The role of diagnostic imaging in small animal endocrine disease

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Submitted in partial fulfilment of the requirements of the degree of Master of Veterinary Science (Clinical)

February 2018

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Abstract

The work reported in this thesis aimed to investigate the role of diagnostic imaging in small animal endocrine disease: adrenal gland ultrasound in dogs and thyroid scintigraphy in severely hyperthyroid cats.

A prospective study evaluated the effect of the patient’s recumbency position on ultrasound derived adrenal gland measurements in dogs with non-adrenal illness. This research determined that the measurement with the best agreement between lateral and dorsal recumbency position was the caudal pole thickness from a longitudinal image plane. Whereas the measurements from the transverse image plane and the length of the adrenal gland had poorer agreement between recumbency positions.

A retrospective review of thyroid scintigraphy findings in severely hyperthyroid cats was compared with the general population of hyperthyroid cats. A greater proportion of the severely hyperthyroid cats demonstrated ectopic thyroid tissue and features which increase the suspicion of thyroid carcinoma. These findings suggest that thyroid scintigraphy is useful in severely hyperthyroid cats and assist with treatment decisions. This work also evaluated the role of thyroid scintigraphy in the calculation of an individualised compared to a standard radioiodine dose. The treatment outcome was assessed by the initial post treatment total T4 and found fewer cats receiving a standard radioiodine dose had a total T4 below the reference range. This finding indicates that further evaluation of radioiodine dosing regime is warranted in severely hyperthyroid cats.

Thus, the ultrasound assessment of dogs with non-adrenal gland illness has been simplified for all veterinarians including those with less ultrasound experience as the current reference ranges of ultrasound derived adrenal gland measurements were validated and there is flexibility of patient recumbency during the ultrasound examination. Also, this work has contributed to the knowledge of the role of thyroid scintigraphy in both the diagnosis and treatment of severely hyperthyroid cats.
Declaration

This is to certify that:

i. the thesis comprises only my original work towards the Masters except where indicated in the Preface

ii. due acknowledgement has been made in the text to all other material used

iii. the thesis is fewer than the maximum word limit in length (23477), inclusive of footnotes but exclusive of tables, maps, bibliographies and appendices.

Anne Marie Rose
Preface

Part of this work (Chapter 3) has been published in the following paper:


Part of this work (Chapter 5, Part 1) was presented at the following conference:

Anne Marie Rose. Thyroid scintigraphy in severely hyperthyroid cats: A description of scintigraphic images and comparison to previous literature. Australian and New Zealand College of Veterinary Scientists College Science Week Radiology Chapter 8 July 2016.

Professional editor Dr Gillian Dite provided copyediting and document formatting services according to standards D and E of the Australian Standards for Editing Practice and the Guidelines for Editing Research Theses from the Institute of Professional Editors.
Acknowledgements

I wish to thank the University of Melbourne U Vet resident research grant program for providing the financial support.
I would like to express my sincere gratitude to my supervisors Dr Thurid Johnstone and Dr Cathy Beck for their guidance, support and perseverance. I would also like to thank Dr Sue Finch for her invaluable assistance.
# Table of contents

Abstract .......................................................................................................................... i  
Declaration ....................................................................................................................... ii  
Preface .......................................................................................................................... iii  
Acknowledgements ......................................................................................................... iv  
Table of contents ............................................................................................................. v  
List of figures ................................................................................................................... viii  
List of tables ................................................................................................................... x  
Abbreviations .................................................................................................................. xi  

Chapter 1. Introduction to the role of diagnostic imaging in small animal endocrine disease .................................................................................................................. 1  

Chapter 2. Literature review: ultrasound assessment of the canine adrenal gland ................................................................................................................................. 5  
2.1. The canine adrenal gland: embryology, anatomy and physiology ........... 5  
2.1.1. Embryology ......................................................................................................... 5  
2.1.2. Anatomy and physiology .................................................................................... 5  
2.2. The role of abdominal ultrasound in the evaluation of the normal canine adrenal gland .............................................................................................................. 7  
2.2.1. Introduction ......................................................................................................... 7  
2.2.2. Subjective features of the normal canine adrenal gland ...................... 8  
2.2.3. Objective features: measurement of the normal canine adrenal gland ......................................................................................................................... 9  
2.2.4. The size of the normal adrenal gland .................................................... 10  
2.3. The role of abdominal ultrasound in the abnormal canine adrenal gland ................................................................................................................................. 11  
2.3.1. Canine hyperadrenocorticism .................................................................. 11  
2.3.2. Effects of medications on ultrasonographic appearance of the adrenal glands ......................................................................................................................... 14  
2.3.3. Other adrenal gland disease which may affect the ultrasonographic appearance of the adrenal glands ...................................................... 15  

Chapter 3. The effect of recumbency position on the ultrasound measurement of the canine adrenal gland in non-adrenal gland illness ........ 17  
3.1. Introduction ............................................................................................................ 17  
3.2. Materials and methods .......................................................................................... 18  
3.2.1. Patient selection ............................................................................................... 18  
3.2.2. Ultrasound equipment and ultrasound examination .................................. 19
3.2.3. Data collection ................................................................. 19
3.2.4. Approach to the data ....................................................... 21
3.2.5. Statistical analysis ........................................................... 22
3.3. Results .............................................................................. 22
  3.3.1. Patient data ................................................................. 22
  3.3.2. Caudal pole thickness from the longitudinal image plane ........ 23
  3.3.3. Equivalent measurements from the transverse image plane ...... 25
  3.3.4. Orthogonal measurements from the transverse image plane ..... 26
  3.3.5. Length of the adrenal gland ........................................... 27
  3.3.6. Comparison between measurements .................................. 28
3.4. Discussion ....................................................................... 29

Chapter 4. Literature review: thyroid scintigraphy in feline hyperthyroidism ......................................................... 32
  4.1. The feline thyroid gland: embryology, anatomy and physiology .... 32
    4.1.1. Embryology ................................................................. 32
    4.1.2. Anatomy ................................................................. 32
    4.1.3. Histology .................................................................. 33
    4.1.4. Hypothalamic-pituitary-thyroid axis............................... 33
    4.1.5. Production of the thyroid hormones ................................ 34
  4.2. Feline hyperthyroidism ..................................................... 34
    4.2.1. Signalment, presentation and clinical signs ....................... 34
  4.3. Aetiology .......................................................................... 35
  4.4. Pathology ......................................................................... 36
  4.5. Diagnosis ......................................................................... 36
    4.5.1. Introduction ............................................................... 36
    4.5.2. Endocrine testing ....................................................... 37
    4.5.3. Thyroid scintigraphy .................................................. 38
    4.5.4. Other diagnostic imaging modalities .............................. 38
  4.6. Treatment ......................................................................... 39
  4.7. Nuclear medicine in diagnosis of feline hyperthyroidism .......... 40
    4.7.1. Basic principles .......................................................... 40
    4.7.2. Acquisition parameters and thyroid scintigraphy in the normal thyroid glands ........................................ 42
    4.7.3. Treatments and other compounds that may interfere with thyroid scintigraphy ........................................... 46
  4.8. Thyroid scintigraphy features in feline hyperthyroidism .......... 47
4.9. Nuclear medicine in the treatment of feline hyperthyroidism .............. 49

Chapter 5. The role of thyroid scintigraphy in severely hyperthyroid cats ................................................................. 57

5.1. Introduction ........................................................................................................................................................................... 57

5.2. Part 1: features of thyroid scintigraphy in severely hyperthyroid (Australian) cats .......................................................... 58
   5.2.1. Materials and methods ............................................................................................................................................. 58
   5.2.2. Results ....................................................................................................................................................................... 62

5.3. Part 2: assessment of the influence of radioiodine dose calculation method on treatment outcome in severely hyperthyroid cats .............. 67
   5.3.1. Material and methods ............................................................................................................................................. 67
   5.3.2. Results ....................................................................................................................................................................... 69

5.4. Discussion ......................................................................................................................................................................... 70

Chapter 6. Concluding remarks and future work ............................................. 77

References ............................................................................................................................................................................. 80

Appendices ........................................................................................................................................................................... 92

Appendix 1. Customised dose group: raw data ................................................. 92
Appendix 2. Standardised dose group: raw data .................................................. 94
List of figures

Figure 3.1. Ultrasound images demonstrating the locations of the length, height and width measurements: (A) dorsal recumbency longitudinal image plane: ultrasound image demonstrating the locations of the length (solid line) and height (dotted line) measurements; (B) dorsal recumbency transverse image plane of the caudal pole: ultrasound image demonstrating the locations of the height (dotted line) and width (solid line) measurements; (C) lateral recumbency longitudinal image plane: ultrasound image demonstrating the location of the length (solid line) and width (dotted line) measurements and (D) lateral recumbency transverse image plane of the caudal pole. ................................................................. 20

Figure 3.2 Graphical display of the Bland–Altman analysis of the caudal pole thickness measurements acquired from the longitudinal image plane of the left and right adrenal gland. The mean difference or the bias is the central solid black line. The 95% upper and lower limits of agreement (ULA and LLA, respectively) are represented by the dotted black lines. ................................................................. 24

Figure 3.3. Graphical display of the Bland–Altman analysis of the caudal pole equivalent measurement acquired from the transverse image plane.... 26

Figure 3.4. Graphical display of the Bland–Altman analysis of the caudal pole orthogonal measurement acquired from the transverse image plane. .... 27

Figure 3.5 Graphical display of the Bland–Altman analysis of the length of the adrenal gland, acquired from the longitudinal image plane............... 28

Figure 3.6. Adrenal gland measurements. Collation of the Bland–Altman analyses comparing the dorsal recumbency with the relevant lateral recumbency and showing the mean differences, upper and lower limits of agreement (all in mm) with 95% confidence intervals............................ 29

Figure 4.1. Thyroid scintigraphy of the normal feline thyroid glands: (A) ventral image of the cervical region with arrows indicating the radionuclide uptake in the zygomatic/molar salivary glands and thyroid glands; (B) ventral image of the cervical region showing the region of interest around the thyroid glands and (C) objective measurements of % TU and T:SG ................................................................. 43

Figure 4.2. Thyroid scintigraphy ventral images of the cervical region demonstrating the patterns of disease in hyperthyroid cats: (A) unilateral, (B) bilateral symmetrical, (C) bilateral asymmetrical and (D) multifocal patterns of disease................................................................. 48

Figure 5.1. Thyroid scintigraphy ventral images of the head and neck showing the line placement between the point of the shoulders: (A) increased radionuclide uptake (IRU) above the line; (B) IRU both above and on the line; and (C) IRU above, on and below the line. ......................... 61

Figure 5.2. Patterns of disease: general population of hyperthyroid cats (yellow) compared with the severely hyperthyroid cats (blue)........................ 65
Figure 5.3. Comparison of subjective features and objective measurements of severely hyperthyroid cats (blue circles) against the published data (yellow dashes) as determined by the one proportion test. ......................... 66
List of tables

Table 2.1. Caudal pole measurements of the normal canine adrenal gland comparing patient recumbency position, image plane, direction of measurement, weight category and measurement ........................................11

Table 3.1. Patient recumbency, image plane and direction of adrenal gland measurements ............................................................................................................................................. 21

Table 3.2. Image plane and direction of adrenal gland measurement relative to the dog’s recumbency position .................................................................................................................................................. 22

Table 3.3. Number of dogs per category ................................................................................................................................................................................................. 23

Table 3.4. Median and range of the caudal pole thickness from the longitudinal image plane ...................................................................................................................... 24

Table 3.5. Caudal pole thickness outliers: signalment and weight ...................................................................................................................................................... 25

Table 4.1. 131I dose determination and treatment outcomes ............................................................................................................................................................ 52

Table 5.1. Descriptive categories of the subjective features of the increased radionuclide uptake .............................................................................................................................................. 60

Table 5.2. Presence of homogeneous and heterogeneous increased radionuclide uptake according to the distribution of patterns of disease ........................................................................ 62

Table 5.3. Subjective features: overall proportion of cats within the descriptive categories .............................................................................................................................................. 64
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Bq</td>
<td>becquerel</td>
</tr>
<tr>
<td>CPT</td>
<td>caudal pole thickness</td>
</tr>
<tr>
<td>DVTDR</td>
<td>dorsoventral thickness difference ratio</td>
</tr>
<tr>
<td>IRU</td>
<td>increased radionuclide uptake</td>
</tr>
<tr>
<td>LLA</td>
<td>lower limit of agreement</td>
</tr>
<tr>
<td>MBq</td>
<td>megabecquerel</td>
</tr>
<tr>
<td>% TU</td>
<td>percentage of technetium uptake</td>
</tr>
<tr>
<td>T3</td>
<td>triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>tetraiodothyronine or thyroxine</td>
</tr>
<tr>
<td>T:B</td>
<td>thyroid to background ratio</td>
</tr>
<tr>
<td>T:SG</td>
<td>thyroid to salivary gland ratio</td>
</tr>
<tr>
<td>ULA</td>
<td>upper limit of agreement</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>U Vet</td>
<td>University of Melbourne Animal Hospital</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>radioiodine</td>
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</table>
Chapter 1. Introduction to the role of diagnostic imaging in small animal endocrine disease

Diagnostic imaging has a role in the investigation of small animal endocrine disease. Ultrasound is commonly used for assessment of the canine adrenal gland, while nuclear medicine in the form of thyroid scintigraphy is often used for diagnosis and pre-treatment assessment in severely hyperthyroid cats. In the diagnosis and therapy of canine adrenal gland disease and feline hyperthyroidism, adrenal gland ultrasound and thyroid scintigraphy, respectively, are pivotal but both techniques require ongoing optimisation.

Spontaneous hyperadrenocorticism is a common disease of middle to older aged dogs (with an incidence of 1–2 cases per 1000 per year) and has been reported in most breeds of dog with a predisposition in poodles, boxers and dachshunds.¹,² Veterinarians have an increasing awareness of hyperadrenocorticism and it is often considered early in the disease process.² A number of endocrine tests are available to veterinary practitioners, but none of these tests are 100% specific for the diagnosis of hyperadrenocorticism.² In this study, the ultrasound examination of the normal canine adrenal gland was undertaken to clarify and simplify the accurate identification of the normal adrenal gland.

Hyperthyroidism is the most common endocrinopathy in cats and is routinely managed by veterinary practitioners.³,⁴ The situation in cats is similar to dogs with hyperadrenocorticism because hyperthyroidism is often identified early in the disease process due to evaluation of tetraiodothyronine or thyroxine (T4) in older or unwell cats.⁴ An important question for veterinary practitioners is when to refer hyperthyroid cats for thyroid scintigraphy, and therefore, both the thyroid scintigraphy features and role of thyroid scintigraphy in treatment are explored here. Thus, this study focuses on the most commonly used imaging modalities in two of the most common endocrine diseases in small animals.
Abdominal ultrasound is the most widely used diagnostic imaging modality for the direct visualisation of the canine adrenal gland because other diagnostic imaging modalities such as radiography, computed tomography and magnetic resonance imaging are limited by a range of factors. The size and radiographic opacity of normal adrenal glands prevents identification on radiographs. The adrenal glands can be seen using computed tomography and magnetic resonance imaging, however there has been less acceptance of these techniques due to the inaccessibility of equipment and the requirement for general anaesthesia for magnetic resonance imaging and the potential need for general anaesthesia for computed tomography.

The incorporation of ultrasound into veterinary practice continues to evolve. Ultrasound is no longer limited to academic institutions and referral hospitals and has become widespread in veterinary practice. However, the identification and the assessment of canine adrenal glands is challenging and further understanding of the ultrasound examination of the canine adrenal gland is sought. This understanding is required to potentially simplify the procedure of the ultrasound examination of the canine adrenal gland to thus assist all veterinary practitioners, including those with less experience.

Clarifying the ultrasonographic appearance of the normal canine adrenal gland is important because adrenal gland disease is commonly encountered in small animal practice. The use of ultrasound to determine the size of the normal canine adrenal gland and to distinguish the normal adrenal gland from bilateral adrenal gland hyperplasia due to pituitary-dependent hyperadrenocorticism has been studied in detail. While there are some characteristic sonographic criteria, there is overlap between the normal adrenal gland and pituitary-dependent hyperadrenocorticism. Identification of adrenal masses via ultrasound is possible, but there are no specific sonographic features for adrenal masses resulting in clinical signs of hyperadrenocorticism. Incidental adrenal gland nodules and tumours of the adrenal medulla may also be seen sonographically.

While subjective features of the normal canine adrenal gland are known (location, shape, margination, echogenicity and echotexture), measurement of the normal canine adrenal gland is less clear and continues to be defined. So far, body weight of the dog and operator experience have been shown to influence
adrenal gland measurement: with increasing body weight, adrenal gland size increases,\textsuperscript{16, 25} and with increasing operator experience, the repeatability and reproducibility of measurements increases.\textsuperscript{16, 26} Ultrasound examination of the canine adrenal gland is complicated,\textsuperscript{24} in part because there are two image planes (longitudinal and transverse), several measurement directions (to assess length, height and width) and three possible recumbency positions (dorsal, left lateral and right lateral).

This study compares adrenal gland measurements in different dog recumbency positions (lateral and dorsal) because it is currently unknown whether recumbency position influences adrenal gland measurements.\textsuperscript{24} If so, this could mean that any adrenal gland measurement is only valid in a certain recumbency position. If there is no impact of recumbency position on adrenal gland measurement, the operator could choose the recumbency position for the examination, which simplifies the procedure. By exploring these variables and their potential impact on ultrasound adrenal gland measurements in normal dogs, examination of the adrenal gland may be able to be simplified for all veterinarians.

Compared with abdominal ultrasound, nuclear medicine is not readily available to practising veterinarians due to advanced training requirements, licensing and the difficulty in accessing equipment.\textsuperscript{27} However, the assessment of the thyroid glands with thyroid scintigraphy is one of the most frequently requested nuclear medicine procedures\textsuperscript{27} and the results are used in the diagnosis and treatment of feline hyperthyroidism.\textsuperscript{27} Thyroid scintigraphy can be used to localise the hyperthyroid tissue and can influence treatment decisions. In particular, thyroidectomy will not be performed if ectopic thyroid tissue is present and cannot be removed surgically.\textsuperscript{28} Thyroid scintigraphy is also essential to customise radioiodine (\textsuperscript{131}I) dose.\textsuperscript{29} The concept of dose customisation was originally broached in the 1980s and 1990s by using a \textsuperscript{131}I tracer dose to calculate an individual \textsuperscript{131}I dose.\textsuperscript{29, 30} The challenges associated with the technique and poor access to thyroid scintigraphy,\textsuperscript{31} led to \textsuperscript{131}I dose determination without thyroid scintigraphy by using either a standardised dose\textsuperscript{32-34} or a clinical scoring system.\textsuperscript{35-37} A perceived disadvantage of these techniques was that a proportion of cats remained hyperthyroid. Additionally the use thyroid scintigraphy to estimate thyroid volume in \textsuperscript{131}I dose calculation had similar treatment outcomes.\textsuperscript{38} Cats that
remained hyperthyroid were generally more severely hyperthyroid before
treatment and received higher $^{131}$I doses during treatment.$^{37,38}$ The development of
iatrogenic hypothyroidism has also been evaluated due to the association with
development of azotaemia and reduced survival in cats with underlying renal
disease.$^{39}$ It is not known whether thyroid scintigraphy dose calculation confers a
better treatment success, particularly in severely hyperthyroid cats. The
administration of $^{131}$I is usually subcutaneous or intravenous and although it is less
common may be per os.$^{38,40-42}$ The administration of $^{131}$I per os is relatively
unique to Australia, so this study may provide further insight into this method.

