Evaluation of Thyroid to Background Ratios in Hyperthyroid Cats

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Abstract

Hyperthyroidism is the most common feline endocrinopathy. ^{131}I is the treatment of choice, and over 50,000 cats have been treated using an empirical fixed dose. Better treatment responses could be achieved by tailoring the dose based on the severity of disease. Scintigraphy is the best method to quantify the severity of the disease. Previously established scintigraphic quantitative methods, thyroid to salivary ratio (T:S ratio) and % dose uptake, are the most widely recognized measurements. Recently, the thyroid to background ratio (T:B ratio) has been proposed as an alternate method to assess function and predict ^{131}I treatment response. The purpose of this study was to determine the best location of a background ROI, which should be reflective of blood pool activity. We also hypothesized that the T:B ratio using the determined background ROI would provide improved correlation to T_4 when compared to T:S ratio and % dose uptake in hyperthyroid cats.

Fifty-six hyperthyroid cats were enrolled. T_4 was used as the standard measure of thyroid function and was obtained prior to thyroid scintigraphy and ^{131}I therapy. Blood samples were collected at the time of scintigraphy and radioactivity within the sample was measured. The plasma radioactivity was compared to the background ROI count densities in 8 anatomic regions using linear regression analysis for 55 cats. One cat was excluded from the study because of an injection error during scintigraphy. T:B and T:S ratios, and % dose uptake on scintigraphy were then compared to serum T_4 by linear regression analysis for 39 cats. Sixteen cats were excluded because of recent methimazole or Y/D diet use, or incomplete data.

The heart ROI correlated best to plasma pertechnetate activity ($r = 0.70$). % dose uptake correlated best to serum T_4 ($r = 0.74$), followed by T:S ratio ($r = 0.66$), followed by the T:B ratio using the heart ROI ($r = 0.59$).

Placing an ROI over the heart is the best method of quantifying plasma radioactivity. T:B ratio using the heart ROI as the background is a good predictor T_4 but percent dose uptake and T:S ratio proved to be better predictors of T_4 than any of the T:B ratios. Therefore, our hypothesis was not supported. The T:B ratio may not provide the best scintigraphic measurement of thyroid function. Hence it is unlikely to accurately predict treatment response to ^{131}I therapy.

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LIST OF ABBREVIATIONS/SYMBOLS

cpm	counts per minute
ROI	region of interest
ROIs	regions of interest
T:B	thyroid to background ratio
T:S	thyroid to salivary ratio
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone; thyrotropin
% dose uptake	percent dose uptake

CHAPTER 1: LITERATURE REVIEW

A. Prevalence and causes of hyperthyroidism

Feline hyperthyroidism was first reported in the literature in 1979. (Peterson 1979)However, earlier reports identified thyroid adenomas as rare entities with Huguenin demonstrating a prevalence of 0.1% in post-mortem exams of 3,000 cats reported at the international conference in Berne in 1927. (Peterson 2006) Since that time the disease had been sporadically reported with gradually increasing prevalence. Alarmingly, over the last 25 years it has become widely recognized as the most common endocrinopathy of domestic cats, with The Animal Medical Center in New York recording 22 cases of feline hyperthyroidism per month in the early 1990's (Peterson 2006). Reports of feline hyperthyroidism are widespread around the globe with incidence rates in Japan around 9 % and nearing 12% in the UK. (Peterson 2012)

The increased reporting of feline hyperthyroid cases could be due in part to the increased awareness and improved access to veterinary care. However, several risk factors have been identified and it is accepted by most that feline hyperthyroidism is a multifactorial disease with environmental, dietary, and genetic contributions. (Mooney 2002, Gunn-Moore 2005) (Peterson and Ward 2007) (Peterson 2012, Scott-Moncrieff 2012) (Peterson 2012) Exposure to goitrogenic compounds through the diet or the environment has been studied extensively with associations made between feline hyperthyroidism and canned cat food, flea control products, lawn care products, use of cat litter, iodine excess or deficiency, selenium excess, increased consumption of soy products, phalates, polybrominated diphenyl ethers, and bisphenols. (Edinboro, Scott-Moncrieff et al. 2004, Scott-Moncrieff 2012, Peterson 2013) (Mensching, Slater et al.

