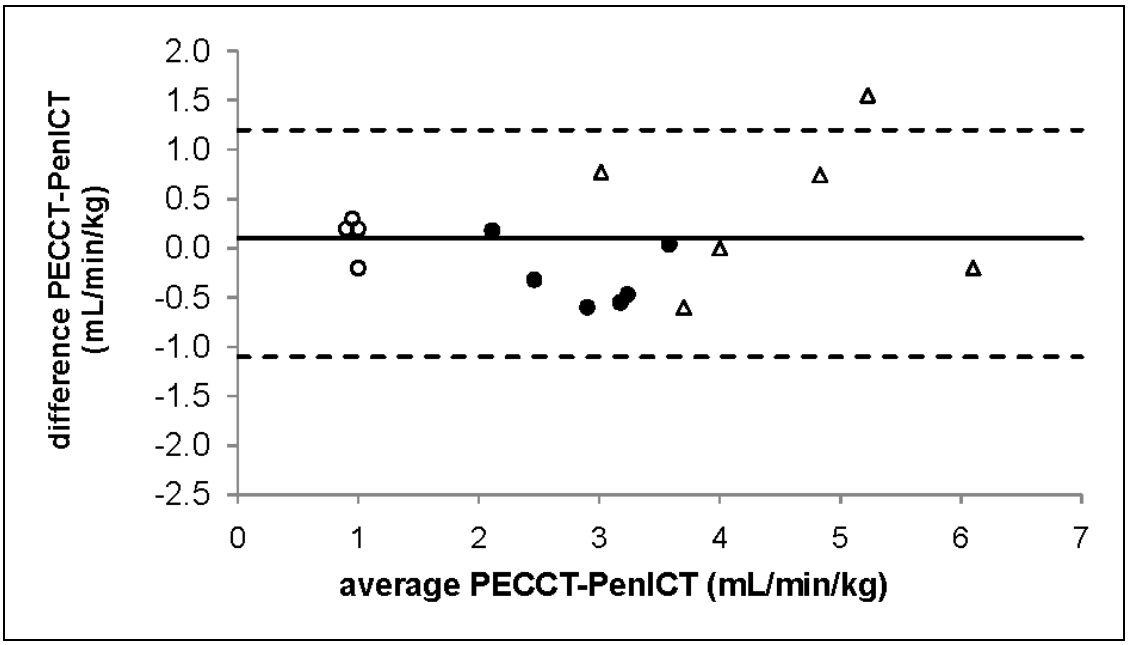


**Figure 2B. Bland-Altman plot of the differences between PECCT and PenICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean differences ± 2*SD.**

**○ cats with CKD, ● H cats, Δ HT cats.**

**PECCT, plasma exogenous creatinine clearance test; PenICT, plasma endo-iohexol clearance test.**

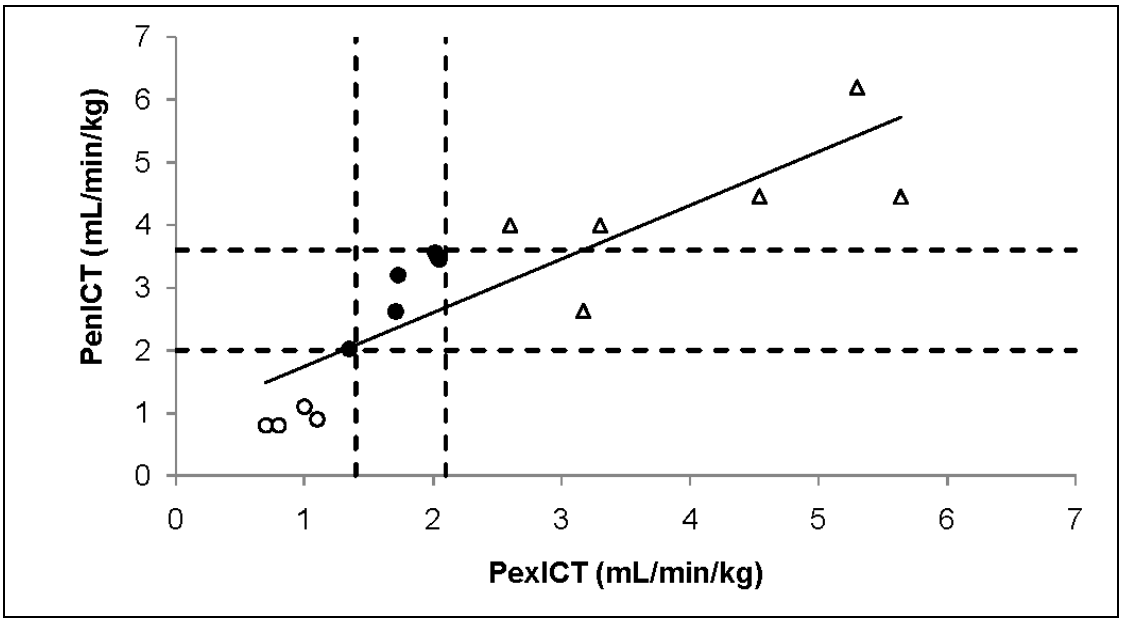


**Figure 3A. GFR calculated by PenICT clearance plotted against GFR values calculated by PexICT. The linear regression equation was $y = 0.856x + 0.888$ ($r^2 = 0.738$). Ranges of H cats for PexICT and PenICT are represented by vertical and horizontal dotted lines respectively.**

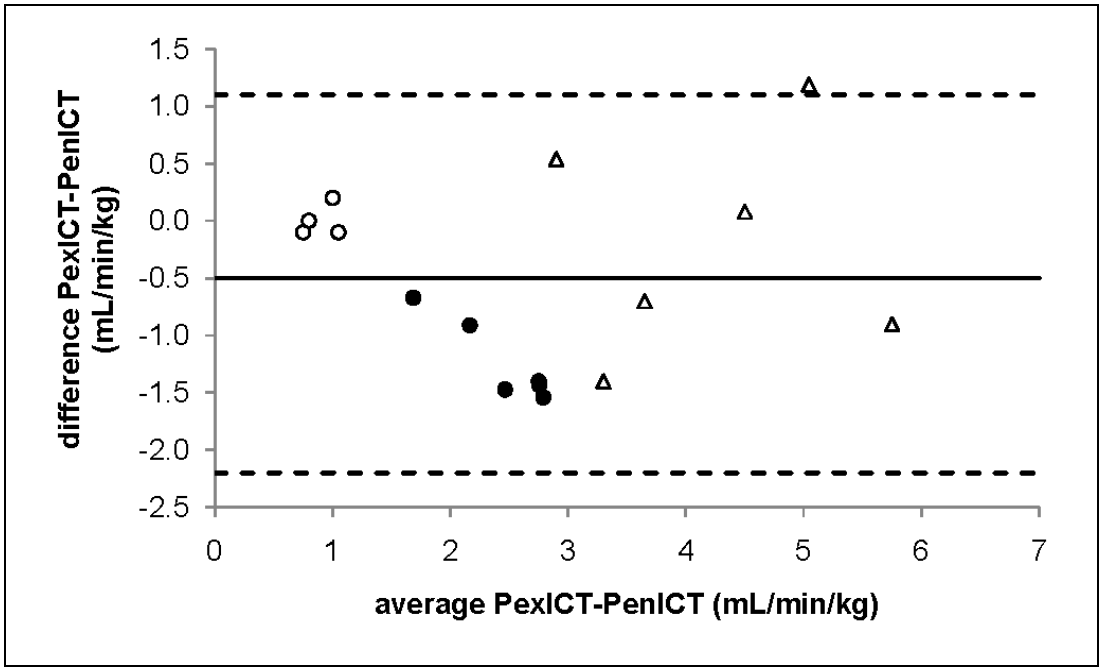


**Figure 3B. Bland-Altman plot of the differences between clearance of PenICT and PexICT against the average value of these clearances. The solid line represents the mean difference. The dotted lines represent the mean differences ± 2*SD.**

**○ cats with CKD, ● H cats, Δ HT cats.**

**PexICT, plasma exo-iohexol clearance test; PenICT, plasma endo-iohexol clearance test.**

**Discussion**

The present study is the first to report the comparison of 3 different GFR techniques in 3 separate groups of cats which are expected to have either low (cats with naturally occurring CKD), normal (H) or high (HT) GFR values, thereby evaluating the GFR techniques over the whole range of GFR expected in cats. Surprisingly, this study showed a significant difference in H cats between PexICT and PenICT and PECCT but not between PenICT and PECCT. Also, there were no differences between GFR methods in HT cats or cats with CKD. Differences between GFR methods in H cats are in accordance with studies described in the literature which compare different clearance techniques.[4,6-8] The difference in GFR assessment between the techniques in only H cats though not in HT cats or cats with CKD can be the cause of the slope approaching the value 1 in the correlation plots. Several studies have compared two GFR techniques in cats with a declined kidney function and described significant differences, albeit other studies found no significant differences nor in H cats nor in cats with a decreased kidney function.[6-10] Recently, our group described the comparison between PexICT, PenICT and PECCT in HT cats before and after radioiodine ($^{131}$I) treatment.[5]

Correlation between PECCT and PexICT (1A) and PenICT (2A) in the low, middle and high GFR values grouped together is acceptable and comparable to values described in the larger group of HT cats.[5] The small bias visible in the Bland-Altman plots 2B and 3B can be caused by an underestimation of PenICT, an overestimation of PECCT and PexICT, or both. PECCT generated systematically higher GFR values compared to PexICT in H and HT cats. PenICT compared to PECCT and PexICT generated higher GFR values in H cats and lower values in HT cats respectively. This is comparable to the findings described earlier in H cats and HT cats before and after $^{131}$I treatment.[4,5]

There is no clear bias visible in the Bland-Altman graph showing the comparison between the PECCT and PexICT (1B), and when bias is visible in comparison between PenICT and PECCT (2B) and PexICT (3B) it is not proven to be statistically significant by a relative systemic error (2A and 3A). The limits of agreement are narrow and mean difference is low, only 0 - 0.5 mL/min/kg. Almost all values are within limits of agreement. The mean difference is lower than the differences between H, HT cat and cats with CKD using PexICT,

PenICT or PECCT described here and therefore not expected to be clinically relevant. This suggests that PexICT, PenICT and PECCT methods can be used interchangeably. However, when GFR is used as part of follow-up of kidney function after treatment of hyperthyroidism, the same clearance technique must be used and preferably PexICT which has the best reproducibility.[4,5] This possibly is caused by the decrease in GFR after treatment, which thereby approaches the range of GFR described in H cats where significant differences between GFR methods are present.

The differences in GFR values according to the technique can be explained by external (marker and method related) and internal (cat and disease status related) factors. Storage time and temperature of plasma samples were similar for PECCT, PexICT and PenICT. Creatinine and iohexol were assayed in different laboratories using different assays, though both assays were validated previously.[4] An interference between creatinine, exo- and endo-iohexol due to the combined use of these analytes as previously performed in dogs and cats[4,6,7,10] is unlikely, because disposition of exo- and endo-iohexol does not seem to be affected by mild to moderate azotemia in cats.[6] Moreover, creatinine is an endogenous compound and the peak concentration observed here in cats with CKD (up to 2077 μmol/L [24 mg/dL]) could be observed in severe azotaemic patients. The AUC extrapolated to infinity has the widest range and highest mean ± SD for all three markers in cats with CKD. However, only in 1/48 pharmacokinetic analyses (16 cats and 3 markers), in a cat with CKD using PECCT, the AUC extrapolated to infinity was higher than 25 % of the whole AUC which suggests the sampling strategy can be considered appropriate in H, HT and moderately azotemic cats. Possibly, in cats with CKD the sampling period has to be prolonged for PECCT as well as for PexICT and PenICT but this needs further research.

Because a combined PEC-ICT was used, factors related to the cats themselves cannot explain the difference in plasma clearance using creatinine, exo- and endo-iohexol. The difference between clearance methods does seems to be related to differences between different physiological conditions and their corresponding different GFR values.

Urinary clearance of inulin is considered the gold standard method,[8,13,14] however it is tedious, time-consuming, stressful and potentially harmful due to the risk of urinary tract inflammation and infection, and are therefore not suitable for practice. Nonetheless, use of a

gold standard method would have been useful to compare the different GFR markers over the range of possible GFR values.

Whatever the cause of the differences between PECCT, PexICT and PenICT, an important clinical aspect of the different markers is the ability to distinguish between the expected different ranges of GFR: low (in cats with CKD), middle (in H cats) and clinically less important high (in HT cats). PexICT is the only clearance method that does not show overlap between low, middle and high GFR values (1A and 3A). Despite the small overlap in GFR values between H and HT cats for PECCT (1A and 2A), this suggests a high sensitivity for small differences in GFR using PECCT. The difference in GFR measured with PECCT between H cats and cats with CKD can also partly be caused by an age-effect, which has been described in healthy cats.[4] Combined with the good reproducibility described for PECCT,[4] this makes the PECCT therefore valuable as a screening test for early detection of renal dysfunction which is usable in practice.

Comparable to results in our study using PECCT and PenICT, a statistically significant difference in GFR value of at least 50 % between the mean GFR values of H cats and cats with a declined kidney function (kidney failure or reduced kidney mass by partial nephrectomy) has been described.[7-10,15,16] These preliminary results may suggest that differences between the lower and normal range of GFR have to be over 50 % to be detected whatever the clearance method used.

Earlier described differences in GFR between H and HT are significant when > 30 %,[17] however unsignificant when < 30 %[18] which is comparable to the results in our study when PexICT, PECCT or PenICT respectively are used. These preliminary results may suggest that differences between the higher and normal range of GFR have to be over 30 % to be detected.

## Conclusion

PexICT, PenICT as well as PECCT can detect large differences in GFR between cats with CKD and HT cats, though only PECCT can detect smaller changes over a large range of GFR between H cats and either cats with CKD or HT cats. Differences between clearance techniques themselves also seem to be correlated to the range of GFR, because only values in the middle range of H cats and not in the lower and higher range of cats with CKD or HT cats respectively, differ between methods.

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# CHAPTER 2

# CHAPTER 3

# EVALUATION OF URINARY RETINOL BINDING PROTEIN AS AN EARLY MARKER OF RENAL DAMAGE IN CATS

# CHAPTER 3

# CHAPTER 3

Introduction to Chapter 3

There is a need in veterinary medicine for a urinary marker to detect early renal damage in hyperthyroid cats. Urinary RBP could be a candidate for this purpose. It is a highly sensitive index of renal tubular damage in humans, because a decrease in tubular function may lead to excretion of RBP in urine. In this chapter, we investigated urinary RBP as a putative marker of renal dysfunction in cats.

In the first section (§ 3.1) we evaluated urinary RBP in healthy cats and cats expected to have tubular dysfunction. In the second section (§ 3.2), we wanted to further investigate urinary RBP in hyperthyroid cats, whether it remained present after treatment, and whether urinary RBP in hyperthyroid cats is possibly linked to serum RBP concentrations.

# CHAPTER 3

# VALIDATION OF URINARY RETINOL BINDING PROTEIN IN CATS

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# CHAPTER 3


**Summary**

The presence of low molecular weight (MW) retinol binding protein (RBP) in urine reflects tubular damage. Therefore, RBP has been used as a renal marker in humans and dogs. Using an anti-human RBP antibody (Ab), this study first demonstrated feline urinary RBP by Western blot analysis and then evaluated its potential as a renal marker in cats by enzyme-linked immunosorbent assay (ELISA).

Urine was taken by cystocentesis, centrifuged and stored at -80 ºC until analysis. Urinary RBP levels were compared in clinically healthy cats (H), chronic renal failure patients (CKD) and cats with hyperthyroidism (HT). The detection of a band at the same position as the human RBP standard with Western blot analysis, indicated that RBP was present in the urine of CKD and HT patients but minimally present in H cats. The data obtained with ELISA were in accordance with these observations. RBP levels were expressed as RBP/creatinine (RBP/c) ratios following normalisation with urinary creatinine. The functional assay sensitivity was 1.37 μg/L RBP. Parallelism between the trend lines of the human RBP standard curve and the curves obtained from sequentially diluted urine samples indicated that feline RBP was recovered. The mean intra-assay coefficient of variance (CV) and the standardised agreement index (AI) regarding repeatability were satisfactory. The RBP/c ratio in all H cats (n = 10) was below the assay sensitivity. The groups of CKD and HT patients had increased mean RBP/c ratios, with large variation in the relative RBP concentrations of individual cats.

In conclusion, RBP is demonstrated for the first time in urine from most CKD and HT patients and the validated ELISA allows its evaluation as a putative renal marker in cats.

## Introduction

In patients at risk of developing renal failure, it is important to apply corrective therapy at an early stage. It is a challenge to assess the onset of a decreased renal function by monitoring sensitive biomarkers.[1] One such candidate biomarker is urinary RBP, which reflects renal damage at the tubular level. With its low molecular weight (MW) of 21 kilodalton (kDa), this protein is freely filtered in the glomerular ultrafiltrate and normally reabsorbed through a megalin-receptor dependent endocytosis mechanism in the proximal tubulus.[2] In small animals, an immunoassay based on cross-reactivity with an anti-human RBP Ab has been successfully used by Raila et al. in plasma and urine of dogs and in cat plasma, but it failed to detect RBP in cat urine.[3-5] The same group recently reported that canine RBP holds promise for the sensitive detection of renal damage.[5,6] Our aim was to assess urinary RBP with Western blot analysis and with an enzyme linked immunosorbent assay (ELISA) validated for the analysis of urine from clinically healthy (H) cats and from cats with either diagnosed or increased risk for renal dysfunction, as a first step in the evaluation of its potential as a putative renal marker.

## Materials and Methods

### Cats

The current study was carried out after approval by the Local Ethical Committee of Ghent University. Thirty-three cats were included: 10 H cats, 10 cats with chronic kidney disease (CKD) and 13 cats with HT. For the H cats, 5 young adult (average age of 2 years) and 5 elderly (average age of 10 years) cats were selected. Cats with CKD (IRIS [www.IRIS-kidney.com] stage II or higher) were included based on symptoms and azotemia (upper reference limit of serum creatinine concentration was 1.5 mg/dl). Cats were excluded when medication that might influence renal function had been recently administered. Inclusion criteria for the HT cats were signs compatible with hyperthyroidism (polyuria/polydipsia, polyfagia, weight loss and tachycardia), an increased serum total T4 (TT4) concentration and an increased uptake of $^{99m}TcO_4^-$ on a scintigraphic thyroid scan. Treatment with antithyroid drugs had to be discontinued at least 3 weeks prior to inclusion.

### Urine samples

All urine samples were taken by cystocentesis. Briefly, animals were placed in a dorsal position and the skin of the abdomen was disinfected. Punction of the bladder was effectuated with a 5 ml syringe and a 22-gauge needle to aspirate the urine. Following centrifugation (3 minutes at 447 x *g*), urine was divided in at least two aliquots of 300 µl and frozen at -80 °C until analysis. The pH value of all urine samples was between 6 and 8, except for one CKD cat with an original urinary pH of 9, this was corrected to 8 with HCl. Blood urea nitrogen, serum creatinine concentration, urinary specific gravity and urinary protein/creatinine ratio were determined for all cats.

### Western blot analysis

Sample volumes of 10 µl urine were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12 % gel. In addition to colorimetric and chemiluminescent markers, two urinary RBP standards (Sigma-Aldrich, Belgium and Immundiagnostik, Germany) were also run. After gel electrophoresis, the separated proteins were electroblotted onto a nitrocellulose membrane with a transfer buffer containing 20 % methanol. Prior to immunodetection, Tris-buffered saline with 0.1 % Tween-20 (TBS-T) containing either 5 % milk powder or 3 % bovine serum albumin (BSA) was used to block

non-specific binding sites on the blot. The membrane was then incubated with a 1:100 diluted polyclonal rabbit anti-human urinary RBP primary Ab (A0040, DakoCytomation, Denmark) at 4 ºC overnight. Following washing with TBS-T, the membrane was incubated with the secondary Abs i.e. 1:1000 diluted goat anti-rabbit IgG horseradish peroxidase (HRP) conjugate or 1:2500 diluted HRP labeled anti-biotine (Bio-Rad Laboratories, Belgium) for the chemiluminescent marker, and again washed with TBS-T. Chemiluminescence was measured on a Gel Doc 2000 system (Biorad) after addition of an in situ mixture of peroxide buffer with luminal enhancer (Supersignal West Dura substrate, Pierce Science, Belgium). The ChemiDoc software (Biorad) was used to analyse the bands.

**ELISA**

A commercial human RBP sandwich ELISA kit (Immundiagnostik AG, Germany) was used. The microtiter plate, pre-coated with the same polyclonal rabbit anti-human RBP Ab as used for Western blot analysis, was washed 5 times with the washing buffer. Wells were filled with 100 µL of 1/10 diluted urine samples or RBP standards (0 µg/L, 1.1 µg/L, 3.3 µg/L, 11 µg/L and 33 µg/L) and the plate was incubated at room temperature for 1 hour on a horizontal mixer. After 5 wash steps, 100 µl of 1/100 diluted peroxidase-labelled rabbit anti-human RBP Ab was added and the plate was incubated for 1 hour on a horizontal mixer, again followed by 5 washings. Every well was then filled with 100 µL of tetramethylbenzidin (TMB) substrate and the colorimetric reaction was stopped after 10 to 12 minutes with 50 µL of stop-solution ($H_2SO_4$) per well. Absorbance of each well was measured using an ELISA plate reader at 450 and also at 600 nm as a reference. The standard curve obtained was used to calculate the RBP concentration in the samples of that plate. Assay sensitivity was calculated based on the standard deviation (SD) between the absorption values of urine samples from H cats to define the lowest RBP concentration that can be reliably distinguished.[5] Recovery of feline RBP was determined by sequential dilution of urine samples and assessment of parallelism with the trendlines of the human RBP standard curve. Samples were also analysed in duplicate on the same day and on different days. The intra-assay coefficient of variance (CV) indicates precision of the method and is defined as the standard deviation of parallel measurements divided by their mean.[7] Day-to-day repeatability of the ELISA was evaluated by determining the standardised agreement index (AI) of parallel samples. The AI is defined as: $AI = 1 - (2*SD_{diff}/mean_{AB})$ ($SD_{diff}$ is the standard deviation of the differences between

parallel samples; $mean_{AB}$ is the mean of parallel samples). A positive AI supports agreement, and a value larger than 0.5 indicates good agreement.[8,9]

## Results

### Western blot analysis

Preliminary experiments for the optimisation of the Western blot conditions showed that blocking with 5 % milk powder yielded better results with respect to background signal than blocking with 3 % BSA. In addition, two urinary RBP standards were evaluated. The RBP standard from Sigma displayed a much stronger signal in this immuno-assay, although it did not yield two pure bands i.e. the expected major band at about 20 kDa (Figure 1) and one at 40 kDa (not shown), likely to be a dimer. The Immundiagnostik urinary RBP standard could not be detected by Western blot analysis (data not shown), although this was not due to a lack of sensitivity because it was positive upon dot spot analysis. A clear band at the same position as that from the RBP standard was observed in urine samples from a CKD and a HT cat, and a weak band in the urine from a H cat (Figure 1).

### ELISA

Urine samples were diluted 10-fold as advised for human urine to minimize the risk of matrix interference. The functional assay sensitivity was calculated based on the absorption values of urine samples from 5 young H cats (the negative control group with respect to renal damage). The average absorption for these negative control samples was 0.085 (n = 5). The corresponding assay sensitivity was 1.37 µg RBP/L urine, defined as the minimum concentration that can be reliably distinguished from the zero standard and that produces an absorbance >10 SD of the negative control samples.[5] The recovery of feline RBP was determined by sequential dilution of feline urine. This approach was chosen because the demonstration of dilutional parallelism provides a valuable alternative if no RBP standard from the species studied is available, as shown by Raila et al.[5] in dogs. The curves obtained by serial dilution of feline urine samples from a HT cat and a CKD cat were parallel to the trendlines of the calibration curves set up with human RBP standards, indicating that the antigen measured in cat urine was indeed RBP and confirming the cross-reactivity with the primary antibody as observed by Western blotting (Figure 2). Samples were analysed in duplicate on the same day (4 H, 4 CKD and 10 HT cats) to determine mean intra-assay CV, and on different days (1 H , 4 CKD and 4 HT cats) to determine day-to-day repeatability expressed by the standardised AI. The mean intra-assay CV was 7 % and the standardised AI was 0.7. Urinary RBP concentrations were normalised by expressing them as RBP/creatinine

(RBP/c) ratios. Results of the RBP ELISA assay and routine biochemical renal function parameters analysed in serum and urine are shown in Table 1. The RBP/c ratio in all H cats (n = 10) was below the assay sensitivity. Patients with CKD and HT had increased mean RBP/c ratios of 1.6 ± 0.5 $x10^{-2}$ µg/mg (n = 10) and 1.4 ± 0.4 $x10^{-2}$ µg/mg (n = 13), respectively.