This study investigates the role of thyroid scintigraphy in the diagnosis
and treatment of hyperthyroidism in a group of severely hyperthyroid cats. The
first part of this study reviews 80 severely hyperthyroid cats and compares the
thyroid scintigraphy features with the published literature.$^{43,44}$ The second part of
this study evaluates the role of thyroid scintigraphy in the treatment of a subset of
severely hyperthyroid cats. The initial treatment response to $^{131}$I dose calculated
by incorporating the percentage of technetium uptake ($% \text{ TU}$) will be compared
with the initial treatment response to a standardised dose.

These investigations of the role of diagnostic imaging in small animal
endocrine disease evaluate common problems in each species, the dog and cat.
The objective is to gain additional insight into the imaging techniques so that
diagnosis and treatment of canine adrenal gland disease and feline
hyperthyroidism can be optimised in the future.
Chapter 2. Literature review: ultrasound assessment of the canine adrenal gland

2.1. The canine adrenal gland: embryology, anatomy and physiology

2.1.1. Embryology

The adrenal gland comprises two distinct components, the medulla and the cortex, each with different functions. They are two separate endocrine organs that join during the embryonic stage to form one structure. The medulla and the cortex are derived from the neural crest ectoderm and the intermediate mesoderm, respectively. Adrenal cortical development occurs late in embryological development. Aggregations of mesoderm tissue arising from regressing mesonephric tubules are located on the ventro-medial aspect of the mesonephros. Cortical development continues with two stages of mesodermal cell proliferation. The first proliferation of mesodermal cells results in development of the foetal cortex and is followed by a second proliferation that ultimately becomes the cortex. Postnatally, the foetal cortex regresses and the cortex differentiates into three distinct hormone-producing zones. The medullary portion of the adrenal gland develops when neural crest cells migrate to the centre of the cortex. The adrenal medulla comprises chromaffin cells and is a modified ganglion of the sympathetic nervous system without axons.\(^{45}\)

2.1.2. Anatomy and physiology

Adrenal glands are paired structures located in the retroperitoneum craniomedial to the kidneys, with the left adrenal gland being slightly larger than the right adrenal gland. The left adrenal gland is at the level of the second lumbar vertebra and is mostly cylindrical with the cranial end being more ovoid in shape and flattened dorsoventrally. The left adrenal gland is bound by the left kidney laterally, aorta medially and the psoas minor muscle dorsally.\(^{46}\) The right adrenal gland is located further cranial than the left adrenal gland and is aligned with the
hilus of the right kidney, approximately level with the thirteenth thoracic vertebra. The right adrenal gland has an angular bend with a longer portion coursing along the caudal vena cava and a shorter portion projecting towards the right kidney. The right adrenal gland is bound medially by the caudal vena cava, dorsally by the psoas minor muscle and crus of the diaphragm, and cranially by the liver. The cranial two-thirds of the right adrenal gland are enveloped by the right lateral lobe of the liver.46

The arterial supply of the adrenal gland is from 20–30 arterioles that enter the adrenal gland from all directions. These small arterioles arise from branches of the abdominal aorta, phrenicoabdominal artery, lumbar arteries and renal artery. Some of the arterioles form an anastomosing network immediately distal to the adrenal gland capsule, whereas other arterioles continue through the cortex and medulla, providing arterial supply directly to the adrenal medulla. The sinusoids of the cortex are supplied by the initial anastomosing network, and a second anastomosing network located deeper in the cortex provides arterial supply to deeper cortical sinusoids. The arterial supply to the medulla arises from two sources: the cortical sinusoids and the medullary arteries. Ultimately, the cortical and medullary sinusoids merge to form the adrenal vein, which exits at the hilus on the medial surface of the adrenal gland. The left adrenal vein enters the left renal vein, which then enters the caudal vena cava, whereas the right adrenal vein directly enters the caudal vena cava.46

The adrenal cortex is divided into three zones: zona glomerulosa, zona fasciculata and zona reticularis. The zona glomerulosa is the outermost zone and produces mineralocorticoids, such as aldosterone, that are important in the regulation of sodium and potassium. The second zone is the zona fasciculata, which produces cortisol and other glucocorticoids, and the innermost zone is the zona reticularis, which produces androgens.46

The hypothalamic-pituitary-adrenal axis is responsible for the production and secretion of cortisol and corticosterone. The stimulation of cortisol production and its release from the adrenal gland originates at the hypothalamus with secretion of corticotrophin-releasing hormone into the hypophyseal portal system. This results in stimulation of the anterior pituitary to release adrenocorticotropic hormone into general circulation. Adrenocorticotropic hormone acts on the adrenal cortex to stimulate production and secretion of cortisol and androgen.
Secreted cortisol inhibits further corticotrophin-releasing hormone and adrenocorticotropic hormone release from the hypothalamus and the anterior pituitary, respectively (negative feedback). Adrenocorticotropic hormone has a short loop feedback and inhibits its own production from the anterior pituitary and a second source of adrenocorticotropic hormone originates from the intermediate lobe B cells under tonic negative regulation from dopamine.\(^{47}\)

The adrenal medulla is a modified ganglion of the sympathetic component of the autonomic nervous system. The preganglionic neurons arise from the celiac, splanchnic and adrenal ganglia and course directly through the adrenal cortex to the medulla. The chromaffin cells of the adrenal medulla are therefore post-ganglionic neurons without axons. This means that the neurotransmitters adrenaline and noradrenaline directly enter the blood vessels rather than act as neurotransmitters at a receptor or synapse. The innervation of the adrenal cortex is poorly understood.\(^{46}\)

### 2.2. The role of abdominal ultrasound in the evaluation of the normal canine adrenal gland

#### 2.2.1. Introduction

The subjective features and objective measurements are assessed during ultrasound of the adrenal glands. The subjective features have been well described, but the objective measurement requires further definition.

The ultrasound appearance of the canine adrenal gland was first described in 1990, and in seven of 10 dogs, at least one or of the adrenal glands was identified as a round to oval hypoechoic structure surrounded by hyperechoic fat.\(^{48}\) Since these first adrenal gland images were acquired, the technology of ultrasound equipment has progressed resulting in better image resolution and potentially improved measurement accuracy, due to higher frequency transducers, post processing techniques and tissue harmonic imaging.\(^{5,49}\)

The use of ultrasound evolved to measuring the size of the adrenal gland, and an upper threshold of 7.5 mm for the height of the left adrenal gland had a sensitivity of 77% and a specificity of 80% for the detection of pituitary-dependent hyperadrenocorticism.\(^{10,11}\) There is overlap in the objective
measurement of adrenal glands in normal dogs and in dogs with pituitary-dependent hyperadrenocorticism.\textsuperscript{5}

While the role of ultrasound assessment in the evaluation of canine adrenal glands has continued to develop since the mid-1990s, there have been conflicting findings in the literature regarding where to measure the adrenal glands, the effect of variability in measurement acquisition and the effect of biological variation.

Adrenal gland measurements can be taken from both the longitudinal and transverse image planes, and the transverse image plane measurements may be acquired from either the cranial or the caudal pole of the adrenal gland. Furthermore, adrenal gland measurements may be acquired in either dorsal or lateral recumbency. Therefore, it is unknown which measurement has the best correlation with adrenal gland size. There is moderate correlation between ultrasound measurements and measurements from gross dissection for the height of the adrenal gland and no correlation for the length or width.\textsuperscript{12}

2.2.2. Subjective features of the normal canine adrenal gland

Subjective ultrasound features are the location, shape, margination, echogenicity and echotexture of the adrenal gland. Typically, vascular landmarks are used to locate the adrenal glands. The left adrenal gland is identified between the left kidney and the aorta, immediately cranial to a hook in the left renal artery and vein. The right adrenal gland is located between the right kidney and aorta, cranial to the right renal artery and vein and the paired coeliac and cranial mesenteric arteries.\textsuperscript{23} The ipsilateral phrenicoabdominal artery and vein course dorsally and ventrally, respectively, across the midline of the relative adrenal gland. The phrenicoabdominal vessels may be identified with colour Doppler ultrasound.\textsuperscript{9}

The left adrenal gland is bilobed in shape with a narrowing in the mid-portion and is described as peanut shaped, whereas the right adrenal gland has an L shape and is described as an arrow head shape. Both adrenal glands are well demarcated because the echogenicity is hypoechoic relative to the surrounding fat and isoechoic relative to the renal cortex. The echotexture within the adrenal glands is variable. The cortex may be hypoechoic against a hyperechoic medulla or there may be a thin hyperechoic band at the corticomedullary junction.\textsuperscript{24}
2.2.3. Objective features: measurement of the normal canine adrenal gland

2.2.3.1. Intra- and inter-observer variability
Ultrasound of the adrenal gland is challenging and several authors, including one of the original reports \(^4\) and more recent publications, \(^13, 26\) have reported that both adrenal glands were not found in all dogs. The patient’s conformation, such as deep-chested dogs and the presence of gas within the gastrointestinal tract contribute to the difficulties in adrenal examination, particularly the right adrenal gland, and changing the patient’s recumbency position is an approach to overcoming these challenges.\(^5\) Inter- and intra-observer variability reduces as experience of the ultrasonographer increases.\(^16, 26\)

The adrenal gland measurement with the least intra- and inter-observer variability is the height of the caudal pole of the left and right adrenal gland when acquired from the longitudinal image rather than a transverse image.\(^50\) The height measurements for the cranial and caudal poles of the adrenal glands were consistently higher on measurements derived from the transverse image plane compared with the longitudinal image plane, probably because transverse measurements overestimate size when the transducer is transecting the adrenal gland at an oblique angle.\(^11, 15, 50\) In the longitudinal image plane, the transducer can be more easily lined up perpendicular to the long axis of the dog, resulting in more reliable measurements.

2.2.3.2. Biological variations: effects of sex, age and body weight
There is conflicting evidence regarding the effects of sex, age and body weight on ultrasound measurements of adrenal gland size. The sex of the dog had either no effect \(^11, 14\) or was associated with greater measurements for a range of individual measurements.\(^15, 25, 26\) Hence, the reliability of these findings is questioned.\(^15, 25, 26\) A weak correlation between sex and increased adrenal gland measurement was identified with the right adrenal gland height in males and length in females,\(^26\) which may be due to the challenges associated with right adrenal gland measurements\(^10, 12\) because there was no physiological basis for this finding.\(^26\) The caudal pole width on a transverse image plane, was greater in female Yorkshire terriers than males, but overestimation of measurements on the transverse image
Finally, the caudal pole thickness (CPT) of the left adrenal gland was significantly greater in males than females in dogs weighing between 12–20 kg and 20–30 kg, but this study population included a large proportion of spayed and neutered dogs.\textsuperscript{25}

The age of the dogs had either no effect on size measurement\textsuperscript{11} or increased age was associated with an increase in the value of specific measurements in studies of the left adrenal gland width,\textsuperscript{14, 26} left adrenal gland length,\textsuperscript{14, 26} dorsoventral measurements in dogs greater than 12 kg,\textsuperscript{25} and a range of height and width measurements in Labrador retrievers and Yorkshire terriers.\textsuperscript{15} However, only one of these studies included healthy dogs.\textsuperscript{15} In addition, some transverse image plane measurements\textsuperscript{15} were included and over-estimation due to obliquity may also have had an effect.\textsuperscript{11, 15, 50}

The biological variant that has gained most interest in the literature is the correlation with body weight. The initial publications showed that the length of the adrenal gland increases with increasing body weight of the dog,\textsuperscript{14, 26} and in Yorkshire terriers, there was an increase not only in length but also in the dorsoventral and mediolateral measurements with increasing body weight.\textsuperscript{15} Dogs have been divided into weight classes to define upper limits for normal adrenal gland size in dogs without adrenal gland disease.\textsuperscript{16, 25}

### 2.2.4. The size of the normal adrenal gland

The dorsoventral measurement of the caudal pole, acquired perpendicular to the longitudinal axis of the adrenal gland, is the most repeatable both between and within observers and is thus the ideal region to acquire the measurement.\textsuperscript{26, 50} The current recommendations for normal adrenal gland size are categorised by weight.\textsuperscript{16, 25} Table 2.1 shows the variation in the dog recumbency position and the direction of the adrenal gland measurement in studies using weight categories. The dorsal recumbency was used to determine that the caudal pole dorsoventral or height measurement is the ideal location for adrenal gland measurement.\textsuperscript{26, 50}

Table 2.1 shows that different recumbencies (lateral\textsuperscript{16} or dorsal\textsuperscript{15}) or a change in recumbency\textsuperscript{25} have been used in the studies to determine the adrenal gland measurements. The direction of measurement (i.e. height) remains the same although the recumbency changes.\textsuperscript{15, 16} However, as the recumbency changed to
include dorsal, oblique dorsal, right and left lateral, the direction of measurement changes between height, oblique height and width. The presumption is that the dog’s recumbency at the time of image acquisition has no effect on the measurement.\textsuperscript{25} However, it is not known whether a dog’s recumbency, either dorsal or lateral, has any effect on ultrasound measurements.\textsuperscript{24}

### Table 2.1. Caudal pole measurements of the normal canine adrenal gland comparing patient recumbency position, image plane, direction of measurement, weight category and measurement.

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient recumbency</th>
<th>Image plane</th>
<th>Direction of measurement</th>
<th>Weight category and measurement (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bento et al\textsuperscript{25}</td>
<td>Dorsal</td>
<td>Longitudinal</td>
<td>Height</td>
<td>(\leq 12 \text{ kg}) (\leq 0.62)</td>
</tr>
<tr>
<td></td>
<td>Dorsal oblique</td>
<td></td>
<td>Oblique height</td>
<td>(\geq 12 \text{ kg}) (\leq 0.72)</td>
</tr>
<tr>
<td></td>
<td>Right lateral</td>
<td></td>
<td>Width</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left lateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soulsby et al\textsuperscript{16}</td>
<td>Lateral</td>
<td>Sagittal</td>
<td>Height</td>
<td>(&lt; 10 \text{ kg}) (\leq 0.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(10–30 \text{ kg}) (\leq 0.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&gt; 30 \text{ kg}) (\leq 0.80)</td>
</tr>
<tr>
<td>De Chalus et al\textsuperscript{15}</td>
<td>Dorsal</td>
<td>Longitudinal</td>
<td>Height</td>
<td>Yorkshire terrier (\leq 0.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Labrador retriever (\leq 0.67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left (\leq 0.54) (\leq 0.79)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right (\leq 0.67) (\leq 0.945)</td>
</tr>
</tbody>
</table>

### 2.3. The role of abdominal ultrasound in the abnormal canine adrenal gland

#### 2.3.1. Canine hyperadrenocorticism

The most common form of naturally occurring hyperadrenocorticism in the dog is pituitary-dependent hyperadrenocorticism, which develops when a pituitary tumour secretes excessive adrenocorticotropic hormone and results in bilateral adrenocortical hyperplasia and excessive secretion of cortisol. The second most common naturally occurring form is adrenal-dependent hyperadrenocorticism, which is a consequence of cortisol-secreting adrenocortical adenomas or carcinomas. Iatrogenic Cushing’s syndrome is also common and develops when
glucocorticoids are administered excessively. Rarely, hyperadrenocorticism
develops because of secretion of adrenocorticotropin hormone from an ectopic site
or because of food-dependent cortisol secretion.47

Ultrasound results are used in conjunction with information gained from
history, clinical examination, and routine and specific endocrine blood testing
(both screening and differentiating tests) to diagnose hyperadrenocorticism and
assist in the differentiation between pituitary-dependent hyperadrenocorticism and
adrenal-dependent hyperadrenocorticism because it is possible to have apparently
normal adrenal glands in dogs with hyperadrenocorticism.2

2.3.1.1. Ultrasound features of canine pituitary-dependent
hyperadrenocorticism

The sonographic changes in pituitary-dependent hyperadrenocorticism are a
significant increase in the median thickness bilaterally (dorsal recumbency:
dorsoventral measurement, perpendicular to the long axis) when compared with
weight- and age-matched healthy dogs.17 In dogs weighing less than 10 kg, a cut-off of 6 mm (lateral recumbency: dorsoventral measurement, perpendicular to the
long axis) differentiates normal dogs from pituitary-dependent
hyperadrenocorticism dogs with a sensitivity of 75% and a specificity of 94%.13

The adrenal glands are symmetrical and retain their normal shape and contour,
appearing subjectively plump.17 Typically, the adrenal glands are homogeneous
and hypoechoic relative to the renal cortex, but a small number of dogs have focal
areas of hyperechogenicity within the adrenal cortex, suggestive of nodular
hyperplasia.17 It is important to note that up to one third of dogs may have one or
both adrenal glands measuring within the normal range, and 5% of dogs with
pituitary-dependent hyperadrenocorticism have nodular hyperplasia with the
potential for asymmetrical adrenal gland enlargement.17

2.3.1.2. Ultrasound features of adrenal cortical tumours

Adrenocortical tumours are variable in appearance and the ultrasound features are
generally the detection of a mass with well-defined margins. The echogenicity can
range from isoechoic, hypoechoic to hyperechoic relative to the renal cortex and
masses may also have a mixed echogenicity.18, 19 The presence of hyperechoic foci
with acoustic shadowing from mineralisation19 and anechoic regions due to
necrosis or haemorrhage has been described. There are no specific ultrasound features that differentiate between adrenocortical adenomas and carcinomas, except that invasion of the caudal vena cava is more likely to be seen with adrenocortical carcinomas. It is also possible to have bilateral adrenal masses or a range of tumour combinations including bilateral adenomas, bilateral carcinomas, adenoma/carcinoma and phaeochromocytoma/adrenocortical tumours.

The excessive cortisol produced by an adrenocortical tumour will result in a reduction in adrenocorticotropic hormone secretion from the pituitary gland. Because adrenocorticotropic hormone is an important trophic factor for normal adrenocortical cells in the zona fasciculata and reticularis, these cells will atrophy in the ipsilateral and contralateral adrenal gland. However, this functional atrophy does not necessarily correspond to a reduction in size of the contralateral adrenal gland on ultrasound. In fact, no significant difference in the length or thickness of the contralateral adrenal gland compared with normal adrenal glands was identified in all dogs in a small case series. The early detection of the adrenal gland lesion may explain this finding.

2.3.1.3. Equivocal ultrasound features in hyperadrenocorticism

The term equivocal adrenal asymmetry defines the presence of asymmetrical adrenal glands in dogs with hyperadrenocorticism where it remains unclear whether the dog has pituitary-dependent or adrenal-dependent hyperadrenocorticism. For example, in pituitary-dependent hyperadrenocorticism, there may be asymmetrical adrenal gland enlargement, while in adrenal-dependent hyperadrenocorticism, the contralateral adrenal gland is similar in size to the adrenocortical tumour.