2012) (Norrgran, Jones et al. 2012) (Peterson and Ward 2007)While these entities alone may not have a direct causal relationship to the development of hyperthyroidism, a synergistic or compounding effect has been proposed. (Peterson 2012)Additionally, epidemiologic studies indicate that it is predominantly a disease of older cats with a mean age of 13 years. (Scott-Moncrieff 2012) It has been estimated that greater than 10% of all geriatric cats will suffer from the disease. (Peterson 2012) Genetically speaking, purebred cats seem to be at decreased risk to develop the disease. (Scott-Moncrieff 2012) Genetic mutations in the TSH receptor or associated G-proteins have been implicated as genetic causes. (Peterson and Ward 2007)

B. Thyroid physiology

The thyroid gland, in it's normal state, functions under the control of the hypothalamic-pituitary-thyroid axis, where by the hypothalamus secretes thyrotropin releasing hormone, which acts on the thyrotroph cells of the pituitary gland to release thyroid stimulating hormone (thyrotropin). At the level of the thyroid follicular cells, TSH is responsible for activating pathways leading to iodide uptake and thyroid hormone synthesis and release. TSH stimulates the Na/I symporter in the thyroid follicular cell membrane to actively trap and concentrate circulating iodide above plasma levels. (Barrett 2003) (Dadachova and Carrasco 2004)This allows the concentration of intracellular iodide, which is needed for thyroid hormone synthesis. This trapping mechanism is the first of two major steps in the production of thyroid hormone synthesis. Due to its valence and size, pertechnetate undergoes the same trapping mechanism as iodide. Pertechnetate is concentrated in the follicular thyroid cells but it does not undergo

the second major step of organification to form thyroid hormone. This trapping mechanism is proportional to the metabolic activity of the thyroid gland. Therefore, the uptake of pertechnetate or radioiodine as measured with scintigraphy is analogous to the metabolic activity of the thyroid gland. (Nieckarz and Daniel 2001) Iodide once trapped by the thyroid gland is oxidized to iodine by the enzyme thyroid peroxidase. (Barrett 2003)At this point oxidized iodine binds with the tyrosyl groups of the thyroglobulin molecule in the process of organification. Following organification the iodotyrosine molecules are coupled with one another to form the major thyroid hormones commonly known as T_4 and T_3. (Barrett 2003) (Broome 2006, Daniel 2006) Dietary iodine or radioiodine are all handled by the thyroid gland in the same manner; that is, trapped, organified, stored, and released in the form of thyroid hormone. Pertechnetate on the other hand is released from the thyroid gland following trapping and is excreted by the kidneys. It is neither organified nor stored by the thyroid gland. (Peterson and Becker 1984) (Daniel 2006) (Datz 1993, Thrall 1995) In health, the hypothalamic-pituitary-thyroid axis is under negative feedback control. When adequate circulating levels of T_4/T_3 are achieved, this negative feedback mechanism results in reduction of TSH directly and indirectly through reduction in TRH. Since TSH is the driving force for iodine uptake and thyroid hormone synthesis, this results in reduced thyroid hormone production.

In cats the dominant secretory output of the thyroid gland is T_4. Much smaller amounts of T_3 are produced by the thyroid glands. In fact, the majority of T_3 in circulation is produced from the conversion of T_4 by the peripheral tissues and not the thyroid. This results in similar intracellular concentrations of T_4 and T3.(Barrett 2003)T_3

is approximately 3-5 times more potent than T_4 at binding to and activating the thyroid hormone receptor. (Mooney 2010) In feline hyperthyroidism, the abnormal follicular thyroid cells function autonomously to concentrate iodide, and produce and secrete thyroid hormone in the absence of TRH and TSH, which have been suppressed based on the negative feedback mechanisms described above. The Na/I symporter is also expressed in the salivary glands, gastric mucosa, and in lactating mammary tissue. (Dadachova and Carrasco 2004) It is this expression, which allows uptake of iodine and pertechnetate and thus visualization of the salivary glands and stomach on scintigraphic images.