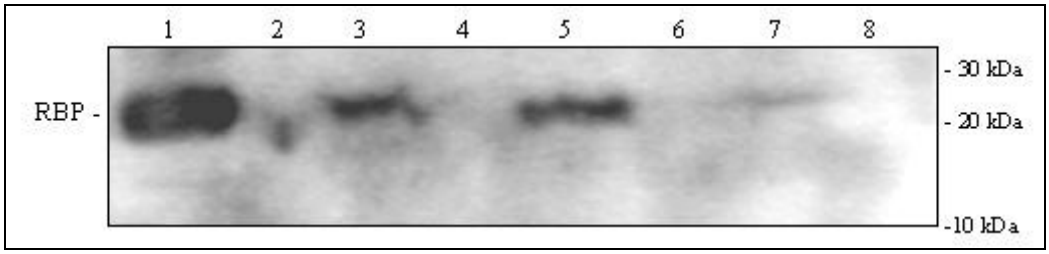


**Figure 1. Representative Western blot following chemiluminescent detection. Lane identification: urinary RBP standard (1), urine from a CKD cat (3), a HT cat (5) and a H cat (7) and empty lanes (2, 4, 6, 8).**

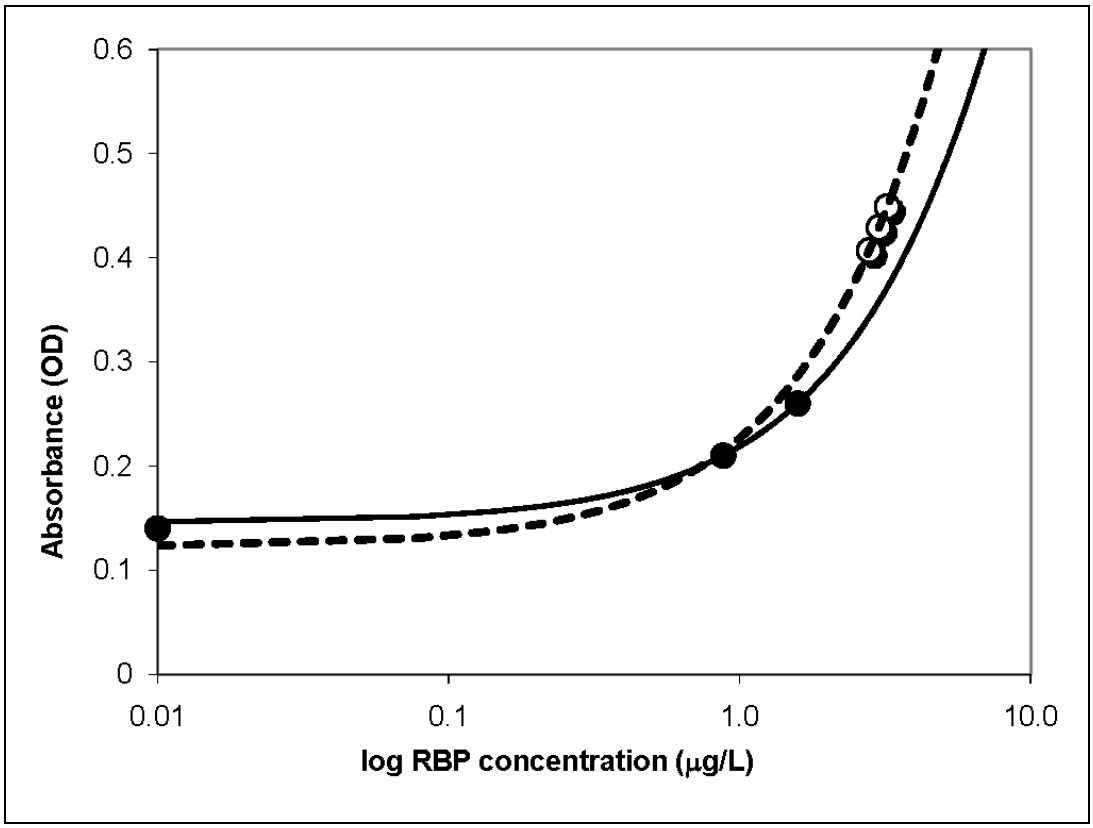


**Figure 2. Recovery of feline urinary RBP from a CKD cat (●, sample A) and a HT cat (○, sample B) demonstrated by the parallelism between the curves of serially diluted cat urine and trendlines from human RBP standards (solid and dotted lines).**

**Table 1. Blood urea nitrogen (BUN, mg/dL), serum creatinine concentration (mg/dL), urinary specific gravity (USG), urinary protein/creatinine (p/c) ratio, mean urinary concentration of RBP (µg/L, n = 2), creatinine (mg/dL) and RBP/c ratio ($x10^{-2}$µg/mg) in 33 cats.**

| Group | BUN | Serum creatinine | USG | p/c ratio | urinary RBP | Urinary creatinine | Urinary RBP/c ratio |
|---|---|---|---|---|---|---|---|
| H | 38.9 | 1.3 | 1.008 | 0.23 | * | 277 | - |
| | 56.9 | 1.3 | 1.012 | 0.00 | * | 259 | - |
| | 47.0 | 1.5 | 1.047 | 0.23 | * | 376 | - |
| | 41.0 | 1.2 | 1.045 | 0.18 | * | 250 | - |
| | 50.0 | 1.4 | 1.048 | 0.09 | * | 380 | - |
| | 43.0 | 1.0 | 1.042 | 0.14 | * | 106 | - |
| | 47.0 | 1.9 | 1.049 | 0.07 | * | 497 | - |
| | 41.9 | 1.9 | 1.050 | 0.06 | * | 580 | - |
| | 47.0 | 1.7 | 1.050 | 0.08 | * | 623 | - |
| | 50.0 | 2.1 | 1.049 | 0.08 | * | 518 | - |
| *mean* | *46.3* | *1.5* | *1.040* | *0.13* | | | |
| *SEM* | *1.7* | *0.1* | *0.005* | *0.02* | | | |
| CKD | 382.2 | 9.6 | 1.017 | 1.75 | 17.8 | 51 | 3.5 |
| | 370.8 | 15.6 | 1.020 | 0.91 | 19.6 | 80 | 2.5 |
| | 390.6 | 17.5 | 1.017 | 0.21 | 15.1 | 130 | 1.2 |
| | 220.8 | 7.6 | 1.010 | 0.88 | 1.9 | 90 | 0.2 |
| | 214.9 | 4.8 | 1.013 | 4.99 | 23.0 | 54 | 4.3 |
| | 231.8 | 4.8 | 1.010 | 0.36 | 1.9 | 48 | 0.4 |
| | 83.9 | 2.2 | 1.015 | 0.23 | 14.1 | 79 | 1.8 |
| | 73.9 | 3.0 | 1.009 | 0.00 | * | 88 | - |
| | 319.7 | 12.4 | 1.010 | 0.25 | 5.6 | 91 | 0.6 |
| | 179.4 | 4.9 | 1.012 | 3.00 | 12.3 | 70 | 1.8 |
| *mean* | *246.8* | *8.2* | *1.013* | *1.40* | | | *1.6* |
| *SEM* | *36.9* | *1.7* | *0.001* | *0.52* | | | *0.5* |
| HT | 71.9 | 1.0 | 1.041 | 1.29 | 23.1 | 204 | 1.1 |
| | 71.9 | 1.5 | 1.022 | 0.40 | 16.4 | 122 | 1.3 |
| | 35.9 | 0.8 | 1.050 | 0.60 | 21.6 | 147 | 1.5 |
| | 44.0 | 0.9 | 1.050 | 0.53 | 24.5 | 126 | 1.9 |
| | 41.0 | 0.6 | 1.050 | 0.46 | * | 219 | - |
| | 71.9 | 2.1 | 1.032 | 0.08 | * | 270 | - |
| | 38.0 | 0.7 | 1.050 | 0.57 | 2.5 | 158 | 0.2 |
| | 44.9 | 1.2 | 1.022 | 0.33 | * | 92 | - |
| | 41.0 | 0.8 | 1.022 | 0.77 | 17.2 | 95 | 1.8 |
| | 39.0 | 1.6 | 1.048 | 0.23 | 15.8 | 323 | 0.5 |
| | 47.9 | 0.8 | 1.048 | 0.57 | 29.3 | 78 | 3.8 |
| | 47.9 | 1.1 | 1.050 | 0.40 | 24.5 | 158 | 1.6 |
| | 47.0 | 0.9 | 1.028 | 0.39 | 8.1 | 19 | 4.2 |
| *mean* | *49.4* | *1.1* | *1.039* | *0.51* | | | *1.4* |
| *SEM* | *3.6* | *0.1* | *0.003* | *0.08* | | | *0.4* |

**H: healthy cats (n = 10), CKD: cats with chronic renal insufficiency (n = 10), HT: hyperthyroid cats (n = 13); µ: mean RBP/c ratio, SEM: standard error of the mean; *: RBP concentration below the assay sensitivity (i.e. 1.37 µg/L); -: ratio can not be calculated.**

## Discussion

RBP is a low MW protein (21 kDa) that is synthetised in the liver and belongs to the superfamily of the lipocalines.[10] It is a specific carrier for the lipophilic vitamin A (retinol) in blood, transporting the retinol ligand from its hepatic storage site to target cells as a holo-RBP-complex. Upon release of its ligand, the uncomplexed RBP can be filtered and reabsorbed in the kidney. According to a comparative immunology study, this systemic transport system is analogous for all Mammalia, including Felidae.[11] However, in the same paper significant immunological differences in RBP among the mammalian orders were described. Interestingly, canine but not feline RBP did display cross-reactivity with a human RBP Ab raised in rabbits. In contrast, another comparative study did observe partial cross-reactivity between a rabbit anti-human Ab and RBP from felids while no cross-reactivity was seen for RBP from dogs.[12] A recent study from Raila et al.[3] showed immunological activity of carnivores with a commercial rabbit anti-human RBP Ab. We suggest that these conflicting literature data may be explained by the different Abs and immunological techniques used in the three studies i.e. radio-immunoassay, radial immunodiffusion and Western blot analysis, respectively. Although the presence of RBP in cat plasma, liver and kidney samples was confirmed in a subsequent paper by Raila et al.,[4] the protein was again not detected by Western blot analysis in feline urine. In two more recent studies from the same group, the authors conclude that in dogs urinary RBP holds promise as a renal marker because increased urinary RBP levels were observed in CKD patients with ELISA and with the innovative protein microchip technology.[5,6] The reported advantages of RBP as a biochemical marker in humans are its relatively constant synthesis rate and its stability, especially with variations in urinary pH.[13,14]

In the current immuno-assay based study, urine from cats belonging to three groups was first analyzed by Western blotting with chemiluminescent detection. In the urine of healthy cats the RBP signal observed was very low in comparison to that in urine from both CKD and HT patients. The detection of RBP in urine from a healthy cat is analogues to the results in healthy humans, who can have detectable amounts of RBP in urine.[15] Feline urinary RBP was present as a band at the same position as a RBP standard purified from human urine. Previous studies by Raila et al.[3,4] have described the attempt to demonstrate RBP in urine of cats and other carnivores, using an antibody raised against human RBP purified from serum

and not from urine as used in the current study. This difference in Abs could be one explanation why RBP was not demonstrated in the urine of cats with Western blot analysis in these earlier studies. Nevertheless, to the best of our knowledge an anti-human serum RBP Ab is not commercially available. Therefore, a more likely explanation for the difference between our results and those from Raila et al. is therefore that the urine samples in their study were chosen at random from patients and did not originate from selected cats with either diagnosed or increased risk for renal damage.

Second, a sandwich ELISA technique was validated to compare the normalized urinary RBP levels in 33 cats. As RBP standards purified from human urine were used for the calibration curve in this quantitative immunoassay and because of the partial cross-reactivity from feline RBP with anti-human RBP,[12] the calculated values should be interpreted as relative concentrations. The ELISA data in the current study were in accordance with the observations made by Western blot analysis. Indeed, the relative RBP levels detected were below the assay sensitivity in all H cats, whereas increased urine concentrations were typically seen in the majority of CKD and HT patients. However, in both of the latter patient groups a large variation in the relative RBP concentration was observed between individual cat urine samples. The anti-human urinary RBP Ab used in the current study reacts with serum, plasma and urinary human RBP. Following additional validation of the immuno-assay for feline serum or plasma, it could therefore be of interest to evaluate whether a similar variation between cats is also present at the systemic level.

In cats, several factors such as muscle mass, GFR and USG could influence urinary creatinine concentration. Further studies are needed to investigate the best way to index urinary RBP concentration taking into account the dilution factor.

## Conclusion

The current study first identified RBP in feline urine with Western blot analysis. Subsequent data obtained with a validated ELISA are in accordance with the Western immunoassay and with observations in other species. The presented findings indicate that feline RBP is released in urine upon renal damage and suggest that RBP might be valuable as a renal marker for cats in a clinical setting.

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# CHAPTER 3

# RETINOL BINDING PROTEIN IN SERUM AND URINE OF HYPERTHYROID CATS BEFORE AND AFTER TREATMENT WITH RADIOIODINE

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# CHAPTER 3

**Summary**

Retinol binding protein (RBP) was previously suggested to be valuable as a renal marker for cats in a clinical setting. We hypothesized that serum and urinary RBP concentrations in hyperthyroid (HT) cats differ from those in healthy (H) cats, and there is influence of radioiodine ($^{131}$I) treatment on serum and urinary RBP concentrations in HT cats.

RBP was measured in urine and serum of 10 HT cats before and after treatment and in serum and urine of 8 H cats. A polyclonal rabbit anti-human RBP antibody was used in a commercial sandwich ELISA that we validated for RBP assessment in feline samples.

There was a significant difference between H and untreated HT cats in urinary RBP/creatinine (uRBP/c) ratio. Serum TT4 concentration and uRBP/c decreased significantly in HT cats at all time points after treatment, and both variables correlated significantly. Serum RBP concentrations from HT cats did not differ significantly from those of H cats and did not change after treatment.

We can conclude from this study that the presence of urinary RBP in HT cats is a potential marker of tubular dysfunction which is correlated to thyroidal status, although independent of circulating RBP concentrations. The decreasing uRBP/c combined with the absence of changes in serum RBP after treatment suggests that the observed tubular dysfunction is mainly reversible upon treatment with $^{131}$I.

## Introduction

Four main types of RBP (named RBP-1 to RBP-4) are described in humans.[1,2] These carrier proteins can be divided into serum RBP and cellular RBPs (CRBPs). Serum RBP and cellular RBPs differ in their primary amino acid sequence, as well as in their secondary and tertiary protein structure.[3] These structural differences affect the way retinol is bound to its specific RBP. The circulating form of RBP is called RBP-4 in humans, while in cats it is named simply serum RBP or urinary RBP [uRBP] because it is not clear which type of RBP is measured. Serum RBP forms with its retinol ligand a holo-RBP complex.[4] Holo-RBP binds physiologically to transthyretin, and this prevents the loss of both serum RBP and its bound retinol through glomerular filtration.[5] Only molecules with a molecular weight smaller than albumin (66 kDa) can be filtered through the glomerular barrier. RBP is a low molecular weight carrier protein of 21 kDa, and the tetrameric transthyretin (TTR) has a molecular weight of 55 kDa. Upon release of its ligand, the uncomplexed apo-RBP no longer has affinity for TTR and can be freely filtered in the glomerular ultrafiltrate and normally reabsorbed through a megalin-receptor dependent endocytosis mechanism in the proximal tubules.[6] However, when tubular function fails, elimination of uRBP shifts from intra-tubular catabolism to urinary excretion.[7] RBP found in urine is modified from RBP in serum by proteolysis at the carboxyl terminus. Urinary RBP can be shown using cross-reactivity with anti-human uRBP antibodies (Abs). This tubular type of proteinuria is a highly sensitive index of renal tubular damage in humans, because a minor decrease in tubular function may lead to RBP excretion in urine.[8,9]

Despite differences in amino acid composition between serum RBP and uRBP, radial immunodiffusion techniques have shown partial but substantial cross-reactivity between feline serum and rabbit anti-human uRBP Abs.[10] Western-blot analysis based on cross-reactivity with an anti-human uRBP antibody (Ab) has confirmed the presence of RBP in both plasma, liver and kidney samples[11] as well as in urine of healthy (H) cats, untreated HT cats and cats with chronic kidney disease (CKD).[12] In the latter study, the same anti-human uRBP Ab was used albeit in a commercially available sandwich ELISA kit[a] to measure relative RBP. Our previous data demonstrate that H cats did not have RBP concentrations above the ELISA limit of quantification (LOQ), whereas increased urinary RBP concentrations were seen in the majority of cats with hyperthyroidism displaying large variations between

individual cats, and in cats with CKD.[12] These data indicate that urinary RBP might be used as a urinary marker to study tubular localization of lesions occurring in kidney damage. Nevertheless, it remains unclear why RBP is present in urine of untreated HT cats.

Plasma concentrations of RBP and transthyretin are decreased in humans with hyperthyroidism, although they remain unchanged during hypothyroidism.[13-15] This former observation can be either explained by an increased plasma turnover of RBP combined with an unchanged RBP synthesis,[16] or by a lower hepatic synthesis of RBP due to decreased serum zinc values.[15] Urinary RBP/creatinine (uRBP/c) ratio's declined in HT humans that became euthyroid, but concentrations varied widely and median values of both HT and euthyroid patients were within reference ranges of control euthyroid subjects.[17]

It can be hypothesized that HT cats have an increased clearance of circulating RBP and that this would lead or contribute to uRBP. Another hypothesis is that uRBP in HT cats is caused by an effect of the hyperthyroid state on tubular function. Effects of hyperthyroidism on other functional parts of the tubules have been reported.[18-21] A decrease in uRBP after treatment of hyperthyroidism can then be expected.

Fractional excretion (FE) of a solute is defined as the fraction of a filtered solute that is excreted in the urine. FE is calculated from the serum and urinary concentrations of this specific solute and creatinine. It is therefore a variable consisting of both systemic and renal components. Consequently, calculating FE for RBP might be helpful in elucidating the possible link between serum and urinary RBP.[22]

The anti-human uRBP Ab reacts with serum, plasma and urinary RBP in humans, and it has been applied in ELISA to measure RBP in both serum and urine.[23,24] However, it has been only qualitatively detected with Western blotting in plasma[11] or sera[10] of cats, and quantitatively with ELISA in urine of cats.[12] No data are available in cats on the influence of treatment for hyperthyroidism on systemic and urinary RBP concentrations. Therefore, the objectives of the current study were to compare serum and urinary RBP concentrations in HT cats and H cats, and to evaluate the influence of radioiodine ($^{131}$I) treatment on these serum and urinary RBP concentrations in HT cats.

## Materials and Methods

This study was carried out after approval by the Local Ethical committee of Ghent University and the care and use of all animals complied with local animal welfare laws, guidelines and policies. Informed consent was obtained from the owners of included HT cats.

### Hyperthyroid cats

Ten HT cats were included in the study and ranged in age from 8 to 16 years (median 13 years) and weighed 2.6 - 5.0 kg (median 3.5 kg). There were 2 castrated male cats and 8 spayed female cats, all domestic shorthair. Cats were included in the study when diagnosed with hyperthyroidism, presented for treatment with $^{131}I$ at the faculty of veterinary medicine of Ghent University (Belgium) and successfully treated for hyperthyroidism 24 weeks after treatment, according to the observed decrease in serum TT4 concentration and amelioration of clinical symptoms.

Diagnosis of hyperthyroidism was based on clinical signs compatible with hyperthyroidism, ie. increased serum TT4 concentration (reference range 1.1-3.5 μg/dL)) and increased thyroidal uptake (ratio thyroid uptake/salivary gland uptake) of pertechnetate ($^{99m}TcO_4^-$). Anti-thyroid drugs had to be discontinued at least 3 weeks prior to inclusion. To assess the clinical condition, cats underwent physical and routine laboratory examinations (CBC, biochemistry, and measurement of serum TT4) and cystocentesis for urinalysis 1 day before and 4, 12 and 24 weeks after $^{131}I$ treatment. At these re-evaluations, serum TT4, serum creatinine, serum RBP, urinary RBP, and urinary creatinine concentrations were measured. Before and after treatment urine specific gravity (USG) and glomerular filtration rate (GFR) were measured using plasma clearance of exo-iohexol (PexICT) as described earlier.[25]

### Healthy cats

Eight healthy cats were included in the study and ranged in age from 2 to 10 years (median 9.5 years) and weighed 2.3 - 5.8 kg (median 4.8 kg). There were 3 spayed male and 5 female (3 spayed, 2 intact) cats, all domestic shorthair. Cats underwent physical and routine laboratory examinations (CBC, biochemistry, and measurement of serum TT4) and cystocentesis for urinalysis. Animals were included only if these examinations showed no clinically significant abnormalities. Serum TT4, serum creatinine, serum RBP, urinary RBP,

urinary creatinine concentration, USG and GFR (PexICT, n = 7) were measured to compare the results with hyperthyroid cats.

**Procedures and RBP analysis**

Blood was taken by jugular venipuncture and urine by cystocentesis, on the same day after the cat was fasted for at least 10 hours. No chemical restraints were used for sampling. Blood samples were allowed to clot for a maximum of 1 hour. Clotted serum and urine were stored at 4 ºC for a maximum of 2 hours. After centrifugation (5 min at 2431 *x g* for serum, 3 min at 447 *x g* for urine), samples were aliquoted and stored at -20 ºC (serum) or -80 ºC (urine). Serum TT4 was measured using a validated chemiluminescent immunoassay (Immulite 2000 Canine total T4 assay, Diagnostic Products Corporation, Los Angeles, USA).Creatinine was measured in serum and urine with a validated spectrofotometric Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Within- and between-run coefficients of variation (CV %) are described in Table 1.

A polyclonal rabbit anti-human uRBP Ab was used in a commercial sandwich ELISA (Immundiagnostik AG, Germany) validated for RBP assessment in feline urine and previously described in detail.[12] Wells were filled with 100 µl of either 1/10 diluted urine samples or 1/200 diluted serum samples. The absorbance of each well was measured using an ELISA plate reader at 450 nm and also at 600 nm as a reference wavelength. Samples producing an absorbance <10 SD of negative control samples were considered as below LOQ (value 0) as described previously by van Hoek et al. (2008). The recovery of RBP was determined by sequential dilution of a serum sample taken in a HT cat before treatment. This approach was chosen because the demonstration of dilution parallelism provides a valuable alternative if no RBP standard from the species studied is available, as shown by Raila et al. in dogs.[26] RBP concentrations were expressed as µg/L in serum and as RBP/c ($10^{-2}$ µg/mg creatinine) ratio in urine. Fractional excretion (FE) of urinary RBP was calculated as the fraction of the amount of urinary RBP filtered through the glomeruli and excreted in the urine with the following formula: (urinary RBP concentration * serum creatinine concentration) / (serum RBP concentration * urinary creatinine concentration).[22]

**Statistical analysis**

Results were analyzed using a linear mixed model (SAS version 9.1, SAS Institute Inc, Cary, IN, USA) with cat as random effect and treatment and time as categorical fixed effects.

The measurements of the HT cats at the different time points were compared with those in H cats at time zero using Dunnett's multiple comparisons technique. Additionally, a multivariate analysis was performed using the same model and introducing BW, age and sex as covariates, in order to adjust for imbalance in these covariates between the HT and H cats. The measurements of the HT cats at the different time points were compared pair wise using Tukey's multiple comparisons technique. All tests were done at a global significance level of 5 % and adjusted P-values (adjusted for multiple comparisons) were reported. Pearson correlation coefficients were obtained for different pairs of variables. Results are expressed as mean ± standard deviation (SD) unless stated otherwise.

**Table 1. Within- and between-run CV for low and high range concentrations of serum creatinine, urine creatinine and serum TT4.**

| | | **Within-run** | | **Between-run** | | |
|---|---|---|---|---|---|---|
| | | **# samples (mean concentration)** | **CV(%)** | **# samples (mean concentration)** | **CV(%)** | **days** |
| Serum creatinine | Low range | 10 (1.3 mg/dL) | 2.2 | 2 - 5 (1.2 mg/dL) | 3.0 | 13 |
| | High range | 10 (4.2 mg/dL) | 1.9 | 2 - 5 (4.0 mg/dL) | 2.3 | 13 |
| Urine creatinine | Low range | 10 (98 mg/dL) | 1.1 | 2 (86 mg/dL) | 2.4 | 13 |
| | High range | 10 (243 mg/dL) | 0.7 | 2 (256 mg/dL) | 2.2 | 13 |
| Serum TT4 | Low range | 10 (2.1 μg/dL) | 4.3 | 1 (2.2 μg/dL) | 7.6 | 12 |
| | High range | 10 (5.0 μg/dL) | 6.0 | 1 (5.1 μg/dL) | 7.0 | 7 |

**CV: coefficient of variation.**

**Results**

First, recovery of feline RBP was demonstrated by the parallelism between the curves of serially diluted HT cat serum before $^{131}I$ treatment and of human RBP standards. This indicates that the antigen measured in feline serum was RBP and confirms the cross-reactivity with the primary anti-human antibody as previously shown for cat urine (Figure 1).[12]

Second, serum RBP and TT4 concentrations, urinary RBP and creatinine concentrations and the uRBP/c ratio, as well as USG and GFR in H cats and in HT cats before and after treatment are described in Table 2. Serum TT4 concentration differed between H and HT cats before treatment ($P < 0.001$), but not 4 weeks ($P = 0.999$), 12 weeks ($P = 1.000$) or 24 weeks ($P = 0.998$) after treatment. Serum RBP did not differ significantly between H cats and HT cats before ($P = 0.984$) or 4 weeks ($P = 0.625$), 12 weeks ($P = 0.857$) or 24 weeks ($P = 0.999$) after treatment. Urinary RBP differed significantly between H and HT cats before treatment ($P = 0.001$) but not at 4 weeks ($P = 0.491$), 12 weeks ($P = 0.385$) or 24 weeks ($P = 0.241$) after treatment. There was a significant difference between H and HT cats in urinary creatinine concentration before treatment ($P = 0.001$), at 4 weeks ($P = 0.016$), 12 weeks ($P = 0.020$) and 24 weeks ($P = 0.008$) after treatment. There was a significant difference between H and HT cats in uRBP/c ratio before treatment ($P = 0.003$), but not at 4 weeks ($P = 0.945$), 12 weeks ($P = 0.796$) or 24 weeks ($P = 0.302$) after treatment. FE was on average higher in HT cats at all time points compared to H cats, however FE did not differ significantly between H cats and HT cats before ($P = 0.131$) or 4 weeks ($P = 0.997$), 12 weeks ($P = 0.895$) or 24 weeks ($P = 0.092$) after treatment. USG did not differ between H and HT cats before ($P = 0.834$), nor 4 ($P = 0.580$), 12 ($P = 0.288$) or 24 ($P = 0.939$) weeks after treatment. GFR differed between H and HT cats at time point 0 ($P = 0.002$), but not at 4 ($P = 0.467$), 12 ($P = 0.310$) or 24 ($P = 0.340$) weeks after treatment.

There was no significant difference between the H cats and HT cats for age, BW or sex. These results were re-analysed with multivariate analysis with age, BW and sex as fixed effects. There were no different results and hence no influence of differences in age, BW or sex between the 2 groups was present.

In HT cats there was a strongly significant decrease in serum TT4 concentration at all time points after $^{131}I$ treatment compared to before $^{131}I$ treatment ($P < 0.001$). No statistically significant differences in serum TT4 concentration were observed between 4 and 12 weeks ($P = 1$), 4 and 24 weeks ($P = 0.986$) or 12 and 24 weeks ($P = 0.996$). Serum RBP concentration

did not change significantly after $^{131}$I treatment (P = 0.799). Compared to pre-treatment values, there was a significant decrease in absolute uRBP concentration 4 weeks (P = 0.004), 12 weeks (P = 0.006) and 24 weeks (P = 0.016) after $^{131}$I treatment. No statistically significant differences were observed between 4 and 12 weeks (P = 0.995), 4 and 24 weeks (P = 0.916) and 12 and 24 weeks (P = 0.977). Urinary RBP remained present in 50 % of the HT cats until 24 weeks after treatment, although 4 of these 5 cats had uRBP/c ratio lower than pre-treatment values. Compared to pre-treatment values, there was a significant increase in urinary creatinine concentration 4 weeks (P = 0.016), 12 weeks (P = 0.008), but not at 24 weeks (P = 0.079) after $^{131}$I treatment. No statistically significant differences were observed between 4 and 12 weeks (P = 0.993), 4 and 24 weeks (P = 0.888) and 12 and 24 weeks (P = 0.754). Compared to pre-treatment values, there was a significant decrease in uRBP/c ratio 4 weeks (P = 0.004), 12 weeks (P = 0.001) but not at 24 weeks (P = 0.084) after $^{131}$I treatment. No statistically significant differences were observed between 4 and 12 weeks (P = 0.986), 4 and 24 weeks (P = 0.580) and 12 and 24 weeks (P = 0.781). No significant change in FE could be detected 4, 12 or 24 weeks after $^{131}$I treatment (P = 0.061). There was no significant difference in USG at 4, 12 or 24 weeks after treatment (P = 0.426). Compared to pre-treatment values, GFR decreased significantly in HT cats at 4 (P < 0.001), 12 (P < 0.001) or 24 (P < 0.001) weeks after treatment. No statistically significant differences in GFR were observed between 4 and 12 weeks (P = 0.158), 4 and 24 weeks (P = 0.203) or 12 and 24 weeks (P = 0.999) after treatment.