The dorsoventral thickness difference ratio is used to determine the presence of equivocal adrenal asymmetry. The maximal dorsoventral dimensions are measured perpendicular to the long axis from the longitudinal image plane of the larger and smaller adrenal glands with the dog in dorsal recumbency. The dorsoventral thickness difference ratio is calculated as:

$$\text{DVTDR} = 2 \times (\text{LDV} - \text{SDV}) / (\text{LDV} + \text{SDV})$$
where DVTDR is the dorsoventral thickness difference ratio, LDV is the larger adrenal gland dorsoventral thickness and SDV is the smaller adrenal gland dorsoventral thickness.\\(^5^\)

Dogs with a dorsoventral thickness difference ratio of $\geq 0.2$, are arbitrarily categorised as having equivocal adrenal asymmetry. Dogs with a dorsoventral thickness difference ratio of $< 0.2$ which indicates symmetrical adrenal glands were further tested for pituitary-dependent hyperadrenocorticism based on unsuppressed plasma adrenocorticotropic hormone levels. Dogs with equivocal adrenal asymmetry and a dorsoventral thickness measurement of $\leq 5$ mm of the smaller adrenal gland were more likely to have adrenal-dependent hyperadrenocorticism. A sensitivity of 100% (95% confidence interval: 82–100%) and specificity of 96% (95% confidence interval: 82–99%) of the 5 mm threshold separates adrenal-dependent hyperadrenocorticism from pituitary-dependent hyperadrenocorticism.\\(^5^)\\(^2\)

### 2.3.2. Effects of medications on ultrasonographic appearance of the adrenal glands

Trilostane is a competitive inhibitor of 3β-hydroxysteroid-dehydrogenase, which blocks the production of glucocorticoids.\\(^4^\)\\(^7\) Between 72–84% of dogs with pituitary-dependent hyperadrenocorticism treated with trilostane will have an increase in adrenal gland size,\\(^5^)\\(^3\),\\(^5^)\\(^4\) with the maximum size identified after 6 weeks of trilostane administration.\\(^5^)\\(^4\) The sonographic appearance alters with greater differentiation between the hypoechoic outer region and the hyperechoic inner region.\\(^5^)\\(^3\) The proposed explanation for these changes is adrenal cortex hypertrophy as the negative feedback to the adrenocorticotropic hormone is abolished and stimulation of the adrenal gland persists.\\(^5^)\\(^3\),\\(^5^)\\(^4\) The adrenal gland becomes irregularly shaped with a heterogeneous echotexture after 1 year of trilostane treatment, and the presence of nodular hyperplasia has been suggested as a potential explanation.\\(^5^)\\(^4\)

Mitotane is a chlorinated hydrocarbon resulting in selective necrosis of the zona fasciculata and reduction in cortisol production.\\(^4^\)\\(^7\) Both gross and histopathological adrenal cortex atrophy occur in clinically healthy dogs receiving mitotane.\\(^5^)\\(^5\) The adrenal gland length and thickness reduces significantly in dogs
with pituitary-dependent hyperadrenocorticism that is treated with mitotane when compared with pre-treatment ultrasound measurements.\textsuperscript{56}

When exogenous glucocorticoids (hydrocortisone 10 mg/kg per os, twice a day for 4 months, then tapered over 1 month) were given to dogs, the dorsoventral dimensions of the cranial and caudal poles of the adrenal glands reduced. The degree of size reduction differed between dogs at 1 month and the dimensions were significantly different to a placebo group at 4 months. Four weeks after treatment ceased, resolution of size changes was seen. Overall, the authors concluded that ultrasound is not recommended as a diagnostic test for iatrogenic hypercortisolism.\textsuperscript{57}

\textbf{2.3.3. Other adrenal gland disease which may affect the ultrasonographic appearance of the adrenal glands}

Dogs with hypoadrenocorticism have a smaller thickness (dorsal recumbency, greatest dorsoventral measurement from the longitudinal image plane) compared with healthy dogs.\textsuperscript{58} A cut-off of 3.2 mm thickness of the left adrenal gland separates hypoadrenocorticism from healthy dogs and dogs with a clinical presentation mimicking hypoadrenocorticism.\textsuperscript{59} Although these findings suggest hypoadrenocorticism, the definitive test remains the adrenocorticotropic hormone simulation test\textsuperscript{59} because a small proportion of dogs (1/30) have normal measurements. These studies did not specify the presence of typical or atypical hypoadrenocorticism.\textsuperscript{58, 59}

The ultrasound characteristics of phaeochromocytomas are variable and there are no specific features that allow ultrasound to make a definitive diagnosis. The size ranges from a small nodule within the margins of the adrenal gland to a large mass (> 20 cm) distorting and expanding outside the expected margin of the adrenal gland.\textsuperscript{18, 22} The echotexture may be heterogeneous, homogeneous, multicystic, multilobular or contain anechoic regions due to necrosis or haemorrhage,\textsuperscript{22} and the echogenicity ranges from hypoechoic to hyperechoic relative to the renal cortex.\textsuperscript{18, 22} Invasion of adjacent vessels, initially the phrenicoabdominal vein and then the caudal vena cava, may also be seen. The portion that enters the vessel (the tumour thrombus) may be present in 50–82\% of
cases. The size of the tumour is no indication of the likelihood to invade adjacent veins, except that small nodules (< 2 cm) are unlikely to be invasive.

Phaeochromocytomas are more likely than adrenocortical carcinomas to invade vascular structures, but this feature should not be relied on to differentiate between tumour types. The presence of mineralisation is uncommon, and although it is less frequent than in cortisol-producing tumours, it should not be relied on as a differentiating feature. Metastasis may occur in up to 40% of cases and abdominal sites include the liver, spleen and adjacent lymph nodes. Phaeochromocytomas are usually unilateral, but bilateral phaeochromocytomas and concurrent tumours in the contralateral adrenal gland and occasionally the same adrenal gland have been reported.

Other adrenal gland neoplasms include non-functional adenomas; carcinomas; metastasis and rare tumours such as myelolipomas. Metastasis to the adrenal glands was identified in 21% of dogs in a retrospective review of post mortem examinations with the common primary tumours being pulmonary, prostatic, gastric and pancreatic carcinoma and melanoma. Non-neoplastic nodules include hyperplasia, lipoma, cysts, abscess or granuloma. Incidental adrenal gland nodules (defined as ≥ 10 mm in diameter) are identified in 4% of dogs (151 of 3748 dogs) undergoing ultrasound without clinical evidence of adrenal gland disease. Of 20 dogs that had an adrenalectomy, one-third of these were malignant and, although not definitive, these lesions were > 2 cm in diameter and more likely to have vascular invasion.
Chapter 3. The effect of recumbency position on the ultrasound measurement of the canine adrenal gland in non-adrenal gland illness

3.1. Introduction

Abdominal ultrasound is readily available to veterinary practitioners, and practitioners with a range of skill levels use ultrasound to identify and assess the adrenal gland. The ultrasound assessment of the adrenal gland includes both subjective features and objective measurements. The aim of this study is to clarify and simplify how the objective measurements are acquired. Simplifying the acquisition of adrenal gland images will assist veterinary practitioners of a range of skills, including those with less experience.

Adrenal glands can be examined in the longitudinal and transverse image plane and the length, height and width may be measured. However, it is not known if recumbency position at the time of image acquisition influences these measurements. There are three patient recumbencies available at the time of image acquisition: left lateral, right lateral and dorsal. The left lateral recumbency enables assessment of the right adrenal gland, the right lateral recumbency enables assessment of the left adrenal gland and both adrenal glands may be assessed from the dorsal recumbency. The choice of recumbency is operator preference, but some patients are more compliant in a specific recumbency position. Therefore, throughout an abdominal ultrasound the recumbency position may change for a patient.

The aim of this study is to determine if the patient’s recumbency position at the time of image acquisition affects adrenal gland measurement.
3.2. Materials and methods

3.2.1. Patient selection

The study was approved by The University of Melbourne Animal Ethics Committee (Number 1413259.2). Patients were prospectively selected from client-owned dogs presenting to The University of Melbourne Animal Hospital (UVet) for clinical evaluation and routine abdominal ultrasound between October 2014 and September 2016. The reason for the abdominal ultrasound was at the discretion of the attending clinician, who had no input as to whether a dog was included in the study at the time of image acquisition. The author was not aware if the dog met the inclusion criteria at the time of image acquisition; information regarding exclusion or inclusion of individual dogs became available during diagnostic work up, which continued after images were acquired.

3.2.1.1. Inclusion criteria

The dogs were then assigned to one of the following three categories:

- Category A – unlikely to have hyperadrenocorticism based on available clinical and clinicopathological information
- Category B – potentially having hyperadrenocorticism due to presenting signs or results of diagnostic work up
- Category C – unlikely to have hyperadrenocorticism, but potential other adrenal gland disease, based on available clinical and diagnostic information.

History, physical examination findings and clinicopathological data at the time of the abdominal ultrasound were examined and only dogs with no evidence of adrenal gland disease (Category A) were included in the study. Dogs with suspected or confirmed adrenal gland disease and dogs that received medication that may influence adrenal gland size (corticosteroids including topical and ocular formulations, mitotane, trilostane, fludrocortisone and desoxycortisone pivalate) were excluded.

The following data were collected from each dog: age, sex, breed, weight, body condition score, reason for abdominal ultrasound, recent medications and diagnosis. The data from all dogs was reviewed a minimum of two months after
collection to ensure additional clinical and clinicopathological information did not alter the dogs’ categorisations. Data from follow-up ultrasound examinations was not included.

3.2.2. Ultrasound equipment and ultrasound examination

To obtain the ultrasound measurements, dogs were restrained manually and the majority were sedated. The measurements were collected by Anne Marie Rose, an experienced sonographer who was aware of the reason for the abdominal ultrasound at the time of data collection. The ultrasound examinations were performed using one of the following machines: Siemens Acuson X300 (Siemens Medical Systems, Malvern, PA, United States of America [USA]), MyLab Twice (Esoate S.p.A, Genova, Liguria, Italy), Philips CX50 (Philips Ultrasound, Bothwell, WA, USA) and Philips Epiq 5 (Philips Ultrasound, Bothwell, WA, USA) predominantly using the manufacturer’s 8–5 MHz curvilinear transducer, and occasionally a 13–5 MHz or 12–5 MHz linear transducer, depending on the size of the dog. Each ultrasound machine had axial and lateral resolution calibrations assessed for measurement accuracy using a phantom (Multipurpose Phantom 539, ATS Laboratories Inc, Bridgeport, CT, USA). Each respective dog had all ultrasound measurements acquired using the same ultrasound machine and transducer. The fur of all dogs was clipped and acoustic coupling gel (Aquasonic®100 ultrasound transmission gel, Parker Laboratories, Fairfield, NJ, USA) was applied. Previously published anatomical landmarks were used to localise the adrenal glands. Images were transferred from the ultrasound machine to the picture archiving communications system (Synapse Fuji Medical Systems Inc., Stamford, CT, USA). Acquired data was transferred to Microsoft Excel spreadsheet.

3.2.3. Data collection

Each dog was scanned in three recumbencies (dorsal, left lateral and right lateral) and a series of adrenal gland measurements was obtained. The length was defined as the longitudinal axis measurement in the cranial to caudal direction. The short axis measurements of height and width were defined, respectively, as the dorsoventral and mediolateral directions. Both the left and right adrenal glands
were examined from the dorsal recumbency, and using the longitudinal image plane, the length and caudal pole height measurements were acquired (Figure 3.1A). The transducer was rotated 90 degrees on the caudal pole to the transverse image plane and the caudal pole height and width of the adrenal glands were measured (Figure 3.1B). The left lateral recumbency was used to examine the right adrenal gland, and using the longitudinal image plane, the length and caudal pole width measurements were acquired (Figure 3.1C). The transducer was rotated 90 degrees on the caudal pole to the transverse image plane and the height and width of the right adrenal gland were measured (Figure 3.1D). Similarly, the right lateral recumbency was used to examine the left adrenal gland and equivalent imaging planes and measurements were acquired. If any other adrenal gland pathology was identified during the ultrasound examination, subjective features and measurements were documented.

Figure 3.1. Ultrasound images demonstrating the locations of the length, height and width measurements: (A) dorsal recumbency longitudinal image plane: ultrasound image demonstrating the locations of the length (solid line) and height (dotted line) measurements; (B) dorsal recumbency transverse image plane of the caudal pole: ultrasound image demonstrating the locations of the height (dotted line) and width (solid line) measurements; (C) lateral recumbency longitudinal image plane: ultrasound image demonstrating the location of the length (solid line) and width (dotted line) measurements and (D) lateral recumbency transverse image plane of the caudal pole.
3.2.4. Approach to the data

The data was collected relative to the patient’s recumbency and the measurements listed in Table 3.1 were acquired.

Table 3.1. Patient recumbency, image plane and direction of adrenal gland measurements.

<table>
<thead>
<tr>
<th>Recumbency</th>
<th>Image plane</th>
<th>Direction of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal†</td>
<td>Longitudinal</td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caudal pole height</td>
</tr>
<tr>
<td>Transverse</td>
<td>Longitudinal</td>
<td>Caudal pole height</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caudal pole width</td>
</tr>
<tr>
<td>Lateral†</td>
<td>Longitudinal</td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caudal pole width</td>
</tr>
<tr>
<td>Transverse</td>
<td>Longitudinal</td>
<td>Caudal pole height</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caudal pole width</td>
</tr>
</tbody>
</table>

* Both the left and right adrenal glands had the same series of measurements acquired in dorsal recumbency
† Right lateral recumbency for the left adrenal gland and left lateral recumbency for the right adrenal gland

The measurements were regrouped according to the direction of adrenal gland measurement (Table 3.2), enabling the data to be approached using six pairs of measurements from the dorsal and lateral recumbencies for each adrenal gland. The CPT is defined as the short axis measurement acquired from the longitudinal image plane as the direction of the measurement changes from height in dorsal recumbency to width in lateral recumbency. There are two groups of measurements that directly compare either the height or the width from the transverse image plane, and two groups of transverse image plane measurements that compare the height in one recumbency against the width in the other recumbency. The final measurement is the length that is only acquired from the longitudinal image plane. Table 3.2 lists the measurement, image plane of the adrenal gland and the direction of each measurement relative to the patient’s recumbency at the time of acquisition. The series of measurements was the same for the left and right adrenal glands.
Table 3.2. Image plane and direction of adrenal gland measurement relative to the dog’s recumbency position.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Image plane</th>
<th>Dorsal recumbency</th>
<th>Lateral recumbency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudal pole thickness</td>
<td>Longitudinal</td>
<td>Height</td>
<td>Width</td>
</tr>
<tr>
<td>Equivalent height measurement†</td>
<td>Transverse</td>
<td>Height</td>
<td>Height</td>
</tr>
<tr>
<td>Equivalent width measurement†</td>
<td>Transverse</td>
<td>Width</td>
<td>Width</td>
</tr>
<tr>
<td>Orthogonal height–width measurement‡</td>
<td>Transverse</td>
<td>Height</td>
<td>Width</td>
</tr>
<tr>
<td>Orthogonal width–height measurement‡</td>
<td>Transverse</td>
<td>Width</td>
<td>Height</td>
</tr>
<tr>
<td>Length</td>
<td>Longitudinal</td>
<td>Length</td>
<td>Length</td>
</tr>
</tbody>
</table>

* Right lateral recumbency for the left adrenal gland and left lateral recumbency for the right adrenal gland
† Equivalent measurement is defined as no change in the direction of the measurement with the change in patient recumbency
‡ Orthogonal measurement is defined as a change in the direction of the measurement with the change in patient recumbency

3.2.5. Statistical analysis

Statistical analyses were performed using Minitab 17 (State College, PA, USA). The level of agreement between measurements in dorsal and lateral recumbency for each of the six measurements for the left and right adrenal gland was determined using the Bland–Altman analysis63 and confidence intervals were calculated for the mean difference, upper and lower limits of agreement.

3.3. Results

3.3.1. Patient data

A total of 119 dogs were initially included in the study, meaning that at the time of image acquisition, the complete set of adrenal gland measurements were acquired (Table 3.3). Of these, only dogs in Category A were included in the analysis reported here.
Table 3.3. Number of dogs per category.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: No evidence of hyperadrenocorticism</td>
<td>100</td>
</tr>
<tr>
<td>B: Potentially hyperadrenocorticism</td>
<td>11</td>
</tr>
<tr>
<td>C: Unlikely to have hyperadrenocorticism but potentially other adrenal gland disease</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
</tr>
</tbody>
</table>

The study group comprised 100 dogs representing 37 breeds and 15 different crossbreeds. Breeds that were represented by more than one dog included Labrador retrievers (n = 10), golden retrievers (n = 7), Cavalier King Charles spaniels and German shepherd dogs (each n = 4), Chihuahua, Jack Russell terrier and Staffordshire bull terrier (each n = 3) and beagle, boxer, Cairn terrier, cocker spaniel, German short-haired pointer, standard poodle (each n = 2). The crossbreeds which were represented by greater than one dog included Maltese terrier crossbreed and Shih Tzu crossbreed (each n = 3), and two for each of the following crossbreeds: Jack Russell terrier, kelpie, Labrador retriever and spoodle. There were 45 neutered females, 43 neutered males, four entire females and eight entire males. The median age was 7 years (range 3 months to 16 years). There were 64 dogs weighing more than 12 kg and 36 dogs weighing less than 12 kg. The median weight was 17.9 kg (range 2.1–47.9 kg). The median body condition score was 6 (range 1–8), based on the 98 dogs in which body condition score was recorded.

3.3.2. Caudal pole thickness from the longitudinal image plane

The CPT is the short axis measurement from the longitudinal image plane when the height in dorsal recumbency is compared with the width in lateral recumbency (Table 3.2). Table 3.4 shows the median and range of the CPT measurements. The Bland–Altman analysis shows that the left adrenal gland CPT is on average 0.10 mm longer in right lateral than dorsal recumbency and the right adrenal gland CPT is on average 0.16 mm longer in left lateral than dorsal recumbency. Thus, the mean difference is biased towards the lateral recumbency for both adrenal glands. The absolute values of the upper limit of agreement (ULA) and lower
limit of agreement (LLA) range between 1.48 mm and 1.95 mm for both the left and right adrenal glands (Figure 3.2).

Table 3.4. Median and range of the caudal pole thickness from the longitudinal image plane.

<table>
<thead>
<tr>
<th></th>
<th>Height (mm)</th>
<th>Width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left adrenal gland</td>
<td>4.8 (2.7, 8.4)</td>
<td>4.9 (2.4, 9.5)</td>
</tr>
<tr>
<td>Right adrenal gland</td>
<td>4.9 (2.8, 10.1)</td>
<td>5.2 (3, 9)</td>
</tr>
</tbody>
</table>

Figure 3.2 Graphical display of the Bland–Altman analysis of the caudal pole thickness measurements acquired from the longitudinal image plane of the left and right adrenal gland. The mean difference or the bias is the central solid black line. The 95% upper and lower limits of agreement (ULA and LLA, respectively) are represented by the dotted black lines.

The Bland–Altman analysis identified nine (4.5%) dogs with measurements beyond the ULA and LLA for either the left or right adrenal gland. Further categorisation using a weight cut-off of 12 kg determined that seven of nine
(77.8%) dogs weighed > 12 kg and two of nine (22.2%) dogs weighed < 12 kg. The signalment and weight of the outliers are listed in Table 3.5.

Table 3.5. Caudal pole thickness outliers: signalment and weight.