C. Systemic manifestations of disease

Systemic manifestations of the disease are gradually progressive and involve many homeostatic functions and most organ systems throughout the body. Clinical disease is most often recognized due to involvement of the cardiovascular, respiratory, renal, and gastrointestinal systems, as well as pathways of metabolism. Commonly, weight loss in the face of polyphagia, is noted by the owner or veterinarian. Additional clinical signs may be present such as polyuria/polydipsia, vomiting/diarrhea, tachypnea/dyspnea, heart murmur, gallop rhythm, tachycardia, systemic hypertension, hyperactivity, changes to the hair coat, and heat and stress intolerance. (Mooney 2010) (Syme 2007, Feldman c 2004) (Peterson 2013)Renal manifestations of thyroid disease are of particular importance.

It has been demonstrated that elevated thyroid hormones can lead to increased cardiac output resulting in increased renal blood flow and increased glomerular filtration.

(Syme 2007) (Adams, Daniel et al. 1997) (van Hoek and Daminet 2009) (Singh, Sharma et al. 1994) The role of thyroid hormone in progression of naturally occurring renal disease is not well understood. However, this relationship has been investigated with experimental kidney disease in rats; thyroidectomy has been shown to slow the progression of experimentally induced renal disease in rats(Tomford, Karlinsky et al. 1981). Renal tubules can be damaged due to hypertrophy and hyperplasia of the tubules seen in the hyperthyroid state. (van Hoek and Daminet 2009)This is thought to have some contribution to progression of renal disease.

Treatment for hyperthyroidism and alleviation of the hyperthyroid state, regardless of treatment type, results in reduction of glomerular filtration rate. The reduction in GFR can result in unmasking of underlying renal disease. The treatment itself does not harm the kidneys.(Adams, Daniel et al. 1997, Adams, Daniel et al. 1997) Demonstrating adequate renal function in the euthyroid state is recommended by some clinicians prior to permanent ablation of the disease with radioiodine treatment. Some clients may not choose the high initial cost of radioiodine therapy in an animal with markedly reduced renal function given the decreased survival time when compared to cats without renal disease and may elect medical management, as it is more cost effective in the short term.

D. Diagnosis

The diagnosis of hyperthyroidism in geriatric cats is based on clinical signs, physical examination, and elevated serum total T_4. (Kurosad, Popiel et al. 2006, Mardell 2013) Some early manifestations of the disease and daily fluctuations in T_4 can result in

T_4 levels within the laboratory reference range and further testing is needed in these cases. (Peterson, Graves et al. 1987) The serum T_3 concentration usually parallels serum T_4. However, in up to 30% of hyperthyroid cats, total T_3 is within the reference range and up to 10% of hyperthyroid cats have a total T_4 within the reference range. (Peterson, Melian et al. 2001) Free T_4 is more sensitive but can be elevated in cats with non-thyroidal illness. (Peterson, Melian et al. 2001) (McLoughlin, Dibartola et al. 1993) The T_3 suppression test and TRH stimulation test can be used to establish a diagnosis in equivocal cases. Thyroid scintigraphy is another test, which provides functional/physiologic information about the thyroid as well as morphologic detail. In some instances this test can assist with discrimination between benign and malignant disease. In cases with equivocal thyroid hormone levels, scintigraphy can accurately diagnose the presence or absence of hyperthyroidism.