The Pearson correlation coefficient (r) was calculated for the HT for all time points and the time points separately. The r, as well as the P-value for significant difference from 0 are described in Table 3. The correlation in HT cats between serum TT4 concentration and uRBP/c is visualized in Figure 2 for the 4 different time points separately.

Finally, 2 HT cats developed CKD (IRIS stage II, serum creatinine 1.6 - 2.8 mg/dL), low urine specific gravity (1.012 and 1.015 respectively) and clinical symptoms. Serum creatinine increased from 0.94 to 2.68 mg/dL and from 0.58 to 1.80 mg/dL, respectively in these cats. Urinary RBP was present in 1 of these cats 24 weeks after treatment. Both cats were also diagnosed with hypothyroidism, i.e. serum TT4 concentration below reference range and no response after rhTSH stimulation.[27]

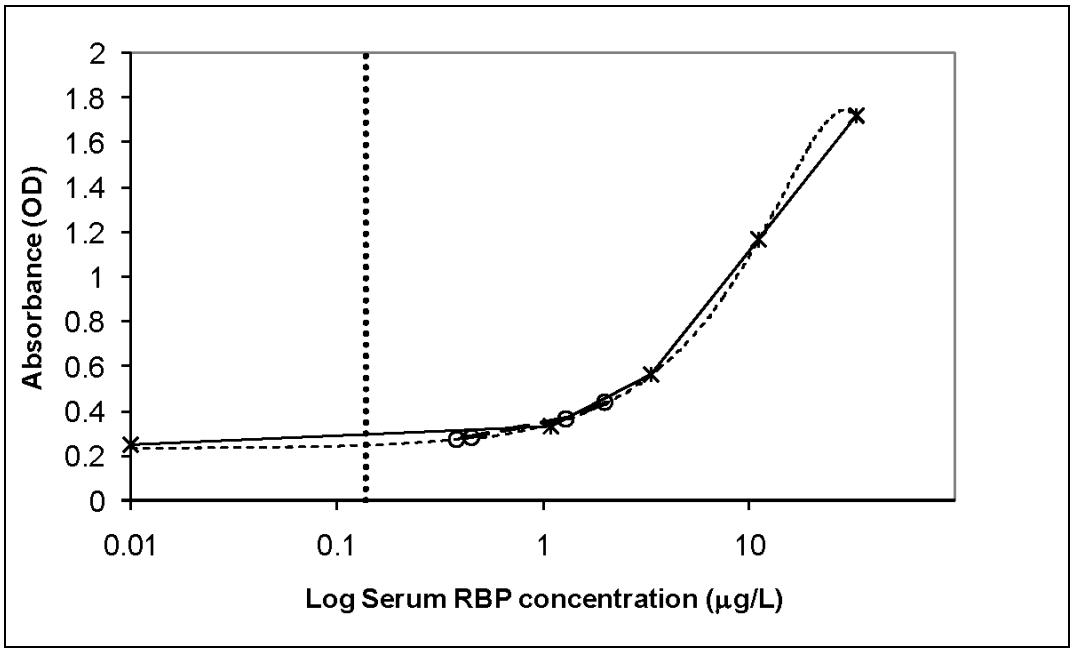


**Figure 1. Recovery of feline RBP from serum of a HT cat before (○) $^{131}$I treatment demonstrated by the parallelism between the curves of serially diluted cat serum (dotted line) and of human RBP standards (*, solid line, with the corresponding trendline as dotted line). The serum sample was diluted 1:100, 1:200, 1:500 and 1:1000. Assay sensitivity (1.37 µg/L) is expressed by the vertical dotted line.**

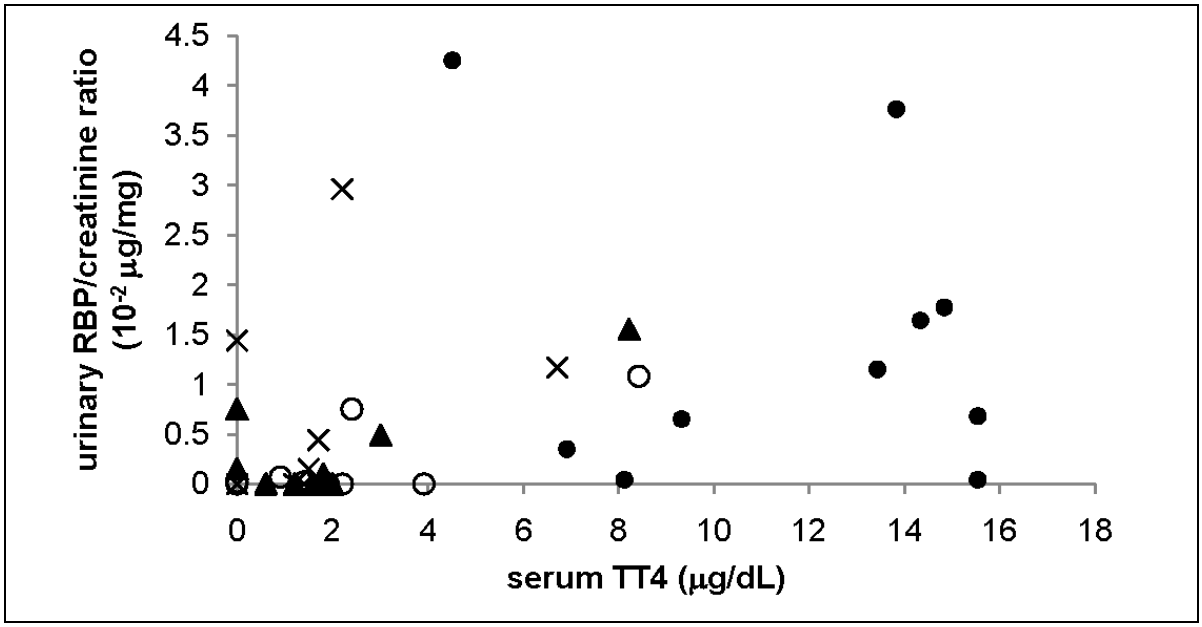


**Figure 2. Correlation between serum TT4 (µg/dL) and uRBP/c ($10^{-2}$ µg/mg) in hyperthyroid cats before (time point 0, ●) and 1 (○), 3 (▲) and 6 (x) months after $^{131}$I treatment. Values were measured in one serum and one urine sample per time point. Corresponding r and P values for significant difference from 0 are presented in Table 3 (bottom row).**

**Table 2. Concentrations (mean and SD (range)) of serum RBP (µg/L), serum TT4 (µg/dL), serum creatinine (mg/dL), urinary RBP (µg/L), urinary creatinine (mg/dL); urinary RBP/c ratio ($10^{-2}$ µg/mg), USG and GFR (mL/min/kg) in H cats (n = 8) and HT cats (n = 10) before (0) and 4, 12 and 24 weeks after $^{131}$I treatment. Median is given in italics for serum TT4, urinary RBP and uRBP/c.**

| Cats | HT<br>0 | HT<br>+4 weeks | HT<br>+12 weeks | HT<br>+24 weeks | H |
|---|---|---|---|---|---|
| Serum TT4 | 11.6 ± 4.0[a]<br>*13.6*<br>(6.9 - 15.5) | 2.1 ± 2.5[b]<br>*1.7*<br>(0 - 8.4) | 2.0 ± 2.4[b]<br>*1.7*<br>(0 - 8.2) | 1.8 ± 1.9[b]<br>*1.6*<br>(0 - 6.7) | 2.0 ± 0.3[b]<br>*2.1*<br>(1.5 - 2.4) |
| Serum RBP | 199 ± 86[a]<br>(115 - 365) | 244 ± 197[a]<br>(111 - 760) | 223 ± 148[a]<br>(94 - 533) | 182 ± 124[a]<br>(127 - 510) | 174 ± 60[a]<br>(70 - 252) |
| Serum creatinine | 0.68 ± 0.14<br>(0.56 - 0.94) | 1.05 ± 0.29<br>(0.56 - 1.49) | 1.38 ± 0.41<br>(0.58 - 2.13) | 1.45 ± 0.54<br>(0.79 - 2.68) | 1.40 ± 0.16<br>(1.22 - 1.71) |
| Urinary RBP | 10.6 ± 8.2[a]<br>*8.45*<br>(0 - 23.5) | 3.0 ± 6.4[b]<br>*0*<br>(0 - 19.5) | 3.5 ± 5.7[b]<br>*1.5*<br>(0 - 18.3) | 4.4 ± 5.4[b]<br>*2.3*<br>(0 - 14.9) | * |
| Urinary creatinine | 117 ± 58[a]<br>(19 - 209) | 186 ± 82[b]<br>(68 - 335) | 192 ± 94[b]<br>(80 - 339) | 171 ± 101[a,b]<br>(25 - 308) | 341 ± 150[c]<br>(106 - 580) |
| Urinary RBP/c | 1.4 ± 1.5[a]<br>*0.9*<br>(0 - 4.3) | 0.2 ± 0.4[b]<br>*0*<br>(0 - 1.1) | 0.3 ± 0.5[b]<br>*0.1*<br>(0 - 1.6) | 0.6 ± 1.0[a,b]<br>*0.1*<br>(0 - 3.0) | - |
| USG | 1.036 ± 0.013[a]<br>(1.014-1.060) | 1.034 ± 0.013[a]<br>(1.015-1.051) | 1.030 ± 0.13[a]<br>(1.015-1.051) | 1.038 ± 19.3[a]<br>(1.012-1.060) | 1.038 ± 0.017[a]<br>(1.008-1.050) |
| GFR | 3.3 ± 1.0[a]<br>(2 - 5.3) | 2.1 ± 0.7[b]<br>(1.2 - 5.3) | 1.6 ± 0.6[b]<br>(0.9 - 3.2) | 1.6 ± 0.6[b]<br>(0.9 - 2.9) | 1.9 ± 0.2[b]<br>(1.6 - 2.1) |

***: RBP concentration below assay sensitivity (1.37 µg RBP/L urine), - : ratio can not be calculated. When the superscripts ([a, b, c]) are different between time points for a specific variable, a statistically significant difference is observed between the values. P values are provided in the results section.**

**RBP: retinol binding protein, USG: urine specific gravity, GFR: glomerular filtration rate.**

**Table 3. Pearson correlation coefficients (r) and corresponding P values for difference from 0, for the comparison of serum RBP with serum TT4 and uRBP/c and the comparison of serum TT4 with uRBP/c. Correlation coefficients were calculated for all time points together, as well as before, 4, 12 and 24 weeks after treatment.**

| | **All time points together** | **0** | **+4 weeks** | **+12 weeks** | **+24 weeks** |
|---|---|---|---|---|---|
| Serum RBP - serum TT4 | r = 0.03<br>P = 0.836 | r = -0.29<br>P = 0.417 | r = 0.83<br>P = 0.003* | r = -0.12<br>P = 0.751 | r = -0.17<br>P = 0.67 |
| Serum RBP - uRBP/c | r = -0.16<br>P = 0.341 | r = -0.48<br>P = 0.155 | r = 0.68<br>P = 0.030* | r = -0.33<br>P = 0.380 | r = -0.28<br>P = 0.464 |
| Serum TT4 - uRBP/c | r = 0.42<br>P = 0.007* | r = -0.19<br>P = 0.594 | r = 0.77<br>P = 0.009* | r = 0.76<br>P = 0.010 | r = 0.25<br>P = 0.485 |

*** : r significantly different from 0, RBP: Retinol binding protein, uRBP/c: urinary RBP/creatinine ratio, TT4: total thyroxine.**

**Discussion**

In this study, we found that untreated HT cats had a significant urinary RBP concentration, and that urinary RBP was globally correlated with serum TT4 concentration. Urinary RBP decreased after $^{131}$I treatment. However, these uRBP/c values were not significantly correlated to serum RBP concentrations either before or after treatment. Moreover, serum RBP concentrations did not significantly differ from concentrations in H cats and were highly variable between HT cats. The observation that uRBP in HT cats is not significantly correlated to the systemic RBP concentration, suggests that it likely reflects dysfunction at the local tubular level. Reversibility of this renal dysfunction is indicated by the decrease in uRBP/c ratios after $^{131}$I treatment. In HT patients, the alteration of tubular function is not unexpected. Indeed, thyroid hormones stimulate active carrier-mediated tubular processes by an increased gene expression, synthesis and activity of carrier proteins like $Na^+$-$K^+$-ATPase and $Na^+/H^+$ exchanger activity in brush border membrane vesicles.[18-20] The metabolic level and reabsorbtive capacity of tubular cells is increased in hyperthyroidism.[21]

In HT cats, several factors such as decreased muscle mass, increased renal blood flow and decreased USG could influence urinary creatinine concentrations. Therefore, these factors could have indirectly contributed to differences observed in uRBP/c ratio. However, the absolute urinary RBP concentration also decreased significantly after treatment. Moreover, there was no difference in USG in the hyperthyroid cats before and after treatment, which strengthens our findings.

The FE of RBP was higher in HT cats before and after treatment compared to H cats, though results did not reach statistical significance. The FE can be increased upon renal dysfunction and more specifically upon tubular impairment. However, normal FE values may be observed in animals with an impaired renal function.[28,29] To the author's knowledge, this is the first study in either human or veterinary medicine investigating the influence of treatment on serum and urinary RBP concentrations in HT patients.

The decrease in feline uRBP/c ratios after radioiodine treatment for hyperthyroidism corroborates the results described in humans.[17] The uRBP/c ratio in humans is higher in

hyperthyroidism than in euthyroidism but varies widely and does not differ from values in control subjects. It is possible that HT humans have a less advanced stage of hyperthyroidism compared to HT cats with less substantial structural tubular damage. Nevertheless, the pathogenesis inducing this change remains unclear also in human endocrinology.

Tubular dysfunction could have different causes in HT cats. First, renal tubules are hypertrophic and hyperplastic during hyperthyroidism, which leads to an increased tubular mass, kidney weight, mitotic index, metabolic level and increased tubular secretory and reabsorbtive capacity.[21,30] This increased functional level could damage or transform tubular cells and affect several processes. Second, if there is a deficiency in the megalin-mediated endocytosis, RBP will not be taken up for recycling and will be lost in the urine.[31] Third, disturbed lysosomal degradation of RBP, subsequent to endocytic uptake, or increased RBP synthesis in tubular cells as part of the stimulated functional level,[6] may lead to increased urinary RBP excretion. Additionally, a higher renal blood flow in HT cats may lead to alterations in renal hemodynamics and may provoke reversible ischemic tubular damage.[32]

Besides local tubular dysfunction, several systemic factors can possibly induce the change in uRBP/c ratios in HT cats. Any change in the affinity between TTR and holo-RBP will lead to an increase in unbound RBP which is susceptible to glomerular filtration. Malnutrition decreases TTR concentrations and thereby increases the glomerular filtration of RBP.[33,34] Undernutrition leading to a low body condition score is common in HT cats. Therefore, although no changes in total serum RBP were observed in our study, we cannot exclude that in untreated HT cats the unbound RBP fraction may be increased and lead to RBP excretion because of the renal threshold.[8,35] To the author's knowledge, such a phenomenon has not yet been described.

Development of post-treatment renal azotemia in HT cats has been described in several independent studies.[36-38] It would therefore be of interest to detect pre-existing though masked renal dysfunction in HT cats. The large individual variation - albeit smaller than in humans[17] - in uRBP/c ratios in HT cats before and after treatment might indicate that this ratio could serve as a potential predictive marker for CKD developing after $^{131}$I treatment. Interestingly, 20 % of the HT patients in the current study developed CKD after treatment. Despite the fact that 2 cats had an increased uRBP/c before treatment, uRBP/c ratio remained

elevated in only one of these two cats until 24 weeks after treatment. A study in a larger number of cats and during a prolonged follow-up period is ongoing. The objective of this latter study is to evaluate the predictive value of different standard and candidate renal markers including uRBP/c for development of post-treatment renal azotemia after treatment.

The current study is the first report on assessment of RBP in serum of cats. Nonetheless, it has to be kept in mind that the reported concentrations are only relative values. Indeed, although the systemic transport system of the holo-RBP-TTR complex is analogous for all Mammalians including Felidae, there are significant immunological differences among mammalian orders. According to one comparative immunology study feline serum RBP and a anti-human serum RBP Ab raised in rabbits failed to show cross-reactivity using a radio-immunoassay.[39] This observation is in contrast to a more recent comparative study using radial immunodiffusion where partial cross-reactivity was seen between a rabbit anti-human Ab and serum RBP from felids.[10] In the latter study, feline serum RBP showed cross-reactivity to both anti-human unbound uRBP Ab as well as anti-human serum TTR-RBP Ab. It is possible that the anti-human Ab not only binds to RBP-4 like in humans, but also to other forms of RBP present in serum of cats.

Besides the described differences between Mammalia, factors related to the assay can also influence the crossreactivity between feline RBP and an anti-human RBP Ab. Affinity of the anti-human uRBP Ab for RBP in serum is lower than for RBP in urine because serum RBP is complexed to TTR while being unbound in urine.[40] This TTR could influence RBP-Ab binding in non-denaturing conditions like in ELISA.[41] Moreover, uRBP is modified by proteolysis at its carboxyl terminus thereby inhibiting binding to TTR, and differences in amino acid composition influence the binding of Abs to the protein.[41] Additional influences on serum RBP assessment are the collection methods for serum or plasma and the fasting versus fed state of the patient.[41] In the current study serum tubes without clot-activator were used, as recommended, and cats were fasted overnight prior to blood collection. Burri et al. found little or no influence of overnight fasting on RBP concentration in humans and rats.[10]

The presented longitudinal study is the first report on quantitative RBP values in serum and urine of H and HT cats before and after treatment of hyperthyroidism. A limitation of our study is the relatively small number of cats. Nonetheless, results of uRBP in HT cats before and after treatment and compared to H cats were significant.

# CHAPTER 3

## Conclusion

The presence of urinary RBP in HT patients is a potential marker of tubular dysfunction which is correlated to thyroidal status, although suggested to be independent of circulating RBP concentrations. Our data suggest that the observed tubular dysfunction is mainly reversible in HT cats after treatment with $^{131}I$. Still, additional data are required to support this hypothesis and investigate the usefulness of RBP as a marker prediction of post-treatment renal azotemia.

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# CHAPTER 4

# FOLLOW UP OF KIDNEY FUNCTION IN HYPERTHYROID CATS AFTER TREATMENT WITH RADIOIODINE

# CHAPTER 4

# CHAPTER 4

Introduction to Chapter 4

Previous studies have evaluated the kidney function after treatment of hyperthyroid cats, but most have focused on glomerular function, over a short term period. We investigated kidney function through measurement of several variables before and after treatment. Regarding these variables, we also assessed the post-treatment time course in cats maintaining a healthy kidney function and cats developing post-treatment renal azotemia, as well as the possible predictive value of these variables for post-treatment GFR and the development of post-treatment renal azotemia.

# CHAPTER 4

# FOLLOW UP OF KIDNEY FUNCTION IN HYPERTHYROID CATS AFTER TREATMENT WITH RADIOIODINE

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# CHAPTER 4


**Summary**

Hyperthyroidism can mask co-existing chronic kidney disease (CKD). Previous studies showed that post-treatment renal azotemia can be predicted by pre-treatment assessment of glomerular filtration rate (GFR). We hypothesized that treatment of hyperthyroidism may have different effects on glomerular and tubular function and these changes might be predicted by additional pre-treatment variables other than GFR.

Serum total T4 (TT4), creatinine and blood urea nitrogen (BUN), blood pressure (BP), body weight (BW), GFR, urine specific gravity (USG), urinary protein/creatinine ratio (UPC) and retinol binding protein/creatinine ratio (uRBP/c) were evaluated before and 1, 4, 12 and 24 weeks post-treatment with radioiodine ($^{131}$I) in 21 non-azotemic hyperthyroid cats. Cats were divided 24 weeks post-treatment into group A (normal kidney function, n = 16) and group B (impaired kidney function, n = 5).

Serum TT4, GFR, UPC and uRBP/c decreased significantly after treatment for the complete group and group A ($P < 0.05$), although GFR and uRBP/c did not change significantly in group B. Serum creatinine and BW increased significantly from 1 week after treatment ($P < 0.05$). There was no change in BUN, USG or BP. Pre-treatment serum TT4, GFR and USG differed significantly between group A and B ($P < 0.05$). GFR at 4 weeks after treatment and maximum decrease in GFR could be partially predicted by a formula using pre-treatment GFR, serum TT4, serum creatinine, BUN and/or USG.

Significant changes in kidney function occur within 4 weeks post-treatment and none thereafter. Pre-treatment measurement of GFR, USG and serum TT4 can have possible predictive value regarding the development of post-treatment renal azotemia.

# CHAPTER 4

## Introduction

Hyperthyroidism is the most diagnosed endocrine disorder in geriatric cats and is reported to affect 0.3 % of all cats with no apparent gender or breed predilection.[1] Chronic kidney disease (CKD) affects 7.7 % of cats over 10 years of age and 15.3 % of cats over 15 years of age.[2,3] Consequently, CKD and hyperthyroidism may occur concurrently in geriatric cats. Prevalence of pre-existing CKD in hyperthyroid cats has indeed been reported to be as high as 40 %.[4,5] Furthermore, hyperthyroidism can mask pre-existing CKD. In up to 39 % [6-9] of treated cats the impaired kidney function becomes only apparent after treatment of hyperthyroidism.

Previous studies have investigated renal function including glomerular filtration rate (GFR) after treatment of hyperthyroid cats.[6,7,10] Boag et al. showed that GFR decreases significantly 1 month after treatment, though remains stable between 1 and 6 months post-treatment.[10]

Reversible treatment with anti-thyroid medication is warranted when a hyperthyroid cat is suspected to have concurrent CKD.[11] Prediction of changes in renal function before starting the treatment would be extremely useful, because early detection of CKD is essential for optimal management of these patients.[12] It has been proposed that cats with normal serum urea (blood urea nitrogen, BUN) and creatinine concentrations combined with urine specific gravity (USG) higher than 1.035 have a reduced risk for development of CKD after treatment.[13] However, BUN and creatinine are insensitive markers of early CKD. Recently, it was also shown that cats with moderately increased serum creatinine concentrations could have normal GFR values.[14] This could be caused by differences in creatinine production rate inducing an increase in serum creatinine concentration while clearance of creatinine is unaffected. A low USG has been suggested to be more specific in predicting CKD, although results were not significant.[7] Nonetheless, cats with USG higher than 1.035 can still develop azotemia after treatment and therefore no suitable cut-off value for USG can be given.[15] Studies which evaluated GFR, BUN and serum creatinine concentrations as well as proteinuria and USG as potential predictors of developing post-treatment CKD found only pre-treatment GFR a sensitive indicator for development of CKD.[5,7,15-17] Nonetheless, pre-treatment GFR was not regarded valuable in two recent studies investigating the predictive

value of GFR for development of CKD despite the finding that pre-treatment GFR was significantly lower in cats developing GFR below reference ranges 6 months after treatment.[10,15]

New variables possibly predicting CKD after treatment in hyperthyroid cats are currently investigated. A new promising urinary marker is urinary retinol binding protein (uRBP). RBP appears in urine when there is a tubular dysfunction or damage. It is present in urine of cats with CKD and to a variable extent in urine of hyperthyroid cats.[18] The uRBP in hyperthyroid cats is not related to serum RBP concentrations before and after treatment of hyperthyroidism.[19] Tubular damage or dysfunction may occur in hyperthyroidism due to hypertrophy and hyperplasia of the tubular cells.[20]

The first objective of the present prospective study was to assess kidney function through measurement of several variables before and after treatment. The post-treatment time course of these variables was then studied in cats which maintained a normal glomerular function and cats developing post-treatment renal azotemia. The second objective was to test the pre-treatment predictive value of any of these variables for the development of renal dysfunction.

## Materials and Methods

### Cats

Twenty-one client-owned hyperthyroid cats treated with radioiodine ($^{131}I$) at the Faculty of Veterinary Medicine of Ghent University (Belgium) were included in the study. Owners signed informed consent previous to inclusion. Age at time of inclusion in the study was 12.6 ± 2.4 years.

Inclusion criteria were a diagnosis of hyperthyroidism (i.e. clinical signs compatible with hyperthyroidism, increased serum total thyroxine (TT4) concentration above reference range values and increased thyroidal uptake of $^{99m}TcO_4^-$. Anti-thyroid medication was ceased at least 3 weeks prior to $^{131}I$ treatment. Cats with concurrent disease, including CKD (IRIS stage II or higher) and neoplasia, were excluded. Cats were fasted for at least 10 hours before the start of the test-day and were fed immediately after the end of the sampling period. Water was offered *ad libitum*. Cats maintained their original diet throughout the study period, except when a renal diet was mandatory based on International Renal Interest Society (IRIS) recommendations (www.IRIS-kidney.com). Cats with serum TT4 below reference range values underwent a TSH stimulation test with 25 μg recombinant human TSH (rhTSH) (Thyrogen®, Genzyme corporation, The Netherlands) IV to test for iatrogenic hypothyroidism. At the end of the study period 24 weeks post-treatment, cats were divided into two groups according to renal status: cats in group A with a normal kidney function based on either serum creatinine concentrations within the reference range (8 - 140 μmol/L) or mild azotemia (< 249 μmol/L, IRIS stage II) though a GFR > 1.2 mL/min/kg, and cats in group B that developed CKD (IRIS stage II or higher) based upon clinical signs (polyuria, polydipsia), development of azotemia (serum creatinine concentration > 140 μmol/L) and GFR ≤ 1.1 mL/min/kg.[21,22] One cat had mild azotemia, a GFR of 1.1 mL/min/kg at 24 weeks after treatment and serum TT4 concentration below reference range. This cat was diagnosed with iatrogenic hypothyroidism and assigned to group A because the azotemia disappeared after thyroxine supplementation. One cat in group B had a GFR of 1.2 mL/min/kg but was also diagnosed with iatrogenic hypothyroidism and after thyroxine supplementation this cat's azotemia increased and GFR decreased to 1.0 mL/min/kg.

**Study design**

The study design is outlined in Table 1. Cats were evaluated 1 - 2 days before treatment, and at 1, 4, 12 and 24 weeks after treatment on a follow-up test-day. Tested variables were body weight (BW), systolic blood pressure (BP), serum TT4, creatinine and urea concentration, GFR, USG (normal USG value defined as USG > 1.030), urinary protein to creatinine ratio (UPC) (proteinuria was defined as UPC > 0.4). and retinol binding protein/creatinine ratio (uRBP/c). Moreover, cardiac auscultation, electrocardiography (ECG), cardiac echography, abdominal ultrasonography, complete blood count (CBC) and urine culture were performed before treatment and at the end of the study (24 weeks). Two years after the study, owners were contacted by phone to document the mortality of the tested animals.

**Procedures**

Systolic blood pressure was measured by Doppler method at the beginning of the test-day after acclimatization to the environment. An average was made of the last 3 values of 5 consecutive measurements.

GFR was measured with plasma exo-iohoxol clearance test (PexICT), as previously described.[21] Briefly, pharmacokinetic analysis was performed using WinNonlin (version 4.0.1, Scientific consulting Inc Apex, NC). Plasma clearance was determined by dividing the dose administered by area under the curve (AUC), and indexed to bodyweight (BW) (mL/min/kg). Range of GFR values found in healthy cats using PexICT is 1.2 - 2.1 mL/min/kg.