<table>
<thead>
<tr>
<th>Signalment</th>
<th>Weight (kg)</th>
<th>Left or right adrenal gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 y 3 m MN† Dogue de Bordeaux</td>
<td>43.2</td>
<td>Left</td>
</tr>
<tr>
<td>3 y 3 m FS‡ American bulldog cross</td>
<td>30–55‡</td>
<td>Left</td>
</tr>
<tr>
<td>10 y 4 m MN spoodle</td>
<td>16.4</td>
<td>Left</td>
</tr>
<tr>
<td>11 y 3 m FS Cavalier King Charles spaniel</td>
<td>11.2</td>
<td>Left</td>
</tr>
<tr>
<td>2 y FS chihuahua</td>
<td>4.3</td>
<td>Left</td>
</tr>
<tr>
<td>4 y 3 m MN Staffordshire bull terrier</td>
<td>28.7</td>
<td>Right</td>
</tr>
<tr>
<td>13 y 10 m FS Belgian shepherd</td>
<td>23</td>
<td>Right</td>
</tr>
<tr>
<td>10 y FS Labrador cross</td>
<td>38</td>
<td>Right</td>
</tr>
<tr>
<td>9 y FS Labrador</td>
<td>29</td>
<td>Right</td>
</tr>
</tbody>
</table>

† MN, male neutered; ‡ FS, female spayed; ‡ exact weight not recorded

3.3.3. Equivalent measurements from the transverse image plane

The caudal pole measurements acquired from the transverse image plane compare the change in recumbency; the direction of measurement is unchanged. Thus, each adrenal gland has two pairs of measurement: height in dorsal versus lateral recumbency and width in dorsal versus lateral recumbency. The measurements are defined as equivalent height or equivalent width measurements, respectively (Table 3.2). The mean difference is towards dorsal recumbency for the right adrenal gland height (0.44 mm) and the left adrenal gland width (0.79 mm) and towards the lateral recumbency for the left adrenal gland height (0.27 mm) and right adrenal gland width (0.59 mm). The absolute values of the upper and lower limits of agreement range between 1.69 mm and 3.38 mm for both the left and right adrenal glands (Figure 3.3).
Figure 3.3. Graphical display of the Bland–Altman analysis of the caudal pole equivalent measurement acquired from the transverse image plane.
The mean difference or the bias is the central solid black line, and the 95% upper and lower limits of agreement (ULA and LLA, respectively) are represented by the dotted black lines.

3.3.4. Orthogonal measurements from the transverse image plane

The caudal pole measurements acquired from the transverse image plane compare both the change in recumbency and the direction of measurement. Therefore, for each adrenal gland, the height in the dorsal recumbency is compared with the width in lateral recumbency, and the width in dorsal recumbency is compared with the height in lateral recumbency. The terms orthogonal height–width and orthogonal width–height are used to define these measurements (Table 3.2). The mean difference is towards the dorsal recumbency for the left adrenal gland height–width (0.01 mm) and width–height (0.42 mm) and towards left lateral recumbency for the right adrenal gland height–width (0.06 mm) and width–height (0.09 mm). The absolute values of the ULA and LLA range between 2.12 mm and 3.12 mm for the left and right adrenal glands (Figure 3.4).
Figure 3.4. Graphical display of the Bland–Altman analysis of the caudal pole orthogonal measurement acquired from the transverse image plane. The mean difference or the bias is the central solid black line, and the 95% upper and lower limits of agreement (ULA and LLA, respectively) are represented by the dotted black lines.

3.3.5. Length of the adrenal gland

The length of the adrenal gland can only be acquired from the longitudinal image plane and therefore the only variant is the comparison between dorsal and lateral recumbency (Table 3.1). The mean difference is towards the dorsal recumbency for the left adrenal gland (0.05 mm) and the right adrenal gland (0.80 mm). The ULA and LLA range between 5 mm and 6.59 mm for the left and right adrenal glands (Figure 3.5). The mean differences, ULA and LLA with 95% confidence intervals are shown in Figure 3.6.
Figure 3.5 Graphical display of the Bland–Altman analysis of the length of the adrenal gland, acquired from the longitudinal image plane. The mean difference or the bias is the central solid black line, and the 95% upper and lower limits of agreement (ULA and LLA, respectively) are represented by the dotted black lines.

3.3.6. Comparison between measurements

The short axis measurement with the lowest limit of agreement is the CPT acquired from the longitudinal axis; all the measurements derived from the transverse image plane have higher limits of agreement (Figure 3.6). Estimates of the mean difference and the limits of agreement for the CPT are all within 2 mm (Figure 3.6). The confidence intervals for the estimates for the CPT are relatively narrow, which indicates that the precision of the CPT estimates is relatively good. In fact, for the CPT, the bounds of the confidence intervals for the limits of agreement are within 2 mm, except for the lower bound for the right adrenal gland dorsal recumbency height compared with the left lateral recumbency width, which is −2.3 mm.
Figure 3.6. Adrenal gland measurements. Collation of the Bland–Altman analyses comparing the dorsal recumbency with the relevant lateral recumbency and showing the mean differences, upper and lower limits of agreement (all in mm) with 95% confidence intervals.

3.4. Discussion

The purpose of the study was to assess whether recumbency position influences measurement of adrenal glands in dogs. The expectation was that recumbency would not influence measurements, but the results show that there is some difference between the measurements in dorsal and lateral recumbency. The measurement with the least variation is the CPT acquired from the longitudinal image plane.

The results support the recent descriptions of ultrasound measurements based on CPT from the longitudinal image plane.\textsuperscript{13, 15, 16} The recently published reference values of the upper limits of normal adrenal gland measurements in dogs with non-adrenal gland illness were derived from dogs in dorsal, lateral and lateral oblique recumbency.\textsuperscript{25} The current study confirms that these reference values remain valid, even though the recumbency positions from which they were derived varied between dogs. However, taking the confidence intervals of the limits of agreement into consideration, the CPT may have up to 1.96 mm and 2.27
mm difference between recumbencies for the left and right adrenal glands, respectively. While the median result for the CPT was within the reference values as shown in Table 3.3, this study did identify dogs with adrenal gland measurements beyond this range. (Table 3.5). Thus, in this study 4.5% of dogs with non-adrenal illness have measurements that suggest hyperadrenocorticism if assessed in one recumbency. These dogs were further categorised according to weight and, the 12 kg cut-off point was chosen. This follows current recommendations regarding adrenal gland size measurements. A great proportion of the outliers (7 of 9 dogs, 77.8%) were dogs weighing more than 12 kg. Thus, if the suspicion of hyperadrenocorticism is low in dogs, particularly in dogs weighing more than 12 kg, it may be of value to confirm the CPT measurement in both recumbencies. This is particularly important because endocrine testing is associated with a higher risk of false positive test results if the dog suffers from non-adrenal illness.

The Bland–Altman analysis determines the mean difference between the two recumbencies. If the mean difference between the dorsal and lateral recumbency is zero then the average measurement is the same irrespective of the patient’s recumbency. However, each of the measurements in the study had some variation in the mean differences. The average CPT measurement was slightly greater in the lateral recumbency than in the dorsal recumbency and this effect was slightly greater for the right adrenal gland than the left adrenal gland. The challenge for acquiring images in the longitudinal plane is to include the entire length of the adrenal gland in the image because this may influence the consistency of CPT measurement, taken perpendicular to the length. The greater mean difference for the right adrenal gland is consistent with the difficulty in assessing the right adrenal gland due to its location and shape.

The mean difference for the transverse image plane measurements were towards the dorsal or lateral recumbencies. This may reflect the effect of obliquity, resulting in overestimation of measurements when rotating the transducer to the transverse image plane. The effect of obliquity should be greater on the width than the height measurement with the patient in dorsal recumbency, and greater on the height than the width measurement when the patient is lateral recumbency. There was no consistency between the expected effect of obliquity and patient recumbency. Thus, the effect of obliquity does not solely explain the variation of
the mean differences, although the absence of obliquity may contribute to the low value of the mean differences demonstrated by orthogonal height–width measurements. There was also variation in the mean difference for the adrenal gland length, which was marginally greater on lateral recumbency, with greater variation for the right adrenal gland. The length of the adrenal gland is known to have the greatest variability, and this study shows that the variability also occurs with changing patient recumbency.

The Bland–Altman analysis does not state that the given limits are acceptable for a clinical situation. There is some evidence to suggest the findings of the current study for the CPT may be clinically acceptable because a mean difference of 0.4–0.7 mm between observers measuring the caudal pole height from the sagittal image plane in lateral recumbency is considered to be clinically insignificant. In the current study, the mean difference between dorsal and lateral recumbency for the CPT was less than these dimensions at 0.1 mm and 0.16 mm for the left and right adrenal glands, respectively.

Whilst four different ultrasound machines were used to acquire the measurements throughout the complete study period, machine-associated measurement differences can be excluded in this study. All measurements in individual dogs (be it in lateral or dorsal recumbency) were taken with the same ultrasound machine. Hence, each dog acted as its own control during the Bland-Altman analysis.

This study is the first to assess a range of adrenal gland measurements within the same dog comparing dorsal and lateral recumbencies. Of all measurements examined, CPT in the longitudinal image plane varied least, suggesting that this measurement should always be included when reference values of adrenal gland measurements are determined. When using CPT, there is greater flexibility regarding patient positioning, without foregoing accuracy of measurements. The results of this study support the applicability of the currently recommended values for the size of adrenal glands in dogs with non-adrenal gland illness, whether these values were derived with patients in either dorsal, lateral or lateral oblique recumbencies.
Chapter 4. Literature review: thyroid scintigraphy in feline hyperthyroidism

4.1. The feline thyroid gland: embryology, anatomy and physiology

4.1.1. Embryology

The primordial thyroid originates as a ventral midline endodermal diverticulum in the foregut between the first and second pharyngeal arches.45 The primordial thyroid migrates caudoventrally to the eventual location of the thyroid glands. As this occurs, the cranial end of the primordial thyroid remains attached to the foregut by the thyroglossal duct and the blind caudal end of the primordial thyroid extends into the mesoderm. Ultimately, the thyroglossal duct disappears and the caudal end of the primordial thyroid forms two discrete thyroid lobes at the ventral trachea.45 The two thyroid lobes are initially connected by an isthmus of glandular tissue but this does not persist in the adult cat.45 In mature cats, there is persistence of a small depression in the tongue, the foramen caecum, which was the origin of the primordial thyroid.45

An understanding of the embryology of the feline thyroid gland is important in the assessment of hyperthyroidism because the ectopic hyperthyroid tissue may develop anywhere along the path of the thyroglossal duct to the normal location of the thyroid glands and may even extend caudally into the thorax.64

4.1.2. Anatomy

The thyroid gland comprises two separate lobes that are located distal to the larynx immediately adjacent to the lateral aspect of the trachea.65 The arterial blood supply is via the cranial thyroid artery, which branches and provides blood supply at various points around the periphery of the thyroid gland. In addition, there are three approaches of the blood supply from the cranial thyroid artery. There are branches that directly supply the cranial pole of the thyroid gland, and there is a branch along the dorsal margin that anastomoses with the caudal thyroid...
artery. There may also be a branch that extends along a portion of the ventral margin of the thyroid gland. Venous drainage occurs via the cranial and caudal thyroid veins.

The cranial and caudal portions of the thyroid gland drain into the medial retropharyngeal and chain of cervical lymph nodes, respectively. The lymphatic drainage may bypass the lymph nodes and drain directly into the venous system at the junction of the internal and external jugular vein or the junction of the subclavian and jugular vein.

### 4.1.3. Histology

The thyroid glands comprise glandular follicles with a circular arrangement of follicle cells, creating an extracellular acinar lumen that contains the colloid where the thyroid hormones are stored. The tissue between the follicles contains interfollicular connective tissue, vasculature, fat and parafollicular cells. The parafollicular cells secrete calcitonin, which is important in calcium regulation.

### 4.1.4. Hypothalamic-pituitary-thyroid axis

Regulation of the thyroid hormones is under the control of the hypothalamic-pituitary-thyroid axis. Thyrotropin-releasing hormone is produced in the median eminence of the hypothalamus and reaches the adenohypophysis (anterior pituitary) via the hypothalamic-hypophyseal portal vessels. Thyroid-stimulating hormone is released in response to thyrotropin-releasing hormone and enters the systemic circulation to the thyroid gland, where it promotes the synthesis and secretion of the thyroid hormones 3,5,3’-triiodothyronine (T3) and T4. The mechanism of thyrotropin-releasing hormone secretion from the hypothalamus is poorly understood. There are negative feedback loops between the thyroid gland and both the hypothalamus and the adenohypophysis. The negative feedback to the hypothalamus is via inhibitory proteins synthesised in response to the thyroid hormones T3 and T4 and reduces thyrotropin-releasing hormone release. The negative feedback loop to the adenohypophysis occurs via T3 and T4 and reduces synthesis of thyroid-stimulating hormone.
4.1.5. Production of the thyroid hormones

Iodine is converted to iodide in the gastrointestinal tract and then enters the systemic circulation. Upon reaching the thyroid gland, two steps are required to form thyroglobulin: iodide trapping and organification. Iodide trapping occurs when iodide reaches a thyroid follicular cell; there is active uptake of the iodide by a thyroid-stimulating hormone-regulated sodium iodide symporter (iodide pump or iodide trap), resulting in the follicular cell concentration of iodide being up to 30 times greater than the plasma concentration. The iodide is then transported into the colloid of the thyroid follicle where it is oxidised by thyroid peroxidase to form iodine.

In the organification process, thyroid peroxidase acts as a catalyst for the attachment of iodine to tyrosine residues of thyroglobulin via one or two potential sites. Monoiodotyrosine forms when one iodine molecule attaches, while diiodotyrosine forms when two iodine molecules attach to the tyrosine. Thyroid peroxidase catalyses T4 formation from the combination of two diiodotyrosine molecules and catalyses T3 formation from the addition of one molecule of monoiodotyrosine to diiodotyrosine. T3 and T4 are stored in the colloid of the thyroid gland. The secretion of the thyroid hormones from the thyroid follicles is stimulated by thyroid-stimulating hormone, which is modulated by negative feedback from free T3 and free T4. T3 and T4 are lipophilic and water-insoluble amino acid hormones and require transport in the blood by carrier proteins as total T4 and total T3. The free (unbound) form of the thyroid hormones, free T3 and free T4, are the physiologically active form and account for less than 1% of the circulating T3 and T4. The free T3 and free T4 forms are able to enter cells to have a biological effect and act as negative feedback to the pituitary and thyroid-stimulating hormone secretion.

4.2. Feline hyperthyroidism

4.2.1. Signalment, presentation and clinical signs

Feline hyperthyroidism is a disease of middle-aged to older cats with an average age of onset at 12–13 years (range 4–22 years). Feline hyperthyroidism is
generally considered an unusual diagnosis in younger cats and occurs in < 5% of cats that are younger than 8 years of age. There is a decreased risk of feline hyperthyroidism in the Burmese, Tonkinese, Persian, Siamese, Abyssinian, British shorthair and Himalayans breeds, and an increased risk in long-haired crossbred cats. Feline hyperthyroidism was first reported in 1979, and the initial cases presented with the characteristic history and clinical signs of the disease, which include weight loss, polyphagia, restlessness, tachycardia, polydipsia, polyuria, vomiting, cardiac murmur, diarrhoea, increased faecal volume, anorexia, tachypnoea and muscle weakness. The less frequently identified features include muscle tremors, congestive heart failure, increased nail growth, dyspnoea, alopecia and ventroflexion of the neck. There has been an increase in both the prevalence and identification of feline hyperthyroidism since it was first reported and it is now the most common endocrine disease in cats.

4.3. Aetiology

The underlying cause of feline hyperthyroidism remains unknown, but as the practices of cat ownership have changed since the disease was first identified just over 35 years ago, there are some environmental factors that may contribute to the development of the disease. These factors may be divided into two categories: nutritional factors and thyroid-disrupting compounds. Nutritional factors may include excess of soy isoflavones (genistein and diadzein), a source of protein used in commercial cat food, and deficiencies of iodine and selenium. The thyroid-disrupting compound bisphenol A, which is used to line metal food tins; polybrominated diphenyl ethers, which are used as flame retardants in textiles, electronics and furniture; and other pesticides, herbicides and goitrogenic compounds have been suggested as contributing to the development of feline hyperthyroidism. Further evaluation of the role of bisphenol A and polybrominated diphenyl ethers in the development of feline hyperthyroidism is required. The underlying principle is that the exposure to these environmental factors is continuous and the additive effect of a number of factors contributes to the alteration in thyroid hormone metabolism. Furthermore, long-term exposure to environmental factors is thought to cause mutations in thyroid-stimulating hormone receptor or G protein genes in some cats.
4.4. Pathology

Irrespective of the underlying aetiology, the result is autonomous growth of the thyroid gland and increased secretion of T3 and T4. The excessive T3 and T4 causes thyrotoxicosis and the resultant clinical presentation of the disease.

The majority of affected thyroid glands have benign disease with malignant tumours sporadic events. The two forms of benign disease are adenomatous hyperplasia and adenoma formation, and there is no difference between the two from a clinical perspective. Both forms may occur within the one thyroid gland. Bilateral thyroid gland involvement occurs in 70% of hyperthyroid cats and unilateral disease occurs in 30%. Thyroid adenomatous hyperplasia results in nodules of hyperplastic tissue ranging in size from 1–30 mm, whereas thyroid adenomas have a thin fibrous capsule, are grossly visible and may compress the adjacent thyroid gland. Larger adenomas may have cystic degeneration, necrosis and mineralisation; occasionally, cystadenomas with large fluid-filled regions occur.

Thyroid carcinomas occur in about 1–3% of cats with feline hyperthyroidism. In cats with long-term disease that is treated with methimazole, there is an apparent greater prevalence of thyroid carcinoma, possibly due the malignant transformation from adenomatous disease. Thyroid carcinomas range from well encapsulated to large locally invasive tumours and may be seen as multiple cervical masses with distant metastasis. Histologically, it can be difficult to differentiate between benign and malignant disease, and the following features may indicate malignant disease: capsular invasion, vascular invasion, extracapsular extension or distant metastasis.

4.5. Diagnosis

4.5.1. Introduction

The goals of the investigation of feline hyperthyroidism are to confirm the diagnosis and to assess the presence of potential complications because concurrent disease may require treatment or may affect treatment decisions.
Hyperthyroidism results in several non-specific findings on haematology, serum biochemistry and urinalysis. Specific endocrine tests or thyroid scintigraphy are commonly used to confirm the presence or absence of hyperthyroidism. Of the endocrine tests, total T4 and free T4 are most commonly measured, while the T3 measurement, T3 suppression test and thyrotropin-releasing hormone stimulation tests are less common.

Whilst the appearance of the hyperthyroid glands can be visualised using ultrasound and computed tomography, thyroid scintigraphy is the diagnostic imaging modality able to evaluate the function in addition to the appearance of the hyperthyroid tissue.

4.5.2. Endocrine testing

The mainstay of feline hyperthyroidism diagnosis is elevated total T4, which is seen in over 90% of hyperthyroid cats with the typical clinical features. Hyperthyroid cats with the total T4 within the reference range may have mild or early disease (70–80%) or concurrent disease (20–30%). The test for free T4 is more sensitive and will identify 95% of hyperthyroid cats with total T4 in the reference range, but it is less specific, and up to 20% of sick euthyroid cats may have elevated free T4. Therefore, free T4 and total T4 are evaluated together. A cat with mild hyperthyroidism will have elevated free T4 and total T4 in the upper third of the reference range, whereas in a cat with non-thyroidal disease, total T4 is in the low- to mid-total T4 range.

Total T3 is generally considered a poor screening test for hyperthyroidism because 42% of hyperthyroid cats have normal total T3. The T3 suppression test is used to distinguish mild hyperthyroidism from euthyroidism, but it is less commonly performed and relies on owner compliance to administer oral T3. The thyrotropin-releasing hormone suppression test also identifies mild hyperthyroidism with similar results to the T3 suppression test. There are disadvantages including the side effects of thyrotropin-releasing hormone, challenges in interpretation in cats with concurrent illness, and the lack of availability and expense of thyrotropin-releasing hormone. Situations in which either the T3 or thyrotropin-releasing hormone suppression tests may be
considered are when repeated total T4 and free T4 results are equivocal, the thyroid gland cannot be palpated or thyroid scintigraphy is not available.  