E. Classification of disease

Hyperthyroidism tends to involve both thyroid lobes in approximately 70% of cases. (Peterson and Ward 2007)A recent large study involving scintigraphy of 917 cats identified 65% of the cases with bilateral thyroid lobe involvement, 30% with unilateral involvement, and nearly 5% with multiple areas of ectopic tissue.(Peterson and Broome 2012) This common bilateral involvement may have important implications for treatment outcome, as cats with bilateral involvement are twice as likely to become hypothyroid following radioactive iodine therapy. (Nykamp, Dykes et al. 2005)From a pathologic standpoint, functional adenomatous hyperplasia (adenoma) affects the thyroid gland in the vast majority of cases (95%) with thyroid carcinoma seen far less often (<5%). Three

classifications of benign disease have been histopathologically described, multinodular adenoma, adenomatous hyperplasia, and benign adenoma, and it is not currently well understood if these 3 benign processes are distinct entities or variation in the terminology used and representative of a similar process. (Feldman c 2004) However, these processes have similar biological behavior. Recent research suggests that long-standing hyperthyroidism with large lesions and very high T_4 values can be a clinical feature seen in cats with suspected malignancy, although this was not compared to cats with suspected benign disease. Histopathology was not performed in this study. However, the criteria for classification as a carcinoma included many of the scintigraphic characteristics of malignancy and a small number of cats (2/35) demonstrated radionuclide uptake to regional lymph nodes on scintigraphy, thus providing substantial evidence in these limited cases (Peterson 2014), although scintigraphy cannot reliably distinguish between benign and malignant disease. This development could suggest malignant transformation. Carcinoma in cats although rare, tends to be aggressive with a high metastatic rate. Metastasis is more common to the regional lymph nodes in cats than to the lungs as seen in dogs. (Barber 2007)

F. Treatment

The goal of therapy for hyperthyroidism is to restore thyroid function to the euthyroid state. Therapies generally involve medical/pharmacologic management, surgical thyroidectomy, iodine restricted diet, and/or radioiodine treatment. Medical management often involves the use of methimazole administered orally or transdermally. Methimazole prevents thyroid hormone synthesis by inhibiting thyroid peroxidase, an

enzyme essential in the pathway of thyroid hormone production. (Trepanier 2007) Methimazole does not reduce the goiter size or eradicate abnormal thyroidal tissue. In fact, autonomously functioning thyroid cells continue to proliferate during methimazole use. Additionally, the medication must be administered at least daily. Side effects of methimazole include gastrointestinal upset, blood dyscrasias, coagulation abnormalities, hepatotoxicity, and facial excoriation. Acquired myasthenia gravis has also been reported. (Trepanier 2007) The benefit of medical management is that it is reversible and can be more closely titrated to maintain adequate level of thyroid hormone. This is especially important in patients with significant underlying renal disease. Additionally, there is a lower initial cost when compared to surgical thyroidectomy and radioiodine therapy. It has been reported that the median survival time using methimazole treatment alone is 2 years. Median survival time is nearly doubled when radioiodine therapy is instituted and an approximately 5-year median survival time is achieved when methimazole is used in combination with radioiodine therapy. (Milner, Channell et al. 2006) Surgery and radioiodine treatment have the common goal of ablating diseased thyroidal tissue while preserving normal thyroidal tissue. At surgery, this is accomplished when presumably a small amount of normal atrophied tissue, ectopic tissue, or the unaffected lobe is left behind, which becomes functional over time. Some report that clinical hypothyroidism is a rare occurrence following surgery.(Mardell 2013),(Mooney 2010) While some clinical studies have reported that hypothyroidism is common following surgery. (Welches, Scavelli et al. 1989) Literature in this area is sparse and different outcomes may have to do with surgical technique, patient population, and bilateral versus unilateral disease. Hypothyroidism can occur in up to 30% of cats

following radioiodine therapy. Iatrogenic hypothyroidism is more likely to occur in patients with bilateral thyroid lesions. (Nykamp, Dykes et al. 2005)Bilateral surgical thyroidectomy can also lead to transient hypocalcemia with removal or disruption of the parathyroid glands. In one study of 86 cats having bilateral thyroidectomy, hypocalcemia occurred in 5 cats. (Naan, Kirpensteijn et al. 2006) Other risks associated with surgery such as hemorrhage or complications secondary to general anesthesia are also considered. Furthermore, one study evaluating 120 hyperthyroid cats identified 22% of cats having hyper functioning thyroidal tissue in an intrathoracic location(Harvey, Hibbert et al. 2009) or in an ectopic location in general and which would require a much more invasive surgery. Other larger studies have identified a lower number of cats (5 %) with ectopic tissue(Peterson 2014) and the author's definition of ectopic tissue may vary.