**Assays**

A polyclonal rabbit anti-human uRBP antibody was used in a commercial sandwich ELISA (RBP4 ELISA kit, Immundiagnostik AG, Bensheim, Germany) validated for RBP assessment in feline urine as described previously by our group.[18] Urinary RBP concentrations were expressed as RBP/creatinine ($10^{-2}$ μg/mg) ratio.

**Statistical analysis**

A mixed model with cat as random effect (Systat version 8.0, SPSS Inc. Chicago IL, USA) was used to test for differences in tested variables among the time-points (i.e. before and 1, 4, 12 and 24 weeks after treatment) at a global significance level of 0.05. Moreover, for each variable, time points before (0) and 1 (not for urinary variables), 4, 12 and 24 weeks

after treatment were compared pair-wise at a Bonferroni-adjusted comparison-wise significance level of 0.05/5 = 0.01 (serum TT4, GFR, serum creatinine, serum urea, BP and BW), or 0.05/4 = 0.0125 (urinary RBP, UPC, USG). A student's t-test was used to compare pre-treatment variables between group A and B to test for a significant effect of group at a significance level of 0.05. The effect of each pre-treatment variable at time point 0 ($variable_0$) on the GFR value at 4 weeks after treatment ($GFR_4$) and the maximum decrease in GFR ($\Delta GFR_{max,}$ expressed in %) was tested using a general linear model at a significance level of 0.05. Results are expressed as mean ± SD unless stated otherwise.

**Table 1. Study design for the follow up of 21 non-azotaemic hyperthyroid cats before and after treatment with radioiodine.**

| | | **Post-treatment (weeks)** | | | |
|---|---|---|---|---|---|
| Procedure | 0 | 1 | 4 | 12 | 24 |
| BW | x | x | x | x | x |
| BP | x | | x | x | x |
| Cardiac examination | x | | | | x |
| Abdominal ultrasound | x | | | | x |
| Serum analysis | x | x | x | x | x |
| CBC | x | | | | x |
| GFR | x | x | x | x | x |
| Urinalysis | x | x | x | x | x |
| Urine culture | x | | | | x |

**0: 1 or 2 days pre-treatment. BW: bodyweight; BP: blood pressure; serum analysis: serum creatinine concentration, BUN; CBC: complete blood count; GFR: glomerular filtration rate; urinalysis: USG, UPC, uRBP/c.**

## Results

### Cats

One cat was lost for follow up 20 weeks after treatment due to euthanasia because of malignant neoplasia of the pleura. One cat failed to have GFR measurement due to aggressive behavior 12 weeks after treatment. Nine of the 21 included cats received anti-thyroid medication previous to treatment. None of the included cats had significant abnormalities on abdominal ultrasound before or 24 weeks after treatment. Alterations seen on cardiac electrocardiography and cardiac ultrasonography compatible with hyperthyroidism improved (n = 10) or remained stable (n = 10) after treatment. None of the cats had an urinary infection (UTI) before treatment. One cat had a UTI 6 months after treatment, though other renal variables showed no abnormalities. Six cats (3 from group A and 3 from group B) had died within two years after treatment, 2 of them (group B) due to renal failure. One cat from group B started a renal diet 3 months after treatment.

The age of the cats at time 0 in group A (11.9 ± 2.2 years) and group B (14.8 ± 1.3 years) differed significantly ($P < 0.05$). There was a significant difference between group A and B in pre-treatment values of serum TT4 ($P = 0.004$), GFR ($P = 0.032$) and USG ($P = 0.001$) but not for the other tested variables.

### Post-treatment time course of the tested variables

Follow up of BW and BP in the complete group and in group A and B separately is shown in Figure 1. BW increased significantly after treatment in the complete group ($P < 0.001$) and group A ($P < 0.001$) and group B ($P = 0.009$) (Figure 1A). BW increased significantly from 1 week after treatment until 24 weeks after treatment for the complete group and group A, though in group B it only increased until 4 weeks after treatment.

BP was $\geq$ 160 mm Hg in 10/21 cats before treatment and in 2/20 cats at 24 weeks after treatment. Of the latter two cats, BP was measured afterwards in their home environment as normotensive. There was no significant change in BP for the complete group, group A and group B (Figure 1B).

Time courses of serum TT4, GFR, serum creatinine and BUN concentration, before and 1, 4, 12 and 24 weeks after $^{131}I$ treatment, for the whole group and groups A and B are shown in figure 2A, 2B, 2C and 2D, respectively. Mean ± SD, range and number of animals

(N) of serum TT4, GFR and uRBP/c for the complete group and group A and B is given in Table 2.

The serum TT4 concentration in the complete group and in groups A and B was significantly lower at all time points after treatment compared to pre-treatment serum TT4 concentration ($P < 0.001$). For groups A and B, the interaction between the time and the group effect was statistically significant ($P = 0.032$). Serum TT4 decreased significantly until 4 weeks after treatment for the complete group and group A ($P < 0.001$), but only until 1 week after treatment for group B ($P < 0.001$). One cat had persistently high serum TT4 of 116 nmol/L, 108 nmol/L, 106 nmol/L and 86 nmol/L at 1, 4, 12 and 24 weeks after treatment, despite improvement of clinical signs. This cat remained included in the study because there were no symptoms of hyperthyroidism 24 weeks after treatment and the cat substantially increased in BW. When the owners were contacted two years after the end of the study period, there were still no signs of recurring hyperthyroidism and the cat showed no clinical symptoms nor had the owners any remarks about the clinical condition of the cat. Serum TT4 was below reference range at 24 weeks post-treatment in 1 cat from group A and 4 cats from group B. Iatrogenic hypothyroidism was diagnosed with a rhTSH stimulation test in the cat from group A and 3 cats from group B.

Serum creatinine increased significantly after treatment in the complete group, group A and group B ($P < 0.001$), and the pattern in increasing serum creatinine differed significantly between group A and B ($P < 0.001$). Serum creatinine increased significantly from 1 week after treatment until 12 weeks after treatment for the complete group and group A, but only until 4 weeks after treatment for group B. At 12 and 24 weeks after treatment 2 cats from group A and all cats in group B were azotemic.

BUN did not change significantly after treatment in the complete group ($P = 0.284$), nor in group A or B separately, and there was no difference in trend between group A and B.

The decreasing pattern in serum TT4 after treatment was comparable to the decreasing pattern in GFR. There was a significant difference in GFR before $^{131}I$ treatment compared to all time points after treatment in the complete group ($P < 0.001$) and in group A ($P < 0.001$) and B ($P = 0.002$). GFR decreased significantly until 4 weeks after treatment for the complete group and group A, but there was no clinical relevant change visible after treatment for group B.

Pre-treatment GFR was comparable to values in healthy cats in 1 cat from group A and 3 cats from group B, all other cats had GFR values higher than described in healthy cats. At

24 weeks post-treatment, GFR remained higher than values in healthy cats in 3 cats from group A, GFR was comparable to values in healthy cats in 11 cats in group A and 1 cat in group B, and GFR was below values in healthy cats in 1 cat from group A and 4 cats from group B.

Time courses of uRBP/c, UPC and USG before and 4, 12 and 24 weeks after $^{131}I$ treatment, for the complete group, groups A and B are shown in Figure 3A, 3B and 3C, respectively.

uRBP/c decreased significantly after treatment for the complete group ($P = 0.001$) and group A ($P < 0.001$) but not for group B ($P = 0.147$), hence the pattern after treatment in uRBP/c differed significantly between group A and B ($P < 0.001$). uRBP/c decreased significantly until 4 weeks after treatment in the complete group and group A. RBP was present in urine of 15 out of 16 cats from group A and all 5 cats from group B before treatment. It remained present at 24 weeks post-treatment in 6 cats from group A and 2 cats from group B.

UPC decreased significantly until 4 weeks after treatment for the complete group ($P < 0.001$), group A ($P < 0.001$) and group B ($P = 0.010$), and the decreasing pattern in UPC differed significantly between group A and B ($P < 0.001$). Pre-treatment proteinuria was present in 13 cats from group A and in all cats from group B. Proteinuria remained present at 24 weeks post-treatment in only 1 cat from group A though in none of the cats in group B.

USG did not change significantly after treatment in the complete group, group A or group B. Pre-treatment USG < 1.030 was present in 2 cats from group A and all cats in group B. At 24 weeks after treatment, 2 cats from group A and 4 cats from group B had USG < 1.030.

**Effect of pre-treatment variables on post-treatment GFR**

Statistically significant effect of pre-treatment variables (GFR, serum TT4, creatinine, BUN and USG) on GFR 4 weeks after treatment ($GFR_4$) and $\Delta GFR_{max}$ and the equation of the corresponding general linear model is given in Table 3. Other pre-treatment variables (BW, BP, uRBP/c and UPC) did not have a significant effect on $GFR_4$ and $\Delta GFR_{max}$. Pre-treatment GFR ($GFR_0$) ($P = 0.001$) explained 48 % of the change observed in $GFR_4$ (Figure 4A), and a model including both serum $creatinine_0$ ($P = 0.003$) and $USG_0$ ($P = 0.006$) could explain 62 % of the variability in $GFR_4$ (Figure 4B). $GFR_0$ ($P = 0.012$) could explain 29 % of the $\Delta GFR_{max}$ (Figure 4C).

**Table 2. Main variables evaluated before and 1, 4, 12 and 24 weeks after treatment: serum TT4, GFR and uRBP/c in the complete group (n = 21), group A ( n = 16) and group B (n = 5).**

| Variable | Time point | Complete group | Group A | Group B |
|---|---|---|---|---|
| Serum TT4 (nmol/L) | 0 | 124.2 ± 53.5 (42.6 - 200) | 137.7 ± 53.4 (42.6 - 200) | 81.0 ± 23.5 (58.1 - 120.0) |
| | 1 | 42.3 ± 29.5 (10.3 - 116.1) | 48.5 ± 30.6 (11.6 - 116.1) | 22.4 ± 11.7 (10.3 - 40.0) |
| | 4 | 18.8 ± 23.4 (6 - 108.1) | 21.8 ± 26.2 (6 - 108.4) | 9.3 ± 5.3 (6.0 - 18.1) |
| | 12 | 20.3 ± 21.2 (6.0 - 105.8) | 23.6 ± 23.3 (6.5 - 105.8) | 9.8 ± 5.5 (6.0 - 18.1) |
| | 24 | 20.4 ± 16.9 (6.0 - 86.4) | 24.1 ± 18.0 (9.0 - 86.4) | 9.4 ± 4.4 (6.0 - 16.8) |
| GFR (mL/min/kg) | 0 | 3.3 ± 1.2 (1.3 - 5.6) | 3.6 ± 1.1 (2 - 5.6) | 2.3 ± 0.9 (1.3 - 3.3) |
| | 1 | 2.4 ± 0.9 (1.1 - 3.8) | 2.7 ± 0.8 (1.4 - 3.8) | 1.8 ± 0.8 (1.1 - 3.1) |
| | 4 | 2.0 ± 0.8 (0.9 - 3.5) | 2.2 ± 0.7 (1.1 - 3.5) | 1.2 ± 0.4 (0.9 - 1.8) |
| | 12 | 1.6 ± 0.6 (0.7 - 3.2) | 1.8 ± 0.6 (1.2 - 3.2) | 1.0 ± 0.3 (0.7 - 1.4) |
| | 24 | 1.6 ± 0.6 (0.8 - 2.9) | 1.9 ± 0.5 (1.1 - 2.9) | 1.0 ± 0.2 (0.8 - 1.2) |
| uRBP/c ($10^{-2}$ μg/mg) | 0 | 2.1 ± 3.7 (0 - 17.2) | 1.3 ± 1.1 (0 - 3.8) | 4.7 ± 7.2 (0.1 - 17.2) |
| | 4 | 0.5 ± 1.2 (0 - 3.6) | 0.2 ± 0.5 (0 - 1.8) | 1.1 ± 2.3 (0 - 3.6) |
| | 12 | 0.4 ± 0.9 (0 - 5.2) | 0.2 ± 0.5 (0 - 1.6) | 0.9 ± 1.5 (0 - 5.2) |
| | 24 | 0.4 ± 0.8 (0 - 3.2) | 0.3 ± 0.8 (0 - 3.0) | 0.7 ± 1.0 (0 - 2.2) |

**Table 3. Prediction of variability in GFR 4 weeks after treatment ($GFR_4$) and in maximum decrease in GFR after treatment ($\Delta GFR_{max}$) with variables measured before treatment ($variable_0$). P values are described in text.**

| GFR | Predicting formula | $R^2$ |
|---|---|---|
| $GFR_4$ | $0.5 + (0.443*GFR_0)$ | $0.48^{\S}$ |
| $GFR_4$ | $3.606 - (0.023*creatinine_0)$ | 0.44 |
| $GFR_4$ | $-30.492 + (0.031 * (USG_0*1000))$ | 0.37 |
| $GFR_4$ | $1.047 + (0.007 * TT4_0)$ | 0.26 |
| $GFR_4$ | $-21.763 - (0.019 * creatinine_0) + (0.024 * (USG_0*1000))$ | $0.62^{\S\S}$ |
| $GFR_4$ | $-19.181 + (0.336 * GFR_0) - (0.019 * (USG_0*1000))$ | 0.59 |
| $GFR_4$ | $1.919 + (0.313 * GFR_0) - (0.014 * creatinine_0)$ | 0.58 |
| $GFR_4$ | $-26.277 + (0.006 * TT4_0) + (0.027 * (USG_0*1000))$ | 0.51 |
| $\Delta GFR_{max}$ | $26.849 + (6.988 * GFR_0)$ | $0.29^{\S}$ |
| $\Delta GFR_{max}$ | $80.642 - (3.605 * BUN_0)$ | 0.20 |

**$^{\S,\S\S}$: Highest $R^2$ and prediction of variability using 1 ($^{\S}$) or 2 ($^{\S\S}$) variables.**

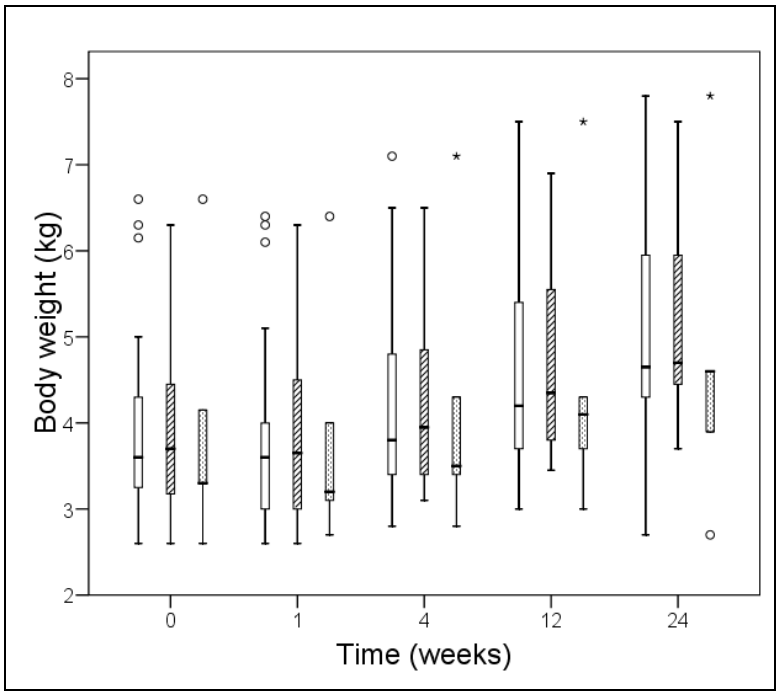


**Figure 1A. Follow up of BW (kg) in the complete group (n = 21), and cats from group A (n = 16) and cats from group B (n = 5).**

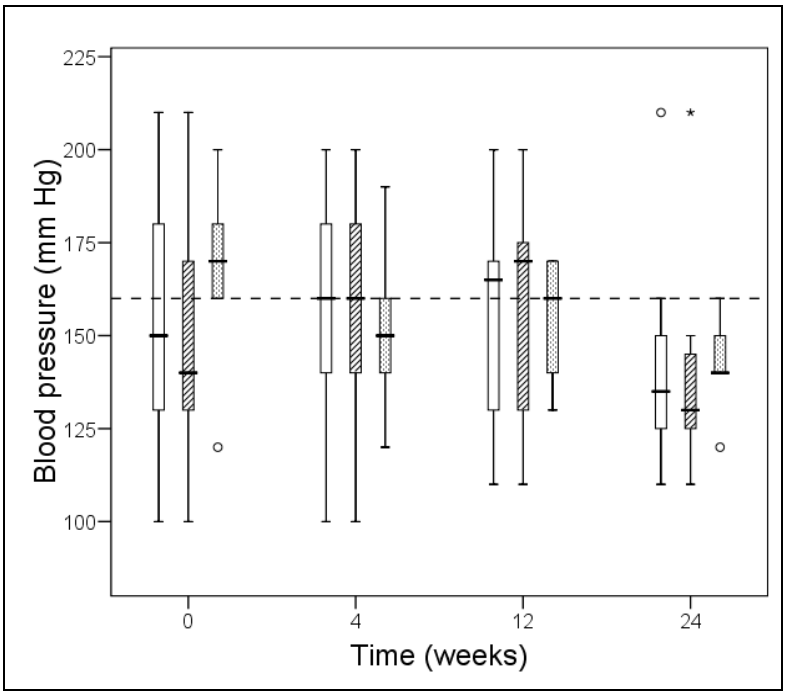


**Figure 1B. Follow up of BP (mm Hg) in the complete group (n = 21), and cats from group A (n = 16) and cats from group B (n = 5). Dotted line in figure 1B (160 mmHg) represents 20 - 40 mm Hg above reference range which is associated with moderate risk of end organ damage according to IRIS guidelines.**

**Blank box: complete group, diagonal line box: group A, dotted box: group B. Horizontal line: median, box: interquartile range, ○: outlier value larger than 1.5*(interquartile range), *: extreme value larger than 3*(interquartile range).**

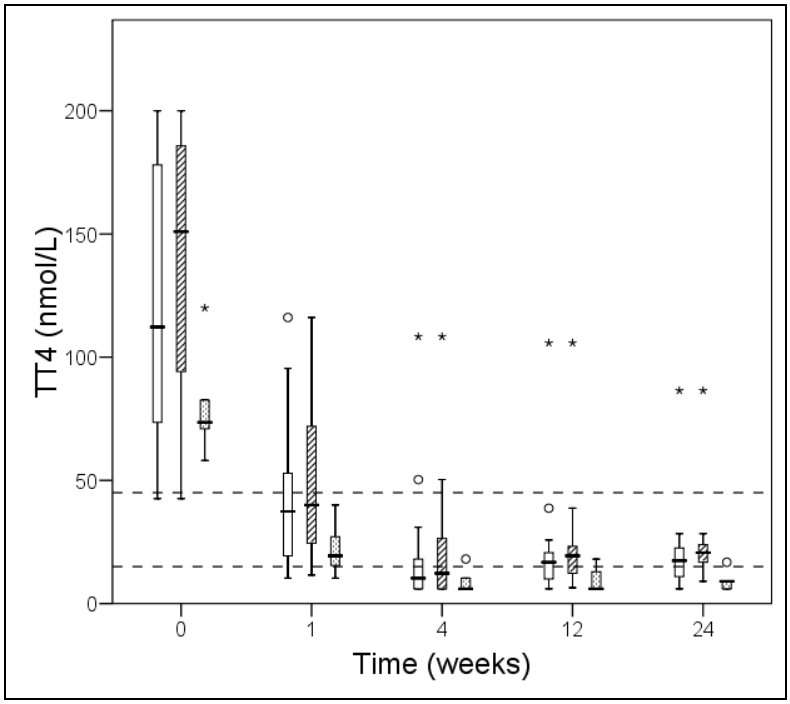


**Figure 2A. Follow up of serum TT4 (nmol/L) in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5), dotted lines represent reference ranges (15 - 45 nmol/L).**

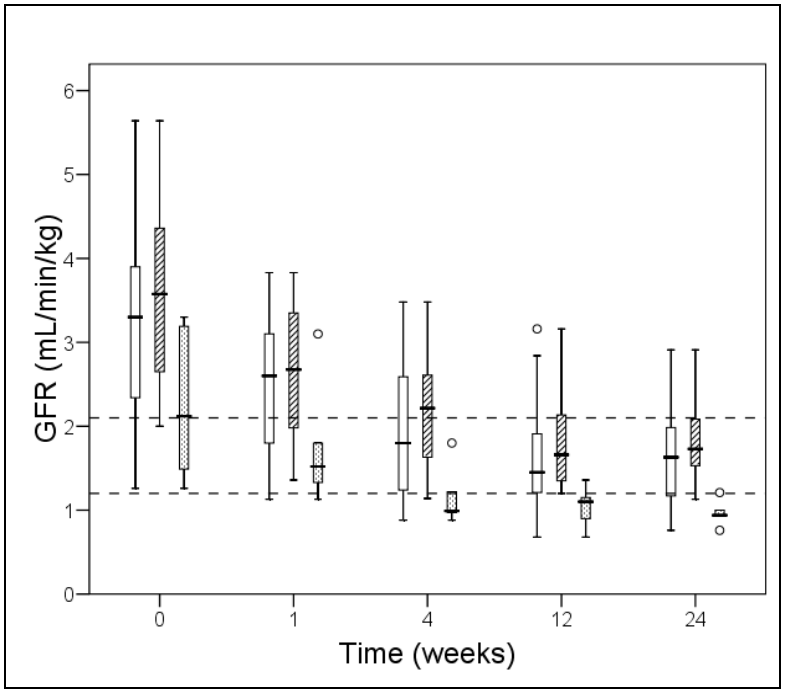


**Figure 2B. Follow up of GFR (mL/min/kg) in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5), dotted lines represent values described in healthy cats (1.2 - 2.1 mL/min/kg).[21]**

**Blank box: complete group, diagonal line box: group A, dotted box: group B. Horizontal line: median, box: interquartile range, ○: outlier value larger than 1.5*(interquartile range), *: extreme value larger than 3*(interquartile range).**

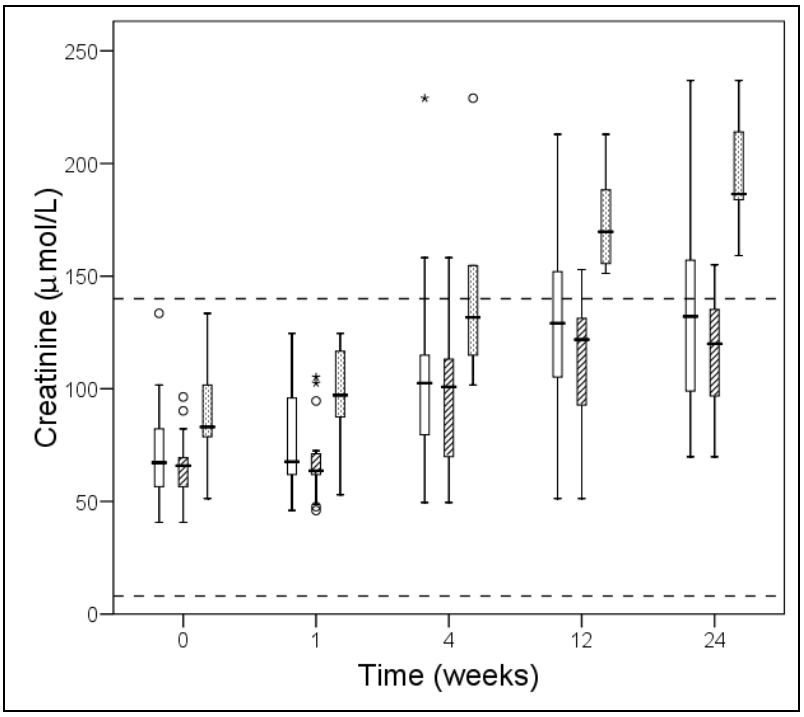


**Figure 2C. Follow up of serum creatinine (µmol/L) in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5). Dotted lines represent reference ranges (8 - 140 µmol/L).**

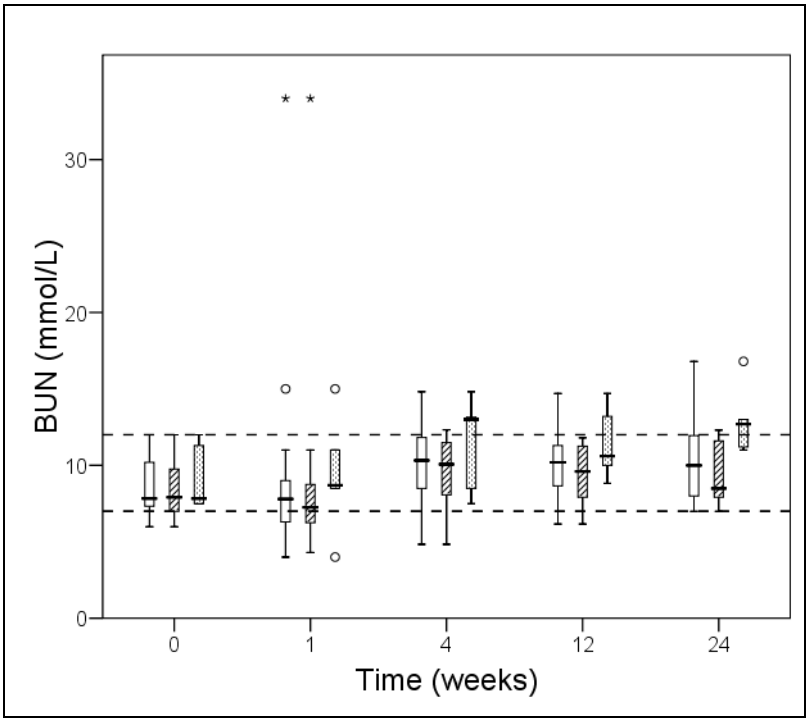


**Figure 2D. Follow up of BUN (mmol/L) in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5). Dotted lines represent reference ranges (7 - 12 mmol/L). Blank box: complete group, diagonal line box: group A, dotted box: group B. Horizontal line: median, box: interquartile range, ○: outlier value larger than 1.5*(interquartile range), *: extreme value larger than 3*(interquartile range).**