4.5.3. Thyroid scintigraphy

Thyroid scintigraphy is the reference standard for thyroid imaging because it provides a visual display of the anatomy and function of thyroid tissue as well as quantitative information about thyroid tissue uptake in the respective animal. Thus, thyroid scintigraphy may diagnose hyperthyroidism before endocrine testing is consistently abnormal. Thyroid scintigraphy is a highly sensitive test to identify hyperthyroidism when total T4 is normal or borderline, and thyroid scintigraphy may diagnose or exclude hyperthyroidism in cats with a high normal or elevated total T4 or free T4 (false positive). Additionally, thyroid scintigraphy provides information such as the presence of ectopic hyperthyroid tissue, features that increase suspicion of thyroid carcinoma, and identification of pulmonary metastasis – that assist in treatment decisions. The basic principles of thyroid scintigraphy interpretation in feline hyperthyroidism and its role in the treatment of feline hyperthyroidism are discussed later in this chapter.

4.5.4. Other diagnostic imaging modalities

The challenges associated with thyroid scintigraphy include accessibility of nuclear medicine facilities, radiation exposure of patients and personnel, and inaccuracy in the determination of the thyroid gland size. Thus, there is continued interest in other imaging modalities for assessment of normal and diseased thyroid glands, particularly ultrasound and computed tomography. The normal thyroid gland is not visualised on radiographs, and it is rare to have displacement of the adjacent trachea, which would indicate thyroid gland enlargement. While the magnetic resonance imaging features of the normal thyroid has been described in dogs, there are no descriptions in the literature of magnetic resonance imaging findings of normal or abnormal thyroid glands in cats.

The sonographic features of the normal and hyperthyroid feline thyroid glands have been described in terms of shape, echogenicity and size. Overall,
Diagnostic imaging in small animal endocrine disease

ultrasound is unlikely to replace thyroid scintigraphy in hyperthyroidism, although due to the ease of availability of ultrasound, it may be useful as an initial screening tool when thyroid scintigraphy is unavailable.\textsuperscript{76} Although ultrasound can distinguish unilateral from bilateral disease, it cannot identify ectopic thyroid tissue.\textsuperscript{76}

Computed tomography is more readily available than thyroid scintigraphy.\textsuperscript{77} Computed tomography has higher spatial resolution than thyroid scintigraphy and does not have waiting periods for radiation safety requirements,\textsuperscript{77} but computed tomography cannot provide functional information.\textsuperscript{77} The normal feline thyroid gland location, shape, volume and attenuation have been described.\textsuperscript{83, 84} The presence of iodine results in the thyroid gland being hyperattenuating relative to the adjacent soft tissue with a Hounsfield unit value of 123.2 (119.4–127.1); thus, thyroid glands are easily identified without contrast.\textsuperscript{83} Following contrast administration (delayed phase), the thyroid gland is homogeneously contrast enhancing with no significant difference in the Hounsfield unit value of 132.1 (127.4–136.8).\textsuperscript{83} The plain computed tomography features of the thyroid gland in feline hyperthyroidism have been described in terms of size, shape, attenuation, delineation and location.\textsuperscript{77} A striking difference between the normal thyroid and the hyperthyroid gland is the pattern of attenuation and reduction in Hounsfield unit values (61.6 ± 29.2).\textsuperscript{77} Plain computed tomography features after achieving euthyroidism with methimazole treatment identified no change to the thyroid gland volume and a reduction in attenuation and heterogeneity.\textsuperscript{85} The computed tomography features of thyroid carcinoma have not been described in detail, with only a partial description of one case within a case series of mediastinal masses.\textsuperscript{86}

4.6. Treatment

Treatment options include medical management, surgical thyroidectomy, dietary manipulation and \textsuperscript{131}I treatment. \textsuperscript{131}I treatment will be discussed later in this chapter. Medical management using methimazole or carbimazole, which reduces the production of thyroid hormones, is not curative and the growth of abnormal thyroid tissue continues.\textsuperscript{4} Methimazole and carbimazole, which is metabolised into methimazole, are thioureylene compounds that inhibit thyroid peroxidase
and, therefore, the synthesis of T3 and T4. The advantages of medical management include the reversibility of treatment and options of oral or transdermal administration. While ≥ 95% of hyperthyroid cats will respond to medical management, it requires once or twice daily administration and ongoing dose evaluation by assessing total T4 concentrations. Side effects have been reported in up to 18% of hyperthyroid cats and include vomiting, anorexia, depression, hepatopathy, blood dyscrasia, and self-induced facial or neck excoriations.

Surgical thyroidectomy may be curative and enables histopathological diagnosis of the underlying thyroid disease. Thyroidectomy in unilateral disease results in rapid resolution, whereas bilateral thyroidectomy may result in complications such as hypoparathyroidism and hypothyroidism. Ectopic tissue, which is present in 4–9% of hyperthyroid cats, may not be identified at surgery, and failure to remove all abnormal tissue can result in recurrence or persistence of hyperthyroidism.

Dietary manipulation through the reduction of dietary iodine (Hill’s Prescription Diet y/d Feline; Hills Pet Nutrition, Sydney, NSW, Australia) limits the synthesis of thyroid hormones but the abnormal thyroid tissue persists. Compared with other treatment options, diet is easy to administer and is not associated with the risks or adverse effects described earlier. Iatrogenic hypothyroidism has not yet been reported in hyperthyroid cats treated with an iodine-restricted diet. While total T4 returns to the reference interval in the majority of cats given an iodine-restricted diet, an improvement of clinical parameters such as body weight or heart rate may not be seen. Hence, it is questionable whether hyperthyroidism is well controlled with this treatment option. Furthermore, strict client and cat compliance is essential for treatment success and the long-term effects of an iodine deficient diet remain unknown.

4.7. Nuclear medicine in diagnosis of feline hyperthyroidism

4.7.1. Basic principles

Nuclear medicine involves the administration of a radiopharmaceutical, that contains a radioactive atom or radionuclide to a patient. Radiopharmaceuticals
have a range of physiochemical forms that enable the radionuclide to localise in specific regions of the body and then emit radiation in the form of gamma rays that travel through the body and are detected by the scintillation camera to create an image. In thyroid scintigraphy this image provides not just anatomical but also functional information. Radioactivity is measured in becquerels (Bq), with one Bq equal to one disintegration per second; the doses used in nuclear medicine are typically in the megabecquerels (MBq). The Bq is the international unit for radioactivity, but historically the unit was the curie, which is still used in the USA, with 1 millicurie equivalent to 37 MBq.

The criteria for choosing the radionuclide is to have a short half-life and emit low-energy gamma radiation because this provides diagnostic information and minimises the radiation dose to the patient. Technetium-99m, which has a half-life of 6 hours and emits gamma radiation at 140 keV, fulfils the above criteria and is the most commonly used radionuclide in thyroid scintigraphy. The radionuclide pertechnetate behaves similarly to iodide due to its valance and size; it is trapped and concentrated within the thyroid follicular cells but does not undergo organification and is not excreted into the colloid.

Because iodide is also trapped and organified by the thyroid gland, it makes sense to explore the isotopes of iodide as radionuclides, in particular $^{123}$I and $^{131}$I. $^{123}$I has a relatively short half-life of 13.3 hours and emits low-energy gamma radiation at 159 keV with no beta radiation emission, but it is expensive and is cost prohibitive for veterinary practice.

Although $^{131}$I is readily available and is cost effective, it does not meet the other criteria for choosing a radionuclide. $^{131}$I has a long half-life of 8.1 days and emits high-energy gamma radiation at 365 keV and beta radiation. The long half-life complicates the timing of the procedure because 24 hours must elapse between the injection and image acquisition, compared with 20 minutes when using technetium pertechnetate. The high-energy gamma and beta radiation contribute to the overall patient radiation exposure; the gamma radiation is ineffectively shielded by the collimators, and although the beta radiation does not contribute to the image, it does contribute to the total body and thyroid radiation dose. The beta radiation emitted by $^{131}$I is used in the treatment of hyperthyroidism. A comparison of thyroid scintigraphy with $^{131}$I and technetium pertechnetate in normal and hyperthyroid cats found the quality of the technetium
pertechnetate images to be consistently equal to or better than the quality of the $^{131}$I images.\textsuperscript{88}

4.7.2. Acquisition parameters and thyroid scintigraphy in the normal thyroid glands

The first description of normal feline thyroid scintigraphy was in 1985,\textsuperscript{89} and the procedure remains similar today. Generally, cats are sedated and given 37–148 MBq of sodium technetium pertechnetate intravenously and static images of the cervical region and thorax are acquired with a low-energy all-purpose collimator 20 minutes after injection for 100 000 counts.\textsuperscript{64} To avoid a misdiagnosis of hyperthyroidism, acquisition of images within 2 hours of injection is essential because the thyroid to salivary gland ratio (T:SG) increases significantly after this time.\textsuperscript{90}

While the standard protocol is intravenous administration of the radionuclide, the relative ease of subcutaneous administration led to evaluation of subcutaneous radionuclide administration. The subcutaneous route has been found to produce accurate and repeatable identification in both normal and hyperthyroid cats with results that are to the intravenous route.\textsuperscript{91}

The collimator is attached to the detector and acts to filter gamma rays en route from the patient to the detector system. The collimator comprises many small holes within lead and allows vertical gamma rays through the collimator while angled gamma rays are absorbed by the collimator and do not reach the detector system. Collimators affect the size, orientation and resolution of the image.\textsuperscript{64} Two collimators are used in thyroid scintigraphy; the most common choice is the low-energy all-purpose collimator, although there are advantages to using a pin-hole collimator.

The low-energy all-purpose collimator acquires an unmagnified image, which usually includes the head, neck, thorax and cranial abdomen.\textsuperscript{92} The limitations of the image are the distribution of radionuclide uptake within the thyroid lobe, definition of the margin of the thyroids and potentially the inability to separate the two thyroid gland lobes.\textsuperscript{92} The pin-hole collimator can acquire a magnified image and only includes the thyroid gland. This results in improved evaluation of the distribution of radionuclide uptake within the thyroid gland lobe.
and visualisation of the edge of the thyroid, but the effects of motion will also be magnified and the use of a pin-hole collimator usually requires general anaesthesia.27 The ideal scenario is being able to use both types of collimators: the pin-hole collimator for the ventral cervical region images and the low-energy all-purpose collimator for the lateral neck, lateral and ventral thorax acquisition. This may be achieved if two cameras are available or by changing the collimator within the study.92

The evaluation of feline thyroid glands uses both subjective assessment of the visual appearance of the thyroid glands and objective data in the form of the T:SG, the thyroid to background ratio (T:B) and % TU. The subjective assessment of the normal feline thyroid gland assesses the pattern of radionuclide uptake. Normal feline thyroid glands have a uniform and homogeneous uptake of the radionuclide, are elongated or ovoid in shape with smooth margins, and are symmetrical in both size and location (Figure 4.1). Normal cats do not have ectopic thyroid tissue.88

![Figure 4.1. Thyroid scintigraphy of the normal feline thyroid glands: (A) ventral image of the cervical region with arrows indicating the radionuclide uptake in the zygomatic/molar salivary glands and thyroid glands; (B) ventral image of the cervical region showing the region of interest around the thyroid glands and (C) objective measurements of % TU and T:SG.](image)

Significant pertechnetate uptake occurs in tissues and organs other than the thyroid gland. The assessment of anatomical detail is limited with thyroid scintigraphy, but by using a multimodality fusion of computed tomography and magnetic resonance imaging with single-photon emission tomography in two cats, four main regions of pertechnetate uptake have been determined: the zygomatic salivary gland, nasal cavity, nasopharynx and soft palate. Minor regions of uptake include the parotid, mandibular and molar salivary glands.93 The increased uptake
in the nasal cavity and nasopharynx may be due to tubuloacinus glands and minor salivary glands, respectively. There is no increased uptake in the head or region of the choroid plexus, but the nasopharyngeal uptake may have been interpreted as the choroid plexus in previous descriptions.\textsuperscript{93}

The T:SG is used to quantify the uptake of pertechnetate and to differentiate between normal and hyperthyroid cats. The T:SG has the highest sensitivity 98.8\% (97.4–99.6) and specificity 100\% (83.2–100) of the quantitative methods to identify hyperthyroid cats.\textsuperscript{94} The region of the zygomatic and molar salivary glands is generally used to calculate the T:SG because the molar salivary gland, which is adjacent to the mandibular molar tooth, is superimposed on the zygomatic salivary gland on the ventral planar image.\textsuperscript{93} Regions of interest are hand drawn around the thyroid glands and the zygomatic/molar salivary glands. The T:SG is calculated by dividing the average thyroid count density from both thyroid glands (total thyroid counts / total thyroid pixels) by the average zygomatic/molar salivary gland count density (total salivary count / total salivary gland pixels).\textsuperscript{89} Initially, thyroid uptake was considered to be similar to salivary gland uptake,\textsuperscript{89} but a later study that included a larger number of confirmed euthyroid cats established the currently accepted normal reference range of 0.48–1.66:1.\textsuperscript{95} This study was recently replicated in a group of 70 normal cats and resulted in a range of 0.5–1.5:1.\textsuperscript{44} The T:SG has no week-to-week variation in normal cats,\textsuperscript{96} and in hyperthyroid cats, the inter- and intra-observer variability is negligible.\textsuperscript{97}

A second ratio used to acquire objective data is to calculate the T:B. The principle of using the background rather than the salivary glands is that the T:SG may be influenced by disease of the salivary glands. The original ratio of 2.76:1 (1.7–4.4) in normal cats was described by Beck et al.\textsuperscript{89} More recently, the T:B was found to be 1.6–6.4:1 in a group of 70 normal cats.\textsuperscript{44} Originally, the background count information was determined by a region of interest in the axilla,\textsuperscript{89} but more recently a range of other locations have been assessed.\textsuperscript{94, 97, 98} A region of interest at the heart had the best correlation with plasma radioactivity,\textsuperscript{98} and a thyroid to heart ratio had the highest sensitivity to diagnose hyperthyroidism compared with other background locations.\textsuperscript{94} The sensitivity of the thyroid to heart ratio was 97.8\% (96.1–98.9) and was less than the T:SG, although this difference was not statistically significant.\textsuperscript{94} The T:B has marked inter- and intra-observer variability
in hyperthyroid cats, although this is reduced using a rectangular shape in the cervical region.\textsuperscript{97} In addition, the T:B ratio may help predict which cats with adenomatous hyperplasia or thyroid adenoma are at risk of treatment failure when treated with standard $^{131}$I therapy.\textsuperscript{99} Cats that failed treatment had significantly higher T:B ratios (median 13:1, range 3.6–73) than successfully treated cats (median 4.4:1, range 1.2–69).\textsuperscript{99}

The % TU of the radionuclide by the thyroid gland can be calculated using either a gamma camera or a thyroid detector probe. These % TU calculations require intravenous administration of the radionuclide\textsuperscript{94} and comparison with a known radionuclide standard or the radionuclide dose prior to administration to take into account the amount of radioactivity being absorbed by the soft tissue in the neck.\textsuperscript{64} Therefore, compared to the T:SG, the % TU is more complicated to calculate.\textsuperscript{94} The % TU has a sensitivity of 97\% (95.1–98.3) and specificity of 100\% (83.2–100)\textsuperscript{94} and good inter- and intra-observer repeatability in hyperthyroid cats.\textsuperscript{97} The mean % TU 20 minutes following intravenous injection in normal cats was originally determined to be 0.64\% with a range of 0.5–1.5\%,\textsuperscript{100} and depending on the measurement technique,\textsuperscript{101} uptakes range from 0.21 ±0.06\%\textsuperscript{90} to 2.23\%.\textsuperscript{102} There is no week-to-week variation in normal cats.\textsuperscript{96}

The same quantitative method can be used with the iodide isotopes of $^{123}$I and $^{131}$I and a thyroid detector probe. The mean radioactive iodine uptake of $^{123}$I has been determined in normal cats to be 19\% at 6 hours and 23.1\% at 24 hours following oral administration, and in hyperthyroid cats to be 54.2\% at 6 hours and 57.8\% at 24 hours following oral administration.\textsuperscript{103} The radioactive iodine uptake of $^{131}$I has been calculated in normal cats at various time points up to 48 hours following oral administration reaching 33\% at 2 hours, plateauing at 21\% between 4 and 24 hours, and finally reducing to 18\% at 48 hours. Comparison of the $^{131}$I uptake and the 20 minute post-injection technetium pertechnetate T:SG identified some correlation between these two techniques in normal cats at the 10 hour radioactive iodine uptake time point.\textsuperscript{104}

The percentage uptake of technetium in the salivary glands ranges between 0.01 and 2.8\% in both normal and hyperthyroid cats, but whether this is of value in the assessment of salivary gland disease or evaluation of the thyroid is unknown.\textsuperscript{96, 97}
4.7.3. Treatments and other compounds that may interfere with thyroid scintigraphy

Methimazole and carbimazole are commonly used to treat hyperthyroidism. These substances block thyroid peroxidase and ultimately the formation of T4, resulting in increased secretion of thyroid-stimulating hormone by the pituitary. Thyroid-stimulating hormone promotes pertechnetate trapping by the normal thyroid, with the potential to falsely diagnose euthyroid cats with hyperthyroidism if they were erroneously treated with methimazole at the time of thyroid scintigraphy. Normal cats being treated with methimazole will have both an increased pertechnetate uptake and increased T:SG, mimicking the thyroid scintigraphy findings in hyperthyroidism. Hyperthyroid cats treated with methimazole for 30 days have been shown to have an increased T:SG and percentage of pertechnetate uptake similar to untreated hyperthyroid cats. Mildly hyperthyroid cats with unilateral increased radionuclide uptake (IRU) have been shown to develop bilateral IRU with methimazole treatment, presumably because of increased pertechnetate trapping caused by methimazole. Currently, it is recommended to cease methimazole treatment at least 7–10 days before thyroid scintigraphy to avoid misdiagnosis of hyperthyroidism due to methimazole.

Iodinated contrast media may inhibit pertechnetate uptake by an incompletely understood mechanism. The sodium iodide symporter within the follicular cell membrane permits iodide and pertechnetate to enter the thyroid follicular cell. The iodide, which originates from the iodinated contrast media, both directly and competitively inhibits the sodium iodide symporter and therefore reduces the uptake. The amount and duration of the uptake inhibition varies depending on the dose and type of iodinated contrast media. The human guidelines are to avoid thyroid scintigraphy for two months after iodinated contrast procedures. In normal cats receiving 880 mg I/kg in the form of iohexol, the T:SG and the % TU were evaluated up to 28 days following contrast media administration. Significant reductions in T:SG at 1 day and % TU at 1, 3 and 14 days after the contrast media administration were identified and a recommendation of avoiding thyroid scintigraphy at 1 to 14 days following contrast media administration was made. However, at no time point were these values below the normal reference range. Iohexol is a nonionic iodinated
contrast media, and while the interference of uptake from ionic iodinated contrast media is not known, it has been suggested that these contrast media may have a greater inhibitory effect on pertechnetate uptake.\textsuperscript{101} There are no reports of the effect of contrast media on thyroid scintigraphy in hyperthyroid cats in the literature.