A prescription diet created by Hill's has recently come on the market for management of feline hyperthyroidism. The y/d diet is both an iodine restricted (Van der Kooij 2014) and iodine deficient (Wedekind, Blumer et al. 2010) diet that when fed exclusively reduces circulating thyroid hormone concentrations and ultimately resolves systemic manifestations of the disease. According to Hill's, the y/d diet contains 0.2 mg/ kg of iodine when fed as directed. In a large multi institutional study approximately 25% of cats remain hyperthyroid on the y/d diet. Additionally, this study did not evaluate side effects beyond 2 months of starting the diet and hypothyroidism is reported with this diet. (Van der Kooij 2014) The long-term effects of feeding a diet deficient in iodine regardless of thyroid state are not well understood. Indirectly, iodine deficiency may be a risk factor for development of feline hyperthyroidism. (Edinboro, Scott-Moncrieff et al. 2010) (Olczak, Jones et al. 2005, Edinboro, Pearce et al. 2013) This is also supported by

the prevalence of goiter seen throughout human history in geographic areas of iodine deficiency. Iodine restriction in humans has led to autonomously functioning goiter. It is conceivable that iodine restriction could create more areas of autonomously functioning thyroid tissue, which have the ability to undergo malignant transformation. In times of iodine deficiency, reduced circulating thyroid hormone results in increased TSH, which is essential to this pathogenesis. TSH acts on the thyroid follicular cells to result hyperplasia and hypertrophy, while increasing secretory activity of the follicular thyroid cells.(Hall 2011) Additionally, hyper functioning tissue will continue to grow in the face of iodine restriction. The length of T_4 suppression following discontinuance of the diet has not been widely reported. Therefore, its impact on interpretation of scintigraphy relative to serum T_4 values as well as the impact of diet use prior to radioiodine therapy is currently unknown.

G. Iatrogenic hypothyroidism and post treatment azotemia

The down side to radioiodine therapy is the high initial cost of therapy, availability, and length of hospitalization. However, given the side effects of methimazole use, decreased survival time with methimazole when compared to radioiodine, and the large number of cats remaining hyperthyroid on dietary management alone, radioiodine therapy is currently recognized as the most practical therapeutic choice where available, and may be the most efficacious with the fewest side effects. Radioiodine therapy is highly effective in resolving hyperthyroidism with a cure rate of nearly 88-98%. (Slater, Komkov et al. 1994, Peterson and Becker 1995, Forrest, Baty et al. 1996, Peterson 2006) (Wallack, Metcalf et al. 2010) It is this high cure rate that results

in the high rate of post-treatment hypothyroidism. Iatrogenic hypothyroidism is one of the most important and common side effects of radioiodine therapy and is becoming a more recognized entity in cats undergoing radioiodine ablation. Approximately, 7-30% of cats become biochemically hypothyroid following radioiodine therapy.(Slater, Komkov et al. 1994, Chun, Garrett et al. 2002, Nykamp, Dykes et al. 2005) (Wallack 2010) (Meric, Hawkins et al. 1986) While clinical signs of hypothyroidism occur in a much smaller proportion of cases, detrimental effects of subclinical hypothyroidism may be greater than previously thought (Peterson 2013) If so, levothyroxine supplementation may be beneficial in biochemical as well as clinical hypothyroidism. Additionally, cats with mild hypothyroidism may fail to develop the classic clinical signs associated with hypothyroidism. Signs of hypothyroidism in cats can be subtle and do not typically involve the signs reported much more commonly in dogs such as alopecia. Hypothyroid cats also may develop a reduced appetite, a sign not commonly see in dogs (Peterson 2013) Onset of hypothyroidism following radioiodine therapy can also be confused with the perceived normal or desired response of radioiodine therapy such as weight gain and decreased activity with resolution of hyperthyroidism. Therefore, recognizing which cats may benefit from thyroid hormone supplementation is not always possible, as there is little research available in this area. Furthermore, a relationship between iatrogenic hypothyroidism and renal azotemia with reduced survival times has recently been suggested. A two part study reports that iatrogenic hypothyroidism is associated with the development of azotemia and that following radioiodine therapy, iatrogenic hypothyroidism with azotemia, contributes to significantly reduced survival times when compared to patients in which euthyroid or non-azotemic hypothyroid states are