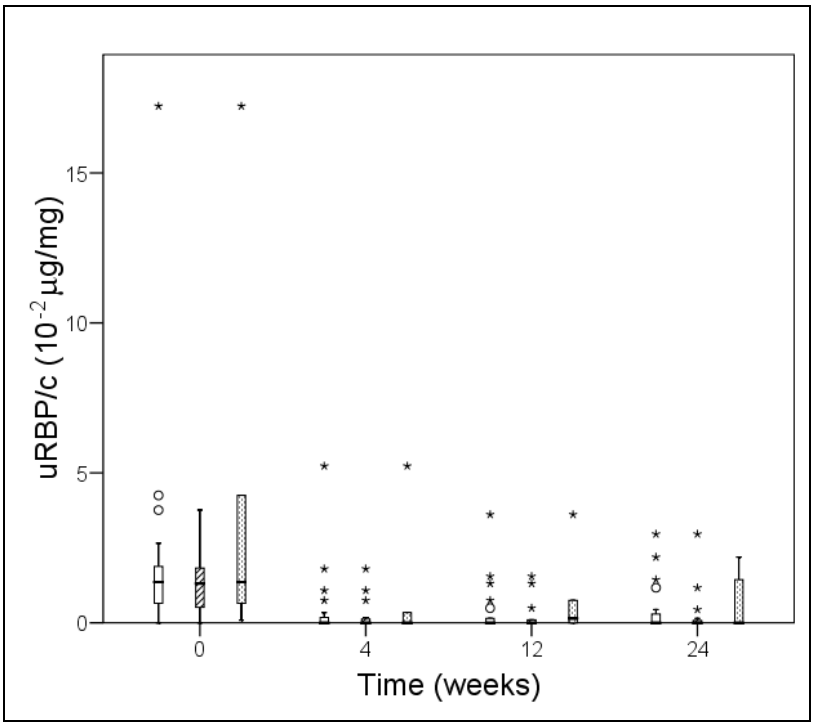


**Figure 3A. Follow up of urinary RBP/c ($10^{-2}$ µg/mg) in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5).**

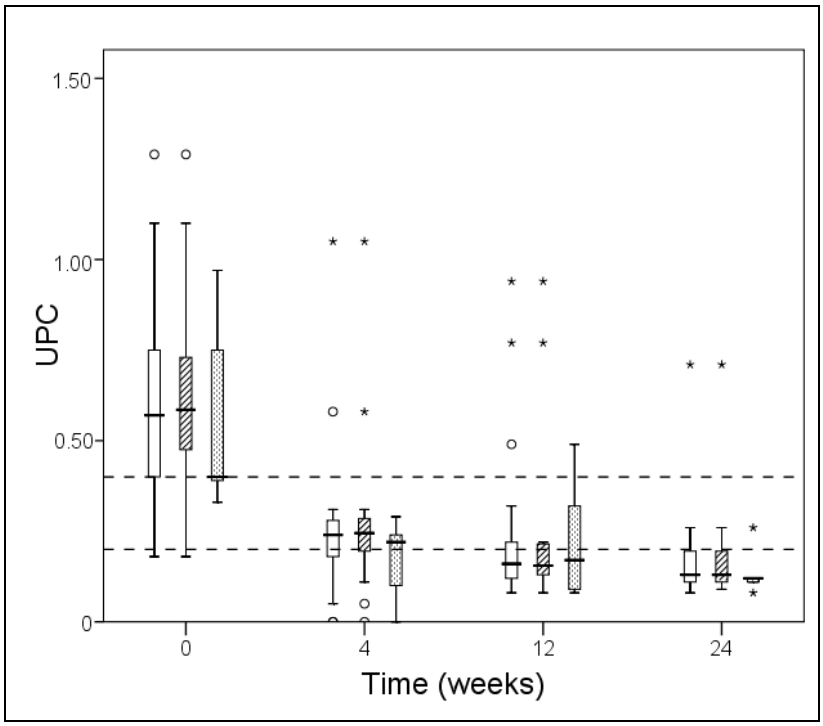


**Figure 3B. Follow up of UPC in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5). Dotted lines represent ranges considered borderline proteinuria according to IRIS guidelines (0.2 - 0.4).**

**Blank box: complete group, diagonal line box: group A, dotted box: group B. Horizontal line: median, box: interquartile range, ○: outlier value larger than 1.5*(interquartile range), *: extreme value larger than 3*(interquartile range).**

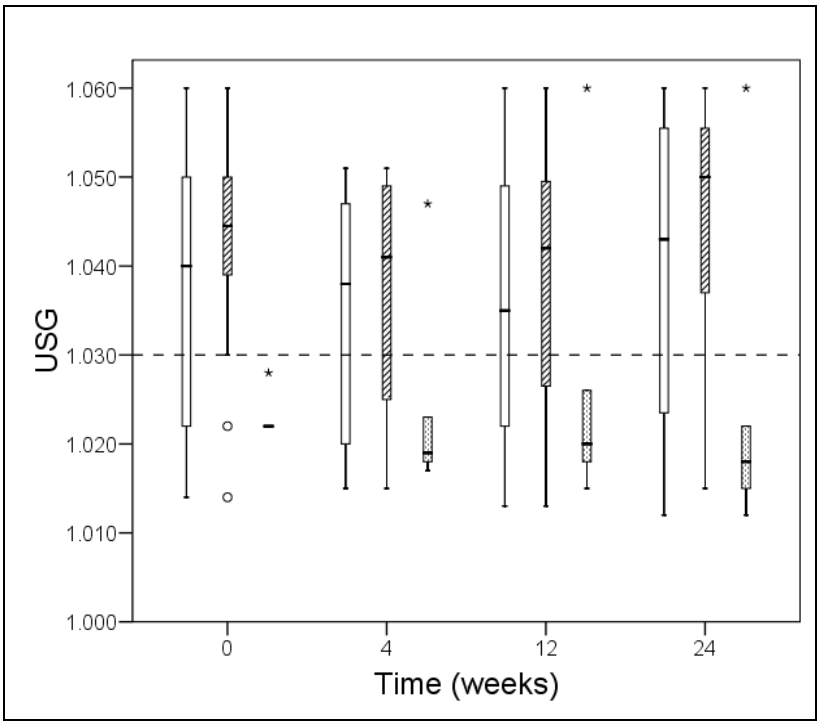


**Figure 3C. Follow up of USG in the complete group (n = 21), and divided in cats from group A (n = 16) and cats from group B (n = 5). Dotted line represents reference value of 1.030.**

**Blank box: complete group, diagonal line box: group A, dotted box, group B. Horizontal line: median, box: interquartile range, ○: outlier value larger than 1.5*(interquartile range), *: extreme value larger than 3*(interquartile range).**

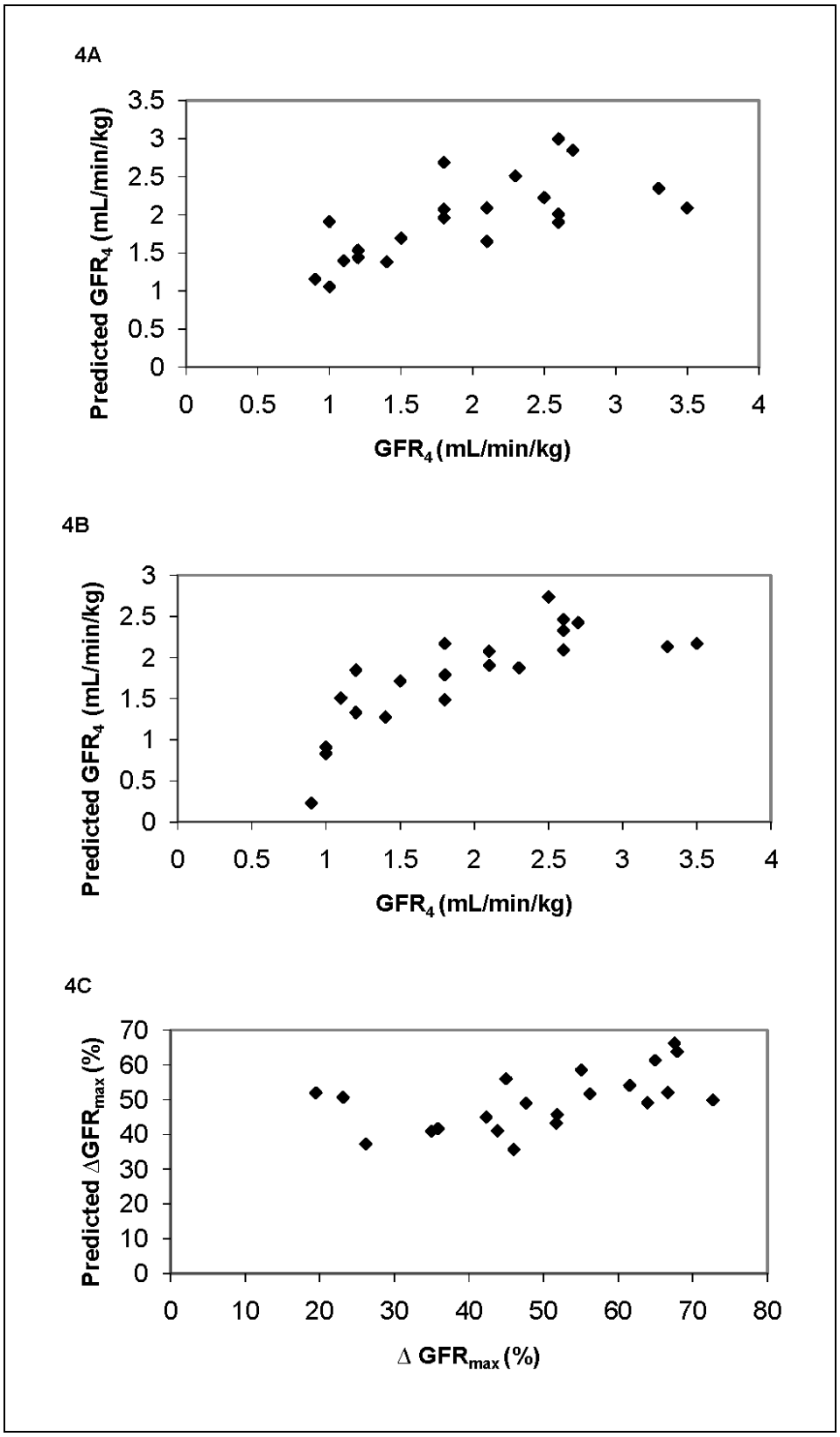


**Figure 4. Predicted values of GFR 4 weeks after treatment ($GFR_4$) using $GFR_0$ (Figure 4A) or $creatinine_0$ and $USG_0$ (Figure 4B) measured pre-treatment, plotted against actual values of $GFR_4$, and predicted values of maximum decrease in GFR after treatment ($\Delta GFR_{max}$) using $GFR_0$ (Figure 4C) plotted against actual values of $\Delta GFR_{max}$. See Table 3 for predicting formula.**

## Discussion

This is the first prospective study evaluating the short- and long-term follow up of routinely used serum and urinary renal variables (BUN, serum creatinine, UPC and USG) as well as of two less common used variables GFR and uRBP/c for glomerular and tubular function, respectively in hyperthyroid cats before and after $^{131}$I treatment. Almost one in five cats developed an impaired kidney function (group B) in contrast to cats maintaining a healthy kidney function (group A), and this percentage is comparable to previously published results.[6-9] The difference in age between cats developing CKD after treatment and cats maintaining a normal glomerular function was described[15] to be due to an increased incidence in CKD with increasing age. The impaired kidney function after treatment can be caused by pre-existing CKD which is masked by the hyperthyroidism, or it can be caused by a resolution of the hyperthyroidism and a fall in the stimulatory effects of hyperthyroidism on kidney function. CKD has a higher prevalence with increasing age.[3] The difference in age between cats maintaining a normal kidney function and cats developing post-treatment renal azotemia suggests that pre-existing CKD could be at least part of the cause of the development of post-treatment renal azotemia.

In this study, 80 % of the cats had pre-treatment GFR values higher than values described earlier in healthy cats.[21] Hyperthyroidism is associated with intrarenal vasodilatation, which combined with increased cardiac output due to positive chronotropic and inotropic effects and decreased systemic vascular resistance,[23] increases renal blood flow, glomerular hydrostatic pressure and GFR.[24] This is enhanced by autoregulatory mechanisms in the kidney that respond to the increased sodium and chloride reabsorption in the tubules leading to an additional increase in GFR.[6,25,26] Our study showed no changes in BP after treatment, suggesting that other mechanisms than peripheral hemodynamic changes must be involved in stimulatory effects of hyperthyroidism on GFR. The hyperthyroidism induced increase in GFR is reversed after treatment in humans and cats.[5-7,27] The present study confirmed the decrease in GFR until 4 weeks after treatment, although no further decrease occurred in the complete group nor in cats from group A. GFR had decreased in the complete group at 4 weeks after treatment by 39 ± 16 % which is comparable to results from other studies,[6,7,28] The between-day coefficient of variation of 8.3 % using PexICT[21] strongly

indicates that the observed decrease in GFR is caused by a change in glomerular function and not by between-day variability.

BW and serum creatinine concentration started to change from 1 week after treatment. The delayed increase in BW appears logical, because metabolic processes have to adjust to the decrease in serum TT4. Nonetheless, serum creatinine concentration is inversely correlated with GFR and is often significantly decreased in humans with hyperthyroidism due to the increased GFR, increased clearance and tubular secretion of creatinine but also to decreased muscle mass.[29,30] The delayed increase in serum creatinine compared to the rapid decrease in GFR after treatment, combined with the fact that renal tubules do not secrete creatinine in cats, suggests that the decreased serum creatinine concentration pre-treatment is more related to the decreased muscle mass than to GFR and enhanced clearance of creatinine. This is supported by the changes in BW which show a comparable trend after treatment as serum creatinine concentration. It was shown previously that a large decrease in GFR can be accompanied by only a small increase in serum creatinine concentration in dogs.[31] Serum creatinine concentration should therefore not be considered as a reliable short-term indicator of deteriorating kidney function in hyperthyroid cats.[10]

There was no change in BUN concentration after treatment in any group which is in accordance with recent studies investigating kidney function after treatment of hyperthyroid cats.[7,10] Nevertheless, an increasing BUN concentration after treatment in hyperthyroid cats has also been described.[5,6,32] These results illustrate that discrepancies exist between BUN and serum creatinine and that the latter is a more appropriate indirect marker of GFR.

Besides serum variables, several urinary variables were evaluated in this study. USG did not change after treatment of the hyperthyroid cats, which is comparable to results described in earlier studies.[7,10] UPC decreased until 4 weeks after treatment in the cats, either developing CKD or not. The decrease in UPC is described in an abstract by Syme and Elliott, which evaluated UPC in cats treated with carbimazole therapy alone or combined with thyroidectomy. However, UPC was only measured before and 6 months after treatment and not earlier.[16] Results from our study suggest reversibility of proteinuria already 4 weeks after treatment in hyperthyroid cats, although hyperthyroidism could be controlled differently with carbimazole or thyroidectomy thereby influencing results. Pre-treatment proteinuria which

reverses after treatment in cats could be a reflection of glomerular hypertension and hyperfiltration, changes in tubular protein handling or a change in the structure of the glomerular barrier.[33] Proteinuria resolves after treatment in cats from group A and B, although BP, GFR and urinary markers of tubular function such as uRBP/c and USG did not change in cats from group B. It seems most likely that the pre-treatment proteinuria is caused by a functional change in the structure of the glomerular barrier regardless of the underlying kidney function, and that this change is reversed after treatment. Whatever the cause of the pre-treatment proteinuria, an important finding is the decrease in proteinuria after treatment and therefore no treatment is indicated.

We have previously reported that the uRBP/c is correlated to serum TT4 concentration and not to serum RBP concentrations in hyperthyroid cats before and after radioiodine treatment.[19] In hyperthyroid cats, it can be therefore hypothesized that uRBP/c is a marker of reversible tubular dysfunction in a healthy kidney (group A), but also a marker of irreversible damage in cats with pre-existing CKD, as uRBP/c levels did not change and remained elevated in group B cats. Hyperthyroidism may have caused an increased functional level of tubular cells together with hypertrophy and hyperplasia, resulting in cell damage and possibly combined with increased RBP synthesis in tubular cells.[20,34]

A decrease in serum TT4 was already visible 1 week after treatment. This was also the case for GFR which supports the fact that TT4 directly and reversibly influences GFR**.** Eighty percent of the cats with post-treatment azotemia and low GFR had serum TT4 concentrations below reference range 24 weeks after treatment. GFR can be reduced in humans, rats and dogs with hypothyroidism[27,35,36] due to systemic and local factors in the kidney, serum creatinine can be increased and ability to concentrate urine impaired. The decreased GFR and increased serum creatinine can be reversed with thyroid hormone replacement.[27,37-39]

The question is raised whether there is a link between the development of post-treatment renal azotemia and low serum TT4 in these cats. Several hypotheses can be proposed.

First, the hypothyroidism could be related to $^{131}$I treatment. In our study, 20 % of the cats developed hypothyroidism (diagnosed with rhTSH stimulation test), which is higher compared to other studies using the same dose estimation of $^{131}$I.[40,41] The administration of the

iodine-containing contrast agent iohexol for measuring GFR could possibly influence treatment outcome, but in a study by Peremans et al. no effects of iohexol on clinical treatment outcome could be found.[42] The difference in pre-treatment serum TT4 concentration between cats from group A and B, with lower values in group B, could be regarded as an actual sign of underlying kidney disease. However, pre-treatment TT4 explained however only 26 % of variability in GFR 4 weeks after treatment by pre-treatment serum TT4 concentration. It is possible that these cats were in a less advanced stage of hyperthyroidism, and that the dose of administered $^{131}$I was too high which caused hypothyroidism. Following this hypothesis, it is also possible that cats with an impaired kidney function previous to $^{131}$I treatment, which can be extrapolated from the decreased GFR pre-treatment in group B compared to group A, have a lower clearance of $^{131}$I. This causes a prolonged effect of $^{131}$I on the thyroid and increases the chance of developing hypothyroidism after treatment. Indeed, human patients with impaired renal function treated for thyroid cancer need a decreased $^{131}$I dose, to avoid hypothyroidism and reach an amount of radioactivity in the thyroid comparable to humans with a normal renal function and receiving a higher dose of $^{131}$I.[43]

Secondly, hypothyroidism, whatever its cause, could contribute to a declining kidney function. Hypothyroidism can cause glomerular lesions such as thickening of the basement membrane and increased mesangial matrix.[39,44] Increased transcapillary leaking of plasma proteins in hypothyroidism can lead to mild proteinuria.[45] Proteinuria has been suggested to cause intrinsic renal toxicity and decreased survival time, although the precise role of proteinuria in the progression of renal disease is uncertain.[46] However, none of the cats in our study from group B were proteinuric after $^{131}$I treatment, and therefore this cause is unlikely. Treatment of hypothyroidism in a human patient with progressive renal failure leads to significant improvement of renal function.[47]

Besides the hypotheses described above, it is also possible that the declined kidney function has a causal effect on the low serum TT4 concentration. A large retrospective study in humans surprisingly found a reduced GFR, as in CKD, to be associated with an increased prevalence of hypothyroidism.[48] The cause is unclear, but there could be a possible role for auto-immune disorders, iodine excess, or retained solutes in the kidney with a potential effect on thyroid function.[48] It is possible that a comparable mechanism is present in cats after

treatment for hyperthyroidism, although the cause-effect relationship remains to be documented.

Finally, CKD can decrease serum TT4 concentrations below reference range values in cats.[49] Hypothetically, the development of CKD and hence euthyroid sick syndrome could decrease serum TT4 surplus to effects of $^{131}$I treatment and cause an earlier plateau in low serum TT4 after treatment in cats with CKD. CKD can suppress serum TT4 concentration (euthyroid sick syndrome) thereby mimicking hypothyroidism.[50]

We can conclude from the hypotheses described above, that a possible causal link between hypothyroidism and CKD after treatment in hyperthyroid cats remains possible, but further research is necessary to elucidate this aspect.

A diagnostic problem can occur in these cats developing post-treatment serum TT4 concentration below reference range and azotemia. There can be iatrogenic hypothyroidism, CKD suppressing serum TT4 concentration, or both. This issue was raised earlier in two studies investigating post-treatment kidney function, although the effect of hypothyroidism on kidney function has not yet been investigated in cats.[5,7] In the study described here, 3 of 5 cats from group B were diagnosed with iatrogenic hypothyroidism 7 to 10 months after treatment by using a rhTSH stimulation test as described by Daminet et al.[51] After supplementation with thyroxine, azotemia and GFR did not improve and therefore the diagnosis of CKD was made in these cats. Serum TT4 concentration had normalized in the 4$^{th}$ cat a few months after the end of the study. Evaluation of kidney function in cats with hypothyroidism has not yet been described.

Another objective of the present study was to determine the relationship between pre-treatment values of tested variables and the development of renal dysfunction. Pre-treatment measurement of GFR is helpful for predicting which cats have clinically significant declines in renal function 30 days after treatment.[5] In our study we found significant differences in pre-treatment values of GFR, USG and serum TT4 concentration between cats from group A and B. The difference in GFR is in accordance with previous findings.[5,10] Also, in our study not only was there a difference in pre-treatment GFR value, pre-treatment GFR explained 48 % of the variability in GFR 4 weeks after treatment. Pre-treatment GFR combined with pre-

treatment USG and serum creatinine explained 59 % and 58 % respectively of the variability in GFR 4 weeks after treatment.

The difference in pre-treatment values of serum TT4 concentration and USG between group A and B described here have not been described earlier to the author's knowledge. A difference in USG had been suggested by Becker et al. (2000) although not statistically significant. Because pre-treatment USG was lower before treatment in the cats that developed post-treatment renal azotemia and USG did not change after treatment in these cats, pre-treatment USG can be considered a valuable predictor of post-treatment changes in kidney function. Variability in GFR 4 weeks after treatment is explained for 37 % by USG alone and for 62 %, 59 % and 51 % when combined with pre-treatment serum creatinine concentration, GFR and serum TT4 concentration, respectively. A cut-off value appropriate for clinical decision making remains however uncertain, because there is still some overlap in pre-treatment values of USG between group A and B.

The difference in pre-treatment value of GFR, serum TT4 and USG between cats from group A and B can be considered predictive for development of CKD, although further research is necessary to develop pre-treatment cut-off values. Nonetheless, it is an interesting finding that 62 % of the variability in GFR 4 weeks after treatment could also be explained by combined measurement of pre-treatment serum creatinine concentration and USG.

Limitations of the present study are the small number of cats, especially in group B. Other studies described that 39 %, 17 % and 37 % of the hyperthyroid cats developed kidney disease.[6,7,10] Nonetheless, results are statistically significant and the number of 5 cats out of 21 developing CKD is comparable to numbers described in literature.[6-9] The results provide information additional to those of previous studies, because glomerular as well as tubular functions were evaluated, in a short- as well as long-term follow up period after treatment.

From the changes in serum TT4 and renal variables after treatment, it can be concluded that most significant changes appear within 4 weeks after treatment, and no important changes occur thereafter. This suggests that an accurate evaluation of kidney function can be made from 4 weeks after treatment onwards. The optimal time point for evaluation of post-treatment kidney function was raised earlier.[10,15,28] In the study by Boag et al., no change in kidney function was seen after more than 1 month after treatment, but the

precise timing was uncertain. The present study is the first showing a difference in time related changes in kidney function between cats maintaining a normal glomerular function and cats developing CKD. Serum TT4 concentration, creatinine concentration, GFR, BW and uRBP/c changed over a longer period of time in the complete group and group A compared to group B. This is important for clinicians to keep in mind when evaluating a cat treated for hyperthyroidism shortly after treatment. If these parameters reach a plateau early after treatment, there seems to be an increased risk of development of CKD, although this needs further research in a larger group of cats.

# CHAPTER 4

## Conclusion

The originality of this study concerning renal function in hyperthyroid cats, lies in the combined evaluation of glomerular as well as tubular function before, but also short and long term after treatment. As a first conclusion, we show for the first time in an evidence based way, that significant changes in kidney function occur within 4 weeks post-treatment of hyperthyroidism and none thereafter, regardless of the degree in declining kidney function. However, treatment of hyperthyroidism had no influence on GFR and tubular function measured with uRBP/c in cats developing post-treatment renal azotemia.

A second conclusion of this study is that pre-treatment measurement of GFR and/or USG as well as serum TT4 concentration can have possible predictive value regarding the development of post-treatment renal azotemia.

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# CHAPTER 5

# EVALUATION OF rhTSH STIMULATION IN CATS

# CHAPTER 5

# CHAPTER 5

Introduction to Chapter 5

A diagnostic challenge can occur in cats that develop post-treatment renal azotemia and serum TT4 below reference range after treatment of hyperthyroidism. A diagnosis of iatrogenic hypothyroidism or a lower serum TT4 due to NTI cannot be made based on a baseline serum TT4 concentration. A diagnostic aid could be stimulation with rhTSH to evaluate thyroid function, combined with thyroid scintigraphy. However, if thyroid scintigraphy is evaluated after rhTSH stimulation, it is necessary to take the effect of rhTSH on healthy thyroid glands into account. In the first section (§ 5.1) study we investigated the change in serum TT4 concentration and T/S uptake ratio in euthyroid cats, following administration of 25 μg rhTSH intravenously. In the second section (§ 5.2), the usefulness of the rhTSH stimulation test as a diagnostic tool for iatrogenic hypothyroidism, in the context of post-treatment renal azotemia, was investigated.

# CHAPTER 5

# SERUM THYROXINE AND THYROID SCINTIGRAPHY IN EUTHYROID CATS

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# CHAPTER 5

**Summary**

This study investigated the thyroidal response to administration of recombinant human thyroid stimulating hormone (rhTSH) by means of serum total thyroxine (TT4) concentration and pertechnetate uptake by the thyroid gland in six healthy euthyroid spayed female cats.

A pertechnetate scan was performed on day 1 to calculate thyroid/salivary gland (T/S) uptake ratio. On day 3, 25 μg rhTSH was injected IV. Six hours later the thyroid scan was repeated as on day 1. Blood was drawn for serum TT4 measurement prior to injection of rhTSH and performance of the pertechnetate scan.

Statistically significant differences in mean serum TT4 concentration, T/S uptake ratio before and 6 hours after rhTSH administration and T/S uptake ratio between left and right lobes were noted. We can conclude that 25 μg rhTSH increases pertechnetate uptake in the thyroid glands of cats, this should be taken into account when thyroid scintigraphy after rhTSH administration is interpreted.

**Introduction**

Evaluation of thyroidal reserve with thyrotropin stimulation (thyroid stimulating hormone, TSH) in cats has gained interest in veterinary medicine, because development of iatrogenic hypothyroidism after treatment of hyperthyroidism with radioiodine ($^{131}I$) can occur in 6 to 30 % of cases.[1-5] The combination of basal serum total T4 (TT4) and endogenous TSH concentration, possibly combined with free T4 (fT4) analysis, is recommended when diagnosing hypothyroidism. Measurement of fT4 is expensive and no feline specific TSH assay is available.[6] Stimulation with recombinant human TSH (rhTSH) could be a simple way to diagnose iatrogenic hypothyroidism in cats. Hyperthyroidism is the most common endocrine disorder in cats and $^{131}I$ treatment is the treatment of choice.[7-9] Another possible application of rhTSH in cats is administration prior to $^{131}I$ treatment of hyperthyroidism to enhance uptake of $^{131}I$ and allow a decrease in effective therapeutic dose.