4.8. Thyroid scintigraphy features in feline hyperthyroidism

There have been several studies describing the scintigraphic appearance of feline hyperthyroid glands.\textsuperscript{43, 44, 88, 100, 108} The most recent publication was in 2014 by Peterson and Broome who studied 2096 consecutive cats\textsuperscript{44} and categorised the scintigraphic findings into four patterns: unilateral disease (31.7% prevalence), bilateral asymmetrical disease (50.6%), bilateral symmetrical disease (12.3%) and multifocal disease (3.9%), with the relative prevalence being similar to other case series.\textsuperscript{44} As the increased T4 results in decreased thyroid-stimulating hormone and suppression of the normal thyroid tissue, the thyroid lesion will have IRU and the normal thyroid tissue will have reduced radionuclide uptake.\textsuperscript{88}

Thus, unilateral disease is described as single thyroid gland involvement with suppression of the contralateral thyroid gland (Figure 4.2A), and bilateral disease has both thyroid lobes involved, either of similar size (symmetrical; Figure 4.2) or dissimilar sizes (asymmetrical; Figure 4.2C). Multifocal disease was uncommon and encompassed ectopic thyroid disease or suspect thyroid carcinoma (Figure 4.2D). In cats with unilateral disease, the left-sided nodules were more common than right-sided nodules.\textsuperscript{44} The appearance of the IRU was described as homogeneous or heterogeneous within each of the four patterns of disease and significant differences were found between the patterns of disease groups. The unilateral disease group had a homogeneous appearance. The bilateral symmetrical disease group had an equivalent distribution of both homogeneous and heterogeneous appearance, whereas the bilateral asymmetrical and multifocal disease groups had heterogeneous appearances.\textsuperscript{44} The prevalence of ectopic thyroid disease was 3.8% and thyroid carcinoma was suspected in 1.7%.\textsuperscript{44} Another study found 23% of cats had ectopic tissue,\textsuperscript{43} which may be explained by the inclusion of cats presented for recurrence of hyperthyroidism following thyroidectomy.
Figure 4.2. Thyroid scintigraphy ventral images of the cervical region demonstrating the patterns of disease in hyperthyroid cats: (A) unilateral, (B) bilateral symmetrical, (C) bilateral asymmetrical and (D) multifocal patterns of disease.

The quantitative features of T:SG, T:B and % TU in hyperthyroid cats are all significantly increased compared with normal cats. Of these, T:SG followed by T:B using the heart region of interest have the greatest sensitivity with a significantly lower sensitivity of the % TU and T:B using the axilla region of interest.94

It is not possible to differentiate adenomatous ectopic thyroid tissue from thyroid carcinoma by thyroid scintigraphy; histopathology is required for a definitive diagnosis.109 However, the following thyroid scintigraphy features may increase the level of suspicion for thyroid carcinoma: heterogeneous IRU with spicular or irregular margins, linear pattern with IRU along the fascial planes and multifocal (> 3) areas of IRU extending beyond the margins of the thyroid glands.64 The presence of diffuse focal pulmonary IRU consistent with pulmonary metastasis is the most definitive thyroid scintigraphy feature of thyroid carcinoma64 but other malignancies such as bronchogenic carcinoma can have the
same appearance. A thyroid carcinoma metastatic rate to lungs and lymph nodes of 70% has been described, but a recent publication found that only 5% of cats with suspected thyroid carcinoma had evidence of metastasis in the medial retropharyngeal lymph nodes and no cats had IRU in the lungs.

4.9. **Nuclear medicine in the treatment of feline hyperthyroidism**

Radioiodine in the form of $^{131}$I is an established treatment for feline hyperthyroidism, which resolves with a single administration of $^{131}$I in approximately 95% of cases. This treatment success rate refers to resolution of hyperthyroidism, whereas the objective of treatment is to restore euthyroidism and avoid hypothyroidism with a single dose of $^{131}$I. When $^{131}$I decays it emits beta radiation that contributes to 90% of local tissue damage and gamma radiation which accounts for the remaining 10%. The beta radiation travels for 2 mm, thus there is limited damage to parathyroid glands, atrophic thyroid tissue and local cervical structures. The dose of radiation delivered to the thyroid gland is not only determined by the dose administered but also by the proportion of the administered dose taken up by the thyroid (radioactive iodine uptake), the half-life of the $^{131}$I and the weight of the thyroid gland. Cats with persistent hyperthyroidism often have larger tumours, more severe clinical signs and higher total T4 levels than those without persistent hyperthyroidism. Thyroid carcinomas are also amenable to $^{131}$I treatment but due to the larger size and less effective concentration and retention of $^{131}$I, a 3- to 10-fold increase in dose is recommended. There are different approaches to the dose determination of $^{131}$I for the benign aetiologies of hyperthyroidism including tracer studies, standardised fixed doses and scoring systems. These studies are summarised in Table 4.1.

The route of $^{131}$I administration may be intravenous, subcutaneous or per os. Comparing intravenous with subcutaneous $^{131}$I administration, there was no difference in the resolution of hyperthyroidism based on the radioactive iodine uptake tracer or individual clinical scoring methods of $^{131}$I dose determination. The subcutaneous route is preferred because it is less stressful for the cat and it is easier to administer with less radiation exposure for personnel. The oral
dose is associated with more cases of persistent hyperthyroidism when compared with intravenous administration,\textsuperscript{38} perhaps due to differences in metabolism,\textsuperscript{42} vomiting\textsuperscript{38} or incomplete ingestion of the dose.\textsuperscript{38, 42} A higher dose for oral administration is recommended.\textsuperscript{42}

Tracer studies are a method of calculating an individualised dose by the administration of an initial tracer dose of $^{131}$I that is used to determine the radioactive iodine uptake and effective half-life of the tracer in conjunction with an estimated thyroid gland size. Although treatment success was achieved using this approach in 82–85% of cases,\textsuperscript{29, 30} it is no longer used\textsuperscript{111} because the effective half-life of the tracer does not correlate with the half-life of the treatment $^{131}$I dose.\textsuperscript{31} This is thought to be due to changes in thyroid physiology after $^{131}$I treatment.\textsuperscript{111}

A common approach is the administration of a standardised dose, irrespective of the severity of the clinical presentation or the size of the thyroid gland.\textsuperscript{40} The doses used are considered moderately high at 148–150 MBq and are administered intravenously with resolution of hyperthyroidism ranging between 84–88%\textsuperscript{32-34} and prevalence of hypothyroidism requiring treatment of between 3–9%.\textsuperscript{33, 34} An oral dose of 185 MBq yielded similar results with 86% and 9% of cats becoming euthyroid and hypothyroid, respectively.\textsuperscript{41} These treatment outcomes are considered similar to the tracer studies method.\textsuperscript{68}

Some hyperthyroid cats were given different doses based on experience or observation,\textsuperscript{42, 112} and scoring systems have also been used to establish an individualised $^{131}$I dose.\textsuperscript{35-38} The scoring systems variably take into account thyroid scintigraphy appearance, clinical signs and results of blood tests and are used to calculate the $^{131}$I dose, with restoration of euthyroidism in 87.5–94.5% of cases.\textsuperscript{35, 36, 42} The cats with post-treatment persistent hyperthyroidism generally had higher scores and thus received higher $^{131}$I doses when using a scoring system approach.\textsuperscript{37, 38} No difference in treatment outcome occurred when doses based on thyroid scintigraphy features of % TU and thyroid size were compared.\textsuperscript{113}

A recent trend has been to evaluate a lower fixed dose in cats with less severe disease.\textsuperscript{114, 115} Of hyperthyroid cats with palpable goitre $\leq$ 20 mm, 97.6% were successfully treated (defined as resolution of hyperthyroidism) with 2% developing persistent hypothyroidism requiring treatment.\textsuperscript{114} The interest in iatrogenic hypothyroidism following $^{131}$I treatment has increased due to the
association with reduced survival time in azotaemic cats. There are challenges with diagnosis of iatrogenic hypothyroidism because many cats do not show clinical signs and total T4 concentrations below the reference range may be seen in sick euthyroid cats. However, a significant reduction in overt and subclinical hypothyroidism at 6 months following $^{131}$I treatment occurred with a low compared with a standard fixed dose.

It is difficult to determine the optimal $^{131}$I dosing regimen from the literature. This is particularly so for cats with severe clinical disease, large thyroid size and high total T4 concentrations because some of these cats remain hyperthyroid irrespective of dose regimen, even after receiving higher $^{131}$I doses. Using higher $^{131}$I doses may also carry a higher risk of iatrogenic hypothyroidism, with a potential negative impact on survival.
Table 4.1. $^{131}$I dose determination and treatment outcomes.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Dose calculation criteria</th>
<th>$^{131}$I dose (MBq)</th>
<th>Method of administration</th>
<th>Number of cats</th>
<th>Treatment outcome</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Turrel et al 1984</td>
<td>Radioactive iodine uptake tracer</td>
<td>Individual</td>
<td>Intravenous</td>
<td>7</td>
<td>Euthyroid 100%</td>
<td></td>
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<tr>
<td>Turrel et al 1984</td>
<td>Radioactive iodine uptake tracer</td>
<td>Individual</td>
<td>Intravenous or subcutaneous (60 cats each)</td>
<td>120</td>
<td>Euthyroid intravenous 85% subcutaneous 84% Hypothyroid 6%</td>
<td>No difference in proportion of euthyroid cats between intravenous or subcutaneous</td>
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<tr>
<td>Theon et al 1994</td>
<td>Fixed</td>
<td>148</td>
<td>Intravenous</td>
<td>62</td>
<td>Euthyroid 84% Hyperthyroid 8% Hypothyroid 8% 2 cats died thus results based on 60</td>
<td>Hypothyroid cats; 3 of the 5 were euthyroid at 6 months and 2 unknown; none required treatment</td>
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<tr>
<td>Meric and Rubin 1990</td>
<td>Fixed</td>
<td>148</td>
<td>Intravenous</td>
<td>193</td>
<td>Euthyroid 80% Hyperthyroid 1% Hypothyroid 9% Within 12 months from treatment</td>
<td>Hypothyroid cats 9%; all clinical and required treatment</td>
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<tr>
<td>Chun et al 2002</td>
<td>Fixed</td>
<td>148</td>
<td>Intravenous</td>
<td></td>
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<tr>
<td>Publication</td>
<td>Dose calculation criteria</td>
<td>$^{131}$I dose (MBq)</td>
<td>Method of administration</td>
<td>Number of cats</td>
<td>Treatment outcome</td>
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<tr>
<td>Craig et al 1993$^{34}$</td>
<td>Fixed</td>
<td>150</td>
<td>Intravenous</td>
<td>66</td>
<td>93% defined as resolution of hyperthyroidism</td>
<td>5 cats hypothyroid &gt; 2 months (8%); 2 required treatment</td>
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<td>88% euthyroid (&gt; 2 months following treatment)</td>
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<td>1 died after injection</td>
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<td></td>
<td>1 persistently hyperthyroid at 9 months and euthanised</td>
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<tr>
<td>Klausner et al 1987$^{41}$</td>
<td>Fixed</td>
<td>185</td>
<td>Per os</td>
<td>23</td>
<td>Euthyroid 86%</td>
<td>2 persistently hypothyroid cats (8 and 11 months); 1 died and 1 responded to thyroxine</td>
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<td></td>
<td>Hyperthyroid 9%</td>
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<td>Hypothyroid 9%</td>
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<td>1 died after injection</td>
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<tr>
<td>Chambers et al 1987$^{112}$</td>
<td>&quot;based on previous experience and appearance of TS&quot;</td>
<td>103.6–329.3</td>
<td>Intravenous</td>
<td>43</td>
<td>All cats had clinical improvement</td>
<td>Only a few cats required treatment for hypothyroidism</td>
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<tr>
<td>Malik et al 1993$^{42}$</td>
<td>Cats with marked thyroid enlargement were given higher doses as study progressed</td>
<td>200–300 250 in 26 cats</td>
<td>Per os</td>
<td>40</td>
<td>36/40 (90%) with normal or reduced total T4</td>
<td>4 remained hyperthyroid</td>
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<td>Euthyroid 35</td>
<td>1 hypothyroid requiring treatment</td>
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<td>Hyperthyroid 4</td>
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<td></td>
<td>Hypothyroid 1</td>
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<td>Publication</td>
<td>Dose calculation criteria</td>
<td>¹³¹I dose (MBq)</td>
<td>Method of administration</td>
<td>Number of cats</td>
<td>Treatment outcome</td>
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<tr>
<td>Jones et al 1991&lt;sup&gt;36&lt;/sup&gt;</td>
<td>Scoring system Severity of clinical signs Size of thyroid Total T4 or freeT4 level</td>
<td>Three categories 39–60; 60–75; 70–100</td>
<td>Intravenous</td>
<td>32</td>
<td>3–4 months following treatment Euthyroid 28/32 (87.5%) 1/28 transient hypothyroidism</td>
<td>3 remained hyperthyroid 1 hypothyroid requiring treatment</td>
</tr>
<tr>
<td>Mooney 1994&lt;sup&gt;37&lt;/sup&gt;</td>
<td>Scoring system Severity of clinical signs Size of thyroid Total T4 level</td>
<td>Three categories ≤ 120; 120–150; ≥ 160</td>
<td>27 cats Intravenous 23 cats Subcutaneous</td>
<td>50</td>
<td>47/50 (94%) Resolution of hyperthyroidism included 28 cats (56% of 50 cats) with hypothyroidism based on total T4</td>
<td>All cats with persistent hyperthyroidism had severe disease Transient hypothyroidism in many cats (56% at 30 days following treatment); none required treatment</td>
</tr>
<tr>
<td>Peterson and Becker 1995&lt;sup&gt;35&lt;/sup&gt;</td>
<td>Scoring system Severity of clinical signs Size of thyroid Total T4 level</td>
<td>Three categories 74–130; 130-167; 167-222</td>
<td>Subcutaneous</td>
<td>524</td>
<td>515/524 euthyroid 94.2% Minimum 3 months following treatment Defined as complete resolution of clinical signs and T4 in reference range</td>
<td>8 (1.5%) remained hyperthyroid Transient hypothyroid in many cats; 11 (2.1%) required treatment</td>
</tr>
<tr>
<td>Publication</td>
<td>Dose calculation criteria</td>
<td>$^{131}$I dose (MBq)</td>
<td>Method of administration</td>
<td>Number of cats</td>
<td>Treatment outcome</td>
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<tr>
<td>Forrest et al 1996$^{38}$</td>
<td>Technetium pertechnetate thyroid scintigraphy used to determine volume Fixed modified dose</td>
<td></td>
<td>Per os Intravenous</td>
<td>80</td>
<td>Significant difference in response to treatment depending on method of administration Oral 75% Intravenous 94.9% Broad range of follow-up times Response to treatment defined as normal total T4 in 65 cats and clinical signs only in 15 cats Cats which remained hyperthyroid came from higher doses thus larger thyroid gland volumes on thyroid scintigraphy and had higher pre-treatment total T4 Did not evaluate proportion of hypothyroid cats</td>
<td></td>
</tr>
<tr>
<td>Morre et al 2017$^{113}$</td>
<td>% TU group Three categories 111, 129.5, 166.5 Size group Mean 159.1 Subcutaneous</td>
<td>% TU 58 Size 25</td>
<td>% TU Hyperthyroid 16% Hypothyroid 21% Size Hyperthyroid 8% Hypothyroid 32</td>
<td>96</td>
<td>6 months following treatment No statistical difference with number of hyperthyroid or hypothyroid cats</td>
<td></td>
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<tr>
<td>Vagney et al 2017$^{115}$</td>
<td>Fixed Palpable goitre ≤ 20mm</td>
<td>123.95 (121 – 127.3)</td>
<td>Intravenous</td>
<td>96</td>
<td>97.6% defined as resolution of hyperthyroidism Hyperthyroid 2% Hypothyroid 2%</td>
<td>2 cats clinically hypothyroid at 50 and 75 days and required treatment</td>
</tr>
<tr>
<td>Publication</td>
<td>Dose calculation criteria</td>
<td>$^{131}$I dose (MBq)</td>
<td>Method of administration</td>
<td>Number of cats</td>
<td>Treatment outcome</td>
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<tr>
<td>Lucy et al 2017$^{114}$</td>
<td>Compared two fixed doses Total T4 51.5–167.3nmol/L</td>
<td>*Low †Standard</td>
<td>Subcutaneous</td>
<td>189</td>
<td>6 months following treatment</td>
<td>6 months following treatment No significant difference in persistently hyperthyroid between dose groups Significant differences in both overt and subclinical hypothyroidism</td>
</tr>
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<td></td>
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<td></td>
<td>150*</td>
<td>Euthyroid 75%* 36%† Hyperthyroid 0%* 3.3%†</td>
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<td>39†</td>
<td>Overt Hypothyroid Defined as low total T4; high thyroid-stimulating hormone 1%* 25%†</td>
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<td>Subclinical Hypothyroid Defined as Normal total T4; high thyroid-stimulating hormone 21% 46†</td>
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</tr>
</tbody>
</table>

* Low-dose 74MBq † Standard-dose 148MBq
Chapter 5. The role of thyroid scintigraphy in severely hyperthyroid cats

5.1. Introduction

Radioiodine therapy is an effective and simple treatment for feline hyperthyroidism and has a reported success rate of between 83–94%, although the reasons for treatment failure remain poorly evaluated.\textsuperscript{40, 68} The optimal dose of\textsuperscript{131}I for the treatment of hyperthyroid cats is controversial, particularly in cats with severe disease.\textsuperscript{37, 38} Furthermore, the negative impact of iatrogenic hypothyroidism on survival in azotaemic cats is becoming realised.\textsuperscript{39} Scoring systems based on clinical assessment or thyroid scintigraphy features have failed to identify the optimal \textsuperscript{131}I dose. This retrospective study has two parts. In part 1, the thyroid scintigraphy features of severely hyperthyroid cats were compared with those of the general population of hyperthyroid cats.\textsuperscript{43, 44} In part 2, the role of thyroid scintigraphy in the treatment of severely hyperthyroid cats was assessed in a retrospective cohort study. Two approaches to \textsuperscript{131}I dose determination in severely hyperthyroid cats were compared: a dose calculated using thyroid scintigraphy features and a standard prescribed dose. \textsuperscript{131}I treatment outcome at 4 weeks following treatment was assessed by total T4. The study objectives were to review the thyroid scintigraphy features of cats with severe hyperthyroidism, identify the thyroid scintigraphy features in severely hyperthyroid cats that differ from the general population of hyperthyroid cats, and identify any differences in treatment outcome between the customised and standardised dose groups. These objectives will clarify the requirement for pre-treatment thyroid scintigraphy in severely hyperthyroid cats.
5.2. Part 1: features of thyroid scintigraphy in severely hyperthyroid (Australian) cats

5.2.1. Materials and methods

5.2.1.1. Patient selection
The study design was a retrospective case series. Study subjects were cats that presented to U Vet for potential treatment of hyperthyroidism with $^{131}$I, had thyroid scintigraphy images available for review and had a documented total T4 concentration > 190 nmol/L before thyroid scintigraphy. Cases were selected by searching the picture archiving and communications system (Synapse™ Fuji Medical Systems, Stamford, CT, USA) between June 2007 and December 2013. This search identified 153 hyperthyroid cats that underwent thyroid scintigraphy. The hospital patient management system (RxWorks™, Eight Mile Plains, Qld, Australia) and archived records from the referring veterinarian at the time of referral were assessed to identify the hyperthyroid cats with a documented total T4 > 190 nmol/L (reference range 9.5–48 nmol/L). These cats were considered severely hyperthyroid and included in part 1 of the study. The following patient data was collated: signalment (age, breed, sex), date of diagnosis, total T4 at diagnosis, previous medical treatment with carbimazole or methimazole, and date of thyroid scintigraphy.