achieved. (Williams, Elliott et al. 2010) This study also demonstrated reduced survival time in azotemic hypothyroid cats compared to non-azotemic hypothyroid cats, whereas no difference in survival time between euthyroid azotemic and non-azotemic cats was demonstrated. This suggests that minimizing iatrogenic hypothyroidism may positively impact survival time following radioiodine therapy.

H. Methods to evaluate treatment response

Several methods have been used in an attempt to achieve a euthyroid response to radioiodine therapy, some with an effort to limit hypothyroidism. Many studies utilizing a modified fixed dosing scheme calculated the radioiodine dose based on scintigraphy. One study administered a modified fixed dose based on the volume of the thyroid lesion(s) identified on scintigraphy. However, this study found that using scintigraphy alone in patients with large thyroid lesions and/or extremely elevated serum T_4 was inadequate. (Forrest, Baty et al. 1996)This study did not evaluate cats for iatrogenic hypothyroidism. Another study of 524 cats utilized a scoring system based on severity of clinical signs, thyroid size based on digital palpation, and magnitude of serum T_4 elevation to calculate the radioiodine dose. This study achieved a response considered good in 94% of cats, with approximately only 2% requiring thyroid hormone supplementation despite 11-16% of the cats having a serum T_4 below the reference interval. (Peterson and Becker 1995) However, based on more recent literature the number of patients requiring thyroid hormone supplementation could be underestimated.(Peterson 2013) Another study found that high pretreatment serum T_4 levels and high scintigraphic T:S ratios did not correlate with serum T_4 levels at standard follow-up time points following radioiodine. (Chun,

Garrett et al. 2002) This study utilized a fixed dosing scheme and approximately 9% of cats required thyroid hormone supplementation following radioiodine therapy. It is important to recognize that in this study many of the cats were receiving methimazole within 10 days of scintigraphy and treatment. This could alter the correlation between scintigraphy and actual thyroid disease as well as response to radioiodine treatment. (Nieckarz and Daniel 2001) (Fischetti, DiBartola et al. 2005) Recently, a study evaluated a less widely used approach to predicting treatment response. In this study the scintigraphic T:B ratio was used to predict treatment response based on administration of a fixed radioiodine dose. This study demonstrated that cats with significantly higher T:B ratios were more likely to be persistently hyperthyroid (6/113 cats) following radioiodine therapy, whereas there was no significant difference in thyroid to salivary ratios seen amongst these cats. (Wallack, Metcalf et al. 2010)This study did not evaluate the development of iatrogenic hypothyroidism as a separate outcome from the euthyroid response. A follow up to this study utilized a scintigraphic T:B ratio cut off to direct modified fixed radioiodine dosing, and has been published only as an abstract. This study identified good treatment response with approximately 3-4% of cats requiring thyroid hormone supplementation for iatrogenic hypothyroidism and only approximately 3% of cats remaining hyperthyroid. However, this study allowed methimazole use within 5 days of scintigraphy and radioiodine treatment. It is also possible that of the reported 18% of cats exhibiting biochemical hypothyroidism, more could have benefited from thyroid hormone supplementation.(Wallack 2010)

With post-treatment hypothyroid rates up to 30%, most studies have not provided an optimal method for radioiodine dose determination in order to eliminate this

complication. While most cats are relatively similar in size, the thyroidal lesions may be vastly different in size, volume, vascular supply, and functional capacity. Therefore, this "one size fits all" fixed dosing scheme is likely overdosing a large number of patients contributing to the high rate of biochemical hypothyroidism identified and increasing need for thyroid hormone replacement. With recognition of the role of hypothyroidism on the metabolic state and its reduction in GFR, the utility of a radioiodine dose tailored to each patient based on the characteristics of the thyroid lesion could be revisited.