The diagnosis of hypothyroidism cannot be made solely based on a low serum TT4 concentration alone, because a variety of non-thyroidal diseases can result in low serum TT4 concentrations.[10] A dynamic thyroid function test may be required when non-thyroidal illness cannot be eliminated. Several protocols for thyroid stimulation have been described in cats using bovine TSH (bTSH) which is no longer commercially available.[11-15]

Recombinant hTSH is a synthetic form of TSH obtained from a line of recombinant Chinese hamster ovary cells.[16] Several studies have evaluated use of rhTSH in dogs.[17-20] To date, only one in-vivo study has described the use of rhTSH in cats: administration of 25 μg rhTSH to euthyroid cats was safe and led to an increase in serum TT4 concentration with a maximum value observed 6 hours after administration.[21]

Metabolic activity of the thyroid gland can be measured with technetium as pertechnetate ($^{99m}TcO_4^-$) uptake. Pertechnetate is actively trapped by the sodium-iodide symporter (NIS) and concentrated in the thyroid in a similar way as iodine, but not incorporated in thyroid hormones. Pertechnetate has ideal imaging characteristics, is readily available, relatively inexpensive, and concentrated in the thyroid and salivary glands which are visible on the scintigraphic thyroid scan. With computer software, regions of interest (ROI) can be drawn around thyroid lobes and salivary glands. The calculated thyroid/salivary

gland (T/S) uptake ratio using pertechnetate is the most commonly used parameter to determine functional status of the thyroid and is significantly correlated with serum TT4 concentration in hyperthyroid cats although not in euthyroid cats.[22,23]

When different methods of thyroid function assessment such as TSH stimulation and thyroid scintigraphy are being evaluated in cats, it is important to know the influence of rhTSH on thyroid scintigraphy. The influence of rhTSH on T/S uptake ratio as part of the evaluation of thyroid function has not yet been investigated in cats. These preliminary data can add value to the interpretation of thyroid scans after rhTSH administration in cats evaluated for thyroid function. Moreover, this would give information whether this parameter of functional status is correlated to serum TT4 concentration in euthyroid cats stimulated with rhTSH in a similar way as in hyperthyroid cats. The objective of this study was to investigate the change in serum TT4 concentration and T/S uptake ratio in euthyroid cats, following administration of 25 μg rhTSH IV.

## Materials and Methods

### Animals

This study was conducted according to guidelines for animal care, with consent of the Ethical Committee of the Faculty of Veterinary Medicine from Ghent University, Belgium. Six healthy euthyroid female spayed cats, with an average age of 2 years and bodyweight (BW) range of 4.0 - 5.2 kg (mean ± standard deviation [SD] 4.7 ± 0.4), were included. To assess the health of the cats, initial screening included physical and routine laboratory examinations (complete blood count [CBC], biochemistry and serum TT4 concentration) and urinalysis (dipstick tests, microscopic analysis, protein/creatinine ratio and urine specific gravity). Cats were included in the study when these examinations showed no abnormalities.

### Experimental design

A prospective study design was used to investigate the influence of rhTSH administration on serum TT4 concentration and pertechnetate uptake. A pertechnetate scan was performed on day 1. A dose of 74 MBq (2 mCi) pertechnetate was injected IV and static images with a set number of 200.000 counts were acquired 30 minutes after injection, with a γ-camera (Toshiba GCA 901A, Exalto SA/NV, Saintes, Belgium) equipped with a low energy high resolution (LEHR) collimator. Cats were fasted for at least 10 hours before the thyroid scan. Cats were held under light anesthesia with propofol (PropoVet$^{TM}$, Propofol 10 mg/ml, Abbott Logistics B.V., Zwolle, The Netherlands) and placed in ventral recumbency over the camera. ROI were manually drawn over the left and right thyroid lobes and ipsilateral salivary glands by the same co-author (E. Vandermeulen) to calculate the thyroid / salivary gland (T/S) uptake ratio in both left and right thyroid lobes. On day 3, 25 μg rhTSH (Thyrogen®, Genzyme corporation, Naarden, The Netherlands) was administered intravenously, which corresponds to a mean dose of 5 μg rhTSH / kg BW in the six healthy cats. The rhTSH had been dissolved in sterile water, divided in aliquots containing 25 μg rhTSH and frozen at -20 $^{o}$C for a maximum of 8 weeks as described by De Roover et al.[24] Aliquots were allowed to thaw at room temperature shortly before injection. Six hours later, the pertechnetate scan was repeated as on day 1.

Two blood samples were taken by jugular venepuncture, before injection of the rhTSH and before the pertechnetate scan, respectively. Serum was collected after centrifugation,

aliquoted and frozen at -20º C until radioactivity had decayed for measurement of TT4 (nmol/L).

**Statistical analysis**

Effect of rhTSH administration on serum TT4 concentration was evaluated by a fixed effects model with period (0 versus 6 h after rhTSH administration) as fixed effect. Effect of rhTSH administration on the T/S uptake ratio was evaluated by a mixed model with cat and lobe as random effects, and rhTSH administration, side (left versus right) and the interaction between rhTSH administration and side as fixed effects. Correlation between the difference in serum TT4 concentration and difference in T/S uptake ratio was quantified by the Pearson correlation coefficient. Results were expressed as mean ± SD. The statistical analysis was done with SAS version 9.1 (SAS Institute Inc., Cary, USA) at the 5 % significance level.

**Results**

Serum TT4 concentration ranged from 12.90 - 25.80 nmol/L before to 49.02 - 65.79 nmol/L (reference values 14.19 - 45.15 nmol/L) after rhTSH administration, and increased significantly ($P < 0.0001$) from 0 hours (19.14 ± 4.65 nmol/L) to 6 hours (54.40 ± 5.91 nmol/L) after rhTSH administration. The ratio between serum TT4 concentration post TSH and baseline serum TT4 concentration was 3.0 ± 0.6.

There was a marginal but significant effect of rhTSH administration ($P = 0.013$) and a significant effect of side ($P = 0.039$) on T/S uptake ratio. There was no significant interaction between the effect of rhTSH administration and the effect of side on T/S uptake ratio ($P = 0.925$). In the left lobe, the T/S uptake ratio increased from 1.12 ± 0.21 nmol/L to 1.27 ± 0.22 nmol/L from 0 to 6 hours after rhTSH administration. In the right lobe, the T/S uptake ratio increased from 0.97 ± 0.10 nmol/L to 1.13 ± 0.17 nmol/L from 0 to 6 hours after rhTSH administration. The increase in T/S uptake ratio for the left and right lobes separately in 6 healthy cats is presented in Figures 1 and 2.

The correlation between difference in serum TT4 concentration and T/S uptake ratio before and after rhTSH administration was -0.28 and did not differ significantly from zero ($P = 0.59$). The correlation between the difference in serum TT4 concentration and difference in T/S uptake ratio before and after rhTSH administration in 6 healthy cats is presented in Figure 3.

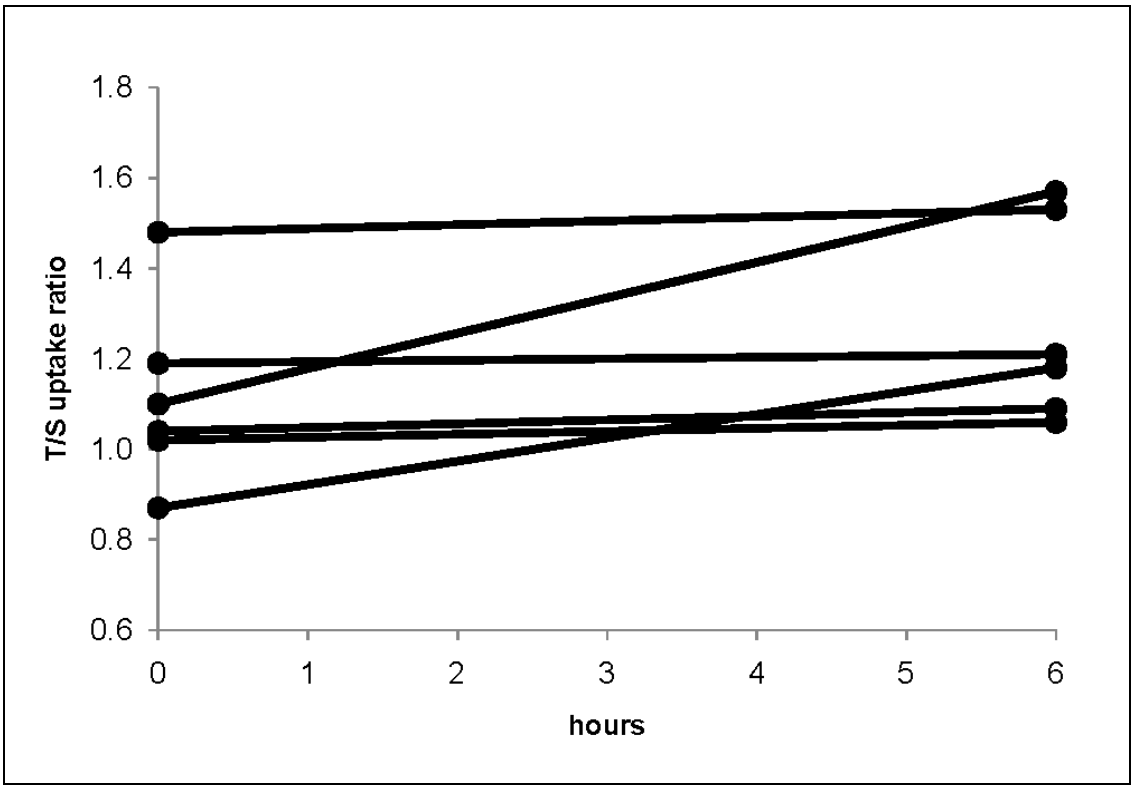


**Figure 1. T/S uptake ratio before (0 hours) and 6 hours after administration of 25 μg rhTSH IV in 6 healthy cats in the left lobe.**

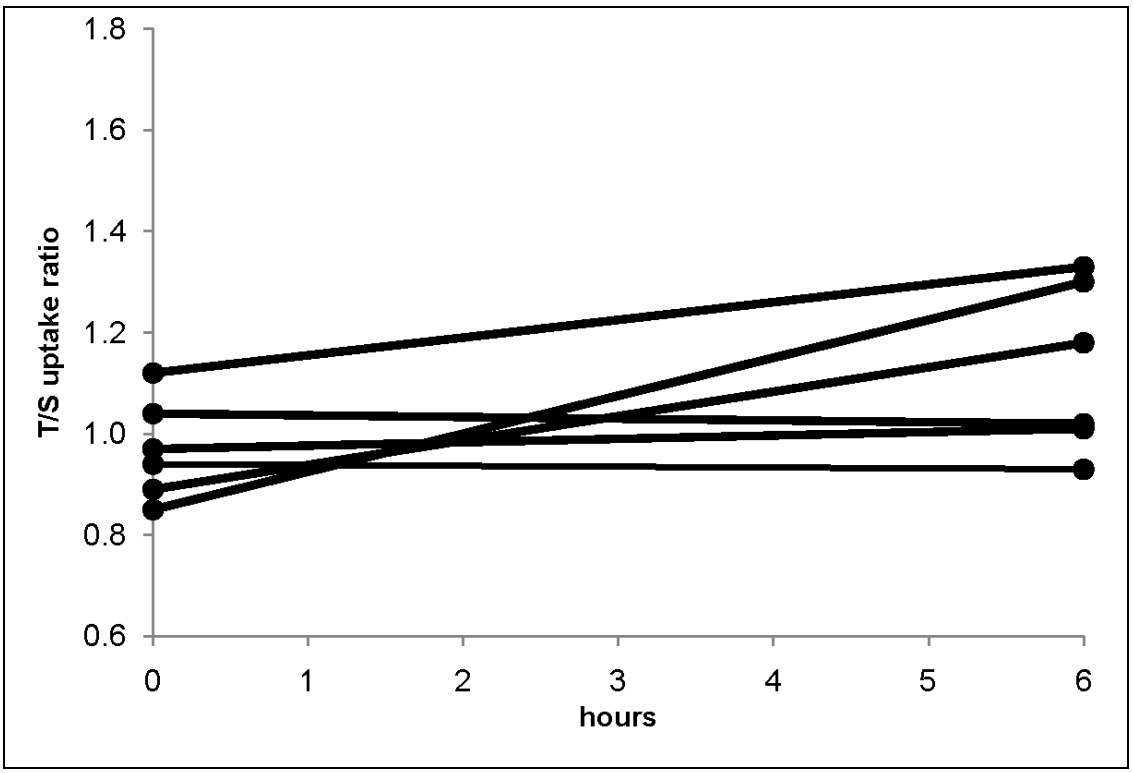


**Figure 2. T/S uptake ratio before (0 hours) and 6 hours after administration of 25 μg rhTSH IV in 6 healthy cats in the right lobe.**

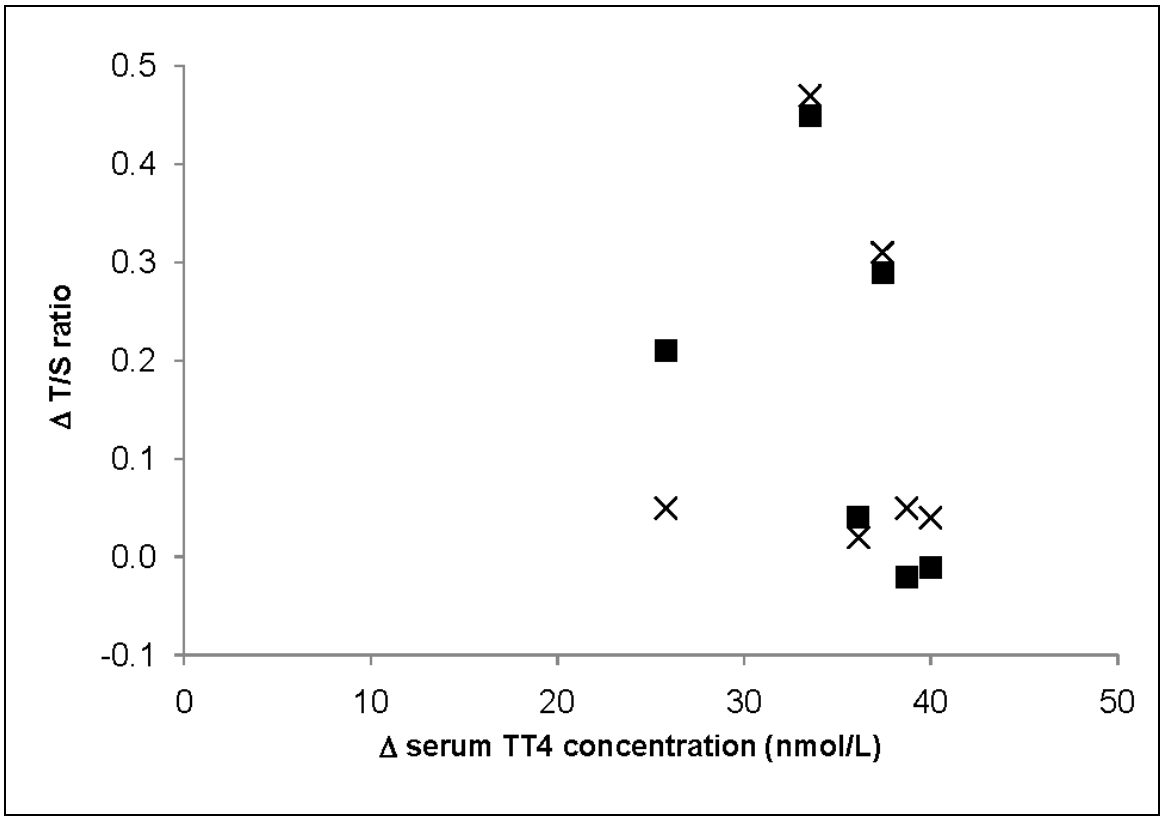


**Figure 3. Correlation between difference in serum TT4 concentration and difference in T/S uptake ratio before (0 hours) and 6 hours after administration of 25 µg rhTSH IV in 6 healthy cats for the left (x) and right (■) lobe in 6 healthy cats.**

# CHAPTER 5

## Discussion

We investigated the influence of 25 μg rhTSH on serum TT4 concentration and pertechnetate uptake by the thyroid glands of 6 healthy euthyroid cats. The effect of TSH on circulating thyroid hormones has been investigated using bTSH and rhTSH in cats.[11-15,21] In this study, a dose of 25 μg of rhTSH caused an increase in serum TT4 concentration 6 hours after administration, which is similar to the findings of Stegeman et al.[21] The 6-hour period after rhTSH administration in the study by Stegeman et al. (2003) and our study is also comparable to TSH stimulation protocols in cats that use bTSH.[11,15]

Follicular cells respond initially to binding of TSH to the TSH receptor by increased endocytosis of colloid and release of preformed thyroid hormone from the colloid in the blood.[25,26] When TSH stimulation persists, there is an increase in expression and functionality of the NIS and an increased organification of iodine into thyroid hormone.[25,27] The effect of rhTSH on the NIS can be measured by the NIS mRNA level correlating to the NIS protein level in the cell. In vitro stimulation of TSH on iodine transport activity in thyrocytes, previously starved from TSH, is partly due to a rapid increase in NIS gene expression after 3-6 hours with a maximum after 24 hours. The gene expression is followed by a relatively slow NIS protein synthesis after 36 hours, which parallels the increased iodine uptake, reaching a maximum after 72 hours.[28] The increased serum TT4 concentration after rhTSH administration can be caused by either an increased release of stored hormone or an upregulation in the production level of thyroid hormones. The time-related effects of TSH on thyroid cells described above make the latter less likely. Moreover, the thyrocytes in the in-vitro study were starved from TSH which is not the case in the euthyroid healthy cats used in this study. Therefore the response in thyrocytes not starved from TSH can be expected to be related to an upregulation of newly formed thyroid hormones and therefore prolonged.

A previously used index of TSH stimulation is the post-TSH / pre-TSH TT4 concentration ratio (post- / pre- TT4 ratio).[11,29] The post- / pre- TT4 ratio had an approximate value of 3 for the dose of 25 μg rhTSH in the study by Stegeman et al. (2003) which is comparable to the mean value of 3.0 ± 0.6 in this study.

Several studies evaluating the use of rhTSH in euthyroid dogs or dogs suspected of hypothyroidism use doses of 50, 75 or 100 μg rhTSH.[17-20] However, when the dose per kg BW is calculated using the mean BW of the dogs, the doses of rhTSH range from 3 to 5 μg/kg BW in these studies. The post- / pre- TT4 ratio in these studies had an approximate value higher than 1.5 (3 or 5 μg/kg BW)[19] or an approximate value of 2 (3 or 4 μg/kg BW)[17,20] or 2.7 (5 μg/kg BW).[20] The cats in this study received a mean dose of 5 μg/kg BW rhTSH which is comparable to doses/BW used in dogs, although the post- / pre- TT4 ratio had a mean value of 3 in this study and the study by Stegeman et al. (2003) which suggests a higher biological activity of rhTSH in cats compared to dogs. The species specific β-subunit of TSH differs in exact amino acid sequence among species, however, biological cross-species reactivity allows TSH of a certain species to stimulate thyroid glands of other species, accompanied by species specific biological differences.[30] The sequence homology of α and β subunits from feline TSH are 96 % and 94 % compared to canine TSH, and 68 % and 88 % compared to human TSH.[31] However, a homologue glycohormone of a specific species can have lower receptor affinity compared to a heterologue glycohormone.[32] This can be caused by differences in glycosylation which alter bioactivity of the hormone.[30,33] The above mentioned reasons could explain the difference in biological effect of rhTSH in dogs and cats, however controlled studies with rhTSH dosed per BW in dogs and cats are needed to evaluate a difference in biological reactivity between these species.

This study is the first report in veterinary medicine showing a marginal effect of rhTSH on T/S uptake ratio by the thyroid. Possibly, at first, the stored TT4 is released from the thyroid and pump mechanisms are only mildly affected, because the dose of rhTSH is possibly insufficient to reach a larger intracellular response. Also, the time interval between injection of rhTSH and image acquisition could be not optimal. Use of the isotope $^{123}$I as a tracer would have allowed us to perform measurements of functional activity post-rhTSH administration for a longer period after administration of the radio-tracer, because $^{123}$I has a half life of 13 hours opposed to 6 hours for pertechnetate.

The time between the scan on day 1 and 3 was more than 60 hours (10 physical half-lives of pertechnetate). Therefore, less than 0.01 % of radioactivity was left which is too small to be of influence. Thyroid imaging was performed 30 minutes after administration of pertechnetate. Nieckarz and Daniel[34] showed that the time from injection to imaging is not

critical if performed within a period of 20 minutes to 2 hours after pertechnetate administration. The LEHR collimator allowed a low dose of 74 MBq pertechnetate with a good thyroid to background distinction, compared to higher doses of 111 - 148 MBq described in the literature where a low energy all purpose (LEAP) collimator is often used.[23,35]

Sodium-iodide symporters are also present in salivary glands. NIS gene expression and NIS protein is found in salivary glands.[36,37] Cells in the salivary gland that express NIS can accumulate though not organify iodide, and TSH exerts no regulatory influence on non-thyroid iodide accumulation.[38] It is, therefore, not expected that TSH administration influences pertechnetate uptake in the salivary gland nor that this is a reason for the marginal increase in T/S uptake ratio. Moreover, Nieckarz and Daniel[34] showed an increased T/S uptake ratio in euthyroid cats made hypothyroid with methimazole, expected to be caused by the increased serum TSH concentration.

No correlation between the difference in serum TT4 correlation and the difference in T/S uptake ratio before and after rhTSH administration could be demonstrated. In the study by Daniel et al.[23] there was a significant difference in T/S uptake ratio between euthyroid and severely hyperthyroid cats, but not between euthyroid and mild hyperthyroid cats. The euthyroid cats in the study reported here showed a mild increase in serum TT4 concentration, which could possibly explain the lack of correlation between the increase in T/S uptake ratio and the increase in serum TT4 concentration in this study.

There was a significant effect of side on the T/S uptake ratio. This difference in T/S uptake ratio between the left and the right thyroid lobe is, however, of limited influence in this study, because the effect of rhTSH on T/S uptake ratio is the same in the left and right thyroid lobe. Asymmetric thyroid lobes on pertechnetate scintigraphy[39] and differences in volume measured with ultrasonography have been described in euthyroid cats but not in euthyroid dogs.[40,41] It is known that in euthyroid humans, the right thyroid lobe is usually larger and more vascularised[42], and this is suggested to be associated with functional asymmetries related to the immune system, hypophysiotrophic neurohormones, neural pathways or a combination of the latter two factors.[43,44]

The study described here could open doors to further research. In humans with nodular goitre the administration of rhTSH has gained major application because administration of rhTSH increases the uptake of $^{131}I$ in the thyroid and changes the regional distribution of $^{131}I$.[45,46] This results in lower therapeutic doses needed and less irradiation to extra-thyroidal tissue.[47-49] A lower efficacious dose of $^{131}I$ in cats with hyperthyroidism will reduce the surface dose-emission rate, urine radioactivity and the duration of isolation for cats treated with $^{131}I$, thereby respecting the As Low As Reasonably Achievable (ALARA) principle.[50,51]

**Conclusion**

We can conclude from this study that the uptake of pertechnetate by the thyroid of euthyroid cats is marginally though significantly increased 6 hours after administration of 25 μg rhTSH, and that this increase is not correlated to the increase in serum TT4 concentration.

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# COMPARISON BETWEEN HEALTHY CATS, CATS WITH NON-THYROIDAL ILLNESS AND CATS SUSPECTED OF IATROGENIC HYPOTHYROIDISM WITH POST-TREATMENT RENAL AZOTEMIA

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# CHAPTER 5

**Summary**

This study investigated the recombinant human thyrotropin (rhTSH) stimulation test in healthy cats (group 1), cats with non-thyroidal illness (group 2) and cats with low serum total T4 (TT4) and renal azotemia after $^{131}I$ treatment (group 3). Serum TT4 concentration before and after, and thyroidal pertechnetate uptake after administration of 25 μg rhTSH IV were measured. Baseline serum TT4 differed significantly between group 1 and 3 but not between group 1 and 2 or 2 and 3. Serum TT4 increased significantly in group 1 and 2 after rhTSH administration. Post-rhTSH serum TT4 differed significantly between group 1 and 3 and group 2 and 3, but not between group 1 and 2. There was no difference between the 3 groups in thyroid / salivary gland uptake ratio (T/S uptake ratio).

We can conclude that stimulation with rhTSH is valuable to differentiate euthyroidism from iatrogenic hypothyroidism in cats.

## Introduction

Treatment with radioiodine ($^{131}$I) is the treatment of choice for feline hyperthyroidism, however iatrogenic hypothyroidism can occur in 6 to 30 % of the cases.[1-3] Another important complication is declining kidney function, because up to 39 % of treated cats develop chronic kidney disease (CKD) after treatment of hyperthyroidism.[4-7] Therefore, it is not unexpected to find serum total T4 (TT4) concentration below reference range combined with renal azotemia in a cat after treatment of hyperthyroidism with $^{131}$I.

When a low circulating serum TT4 concentration is measured in cats treated with $^{131}$I, non-thyroidal illness (NTI) must be considered, especially CKD, before a diagnosis of iatrogenic hypothyroidism can be confirmed.[8] Several NTIs such as diabetes mellitus (DM), hepatic insufficiency and CKD can decrease serum TT4 concentrations.[9]

Cats with serum TT4 below reference range and post-treatment renal azotemia represent a diagnostic challenge because it is not clear whether these cats have iatrogenic hypothyroidism or a low serum TT4 due to NTI. Also because of the interplay between thyroid status and renal function, definitive diagnosis of hypothyroidism is warranted. In hypothyroid humans, rats and dogs, glomerular filtration rate is decreased, and serum creatinine is increased, respectively.[10-13]

Thyroid function can be assessed with serum free T4 after equilibrium dialysis, which has a low specificity,[14] or endogenous serum TSH concentration. Feline TSH measurement however, is not available in cats. A canine TSH assay was recently validated for use in healthy cats, hyperthyroid cats and cats with hyperthyroidism and chronic kidney disease.[15] However, accuracy of this assay remains unclear in the higher range of TSH concentrations expected in hypothyroid cats. Moreover, only a 1$^{st}$ generation assay is available.

Another method which could proof valuable for measuring thyroid function, is stimulation of thyroid tissue with TSH. The possibility of prolonged storage of rhTSH[16] and the biological activity described in cats[17] opened doors to the use of rhTSH in feline medicine. Recent studies described the stimulatory effect of 25 µg recombinant human TSH (rhTSH) on serum TT4 concentration and thyroid scintigraphy in healthy cats and uptake of radioiodine $^{123}$I in hyperthyroid cats.[17-19]

When TSH stimulation is performed, thyroid function can be measured with serum TT4 concentration as a representative of thyroid hormone synthesis and release of preformed hormones. Metabolic activity of the thyroid gland can be measured with technetium as pertechnetate ($^{99m}TcO_4^-$) uptake. Pertechnetate is trapped by pump mechanisms in thyroid cells, the Sodium-Iodide symporters (NIS). It is concentrated in the thyroid but not incorporated in thyroid hormones. With computer software, regions of interest (ROI) can be drawn around thyroid lobes and salivary glands. The calculated thyroid/salivary gland (T/S) uptake ratio using pertechnetate is the most commonly used parameter to determine functional status of the thyroid in healthy and hyperthyroid cats.[20,21] When thyroid scintigraphy is performed after TSH administration, a marginal but significant increase in T/S uptake ratio, which has been described in healthy euthyroid cats, must be taken into account.[18]

The objectives of this study were to evaluate thyroid function with rhTSH stimulation followed by thyroid scintigraphy, in cats with a serum TT4 below reference range combined with renal azotemia after $^{131}I$ treatment, compared with cats with non-thyroidal illness and a group of healthy control cats.