5.2.1.2. Thyroid scintigraphy protocol
Medical therapy for hyperthyroidism was withdrawn for a minimum of 7 days. Thyroid scintigraphy was performed under sedation after intravenous injection of 37–150 MBq technetium pertechnetate. Ventral, right and left lateral images of the cervical region and thorax were acquired at 20 minutes with an Argus Epic Gamma camera (Philips Healthcare, North Ryde, NSW, Australia) using a low-energy all-purpose collimator. Each image was acquired for 400 000 counts and a 256 × 256 × 16 matrix was used. The images were processed with a Pegasys Ultra High Tier imaging system (Philips Healthcare, North Ryde, NSW, Australia). The ventral images of the cervical region and the thorax were displayed in two formats: a higher level of windowing with the background shape of the cat visible at 2× magnification and a lower level of windowing without magnification. The
right and left lateral images of the cervical region and the thorax were displayed with and without magnification. The T:SG and % TU calculations were performed by Tanya Puksman, a nuclear medicine technologist. The T:SG was determined from the ventral image as the thyroid glands and salivary glands are better delineated. The % TU used counts from the syringe containing the radionuclide pre- and post-injection and regions of interest manually drawn around of all areas of IRU and a background region close to the thyroid. The % TU was calculated by the software and expressed as a percentage of the injected dose. The images including the T:SG and % TU values were transferred to the picture archiving communications system and viewed on a dedicated workstation (Multisync LCD 2190UXp, NEC, Chicago, IL, USA).

5.2.1.3. Data collection of the thyroid scintigraphy features of severely hyperthyroid (Australian) cats

Thyroid scintigraphy: subjective features

The thyroid scintigraphy studies were interpreted by Anne Marie Rose and a consensus of the subjective features was reached with Cathy Beck and Dayle Tyrrell.

The pattern of disease was categorised as unilateral, bilateral symmetrical, bilateral asymmetrical and multifocal, in accordance with previous studies (Figure 4.2). All images and the higher windowing level were used. The characteristics of appearance, margin, subjective intensity relative to the zygomatic/molar salivary glands and shape were documented for each IRU and are presented in

Table 5.1. If disease pattern was determined to be bilateral or multifocal, IRU was classified as homogeneous in appearance only if all IRU were homogenous in appearance. If one region of IRU was heterogeneous in appearance, overall IRU appearance was classified as heterogeneous. Similarly, overall margins were only classified as smooth if margins of all individual IRU were smooth and the overall intensity of the IRU was only classified as greater than that of the salivary glands if all IRU met this particular criterion. The shape of the IRU was determined as a percentage of all the regions of IRU overall and within a group with a particular pattern of disease. For the bilaterally asymmetrical disease group, IRUs representing the left and right thyroid were also
separately categorised, resulting in several combinations of shapes of IRU. Each IRU was evaluated separately for the subjective characteristics for the cats with multifocal patterns of disease. The images with the lower level of windowing were used to assess the appearance of the IRU, and all images were used to assess the heterogeneity of the IRU. The lower level of windowing was preferred as these images were not magnified and the grey scale was optimised to assess the appearance of the IRU as either heterogeneous or homogeneous.

Table 5.1. Descriptive categories of the subjective features of the increased radionuclide uptake.

<table>
<thead>
<tr>
<th>Subjective feature</th>
<th>Description of feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Homogeneous</td>
</tr>
<tr>
<td></td>
<td>Heterogeneous</td>
</tr>
<tr>
<td>Margin</td>
<td>Smooth</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
</tr>
<tr>
<td>Intensity</td>
<td>&gt; SG*</td>
</tr>
<tr>
<td></td>
<td>&lt; SG*</td>
</tr>
<tr>
<td>Shape</td>
<td>Ovoid</td>
</tr>
<tr>
<td></td>
<td>Lobulated</td>
</tr>
<tr>
<td></td>
<td>Elongated</td>
</tr>
<tr>
<td></td>
<td>Ill-defined</td>
</tr>
</tbody>
</table>

SG* = salivary gland

The location of each IRU was determined using the ventral image of the head and neck. A line was drawn between the points of the shoulders and the IRU were described as being above the line, on the line or below the line, which was classified as being within the neck, at the thoracic inlet or within the thorax respectively, or potentially multiple locations (Figure 5.1). The number of IRU was determined and then categorised into one of the following three groups: one, two, or three or more IRU. All images were used to identify both the ectopic tissue and number of regions of IRU.
Ectopic tissue was defined as IRU in more than two foci or the presence of one or more areas within the thorax. The location was recorded as being either in the lingual or sub-lingual region or within the mediastinum. The cat was suspected to have thyroid carcinoma based on the previously described features. The presence of pulmonary metastasis was recorded.

**Thyroid scintigraphy: objective measurements**

Measurements of the % TU and T:SG were retrieved from the thyroid scintigraphy images on the picture archiving communications system.

### 5.2.1.4. Comparison of thyroid scintigraphy features with the general population of hyperthyroid cats

A range of thyroid scintigraphy features from the severely hyperthyroid cats were compared with thyroid scintigraphy features from the published data of the general population of hyperthyroid cats. The distribution of the patterns of disease and the proportions of the heterogeneous regions of IRU was compared with the published data. The number of regions of IRU, presence of ectopic tissue and location of IRU was also compared.

**Statistical analysis**

Statistical analyses were performed using Minitab version 17 and 18 (State College, PA, USA). The patterns of disease were compared using the Pearson’s chi-square test of independence and the thyroid scintigraphy features were
compared using the one proportion test. All analyses used the value of $P < 0.05$ as significant.

5.2.2. Results

5.2.2.1. The features of thyroid scintigraphy in severely hyperthyroid (Australian) cats

Patient data
Seventy-nine severely hyperthyroid cats met the selection criteria. The median age of the 77 cats with known ages was 12.5 years (5–15.3 years) and 2 (2.53%) cats were acquired as strays and had unknown ages. Sixty-two (78.48%) were domestic short-haired, 7 (8.87%) were domestic medium-haired and 6 (7.59%) were domestic long-haired cats. The other breeds included 1 (1.25%) each of Chinchilla, British short-haired, Persian cross and Russian blue cross. Forty-three (54.43%) cats were spayed females, 33 (41.77%) were castrated males and 3 (3.80%) were entire females.

5.2.2.2. Thyroid scintigraphy: subjective features

Pattern of disease
The appearance of the IRU were evaluated according to the patterns of disease (Table 5.2).44

Table 5.2. Presence of homogeneous and heterogeneous increased radionuclide uptake according to the distribution of patterns of disease.

<table>
<thead>
<tr>
<th>Pattern of disease</th>
<th>Number of cats</th>
<th>Homogeneous IRU*</th>
<th>Heterogeneous IRU*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral</td>
<td>9 (11.25%)</td>
<td>66.7%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Bilateral symmetrical</td>
<td>1 (1.25%)</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Bilateral asymmetrical</td>
<td>60 (75.95%)</td>
<td>13%</td>
<td>87%</td>
</tr>
<tr>
<td>Multifocal</td>
<td>9 (11.25%)</td>
<td>11.1%</td>
<td>88.9%</td>
</tr>
</tbody>
</table>

* IRU = increased radionuclide uptake
The findings of the margins, intensity and shape are presented in Table 5.3. Seventy-six (96.20%) cats had smooth margins of all regions of IRU and 3 (3.80%) cats had irregular margins. Two of these cats had bilateral asymmetrical disease and irregular margins to the left regions of IRU. These IRU were also heterogeneous and ill-defined. The third cat with irregular margins was from the multifocal disease group and had three regions of IRU, one of which regions had irregular margins and a lobulated shape that was located at the thoracic inlet.

Sixty-nine (87.34%) cats had IRU intensity judged subjectively as greater than the zygomatic/molar salivary glands, 10 (12.66%) cats had a reduced IRU intensity including 6 cats from the bilaterally asymmetrical group (10% of bilaterally asymmetrical cats) and 4 cats from the multifocal disease group (44.4% of multifocal disease group cats). While the other subjective features from the bilaterally asymmetrical group were variable, all the regions with reduced intensity within the multifocal disease group were also heterogeneous, smooth margined, ill-defined and located in the thorax, except for one located in the neck.

There were 165 IRU in the 79 cats. The shape of the IRU was equally distributed between the ovoid, lobulated and elongated shapes (33.3% each) in the unilateral group. The cat with bilaterally symmetrical disease had an elongated shape to both regions of IRU. The most frequent shape of the IRU (48/120 IRU, 40%) in the bilaterally asymmetrical group was the elongated shape with 21 (35%) of cats in this group having either one or both IRU elongated. The ovoid shape was present in 16.67% of all IRU in this group and the lobulated and ill-defined shapes were seen in 21.67% of all IRU in this group. Furthermore, cats were categorised in the following categories: ovoid–ovoid (2 cats, 3.33% of the group), lobulated–lobulated (5 cats, 8.83%), elongated–elongated (9 cats, 15%), ovoid–lobulated (2 cats, 3.3%), lobulated–elongated, (7 cats, 11.67%), elongated–ill-defined (14 cats, 23.33%), ovoid–elongated (9 cats, 15%), lobulated–ill-defined (7 cats, 11.67%) and ovoid–ill-defined (5 cats, 8.33%). No cat in the bilateral asymmetrical disease group had two ill-defined regions of IRU. The distribution of IRU within the multifocal disease group was ovoid (26.5%), lobulated (17.6%), elongated (17.6%) and ill-defined (38.3%).
Table 5.3. Subjective features: overall proportion of cats within the descriptive categories.

<table>
<thead>
<tr>
<th>Subjective feature</th>
<th>Description of feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margin</td>
<td>Smooth</td>
</tr>
<tr>
<td></td>
<td>96.20%</td>
</tr>
<tr>
<td>Intensity</td>
<td>&gt; SG</td>
</tr>
<tr>
<td></td>
<td>87.34%</td>
</tr>
<tr>
<td>Shape</td>
<td>Ovoid</td>
</tr>
<tr>
<td></td>
<td>19.39%</td>
</tr>
<tr>
<td></td>
<td>Elongated</td>
</tr>
<tr>
<td></td>
<td>35.76%</td>
</tr>
</tbody>
</table>

The location of the IRU was categorised for each of the pattern of disease groups. The unilateral group had an IRU region in the neck (66.7%) or thoracic inlet (33.3%). The cat with the bilaterally symmetrical disease had both regions of IRU in the neck. Most cats in the bilateral asymmetrical group had both regions of IRU located in the neck (91.6%) and the remainder (8.4%) had one IRU in the neck and the other at the thoracic inlet. The multifocal disease group had IRU regions in the neck and thoracic inlet (11.1%); neck and thorax (55.5%); and neck, thoracic inlet and thorax (33.3%).

Nine cats had three or more regions of IRU and met the definition of ectopic IRU. One cat was excluded due to the presence of pulmonary metastasis, and therefore, eight (12.66%) cats had ectopic IRU. All ectopic tissue was located at the thoracic inlet (1 cat), within the thorax (4 cats) or both (3 cats). No ectopic tissue was in the lingual or sublingual region.

Two (2.5%) cats had definite thyroid carcinoma. One cat had definitive thyroid scintigraphy features of thyroid carcinoma as defined by the presence of pulmonary metastasis. The IRU representing the thyroid glands was multifocal and extended from the neck, through the thoracic inlet and into the thorax. It was heterogeneous in appearance, lobulated and ill-defined in shape with smooth margins. The second cat did not have thyroid scintigraphy features classified as suspicious of thyroid carcinoma and was diagnosed by histopathology. The thyroid scintigraphy features of this cat were three regions of IRU: two in the neck were ovoid, homogeneous and smooth margined, and the third was a small ectopic region in the thorax. A third cat was classified as suspicious of thyroid carcinoma because there was multifocal pattern of disease with four regions of IRU that
included one region beyond the margins of the thyroid glands and located within the thorax with a heterogeneous appearance and ill-defined in shape with smooth margins. Histopathology was not performed.

5.2.2.3. Thyroid scintigraphy: objective measurements

The median and range of % TU was 10.46% (2.60–37.41%) and the median and range of T:SG was 6.47 (1.82–21.90).

5.2.2.4. Comparison of thyroid scintigraphy features of severely hyperthyroid cats with the general population of hyperthyroid cats

Patterns of disease

The distribution of the patterns of disease of the severely hyperthyroid cats was significantly different (chi-square = 37.29, df=3, \( P < 0.001 \)) when compared with the published data (Figure 5.2).\(^{44}\) This difference arose because unilateral and bilateral symmetrical disease were less common in severely hyperthyroid cats studied here, while multifocal and bilateral asymmetrical disease were more common.

![Patterns of disease](image)

Figure 5.2. Patterns of disease: general population of hyperthyroid cats (yellow) compared with the severely hyperthyroid cats (blue).

Thyroid scintigraphy: subjective features

Figure 5.3 illustrates the comparison of the subjective features with the published data.\(^{44}\) The proportion of cats with heterogeneous appearance of the regions of
IRU was significantly higher in the study population compared when compared to the reference population ($P < 0.001$). The proportion of cats with three or more regions of IRU and ectopic tissue were also both significantly higher in the study population compared to the reference population ($P = 0.003$ and $P = 0.001$, respectively). The location of the regions of IRU was not significantly different for the neck ($P = 0.413$), thoracic inlet ($P = 0.631$) and thorax ($P = 0.630$).

Figure 5.3. Comparison of subjective features and objective measurements of severely hyperthyroid cats (blue circles) against the published data (yellow dashes) as determined by the one proportion test.
5.3. Part 2: assessment of the influence of radioiodine dose calculation method on treatment outcome in severely hyperthyroid cats

5.3.1. Material and methods

5.3.1.1. Patient selection
The study was designed as a retrospective cohort study. All included cats had severe hyperthyroidism with total T4 > 190 nmol/L at the time of diagnosis or immediately before therapy. Total T4 concentrations were determined at U Vet or by the referring veterinarian. Depending on the method of analysis used, actual numerical values or set concentrations (total T4 > 190 nmol/L) were recorded.

The treatment group (customised dose group) of cats was selected from the 79 cats included in Part 1 of this study. These cats received an individual $^{131}$I dose that was calculated based on thyroid scintigraphy features and % TU. Dose calculation was at the discretion of the attending medicine clinician. A prerequisite to using calculated doses was that the cat had apparently benign thyroid disease, as determined by thyroid scintigraphy. The formulas used to calculate the actual individual $^{131}$I oral doses were as follows:

- unilateral homogeneous thyroids – dose = $(328 \times \%\ TU + 148) \times 1.25$
- bilateral symmetrical and homogeneous thyroids – dose = $(270 \times \%\ TU + 151) \times 1.25$
- bilateral asymmetrical or heterogenous thyroids – dose = $(388 \times \%\ TU + 144) \times 1.25$.

These doses were increased by 25% if the cat had previously received methimazole or carbimazole over $\geq$ 6 months.

The control group (standardised dose group) consisted of cats referred to U Vet between 2011 and 2015 for evaluation for treatment of feline hyperthyroidism with $^{131}$I therapy. Cats were identified by reviewing the hospital patient management system and the radiopharmaceutical log book. These cats did not have thyroid scintigraphy performed and were given a standard $^{131}$I dose.

All cats were treated per os with sodium iodide therapy capsules (ANSTO Health, Lucas Heights, NSW, Australia) according to the U Vet protocol. This
protocol required that cats had not received medical treatment for hyperthyroidism in the previous 7 days. Each cat in the standardised dose group received a capsule of 150 MBq as was measured just before dispatch from ANSTO. After dispatch, natural decay of $^{131}$I occurred. For the customised dose group, natural decay of $^{131}$I from dispatch to administration was estimated. The doses were prepared at ANSTO so that the actual dose at the time of administration was identical or very close to the calculated dose. The actual $^{131}$I dose each cat received was measured according to the U Vet protocol on the day of administration.

All cats studied had total T4 concentration measured between 4 and 11 weeks following $^{131}$I treatment, referred to later in this study as the post-treatment total T4 concentration. The earliest total T4 value was used in any cat with more than one total T4 measurement within this time frame. Treatment outcome was defined by the post-treatment total T4 concentration. A cat was considered persistently hyperthyroid if the total T4 concentration was > 60 nmol/L, euthyroid if the total T4 concentration was 15–60 nmol/L and potentially hypothyroid if the total T4 was < 15 nmol/L.

The following patient data was collated from the patient records or by contacting referring veterinarians: signalment (age, breed, sex), date of diagnosis, total T4 concentration at diagnosis and following $^{131}$I treatment, previous medical treatment with carbimazole or methimazole, other treatments for hyperthyroidism and actual $^{131}$I dose.

5.3.1.2. Statistics
The statistical analyses were performed using Minitab version 18 (State College, PA, USA) and SPSS (IBM Corporation, Armonk, New York, USA). The Fisher’s exact test and Pearson’s chi-squared test of independence were used to identify differences in proportions between cat breed and sex, respectively. Medians and ranges were calculated for age groups and pre- and post-treatment total T4 concentrations, because data was not normally distributed when reviewing histograms. The Wilcoxon rank-sum test was used to identify potential differences between groups based on the age of the cats, pre- treatment total T4 concentrations and $^{131}$I doses that cats received. Only cats with numerical values for pre- and post-treatment total T4 concentrations or ages were included in this analysis. The Pearson’s chi-squared test of independence was used to assess the
response to treatment between the customised and standardised dose groups. The null hypothesis was that treatment outcome (hyperthyroid, euthyroid or potentially hypothyroid) was not dependent on the method of dose calculation. Associations were considered significant at $P < 0.05$.

5.3.2. Results

5.3.2.1. Patient data

Forty-nine cats met the selection criteria for the customised dose group. Fifty of the 79 severely hyperthyroid cats had total T4 concentration measurements available following $^{131}$I treatment, but one cat was found to have thyroid carcinoma and pulmonary metastasis on thyroid scintigraphy and was excluded.

The median age of the 47 cats with known ages was 12.1 years (range 5–15.3 years). Two (4%) cats were strays with unknown ages. Thirty-seven (75.6%) cats were domestic shorthairs, 5 (10.2%) cats were domestic medium haired, 5 (10.2%) cats were domestic long-haired, 1 (2.0%) cat was a Chinchilla and 1 (2.0%) cat was a Russian blue cross. Twenty-eight (57.1%) cats were spayed females and 21 (42.9%) cats were castrated males. Forty-seven (95.9%) cats had previous medical management and no cat had prior thyroid surgery. Forty-one (83.7%) cats had a total T4 concentration performed at U Vet immediately prior to the thyroid scintigraphy, 7 (14.3%) cats had numerical values provided by the referring veterinarian and one cat had total T4 > 190 nmol/L. The median total T4 concentration of 42 cats that had T4 assessed at U Vet immediately before thyroid scintigraphy was 294 nmol/L (range 44–742 nmol/L). The cats that had a total T4 concentration <190 nmol/L documented immediately before thyroid scintigraphy had total T4 concentrations >190 nmol/L recorded in the months prior at the referring veterinarians. The median of the % TU and T:SG of all 49 cats were 9.94% (range 2.65–29.79) and 6.15 (range 1.87–21.9), respectively. All cats had a post-treatment total T4 concentration measured; 37 cats had a numerical value with a median of 16 nmol/L (range 6.5–87 nmol/L). Twelve cats had total T4 concentrations < 10 nmol/L. Appendix 1 displays the raw data.