## Materials and Methods

### Cats

This study was conducted according to guidelines for animal care, with consent of the Ethical Committee of the Faculty of Veterinary Medicine from Ghent University, Belgium. Owners of the cats with NTI and treated hyperthyroid cats with serum TT4 below reference range combined with post-treatment renal azotemia signed an informed consent form.

Six healthy euthyroid female spayed cats, with an average age of 4 years and bodyweight (BW) range of 4.0 - 5.2 kg (mean ± standard deviation [SD] 4.7 ± 0.4), were included (group 1, H cats). To assess the health of these cats, initial screening included physical and routine laboratory examinations (CBC, biochemistry and serum TT4 concentration) and urinalysis (dipstick tests, microscopic analysis, protein/creatinine ratio and urine specific gravity). Cats were included in the study when these examinations showed no clinically significant abnormalities.

Five cats with diseases reported as NTI[9] (group 2, NTI cats), all patients from the Veterinary Clinic of Small Animal Medicine, Ghent University, were included. Abnormal findings on physical and routine laboratory examination had to be compatible with the established disease and no other significant abnormalities could be present. The diseases in the cats with NTI were CKD (IRIS [International Renal Interest Society, www.iris-kidney.com/guidelines/en/staging_ckd.shtml] stage II, n = 1), DM (n = 1) and severe chronic gingivitis and stomatitis (n = 3). The cats had an average of 9 years and BW range of 2 - 6 kg (mean ± SD 4.2 ± 1.8).

The group of cats with low serum TT4 concentration and renal azotemia were included from a larger group of hyperthyroid cats that were evaluated at 1, 3 and 6 months after $^{131}$I treatment. At evaluation, serum TT4 and creatinine concentration, as well as urinary specific gravity (USG) were measured. Glomerular filtration rate (GFR) was measured before and 1, 3 and 6 months after treatment with the plasma exo-iohexol clearance test (PexICT) as described earlier.[22] Four cats with a documented serum TT4 concentration below reference range and post-treatment renal azotemia at 3 and 6 months after treatment, and at 7 - 8.5 months after $^{131}$I treatment when the study was performed, were included (group 3, low T4-RA cats). Average age was 14.5 years and BW range of 3.0 - 8.0 kg (mean ± SD 5.8 ± 2.3).

Renal azotemia was defined as serum creatinine concentration 140 - 249 μmol/L (IRIS stage II) or 250 - 439 μmol/L (IRIS stage III).

**Experimental design**

A prospective study design was used to investigate the influence of rhTSH administration thyroid function measured with serum TT4 concentration and pertechnetate uptake on thyroid scintigraphy. Twenty five μg rhTSH (Thyrogen®, Genzyme corporation, Naarden, The Netherlands) was administered intravenously. The rhTSH had been dissolved in sterile water, divided in aliquots containing 25 μg rhTSH and frozen at -20 ºC for a maximum of 8 weeks as described by De Roover et al.[16] Aliquots were allowed to thaw at room temperature shortly before injection. Six hours later, a pertechnetate scan was performed as described earlier.[18] A dose of 74 MBq (2 mCi) pertechnetate was injected IV (5.5 hours after administration of rhTSH) and static images with a set number of 200.000 counts were acquired 30 minutes after injection, with a gamma camera (Toshiba GCA 901A, Exalto SA/NV, Saintes, Belgium) equipped with a low energy high resolution (LEHR) collimator. Cats were fasted for at least 10 hours before the thyroid scan. Cats were held under light anesthesia with propofol (PropoVet™, Propofol 10 mg/ml, Abbott Logistics B.V., Zwolle, The Netherlands) and placed in ventral recumbency over the camera. Regions of interest (ROI) were manually drawn over the left and right thyroid lobes and ipsilateral salivary glands by the same co-author (E. Vandermeulen) to calculate the T/S uptake ratio in thyroid lobes.

Two blood samples were taken by jugular venipuncture, within 5 minutes prior to injection of the rhTSH and the pertechnetate scan respectively. Serum was collected after centrifugation, aliquoted and frozen at -20 ºC until radioactivity had decayed for measurement of TT4. The TT4 was measured with a validated chemiluminescent immunoassay (Chemiluminescent Immulite 2000, DPC, Los Angeles USA [nmol/L]). Intra- and inter-assay coefficient of variation were 4 and 8 % for the lower range (2 μg/dL) and 6 and 7 % for the higher range (5 μg/dL) of serum TT4, respectively. A serum sample with high TT4 concentration was diluted with a serum sample with low TT4 concentration, which showed a linear dilution curve.

**Statistical Analysis**

Statistical analysis was performed with S-Plus version 8.0 (Insightful Corporation, Seattle, USA). The effect of rhTSH on serum T4 concentration was analysed using a paired sample T-test for each of the 3 groups. Differences in serum TT4 concentration pre- and post rhTSH administration, absolute and relative increase in serum TT4 concentration after rhTSH

administration and T/S ratio were analysed with analysis of variance (ANOVA). Global significance level was 5 %. Results were expressed as mean ± SD.

**Results**

There was no significant difference between groups for BW or dose/kg BW. There was a significant difference in age between group 1 and 3, but not between groups 1 and 2 or 2 and 3. All cats in group 3 had signs suggestive of hypothyroidism when the study was performed (increase in BW, scaly fur, lethargic). All cats from group 3 had renal azotemia stage II at 3 and 6 months after treatment with radioiodine. At the time of the study, cats from group 3 had renal azotemia IRIS stage II (n = 3) or III (n = 1). The cat with CKD from group 2 had renal azotemia stage II.

Serum TT4 (pre- and post-rhTSH administration, absolute increase and relative increase as well as post-rhTSH / pre-rhTSH serum TT4 ratio [post- / pre- TT4 ratio]) and T/S ratio, for the 3 groups, are described in Table 1. Serum TT4 concentration increased significantly in group 1 ($P < 0.001$) and group 2 ($P = 0.014$) but not in group 3 ($P = 0.183$) after rhTSH administration. There was a significant difference ($P = 0.007$) in pre-rhTSH serum TT4 concentration between group 1 and 3 but not between group 1 and 2 or group 2 and 3. There was a significant difference between group 1 and 3 and between group 2 and 3 in post-rhTSH serum TT4 ($P < 0.001$), absolute increase in serum TT4 concentration ($P = 0.001$) and relative increase in serum TT4 concentration ($P < 0.001$), though there was no difference for these variables between group 1 and 2. There was no difference between the groups for T/S uptake ratio after rhTSH administration ($P = 0.07$).

All significant results had a power > 90 %. Power was > 80 % for the non-significant difference in basal serum TT4 concentration between group 2 and 3, and for the non-significant difference in T/S pertechnetate uptake ratio between group 1 and 3.

Based on the marginal increase in serum TT4 concentration, compared to the healthy cats and cats with NTI, it was concluded that all 4 cats from group 3 had iatrogenic hypothyroidism. Treatment with levothyroxin 10 - 20 μg/kg/day (Forthyron, Eurovet Animal Health B.V., Bladel, The Netherlands) was started. Cats were re-evaluated when euthyroidism was reached. One owner denied more than 1 follow-up, and therefore this cat could not be re-evaluated when euthyroid. Serum concentration of TT4, creatinine, GFR and USG of cats in group 3 before and 1, 3 and 6 months after radioiodine treatment, as well as after levothyroxin supplementation, is presented in Table 2.

**Table 1. Serum TT4 concentration pre- and post-rhTSH administration, absolute and relative increase in serum TT4 concentration and T/S ratio after rhTSH administration in healthy cats (Group 1), cats with non-thyroidal illness (Group 2) and cats with serum TT4 below reference range and renal azotemia after $^{131}$I treatment (Group 3).**

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Serum TT4 pre-rhTSH (nmol/L) | 19.1 ± 4.6[a] (12.9 - 25.8) | 15.5 ± 3.0[a,b] (11.6 - 18.1) | 9.1 ± 3.7[b] (5.9 - 12.9) |
| Serum TT4 post-rhTSH (nmol/L) | 54.4 ± 5.9[a] (49.0 - 65.8) | 55.0 ± 23.1[a] (33.5 - 92.9) | 9.7 ± 4.4[b] (5.9 - 14) |
| Serum TT4 absolute increase (nmol/L) | 35.3 ± 5.1[a] (25.8 - 40) | 39.5 ± 21.2[a] (21.9 - 74.8) | 0.6 ± 0.7[b] (0 - 1.3) |
| Serum TT4 relative increase (%) | 195.8 ± 64.1[a] (111 - 300) | 250.0 ± 103.9[a] (146 - 414) | 5.0 ± 5.8[b] (0 - 11) |
| Post- / Pre- T4 ratio | 2.8 ± 1.3 (2.1 - 4.0) | 3.6 ± 7.6 (2.9 - 5.1) | 1.1 ± 1.2 (1.0 - 1.1) |
| T/S uptake ratio post-rhTSH | 1.2 ± 0.2[a] (1.0 - 1.4) | 1.1 ± 0.3[a] (0.8 - 1.5) | 0.8 ± 0.1[a] (0.6 - 0.9) |

**TT4: total T4, rhTSH: recombinant thyroid stimulating hormone, Post- / Pre- T4 ratio: post-rhTSH stimulation / Pre-rhTSH stimulation serum TT4 concentration ratio, T/S uptake ratio: thyroid/ salivary gland uptake ratio.**

**If superscripts ([a, b, c]) differ between columns for a variable, a significant difference was noted (P values are provided in the text).**

**Table 2. Mean ± standard deviation (range) of serum concentration of TT4 (nmol/L), creatinine (μmol/L), GFR (mL/min/kg) and USG in cats from group 3 before (0), 1, 3 and 6 months after treatment with $^{131}$I, and after euthyroidism was re-established.**

| Group 2 | 0 | 1 month | 3 months | 6 months | Euthyroidism (n = 3) |
|---|---|---|---|---|---|
| Serum TT4 | 83.4 ± 26.4 (58.1 - 120.0) | 8.4 ± 9.7 (0 - 18.1) | 5.8 ± 6.8 (0 - 12.9) | 4.5 ± 5.2 (0 - 9.0) | 37.4 ± 9.7 (28.4 - 47.7) |
| Serum creatinine | 83 ± 22 (51 - 101) | 136 ± 26 (101 - 158) | 162 ± 18 (151 - 188) | 183 ± 38 (152 - 236) | 161 ± 32 (126 - 187) |
| GFR | 2.7 ± 0.6 (2.1 - 3.3) | 1.3 ± 0.3 (1.0 - 1.8) | 1.2 ± 0.2 (0.9 -1.4) | 1.1 ± 0.1 (0.9 - 1.2) | 1.1 ± 0.1 (1.1 - 1.2) * |
| USG | 1.030 ± 0.02 (1.022 - 1.060) | 1.030 ± 0.01 (1.018 - 1.047) | 1.040 ± 0.02 (1.015 - 1.048) | 1.030 ± 0.02 (1.012 - 1.060) | 1.040 ± 0.02 (1.014 - 1.060) |

**TT4: total T4, GFR: glomerular filtration rate, USG: urine specific gravity, UPC: urinary protein/creatinine ratio.**

*** n = 2**

**Discussion**

We investigated the rhTSH stimulation test for the evaluation of thyroid function in cats suspected of iatrogenic hypothyroidism and showing renal azotemia after treatment of hyperthyroidism with $^{131}$I (low T4-RA), by comparing them to H cats and cats with NTI without history of thyroid problems. The cats with NTI were included in the study to evaluate the effects of NTI on the results of the rhTSH stimulation test.

There was no difference in serum TT4 concentration pre-rhTSH stimulation between H cats and NTI cats. Although chronic gingivitis and periodontal disease have been described as NTI, it can be expected that these diseases are less severe NTI compared to CKD and DM in the other 2 cats.[9] This could have lead to a smaller and therefore non-significant difference in serum TT4 between group 1 and 2. Indeed, ranges of serum TT4 concentration were comparable for group 1 and 2 (Table 1). On the other hand, group size might have been to low to detect a significant difference in serum TT4 concentration.

There was no difference between the NTI cats and the low T4-RA cats in basal serum TT4 concentration, which is a more important finding. This underlines the need for further evaluation of thyroid function in cats suspected of iatrogenic hypothyroidism and renal azotemia after $^{131}$I treatment. A diagnosis of hypothyroidism cannot be made based solely on the basal serum TT4 concentration.

In several species, hypothyroidism can affect kidney function.[10-13] Although this has not yet been investigated in cats, the presence of kidney disease and potential deleterious effects of the previous hyperthyroidism on kidney function merit attention. An early diagnosis of hypothyroidism in these cats is important. Untreated hypothyroidism can have long term effects on kidney function and is associated with congestive heart failure in humans.[23,24] Stimulation with rhTSH has not yet been investigated as a diagnostic test in cats suspected of iatrogenic hypothyroidism. A recent study evaluated rhTSH stimulation in healthy dogs, euthyroid sick dogs and hypothyroid dogs and established the following criteria: dogs were euthyroid if post-TSH serum TT4 concentration was equal or exceeded 40 nmol/L or if the increment of post-TSH serum TT4 concentration was at least 20 nmol/L.[25] All of the cats from group 1 and 4 cats from group 2, though none of the cats from group 3, met these

criteria. These criteria are arbitrary in cats and need further validation but the results in the study using these criteria are promising.

Thyroid stimulation can be indexed by the post- / pre-TT4 ratio.[26,27] In dogs with hypothyroidism this ratio has an approximate value of 1.[25,28] This is comparable to the results in our study for the cats from group 3, but lower than the results for the cats from group 2.

In the study described here, there was no difference in post-rhTSH T/S uptake ratio between the groups. This suggests that assessment of thyroid function with thyroid scintigraphy after rhTSH stimulation is not accurate in cats with low serum TT4 concentration combined with renal azotemia. However, this does not exclude the possibility of a difference in T/S uptake ratio before stimulation with rhTSH between the groups. When different methods of thyroid function assessment such as TSH stimulation combined with serum TT4 concentration followed by thyroid scintigraphy are being evaluated in cats, the influence of rhTSH on thyroid scintigraphy must be taken into account. In a recent study by the same group, a marginal but significant effect of rhTSH on T/S uptake ratio by the thyroid gland was described in healthy cats.[18] It remains possible that basal T/S uptake ratios differed between groups but that this difference was masked by possible effects of rhTSH on remaining functional thyroid tissue in the glands of the low T4-RA group. Indeed, in dogs thyroid scintigraphy had the highest discriminatory power with primary hypothyroidism and non-thyroidal illness in one study.[29]

Follicular cells respond initially to binding of TSH to the TSH receptor and release of preformed thyroid hormone from the colloid in the blood.[30] When TSH stimulation persists, there is an increase in expression and functionality of the Sodium/Iodide Symporter (NIS) and an increased organification of iodine into thyroid hormone.[31] In cats with hypothyroidism, there can be no release of preformed hormones, because production level is too low. However, it remains possible that there is room for increase in production level because there are still remaining NIS taking up pertechnetate, as seen from the low but present T/S uptake ratio in the low T4-RA group. Theoretically, if stimulation with TSH persists, which is expected in primary hypothyroidism, this would qualitatively and quantitatively increase NIS. This suggests that not only there is insufficient amount of functional thyroid cells producing T4 which causes hypothyroidism, there is also a functional decrease in response to TSH of the

remaining NIS which thereby maintains the hypothyroid state. Further studies are needed to investigate the mechanisms of iatrogenic hypothyroidism in cats.

After the study, treatment with levothyroxin was started in all four cats from group 3 with iatrogenic hypothyroidism and renal azotemia (Table 2). After euthyroidism was reached, serum creatinine decreased and GFR increased in one cat. The other 2 cats showed increased serum creatinine levels, and the diagnosis of concurrent CKD in these cats was confused based on the worsening kidney function despite improvement of serum TT4 concentration. Hypothyroidism can cause glomerular lesions such as thickening of the basement membrane and increased mesangial matrix.[32,33] Indeed, treatment of hypothyroidism in a human patient with progressive renal failure can lead to significant improvement of renal function.[34] Evaluation of kidney function in cats with iatrogenic hypothyroidism has not yet been described. The concurrent hypothyroidism and CKD can be a coincidence in this small number of cats but the question is raised whether there is a link between the development of CKD after treatment with radioiodine and hypothyroidism in these cats.

Limitations of this study are the small number of cats investigated. However, results were significant and power of the statistical analysis sufficient.

Cats with low serum TT4 concentration combined with renal azotemia after treatment of hyperthyroidism represent a diagnostic challenge. It can be unclear whether these cats have truly iatrogenic hypothyroidism or low serum TT4 concentration due to NTI, based on clinical symptoms and basal serum TT4 measurement alone. A reliable evaluation of thyroid function is essential, because iatrogenic hypothyroidism can potentially be deleterious on kidney function. We can conclude from this study that stimulation with a rhTSH stimulation test measured with serum TT4 concentration is able to differentiate euthyroidism from iatrogenic hypothyroidism in cats.

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# CHAPTER 5

# GENERAL DISCUSSION

Declining kidney function is a common complication of $^{131}$I treatment and an early assessment of development of chronic kidney disease (CKD) is essential. It was important to first investigate methods which could give additional information about the status of kidney function in cats and to investigate whether these methods could aid in the early assessment of declining kidney function after $^{131}$I treatment. The extensive linkage between thyroid and kidney function is described in Chapter 1.

The main objective of this thesis was to gain insights into kidney function of hyperthyroid cats, by evaluating several stepping stones that could help to understand the declining kidney function in hyperthyroid cats after $^{131}$I treatment.

## 1. Evaluation of plasma clearance methods (Chapter 2)

Because one of the aims of the thesis was to evaluate kidney function in hyperthyroid cats, we first investigated suitability of different plasma clearance methods for glomerular filtration rate (GFR) measurement. Important characteristics determining the usefulness of a method for GFR measurement are not only convenience and availability but also accuracy, precision and reproducibility, and ability to distinguish between a wide range of GFR values. The traditional gold standard is urinary clearance of inulin. However, urinary clearance techniques are highly cumbersome, stressful and potentially harmful for the patient. Urinary clearance of inulin is comparable to urinary clearance of exogenous creatinine and plasma clearance of iohexol in cats.[1-3] In Chapter 2 (§2.1, §2.2 and §2.3), we evaluated plasma clearance of exo-iohexol (PexICT), endo-iohexol (PenICT) and exogenous creatinine (PECCT) for their potential application in research and practice conditions.

### *Precision*

We investigated precision because this has important implications when GFR is used in the follow up of patients. Reproducibility of repeated measures was investigated in young adult and aged healthy cats (§ 2.1). The between-day coefficients of variation were < 22 % for all markers. This variation is not excessively high and means a change in GFR of 22 % (for instance a decrease from 2.5 to 2.0 mL/min/kg) can be due to between-day variability and not to a biologic change. However, any change higher than 22 % can be considered clinically relevant. This is more sensitive than the routine indirect parameters of renal function such as serum creatinine concentration, which change when at least two thirds to three quarters of

renal function is impaired. Therefore, the reproducibility for all markers was considered sufficient for screening patients for early renal dysfunction. However, PexICT had the best reproducibility and is therefore most suited in research conditions. Apparently, factors related to individual cats have more influence on the PECCT and PenICT because variance between repeated measures in the same cat was larger compared to PexICT.

***Similarity and distinguishment***

Besides precision, we also evaluated these methods for their similarity when evaluated concurrently in the animal. The ability to distinguish between different ranges of GFR expected in cats caused by pathological conditions or age was investigated in § 2.1 and § 2.3. This distinguishment was also investigated for different GFR values measured over a time period in which the GFR is expected to change after treatment of hyperthyroidism (§ 2.2). There was no difference between the different clearance methods in cats with CKD, but there were differences in GFR between healthy and hyperthyroid cats although differences between clearance methods differed between the groups. For instance PenICT generated GFR values higher than PexICT and PECCT in healthy cats, albeit lower than these methods in hyperthyroid cats. Also, the amount of difference between the methods was not consistent over the range of GFR measured. These findings suggest that the difference between clearance methods does not seem to be related to pathophysiological differences between cats per se, but more to differences between different pathophysiological conditions and their corresponding different GFR values. Moreover, these findings suggest that for a single GFR evaluation as part of evaluation of the complete clinical status of a geriatric cat, any of the three clearance methods can be used, especially in cats with CKD. However, this is not the case when cats are screened for kidney dysfunction, and evaluated repeatedly over a period of time in which the GFR is expected to change. It is then important to be able to distinguish between clinically different GFR values. This distinguishment is associated with the precision. In theory, any of the three clearance methods can be used because all three methods indicated the same trend in hyperthyroid cats with decreasing GFR until 4 weeks after $^{131}$I treatment with very little decline thereafter (§ 2.2). Also, in the population consisting of cats expressing GFR over the complete range expected in cats, limits of agreement were narrow and mean difference was low when the three methods were compared (§ 2.3). However, the same method has to be used in monitoring kidney function because at all time points after treatment there was a significant difference between GFR values using the three methods.

PexICT showed the best reproducibility and there was no overlap between ranges of GFR values in cats with CKD, healthy cats and cats with hyperthyroidism, which makes it suitable for application in a research setting. PECCT was the only clearance method that could distinguish in GFR between cats with CKD, healthy cats and cats with hyperthyroidism, as well as between young adult and aged healthy cats. Reproducibility of PECCT was acceptable and creatinine analysis can be performed using routine devices for biochemical analysis, which makes it suitable for application in referral practice. However, PECCT was not able to distinguish between GFR values measured 1 week and 4 week after $^{131}$I treatment of hyperthyroid cats, which suggests it might be less sensitive to small changes in GFR compared with PexICT and PenICT.

***Accuracy***

Another important characteristic is accuracy which can be evaluated by comparison to the gold standard method: urinary clearance of inulin. However, clearance of iohexol as well as exogenous creatinine have been proposed as adequate alternatives for urinary clearance methods in cats.[1-5] Use of a gold standard method would have been useful to compare different GFR markers over the range of possible GFR values and this is a limitation of the studies evaluating GFR methods described here. Indeed, precision may not be relevant for methods which have poor accuracy. On the other hand, an accurate method with a poor reproducibility or lacking ability to distinguish between different GFR values can not be acceptable for GFR assessment when monitoring kidney function. A compromise between a good accuracy and a correct reproducibility and ability to distinguish has still to be found. Because of the findings described in Chapter 2 (§ 2.1, § 2.2 and § 2.3), we decided to use the PexICT in the following chapter on the long-term follow up of kidney function in hyperthyroid cats.

**2. Evaluation of urinary retinol binding protein (RBP) as a marker of kidney function (Chapter 3)**

In patients at risk of developing kidney failure, such as cats treated for hyperthyroidism, it is important to apply corrective therapy at an early stage of kidney disease. Previous studies have shown that only GFR was predictive for development of CKD.[6,7] The use of RBP as a putative urinary marker was investigated in Chapter 3 (§ 3.1 and § 3.2). Renal tubules are hypertrophic and hyperplastic in hyperthyroidism.[8] Tubular cells can

be damaged due to the hypertrophy and hyperplasia, or are debilitated due to the increased functional level. While other studies focused on glomerular changes in hyperthyroid cats, we also wanted to investigate tubular function in hyperthyroid cats as part of a total assessment of kidney function. The onset of decreased kidney function can be assessed by monitoring sensitive biomarkers, such as urinary RBP which reflects renal damage at the tubular level. Our first aim in this area was to assess urinary RBP with Western blot analysis with an ELISA validated for the analysis of urine from clinically healthy cats and from cats with either diagnosed (cats with CKD) or increased risk of kidney dysfunction (hyperthyroid cats) (§ 3.1). The detection of a band at the same position as the human RBP standard with Western blot analysis, indicated that RBP was present in the urine of cats with CKD or hyperthyroidism, but minimally present in healthy cats. In urine of healthy cats, the RBP signal observed was very low in comparison to that in urine from both cats with CKD or hyperthyroidism. Indeed, the relative RBP concentrations detected with ELISA were below the assay sensitivity in all healthy cats, whereas increased urinary RBP concentrations, with a large variation between individual cat samples, were typically seen in the majority of cats with CKD and hyperthyroid cats. The healthy cats consisted of young adult as well as aged cats, and therefore a physiologic decrease in tubular function due to increasing age could not be established. Serum creatinine, USG and UPC varied widely in cats with CKD. This could express different degrees of advanced kidney failure, and could account for the large variation in urinary RBP found in cats with CKD. Urinary RBP concentrations also vary widely in hyperthyroid humans before, as well as after, treatment. However, they do not differ from concentrations in healthy control subjects. This suggests that tubular damage is more severe in hyperthyroid cats than in humans. Another possible cause for the smaller difference in urinary RBP between hyperthyroid and healthy humans, could be the decreased plasma RBP concentrations described in hyperthyroid humans.[9,10]

We found urinary RBP in hyperthyroid cats (§ 3.1), although the reason for this was unclear. Therefore, we evaluated whether the same variation as in *urinary* RBP was present in *serum* RBP in hyperthyroid cats and healthy cats. We also investigated influence of treatment of hyperthyroidism on serum and urinary RBP in hyperthyroid cats (§ 3.2). Nonetheless, when urinary and serum RBP are evaluated, it has to be kept in mind that the reported concentrations are relative values. Urinary RBP decreased after treatment. However, it was not correlated to serum RBP concentrations. From these results it is therefore suggested that

urinary RBP in hyperthyroid cats reflects dysfunction at the local tubular level and is not caused by changes in serum RBP concentrations. This dysfunction is also suggested to be reversible when euthyroidism is restored.

### 3. Long term effects of $^{131}$I treatment on kidney function and possible prediction of post-treatment renal azotemia (Chapter 4)

Evaluating renal function in a hyperthyroid cat is important but difficult at the same time. Measurement of GFR for instance, could be interesting to detect subclinical kidney disease before a definitive treatment of hyperthyroidism is performed and predict which cats may develop renal azotemia after treatment of hyperthyroidism. This early detection of an underlying kidney disease could influence the choice of therapy of hyperthyroidism. It would also allow early treatment of kidney disease and monitoring, and thus an increase in patient's well being.

A study investigating short as well long term effects of treatment of hyperthyroidism with $^{131}$I on the glomerular, as well as tubular function, in cats had not yet been performed. We investigated kidney function through measurement of several variables, including GFR and urinary RBP which were validated in the previous chapters, before and after treatment. Further, the post-treatment time course of these variables in cats which maintained a normal kidney function and cats developing renal azotemia was assessed. Finally, we tested possible pre-treatment predictive value of any of these variables for the development of post-treatment renal azotemia and GFR (Chapter 4).

#### *Renal differences between cats developing post-treatment renal azotemia and cats maintaining a healthy kidney function*

In our study, almost one in five cats developed post-treatment renal azotemia, and this percentage is comparable to previously published results.[7,11-13] There was a significant decrease in GFR, UPC and uRBP/c for the complete group and cats maintaining a healthy kidney function already 1 week after treatment, until 4 weeks after treatment. In contrast, GFR and uRBP/c did not change in cats developing post-treatment renal azotemia. Pre-treatment values of BW and serum creatinine did not differ with values measured 1 week after treatment. Serum creatinine did not increase statistically significant after 4 weeks post-treatment in the cats developing post-treatment renal azotemia, however an increase remains visible (figure 2C, Chapter 4). It is possible that the number of cats was too small to generate

significant findings. If the increase in serum creatinine would be significant after 4 weeks in the cats developing post-treatment renal azotemia, there is still no significant decrease in GFR visible in these cats. These findings suggests that the decreased serum creatinine concentration pre-treatment is more related to the decreased muscle mass than to GFR and enhanced clearance of creatinine, and that the increased serum creatinine after treatment cannot completely be regarded as representative for a decreased kidney function. Therefore, as suggested in an earlier study,[14] serum creatinine should not be considered a reliable short-term indicator of deteriorating kidney function in hyperthyroid cats.

Pre-treatment proteinuria decreased after treatment regardless the development of post-treatment renal azotemia or not and therefore treatment for proteinuria is not indicated in these cats. Pre-treatment proteinuria is suggested to be caused by a functional change in the structure of the glomerular barrier which is reversed after treatment.[15] Indeed, BP, GFR and markers of tubular function did not change after treatment in cats developing post-treatment renal azotemia. Other suggested causes for proteinuria in hyperthyroidism, like glomerular hypertension and hyperfiltration or changes in tubular protein handling, are therefore less likely to cause pre-treatment proteinuria, although this was not investigated.

We found significant differences in pre-treatment values of GFR, USG and serum TT4 concentration, between cats maintaining a healthy kidney function and cats developing post-treatment renal azotemia. These findings can be considered predictive for development of post-treatment renal azotemia. The difference in GFR is in accordance with previous findings.[6,14] Besides the difference in pre-treatment GFR value, pre-treatment GFR explained 48 % of the variability in GFR 4 weeks after treatment, and explained 59 % and 58 % respectively of the variability in GFR 4 weeks after treatment when combined with pre-treatment USG or serum creatinine.

In contrast to GFR, the findings regarding pre-treatment values of serum TT4 concentration and USG have not yet been described. A difference in USG had been suggested by Becker et al.[7] although not statistically significant. Also, variability in GFR 4 weeks after treatment is explained for 37 % by USG alone and for 62 %, 59 % and 51 % when combined with pre-treatment serum creatinine concentration, GFR and serum TT4, respectively. The lower pre-treatment serum TT4 concentration in cats developing post-treatment renal azotemia compared to cats maintaining a healthy kidney function, could be regarded as an actual sign of underlying kidney disease, because kidney disease can act as a non-thyroidal illness (NTI) and suppress serum TT4 concentration. Further research is necessary to develop

pre-treatment cut-off values for GFR, USG and possibly serum TT4 to be able to predict which cat has an increased risk for post-treatment renal azotemia.

An overall limitation of the study was the small number of cats evaluated, especially the number of cats developing post-treatment renal azotemia. Other studies showed that 39 %, 17 % and 37 % of treated hyperthyroid cats developed kidney disease.[7,11,14] Nonetheless, results are statistically significant and the number of 5 cats out of 21 developing post-treatment renal azotemia after treatment of hyperthyroidism is comparable to numbers described in the literature.[7,11-13]

The originality of our studies concerning renal function in hyperthyroid cats, lies in the combined evaluation of glomerular as well as tubular function before, but also short- and long-term after treatment. Moreover, we evaluated routinely used serum and urinary renal variables (BUN, serum creatinine, urine protein/creatinine ratio (UPC) and urine specific gravity (USG)) as well as two less common used variables (GFR and uRBP/c) for glomerular and tubular function. Previous studies focused on glomerular function, and most of these only measured GFR at one time point (6 days, 30 days or 6 weeks) after treatment and are therefore less extensive compared to our study.[6,7,11] In the study described in Chapter 4, we show for the first time in an evidence based way, that significant changes in kidney function occur within 4 weeks post-treatment and remain stable thereafter, regardless of the degree in declining kidney function. Therefore, we can recommend an accurate assessment of kidney function 1 month after treatment with $^{131}$I.

### 4. Evaluation of rhTSH stimulation test to measure thyroid function in cats with post-treatment renal azotemia suspected of iatrogenic hypothyroidism (Chapter 5)

At the end of the study described in Chapter 4, 4 out of 5 cats with post-treatment renal azotemia also had serum TT4 values below the reference range. This represented a diagnostic challenge as it was not clear whether these cats had iatrogenic hypothyroidism or a low serum TT4 due to NTI. Also because of the interplay between thyroid status and renal function, definitive diagnosis of hypothyroidism was warranted. Indeed, iatrogenic hypothyroidism occurs in 6 to 30 % of hyperthyroid cats treated with $^{131}$I and could contribute to a declining kidney function.[16-18,18,19] On the other hand, CKD, which develops in up to 39 % of hyperthyroid cats after treatment,[11] can act as a NTI and can suppress serum TT4 below reference ranges in cats.[20,21] Either one or both of these conditions can be present in these cats

with post-treatment renal azotemia and suspected of iatrogenic hypothyroidism and a definitive diagnosis is warranted.

Thyroid function can be assessed with serum free T4 after equilibrium dialysis, which has a low specificity,[22] or endogenous serum TSH, however feline TSH measurement is not available. Another method in cats which could proof valuable for measuring thyroid function, is stimulation of thyroid tissue with TSH. The possibility of prolonged storage of rhTSH[23] and the biological activity described in cats[24] opened doors to the use of rhTSH in feline medicine. When stimulation with TSH is performed, thyroidal response could be measured with serum TT4 concentration or thyroid scintigraphy. However, in the latter case the influence of TSH stimulation on thyroid/salivary gland uptake ratio (T/S uptake ratio) in thyroid scintigraphy in a normal functioning thyroid must be taken into account, though this had not yet been investigated.

In Chapter 5, we first investigated the influence of rhTSH administration on serum TT4 concentration and on thyroid scintigraphy in healthy cats (§ 5.1), before we investigated the application of rhTSH for evaluation of thyroid function in cats developing serum TT4 below reference range and azotemia after treatment of hyperthyroidism (§ 5.2).

In the healthy cats, there was a significant increase in serum TT4 and a marginal but significant increase in T/S uptake ratio after rhTSH administration. The only small increase in T/S uptake ratio after rhTSH administration can be caused by an insufficient dose to reach an effect on the pump mechanism that would strengthen the increase in T/S uptake ratio. Also, the time interval between rhTSH administration and image acquisition could have been to short to establish a profound increase in T/S uptake ratio. However, the small increase in T/S uptake ratio after TSH stimulation must be taken into account when thyroid function is evaluated with thyroid scintigraphy.

There was no difference in baseline serum TT4 concentration between cats with NTI and cats with low serum TT4 concentration and renal azotemia. This confirms the need for an accurate thyroid evaluation test because the diagnosis of iatrogenic hypothyroidism could not be made based on baseline serum TT4 concentration. Serum TT4 increased significantly after rhTSH administration in the healthy cats and in the cats with NTI but not in the cats suspected of hypothyroidism. There was no difference in T/S uptake ratio after rhTSH stimulation between the 3 groups. This shows that the rhTSH stimulation test with measurement of serum TT4 can differentiate between euthyroid and hypothyroid cats. From our study, it was not

possible to conclude whether scintigraphy alone could be used for measurement of thyroid function in these cats.

With the use of rhTSH stimulation, the cats with post-treatment renal azotemia and serum TT4 below reference range were diagnosed with iatrogenic hypothyroidism. The question could be raised whether there is a link between the development of post-treatment renal azotemia and iatrogenic hypothyroidism in these cats. On one hand, hypothyroidism could contribute to the development of declining kidney function or could just cause a reversible decreased GFR and hence renal azotemia. On the other hand, it is possible that cats with an impaired kidney function previous to $^{131}$I treatment, which can be extrapolated from the lower pre-treatment GFR, have a lower clearance of $^{131}$I. This could cause a prolonged effect of $^{131}$I on the thyroid and thereby increase the chance of developing iatrogenic hypothyroidism. A possible causal link between hypothyroidism and CKD after treatment in hyperthyroid cats remains possible, but further research is necessary to elucidate this aspect. Definitive diagnosis of hypothyroidism, especially in this context, seems warranted and our results suggest that rhTSH stimulation is an appropriate test.

**Conclusion**

In this thesis, we have gained significant insight into the kidney function of hyperthyroid cats. We investigated suitable GFR methods as well as a method for direct evaluation of tubular function. Clearance of exo-iohexol revealed to be the most precise method, and this was therefore used in the further studies to measure GFR. Urinary RBP indicated tubular dysfunction in hyperthyroid cats, and this dysfunction was suggested to be reversible after $^{131}$I treatment.

The declining kidney function after treatment stabilized within 4 weeks after $^{131}$I treatment, and therefore we can recommend that an accurate evaluation of kidney function can be made at that time. Prediction of development of post-treatment renal azotemia might be possible with pre-treatment measurement of GFR, USG and serum TT4. When there is development of post-treatment renal azotemia combined with low serum TT4, a definitive diagnosis of iatrogenic hypothyroidism can be made using the rhTSH stimulation test.

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# SUMMARY

# SUMMARY

Hyperthyroidism and chronic kidney disease (CKD) are both frequently encountered diseases in geriatric cats. An important problem in hyperthyroid cats is the declining kidney function after treatment, whatever the treatment method used. There is a strong need to be able to predict which hyperthyroid cats develop CKD after treatment, and to be able to detect a declining kidney function early after treatment. The first chapter of this thesis reviews the literature on thyroid function and feline hyperthyroidism, the effect of thyroid hormones on kidney function, and the different methods available to evaluate kidney function. Further, diagnostic challenges encountered in cats with concurrent non-thyroidal illness are reviewed.

In this thesis several aspects that could lead to improved insight into kidney function of hyperthyroid cats, before as well as after treatment, were evaluated. First, suitability of plasma clearance methods for measuring glomerular filtration rate (GFR) in cats was assessed (Chapter 2). The reproducibility of plasma clearance of exogenous creatinine, exo-iohexol and endo-iohexol, and differences in GFR using these methods between different ages, were investigated in healthy young adult and elderly cats (§ 2.1). Globally, the methods differed significantly in GFR assessment. Clearance of exo-iohexol showed the best reproducibility, and was used in our further study on the long term follow up of kidney function in treated hyperthyroid cats (Chapter 4).

Besides reproducibility, the ability to distinguish between GFR over a period of time in which GFR is expected to change after $^{131}$I treatment of hyperthyroid cats was investigated (§ 2.2). Globally, the GFR methods resulted in different GFR results. However, GFR results were the same at all time points among the different methods and all three techniques indicated decreasing GFR after $^{131}$I treatment. The decrease in GFR stabilized 4 weeks after treatment, with very little decline afterwards. Our results showed it was mandatory to use the same GFR method in follow-up studies.

This ability to distinguish between GFR values was also investigated between GFR ranges that can be expected: low, normal and high, respectively (§ 2.3). There was only a difference in GFR between the methods in healthy cats, and not in cats with CKD or hyperthyroidism. For all three methods, GFR differed between cats with CKD and cats with hyperthyroidism, although GFR values differed only for exo-iohexol and creatinine between healthy and hyperthyroid cats, and for endo-iohexol and creatinine between cats with CKD

and healthy cats. This showed that apparently only clearance of exogenous creatinine could detect the smaller differences between different ranges of GFR. Differences between clearance techniques seem to be correlated to the range of GFR in which it is used.

In patients at risk for developing kidney disease, such as hyperthyroid cats, it is important to apply corrective therapy at an early stage. An early detection of decreased kidney function can be assessed by monitoring sensitive urinary biomarkers, such as urinary retinol binding protein (RBP) which reflects damage or dysfunction at the tubular level. Urinary RBP was first evaluated in healthy cats and cats expected to have tubular dysfunction (§ 3.1). Urinary RBP was undetectable in healthy cats, although it was present in variable amounts in cats with CKD or hyperthyroidism.

In search of the cause of urinary RBP in hyperthyroid cats, we further investigated whether this remained after treatment with $^{131}$I, and whether urinary RBP was linked with serum RBP concentration (§ 3.2). In cats that did not develop post-treatment renal azotemia, the urinary RBP decreased after treatment. It was correlated to serum TT4 concentration, but not to serum RBP concentration. These results suggest that the urinary RBP found in hyperthyroid cats is indicative of a reversible tubular dysfunction when a healthy kidney function is maintained after treatment.

While earlier studies focused on glomerular function after treatment of hyperthyroid cats, over only a short term period, this thesis investigated the influence of $^{131}$I treatment on glomerular as well as tubular kidney function over a long term period. Further, possible prediction of post-treatment renal azotemia and GFR with several variables measured pre-treatment was assessed (Chapter 4).

There was a significant decrease in serum TT4, GFR, UPC, and urinary RBP in cats maintaining a healthy kidney function after treatment. However, GFR and urinary RBP did not change in cats developing post-treatment renal azotemia. After treatment, there was no change in BUN, USG or BP. The most important changes in variables occurred within 4 weeks after treatment, regardless of the development of CKD.

Pre-treatment serum TT4, GFR and USG differed significantly between cats maintaining a healthy kidney function and cats developing post-treatment renal azotemia.

GFR at 4 weeks after treatment and the maximum decrease in GFR could be predicted by a formula using pre-treatment GFR, serum TT4, serum creatinine, BUN and/or USG.

At the end of the study described in Chapter 4, several cats had post-treatment renal azotemia, but also serum TT4 concentrations below reference range. This represented a diagnostic challenge, as it was not clear whether these cats had truly iatrogenic hypothyroidism or a lower serum TT4 concentration due to NTI. We investigated whether measurement of thyroid function after stimulation with rhTSH using serum TT4 and thyroid scintigraphy, could discriminate between cats with iatrogenic hypothyroidism and cats with NTI. First, the influence of rhTSH on thyroid scintigraphy was investigated in healthy cats (§ 5.1). After this, serum TT4 and thyroid scintigraphy after rhTSH stimulation in healthy cats, cats with NTI and cats with post-treatment renal azotemia suspected of hypothyroidism was evaluated (§ 5.2). In the healthy cats, there was a significant increase in serum TT4 after stimulation with rhTSH. Also T/S uptake ratio increased after stimulation with rhTSH, and the increase was marginal but significant. This should be taken into account when these variables are evaluated after rhTSH stimulation in measurement of thyroid function. There was a significant increase in serum TT4 concentration after rhTSH stimulation, in healthy cats and cats with NTI, though not in cats suspected of hypothyroidism. Serum TT4 after rhTSH administration, though not T/S uptake ratio, differed between the cats suspected of hypothyroidism and the healthy cats and cats with NTI, respectively. These results showed that stimulation with rhTSH was valuable to differentiate euthyroidism from hypothyroidism in cats.

In conclusion, the present thesis allowed to gain several new insights into kidney function in hyperthyroid cats.

- Reproducibility and precision was the highest for plasma clearance of exo-iohexol which made it most suitable for research environments.
- Reproducibility and distinguishment of plasma clearance of exogenous creatinine were sufficient. Because creatinine analysis can be performed using routine devices for biochemical analysis, it is an applicable clearance method in veterinary practice.
- Any of the three evaluated plasma clearance methods could be used for measuring GFR. However, when GFR was measured repeatedly as part of follow up of kidney function, it was important to use the same GFR method every time.

- Urinary RBP was indicative of reversible tubular dysfunction in hyperthyroid cats.
- An accurate evaluation of kidney function could be made 1 month after $^{131}I$ treatment of hyperthyroid cats.
- Post-treatment renal azotemia and GFR could possibly be predicted by pre-treatment GFR measurement as well as pre-treatment serum TT4, serum creatinine, BUN and/or USG.
- Evaluation of thyroidal reserve in cats with post-treatment renal azotemia suspected of iatrogenic hypothyroidism can reliably be performed by rhTSH stimulation.

# SAMENVATTING

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Hyperthyroïdie en chronische nierziekte (CNZ) zijn beide frequent voorkomende ziektes bij oude katten. Bovendien zorgt elk type behandeling van hyperthyroïdie voor een daling van de nierfunctie en is het dus van groot belang dat voorspeld zou kunnen worden welke hyperthyroïde katten CNZ ontwikkelen na behandeling. Het is tevens belangrijk dat een dalende nierfunctie na behandeling vroeg wordt opgespoord. In het eerste hoofdstuk van deze thesis wordt een literatuur overzicht gegeven over schildklierfunctie en hyperthyroïdie bij de kat, de effecten van schildklierhormonen op de nierfunctie en de verschillende beschikbare methoden om nierfunctie te evalueren. Tevens worden de diagnostische uitdagingen besproken bij katten met een gelijktijdige niet-schildklier gerelateerde ziekte.

In deze thesis werden verschillende aspecten geëvalueerd die zouden kunnen leiden tot inzichten in de nierfunctie van hyperthyroïde katten, voor maar ook na behandeling. Eerst werd de geschiktheid van plasma klaringsmethoden voor meting van glomerulaire filtratie snelheid (GFS) bij de kat geëvalueerd (Hoofdstuk 2). De reproduceerbaarheid van plasma klaring van exogeen creatinine, exo-iohexol en endo-iohexol, en verschillen in GFS tussen verschillende leeftijden door gebruik van deze methoden, werden onderzocht in gezonde jong volwassen en oude katten (§ 2.1). Globaal gezien was er een significant verschil in GFS meting. De klaring van exo-iohexol toonde de beste reproduceerbaarheid, en daarom werd deze methode verder gebruikt in de studie naar lange termijn opvolging van de nierfunctie bij hyperthyroïde katten na behandeling.

Naast reproduceerbaarheid, werd ook het vermogen onderzocht om onderscheid te maken tussen waarden van GFS, gemeten over een tijdsperiode waarin werd verwacht dat de GFS verandert, namelijk bij hyperthyroïde katten voor en na behandeling met $^{131}$I (§ 2.2). De daling in GFS stabiliseerde 4 weken na behandeling, met slechts een zeer kleine daling na deze periode. Onze resultaten toonden aan dat het noodzakelijk was om dezelfde GFS methode te gebruiken voor opvolging van de nierfunctie.

Dit vermogen om onderscheid te maken tussen GFS waarden werd ook onderzocht bij verschillende GFS waarden die verwacht kunnen worden: laag, normaal en hoog (§ 2.3). Er was enkel een verschil in GFS waarde tussen de 3 klaringsmethoden bij gezonde katten, en niet bij katten met CNZ of hyperthyroïdie. Voor alle drie de methoden was er een verschil in GFS tussen katten met CNZ en katten met hyperthyroïdie, maar er was enkel een verschil in

GFS voor exo-iohexol en creatinine tussen gezonde katten en hyperthyroïde katten, en voor endo-iohexol en creatinine tussen gezonde katten en katten met CNZ. Dit toonde aan dat blijkbaar alleen klaring van exogeen creatinine een klein verschil tussen verschillende GFS waarden kon detecteren. Verschillen tussen klaringsmethoden lijken gecorreleerd te zijn aan het bereik waarbinnen GFS wordt gemeten.

Bij patiënten die een risico lopen op het ontwikkelen van nierfalen, zoals hyperthyroïde katten, is het belangrijk om corrigerende therapie toe te passen in een vroeg stadium. Vroege detectie van een gedaalde nierfunctie kan worden uitgevoerd door het opvolgen van gevoelige urinaire biomerkers, zoals urinair retinol bindend proteïne (RBP) dat schade of verminderde functie op het tubulaire niveau reflecteert. In Hoofdstuk 3 werd eerst het urinair RBP geëvalueerd bij gezonde katten en katten waarvan een verminderde tubulaire functie verwacht kan worden (§ 3.1). Urinair RBP was niet detecteerbaar bij gezonde katten, maar het was aanwezig in variërende mate bij katten met CNI of hyperthyroïdie.

Om de oorzaak van de aanwezigheid van urinair RBP bij hyperthyroïde katten verder te onderzoeken, werd onderzocht of het urinair RBP detecteerbaar bleef na behandeling met $^{131}$I en of het gerelateerd was aan serum RBP (§ 3.2). Bij katten die geen renale azotemie ontwikkelden na behandeling, daalde het urinair RBP. Het was gerelateerd aan serum totaal T4 (TT4) concentratie, maar niet aan serum RBP concentratie. Deze resultaten suggereerden dat het urinair RBP bij hyperthyroïde katten indicatief was voor een reversibele vermindering van de tubulusfunctie mits een gezonde nierfunctie na behandeling behouden blijft.

Terwijl vroegere studies zich toespitsten op glomerulaire functie na behandeling van hyperthyroïde katten, over een korte termijn, werd in dit proefschrift de invloed van $^{131}$I behandeling op de glomerulaire en tevens de tubulaire functie onderzocht en dit over lange termijn. Tevens werd de mogelijkheid om renale azotemie en GFS na behandeling te voorspellen onderzocht, aan de hand van verschillende variabelen, gemeten voorafgaand aan de behandeling (Hoofdstuk 4). Er was een significante daling in serum TT4, GFS, urinaire proteïne / creatinine ratio (UPC) en urinair RBP bij katten die een gezonde nierfunctie behielden na behandeling. Niettemin was er geen verandering in GFS en urinair RBP bij katten die renale azotemie ontwikkelden na behandeling. Na behandeling was er geen verandering in BUN, USG of bloeddruk. Er was een significant verschil in serum TT4, GFS

en urinair soortelijk gewicht (USG) tussen katten die een gezonde nierfunctie behielden en katten die renale azotemie ontwikkelden na behandeling. De GFS op 4 weken na behandeling en de maximale daling in GFS kon mogelijk worden voorspeld door een formule die gebruik maakt van GFS, serum TT4, serum creatinine, BUN en/of USG, gemeten voor de behandeling.

Aan het eind van de studie beschreven in Hoofdstuk 4, waren er verschillende katten met renale azotemie na behandeling, gecombineerd met een serum TT4 concentratie lager dan de referentiewaarde. Dit zorgde voor een diagnostische uitdaging, omdat het niet duidelijk was of deze katten werkelijk iatrogene hypothyroïdie hadden of dat de lagere serum TT4 concentratie veroorzaakt werd door een ziekte niet gerelateerd aan de schildklier. We wilden onderzoeken of evaluatie van schildklierfunctie gemeten met serum TT4 en schildklierscintigrafie na stimulatie met recombinant humaan thyrotropine (rhTSH), een onderscheid kon maken tussen katten met iatrogene hypothyroïdie en katten met een niet-schildklier gerelateerde ziekte. Omdat de invloed van rhTSH op schildklierscintigrafie nog niet was onderzocht, werd eerst de invloed van rhTSH stimulatie op schildklierscintigrafie onderzocht bij gezonde katten (§ 5.1). Hierna werd serum TT4 en schildklierscintigrafie geëvalueerd na rhTSH stimulatie bij gezonde katten, katten met een niet-schildklier gerelateerde ziekte, en katten met renale azotemie na behandeling die tevens verdacht werden van hypothyroïdie. Bij de gezonde katten was er een significante stijging van serum TT4 na stimulatie met rhTSH. Tevens was er een milde doch significante stijging van de thyroid/speekselklier (T/S) opname ratio. Dit moet in acht worden genomen als deze variabelen worden geëvalueerd na rhTSH stimulatie bij de meting van schildklier functie. Er was een significante stijging van de serum TT4 concentratie na rhTSH stimulatie bij de gezonde katten en katten met een niet-schildklier gerelateerde ziekte, maar niet bij de katten verdacht van hypothyroïdie. Serum TT4 maar niet de T/S opname ratio verschilde tussen de gezonde katten en katten verdacht van hypothyroïdie. Deze resultaten toonden aan dat stimulatie met rhTSH waardevol was om een normale schildklierfunctie te kunnen onderscheiden van hypothyroïdie bij de kat.

# SAMENVATTING

In conclusie, in deze thesis werden verschillende inzichten verkregen omtrent de nierfunctie bij hyperthyroïde katten, zowel voor als na $^{131}$I behandeling.

- Reproduceerbaarheid of precisie was het hoogst voor plasma klaring van exo-iohexol wat het geschikt maakt voor onderzoeksdoeleinden.
- Reproduceerbaarheid en onderscheidend vermogen van plasma klaring gemeten met exogeen creatinine waren voldoende. Omdat creatinine bepalingen gedaan kunnen worden met routine apparaten voor biochemische analyses, is dit een klaringsmethode die toepasbaar is in de veterinaire praktijk.
- Elk van de 3 onderzochte klaringsmethoden kon gebruikt worden als onderdeel van de opvolging van nierfunctie, maar het was hierbij van belang om telkens dezelfde methode te gebruiken.
- Urinair RBP was indicatief voor een reversiebele verminderde tubulaire functie in hyperthyroïde katten.
- Een accurate evaluatie van de nierfunctie kon gebeuren vanaf 4 weken na behandeling met $^{131}$I.
- Renale azotemie en GFS na behandeling konden mogelijk voorspeld worden door het meten van GFS, serum TT4, serum creatinine, BUN en/of USG voorafgaande behandeling.
- Als er na behandeling ontwikkeling is van renale azotemie gecombineerd met een lage serum TT4 concentratie, kon een definitieve diagnose van iatrogene hypothyroïdie worden gesteld met de rhTSH stimulatie test.

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Onderstaand recept is voor 1 persoon. Dat wil echter niet zeggen dat dit ook door slechts 1 persoon bereid kan worden. Het vergt veel onmisbare hulp van anderen, waarvoor ik zeer dankbaar ben. De totale bereidingstijd is lang maar het resultaat is onvergetelijk. Soms moet het zachtjes pruttelen, soms kookt het over, maar het ruikt altijd overheerlijk.

**Recept voor Doctoraat.**

**Bereidingsduur: 4 jaar**

**Aantal personen: 1**

**Benodigdheden.**

Zoals elk gerecht staat of valt met de kwaliteit van de ingrediënten, wordt ook de basis van een doctoraat gevormd door de aandacht voor de benodigdheden. De katten zijn de basis van de studie en de dieren waarvoor je het uiteindelijk allemaal doet. Zij maken het werk waardevol.

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# DANKWOORD

# CURRICULUM VITAE

Ingrid van Hoek werd geboren op 24 mei 1977 te Tilburg in Nederland.

Zij behaalde in 1997 haar VWO diploma aan het DAC-ROC departement atheneum. Daarna starte zij haar studies Diergeneeskunde aan de Universiteit Antwerpen waar zij in 2001 haar Kandidatuurs Diploma behaalde.

Ze vervolgde haar studies Diergeneeskunde aan de Universiteit Gent en behaalde in 2004 met onderscheiding haar Dierenarts Diploma in de afstudeerrichting Onderzoek en Industrie.

Geboeid door het wetenschappelijk onderzoek waarmee zij in haar afstudeerjaar kennis had mogen maken, startte zij op 1 januari 2005 met een doctoraatsstudie over nierfunctie bij hyperthyroïde katten op de dienst Interne Geneeskunde van de Vakgroep Geneeskunde en Klinische Biologie van de Kleine Huisdieren aan de Faculteit Diergeneeskunde van de Universiteit Gent. Deze studie werd gefinancierd door het Bijzonder Onderzoeks Fonds van de Universiteit Gent.

Ingrid van Hoek is auteur of mede-auteur van 18 wetenschappelijke abstracts op nationale en internationale congressen en 11 wetenschappelijke publicaties. Zij nam actief deel aan meerdere nationale en internationale congressen. Tevens was zij in 2008 local chairperson voor het congres van de European College of Veterinary Internal Medicine (ECVIM) in Gent.

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