Nineteen cats met the selection criteria for the standardised dose group. The median age of the 18 cats with known ages was 11.8 years (7.4–15 years), and 1 cat was a stray with unknown age. Eighteen cats were domestic short-haired
(94.7%) and 1 (5.3%) cat was a Russian blue cross. Thirteen (68.4%) cats were spayed females and 6 (31.6%) cats were castrated males. Sixteen (84%) cats had previous medical management and no cat had prior thyroid surgery. Seven (36.8%) cats had a numerical value for the total T4 concentration, with a median of 199 nmol/L (range 193–330 nmol/L). The remaining 12 (63.15%) cats had total T4 > 190 nmol/L. All cats had a post-treatment total T4 concentrations measured; 14 cats had a post-treatment numerical value with a median of 28 nmol/L (range 17–200 nmol/L), one cat had a total T4 value > 129 nmol/L and three cats had a total T4 value < 6.5 nmol/L. Appendix 2 displays the raw data.

There was no significant difference between the customised and standard dose groups for median age ($P = 0.21$), domestic or purebred ($P = 1$), and sex ($P = 0.43$). The customised dose group had a significantly higher pre-treatment total T4 concentration ($P = 0.019$). The customised dose group received a median $^{131}\text{I}$ dose of 220 MBq (range 166–315 MBq) and the standardised dose group received a median $^{131}\text{I}$ dose of 131.6 MBq (range 126.2–139.2 MBq). The customised dose group received a significantly higher $^{131}\text{I}$ dose compared to the standardised dose group ($P<0.001$).

### 5.3.2.2. Treatment outcome

Following $^{131}\text{I}$ treatment, 27 (39.7%) of the hyperthyroid cats became euthyroid: 16 (32.7%) in the customised dose group and 11 (57.8%) in the standardised dose groups. Hyperthyroidism persisted in 4 (8.1%) cats in the customised dose group compared with 4 (21.1%) cats in the standardised dose group. Total T4 concentrations below the reference interval were seen in 29 (59.2%) cats in the customised dose compared with 4 (21.1%) cats in the standardised dose group.

There was a significant difference in the treatment outcome between the customised and standardised dose groups (chi-square= 8.232, df = 2, $P < 0.016$). This effect was due to fewer of the cats receiving the standardised dose having a post-treatment total T4 < 15 nmol/L.

### 5.4. Discussion

The comparison of thyroid scintigraphy features of severely hyperthyroid cats identified differences with the general population of hyperthyroid cats and
included features that affect treatment decisions. There was also a difference in the 4 weeks following $^{131}$I treatment due to fewer of the hyperthyroid cats receiving the standardised dose becoming hypothyroid.

Part 1 of the study was the first evaluation of thyroid scintigraphy features in severely hyperthyroid Australian cats. The benefit of thyroid scintigraphy is the direct visualisation of the hyperthyroid tissue, which confirms hyperthyroidism and the appearance and location of the abnormal thyroid tissue.\textsuperscript{64, 111} The appearance of IRU in this study was consistent with descriptions of the subjective features of hyperthyroidism including smooth margins, increased intensity relative to the uptake of zygomatic/molar salivary glands and elongated shape. There were also some variations including irregular margins, decreased intensity and lobular shapes.\textsuperscript{64, 111} This study of Australian hyperthyroid cats did not include any cats with previous thyroidectomy and thus the reason for presentation is similar to the general population of hyperthyroid cats in the USA.\textsuperscript{44} The situation is different in the UK, where 23% of hyperthyroid cats present with a history of previous thyroidectomy resulting in a greater expectation of ectopic IRU on thyroid scintigraphy.\textsuperscript{43}

This study also compared the features of severely hyperthyroid cats to the features seen in the general population of hyperthyroid cats. The differences in the proportions of unilateral, bilateral symmetrical and bilateral asymmetrical disease may be explained by differences in severity of disease and may not affect treatment decisions. The greater proportion of multifocal disease in severely hyperthyroid cats is noteworthy because this pattern is seen in both ectopic disease and the suspicion of thyroid carcinoma. This study found a significantly higher proportion of cats with ectopic tissue in the severely hyperthyroid group, suggesting that thyroid scintigraphy should be performed in severely hyperthyroid cats under consideration for treatment with thyroidectomy because surgery would not be curative in these cats.\textsuperscript{43} This study also found a significantly higher proportion of a heterogeneous appearance to the IRU compared to the reference population. Particularly cats in the multifocal group were affected, which raises the suspicion of thyroid carcinoma in these cats\textsuperscript{64} and supports the notion that thyroid scintigraphy may assist to identify possible thyroid carcinoma. Two (2.5%) cats had definitive evidence of thyroid carcinoma, which is consistent previous studies.\textsuperscript{88, 110} One cat had pulmonary metastasis, which is a definitive
thyroid scintigraphy feature, and the second cat was diagnosed by histopathology. The difference in the thyroid scintigraphy features of the thyroid glands in these two cats illustrates the inability of thyroid scintigraphy to definitively identify thyroid carcinoma.43, 64

There are limitations to this descriptive part of the study. The images were acquired using a low-energy all-purpose collimator rather than the ideal scenario of both low-energy all-purpose and pin-hole collimators. The low-energy all-purpose collimator has reduced resolution and thus the patterns of disease categorisation may be altered with the high resolution of the pin-hole collimator. However, the collimator used was consistent for the comparison study as the published data from the general population of hyperthyroid cats was also acquired with a low-energy all-purpose collimator.44 Additionally, comparing two populations of hyperthyroid cats retrospectively has limitations. The two populations may differ in age, breed, sex and previous treatment. These parameters could not be matched. Cats were also diagnosed in different facilities, with thyroid scintigraphy images acquired using independent protocols and over different time frames. These factors may have influenced the results of the comparative assessment.

Part 2 of the study evaluated cats following $^{131}$I treatment. The results suggest that the method of dose calculation matters when considering outcome. The study found that cats receiving a standardised dose significantly less often had total T4 concentrations < 15 nmol/L following treatment. Thus, there were significantly fewer potentially hypothyroid cats in the immediate post-treatment period in this group.

Iatrogenic hypothyroidism is recognised as a complication of $^{131}$I treatment and may be permanent or transient. Originally, permanent iatrogenic hypothyroidism was considered an uncommon occurrence, rarely causing clinical signs that required treatment.79 Recently, iatrogenic hypothyroidism and subclinical iatrogenic hypothyroidism have been found to be more common than originally thought.28, 79 Cats with bilateral thyroid disease are twice as likely to develop permanent iatrogenic hypothyroidism than cats with unilateral disease.108 In the bilateral disease pattern, more of the normal thyroid tissue is in proximity to the hyperthyroid tissue and is therefore susceptible to destruction from the beta radiation.28 Thyroid scintigraphy was not performed in cats in the standardised
dose group, and therefore, this study could not evaluate whether disease pattern influenced treatment outcome. It is possible that bilateral disease was more common in cats receiving the customised dose, although Part 1 of this study indicates that bilateral disease is generally common in cats with total T4 concentrations >190 nmol/L. This concurs with the literature\textsuperscript{44} and suggests that the majority of cats in both groups suffered from bilateral disease.

Transient hypothyroidism is common in the immediate post-treatment period.\textsuperscript{79} This is due to normal thyroid tissue becoming atrophied as a consequence of the negative feedback on thyroid-stimulating hormone by elevated thyroid hormones in the hyperthyroid state. Transient hypothyroidism typically resolves by 3–6 months following treatment.\textsuperscript{40} It is possible that the proportions of cats with transient hypothyroidism differed between groups assessed here, with cats in the customised dose group being affected more often.

The identification and potential treatment of iatrogenic hypothyroidism has recently gained attention in the literature.\textsuperscript{39} Hypothyroidism is associated with a reduction in renal glomerular filtration rate and may result in azotaemia. Cats that suffer from hypothyroidism and azotaemia have been shown to have shorter survival times than non-azotaemic cats.\textsuperscript{39} Previous studies do not differentiate between transient or permanent hypothyroidism, yet to avoid potential further renal damage iatrogenic hypothyroidism is best avoided.\textsuperscript{39} The challenge remains to diagnose iatrogenic hypothyroidism or subclinical hypothyroidism, both of which may not be associated with clinical signs. Weight gain and a decrease in activity, which are non-specific signs of hypothyroidism, are expected as hyperthyroidism resolves.\textsuperscript{28, 79} Lastly, diagnosis of hypothyroidism cannot rely on total T4 concentrations alone because total T4 concentrations below the reference interval may also be present in cats with non-thyroidal illness. Permanent iatrogenic hypothyroidism requires at least three of the following findings: low or low-normal total T4, free T4 or total T3 and elevated thyroid-stimulating hormone.\textsuperscript{28, 79} However, in transient hypothyroidism, thyroid-stimulating hormone may remain low for up to 3 months.\textsuperscript{39} In these cats, thyroid scintigraphy could be used to diagnose hypothyroidism.\textsuperscript{79}

This study only assessed total T4 concentrations in the immediate post-treatment period. Hence, it cannot be determined whether cats with a total T4 concentration < 15 nmol/L suffered from transient or permanent iatrogenic
hypothyroidism. In some cats, total T4 could have been low because of concurrent non-thyroidal illness. However, it is unlikely that non-thyroidal illness affected many cats with a total T4 below the reference interval because $^{131}$I therapy at U Vet is generally only performed in cats that are otherwise healthy, as determined by examination and review of recent blood and urine results.

Although a significantly higher proportion of cats in the customised dose group became potentially hypothyroid in the immediate post-treatment period, it cannot be concluded that giving a standardised dose is superior to giving a dose that was calculated based on thyroid scintigraphy features. Cats in the customised dose group had significantly higher total T4 concentrations before treatment than cats in the standardised dose group. Thus, cats in the customised dose group may have suffered from more severe disease. Despite this difference, the persistence of hyperthyroidism in the immediate post-treatment period was comparable between groups with 8% and 21% of cats being affected by persistent hyperthyroidism in the customised and standardised dose groups, respectively.

The proportion of persistently hyperthyroid cats in the customised dose group is comparable to proportions reported in the general population (5%), although persistent hyperthyroidism is more commonly seen in cats that suffer from more severe disease. Thus, dose calculation based on thyroid scintigraphy features warrants further evaluation. A recent study determined that % TU has the highest correlation with total T4 concentrations and predicts volume and metabolic activity of the thyroid gland in hyperthyroidism best. In humans, % TU is a good predictor of the resolution of hyperthyroidism using a fixed dose of $^{131}$I, though further differentiations between euthyroidism and hypothyroidism were not made. Another feature used in humans is the assessment of rapid $^{131}$I turnover, determined by the 4- to 24- hour uptake ratio. A rapid $^{131}$I turnover equates to shortened resident time in the thyroid gland and is a predictor of treatment failure defined as persistence of hyperthyroidism. The customised dose used in this study was based on the visual features and the % TU and this resulted in higher median $^{131}$I doses compared with the standardised dose group. While persistence of hyperthyroidism was uncommon, the calculated $^{131}$I dose appears to have over-compensated for the significantly greater median total T4 concentrations in the customised dose group, with 59.2% of those cats having post-treatment total T4 concentrations below the reference interval. Further
optimisation of the dose calculation based on thyroid scintigraphy features is needed to increase the proportion of cats that achieve euthyroidism in the immediate post-treatment period.

The oral route of $^{131}\text{I}$ administration is common in Australia but less common in other countries. The proportion of cats that achieved euthyroidism in this study (39.7%) was lower than that reported in other studies where $^{131}\text{I}$ was administered per os (75–87.5%). This could be because hypothyroidism was not considered as a negative treatment outcome or because the populations differed in severity of disease before treatment. Furthermore, the follow-up period differed between studies and the rate of euthyroidism in this study population may increase as transient hypothyroidism or persistent hyperthyroidism resolve in the 3–6 months following treatment.

There are limitations to this second part of the study. The timing of drug withdrawal prior to thyroid scintigraphy was kept reasonably short (approximately 7 days) in line with previous publications and to avoid exacerbation or recurrence of hyperthyroidism. However, in choosing this time frame, thyroid scintigraphy may have overestimated disease severity, because uptake of a radionuclide may be enhanced up to 14 days post methimazole withdrawal with a peak effect noticed between 4-9 days. This potential rebound effect could have influenced $^{131}\text{I}$ dose calculations in some cats and may have led to higher $^{131}\text{I}$ doses than needed in these cats. In turn, this could have led to a higher incidence of hypothyroidism in the customised dose population. The other study limitations are largely due to its retrospective nature. The post-treatment outcome measure of total T4 concentration is inadequate to confirm hypothyroidism and the post-treatment time point of approximately 4 weeks may include cats with transient hypothyroidism. Ideally, the cats should have been reassessed 3–6 months post treatment but follow-up at 4 weeks was chosen because it was anticipated that few cats would have had sufficient information available at 3–6 months following treatment. Even at approximately 4 weeks following treatment, total T4 concentrations were not available in many cats, which resulted in small group sizes for analyses. This reduces the power of this study. Further limitations of this study were that data was missing for some cats, the time span between measurement of pre-treatment and post-treatment total T4 concentration and $^{131}\text{I}$ treatment was not standardised, the method with which total T4 was measured
Diagnostic imaging in small animal endocrine disease

was not standardised and, due to the nature of the assays used, total T4 concentrations of many cats were not quantitated (i.e. they were recorded as > 190 nmol/L).

Thyroid scintigraphy is recommended in hyperthyroid cats with total T4 concentrations above 190 nmol/L because this population is more likely to have subjective features such as multifocal disease or heterogeneous appearance of the thyroid than the general hyperthyroid population. This in turn may influence decisions regarding further work up and treatment of hyperthyroidism. Treatment based on doses calculated using subjective thyroid scintigraphy features and % TU resulted in a greater proportion of cats with post-treatment total T4 concentrations below the reference interval compared with a group receiving a standardised dose, but it cannot be concluded that $^{131}$I treatment based on calculated doses is inferior to standard dose administration. The true value of dose calculation based on thyroid scintigraphy features remains to be determined and further optimisation of the dose calculation method used at U Vet is required to achieve a higher rate of euthyroidism in the immediate post-treatment period.
Chapter 6. Concluding remarks and future work

This study aimed to extend the knowledge regarding diagnostic imaging techniques commonly used in small animal medicine. The study focused on adrenal glands in dogs and thyroids in cats because these organs are associated with some of the most common endocrine disorders encountered in these species. At the same time, imaging plays an integral role in the diagnostic work up of canine adrenal and feline thyroid conditions.

Abdominal ultrasound is becoming more commonplace in general practice. Adrenal glands are challenging to examine and the examination is further complicated by image plane, direction of measurement and patient recumbency position. The purpose of this study was to assess whether recumbency position affects adrenal gland measurement. This study found that the CPT measurement acquired from the longitudinal image plane had the least variation between lateral and dorsal recumbency position for the left and right adrenal glands. This finding supports the notion that the CPT acquired from the longitudinal image plane is one of the most reliable measurements and should always be taken when the adrenal is objectively assessed, and validates the current reference ranges determined from dogs in dorsal, lateral and lateral oblique recumbency positions.

By comparing measurements taken from multiple recumbencies the project aimed to optimise the abdominal ultrasound examination of the adrenal gland. The data presented does not only clarify the role of recumbency on measurements, but also confirms that there is flexibility in recumbency position at the time of the ultrasound examination. This flexibility is of benefit to all veterinary practitioners, particularly those with less experience. Finally, even though recumbency does not markedly affect CPT measurement accuracy in the majority of cases, it must be noted that there are few dogs (<5%), particularly those weighing more than 12 kg, where CPT measurements may differ between recumbencies. Thus, if the first measurement is questionable, particularly if it
does not agree with the clinical picture or if examination of the dog was difficult, it is recommended to check the measurement in a different recumbency.

Future work could evaluate the CPT of the adrenal gland in normal dogs against the aorta within the same dog because the aorta has traditionally been used to generate a ratio against normal structures in abdominal ultrasound and echocardiography.\textsuperscript{118,119} This work could then be extended to dogs that have no evidence of hyperadrenocorticism but suffer a mild acute illness to determine the usefulness of a ratio against the aorta in a clinical setting by assessing the ratio at presentation and on recovery. Finally, the effect of recumbency on the CPT of adrenal glands in dogs with hyperadrenocorticism could be assessed.

Nuclear medicine facilities are less available to veterinary practitioners, but thyroid scintigraphy has a role in the evaluation of hyperthyroid cats and the findings influence treatment options.\textsuperscript{4,28} This study found that some thyroid scintigraphy features in severely hyperthyroid cats differ from the general population of hyperthyroid cats. Of interest are the presence of ectopic hyperthyroid tissue and the heterogeneous appearance of the regions of IRU because these findings affect treatment decisions and increase the suspicion of thyroid carcinoma, respectively. Thyroid scintigraphy should be considered in severely hyperthyroid cats. The limitation in this comparative study of thyroid scintigraphy features was that the two groups of cats came from different populations. Future work comparing cats from the same population could overcome these differences such as age, breed and sex, previous treatment, the time frames of the study and differences in thyroid scintigraphy facilities and protocols.

The treatment objective of \textsuperscript{131}I is to restore euthyroidism after one treatment without inducing hypothyroidism. However, the optimal \textsuperscript{131}I dosing regime remains to be determined. This study assessed the initial treatment outcome in hyperthyroid cats with total T4 > 190 nmol/L, comparing a customised dosing regime calculated using thyroid scintigraphy to a standard dosing regime retrospectively. The finding that significantly fewer cats receiving the standard dose had total T4 concentrations below the reference interval warrants further evaluation. In particular, it is suggested that further evaluation and optimisation of the dose calculation method used at U Vet is indicated in order to achieve a higher rate of euthyroidism in the immediate post treatment period. However, this study
Diagnostic imaging in small animal endocrine disease

was performed retrospectively and, among other limitations, cases in respective groups could not be matched. Furthermore, the study only evaluated the immediate post-treatment outcome. Ideally, findings should be confirmed in a prospective randomised study that includes evaluations of the cats at 3 and 6 months post-treatment because transient hypothyroidism that may occur after $^{131}$I treatment should have resolved.\(^{28,79}\) Additionally, any cats with total T4 concentrations persistently below the reference interval require more vigorous evaluation for permanent iatrogenic hypothyroidism with concurrent measurement of free T4, total T3 and thyroid-stimulating hormone concentrations of thyroid scintigraphy to assess the prognostic impact of hypothyroidism after $^{131}$I. Future work evaluating dose comparison methods should include thyroid scintigraphy in both the customised and standardised dose groups, which would further clarify the impact of % TU in the $^{131}$I dose calculation.

The objectives of this project were to evaluate the effect of patient recumbency position on ultrasound derived adrenal gland measurements and to explore the role of thyroid scintigraphy in the diagnosis and treatment of severely hyperthyroid cats, and these objectives were achieved. Thus, the results of this project help to optimise the technique of adrenal ultrasonography and contribute the knowledge regarding thyroid scintigraphy in diagnosis and treatment of severe feline hyperthyroidism.
Diagnostic imaging in small animal endocrine disease

References


Diagnostic imaging in small animal endocrine disease


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Diagnostic imaging in small animal endocrine disease


Appendices

Appendix 1. Customised dose group: raw data

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## Appendix 2. Standardised dose group: raw data

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<td>20</td>
<td>128.5</td>
</tr>
<tr>
<td>19</td>
<td>&gt;190</td>
<td>25</td>
<td>130.8</td>
</tr>
<tr>
<td>Median and range</td>
<td>(193 – 330)*</td>
<td>(17- 200)*</td>
<td>(126.2 – 139.2)</td>
</tr>
</tbody>
</table>

*Median and range only included cats with numerical value
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Title:
The role of diagnostic imaging in small animal endocrine disease

Date:
2018

Persistent Link:
http://hdl.handle.net/11343/216875

File Description:
Thesis The role of diagnostic imaging in small animal endocrine disease

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