I. Scintigraphy and quantifying disease

Thyroid scintigraphy is most commonly performed using pertechnetate in practice due to its exquisite affinity for the thyroid gland creating excellent target to background for image purposes. Its short half-life allows for a short period of isolation following imaging. Additionally, the predominant 140 keV gamma emissions make it ideal for image capture using a gamma camera. Pertechnetate, when injected intravenously rapidly equilibrates in the vascular and extracellular fluid space. Due to its "pseudohalogen" (Daniel 2006)characteristics it is also concentrated above plasma levels via the sodium/iodine symporter of the thyroid follicular cells under the influence of TSH in the normal state. This process, known as trapping, is the first main step in the formation of thyroid hormone. In the hyperthyroid state autonomously functioning follicular cells of the thyroid act independent of TSH to concentrate pertechnetate or iodide above plasma levels and proportional to the severity of the disease. In contrast, non-diseased portions of the thyroid gland are suppressed and iodine/pertechnetate trapping is diminished due to the inherent absence of TSH related to previously discussed negative feedback

mechanisms associated with high circulating T_4. Ectopic hyperplastic or disseminated malignant hyper functioning thyroid tissue may also be identified on the images. Therefore, scintigraphy provides important morphologic and pathophysiologic information, which cannot be obtained from any other diagnostic method.

While thyroid scintigraphy is an accurate method to diagnose hyperthyroidism it also provides insight in the metabolic activity of the gland, which could be used to determine the optimal radioiodine dose for effective treatment. The most similar human counterpart to feline hyperthyroidism is toxic nodular goiter (Peterson and Ward 2007, Wakeling, Smith et al. 2007) In humans with toxic nodular goiter the scintigraphic characteristics such as size, volume, and tumor heterogeneity are used to aid radioiodine-dosing schemes. (Sarkar 2006) Three measurements are widely reported in the literature for scintigraphically quantifying thyroid disease. The T:S ratio is a simple reproducible method to quantify thyroid disease and has demonstrated good correlation with pre-treatment serum T_4 in cats. (Daniel, Sharp et al. 2002, Peterson 2006, Feeney and Anderson 2007, Shiel and Mooney 2007) Given the normal expression of the Na/I symporter under constitutive regulation in salivary tissue, (Portulano, Paroder-Belenitsky et al. 2014) the salivary gland is readily identifiable on an image and can be utilized as a standard for comparison with thyroidal radionuclide uptake. A ratio of scintigraphic count density in the thyroid gland is compared to scintigraphic count density in the salivary glands to generate the T:S ratio. In cats this uptake in the salivary glands is seen almost exclusively in the salivary glands of the soft palate, namely the zygomatic salivary glands.(Cohen 1959) However, this measurement assumes that the salivary gland is normal and predictable in every patient. Another technique, the percent dose uptake,

correlates with serum T_4, although this relationship may not be as strong as that for T:S ratio. (Mooney, Thoday et al. 1992, Daniel, Sharp et al. 2002)The percent dose uptake is more tedious to calculate, as corrections must be made for decay of the radionuclide, superimposed background activity on the image, and depth of thyroidal tissue, which may not be constant in every patient. With this method the amount of injected radionuclide is measured and the percentage of the injected dose concentrated by the thyroid gland is obtained from the scintigraphic image following corrections listed above. The third method, T:B ratio, is calculated from the scintigraphic image in a similar fashion to the T:S ratio. However, with this method, a region of interest is drawn most commonly in the axillary area to obtain background count density, instead of the salivary count density. The ratio is then created comparing thyroid count density to background count density on the image. The T:B ratio has demonstrated correlation to inadequate radioiodine treatment response, namely persistent hyperthyroidism. Additionally, in a recent study of 2096 hyperthyroid cats, there is significant correlation between T:B ratio and pre-treatment serum T4.(Peterson 2014) However, correlation to iatrogenic hypothyroidism after radioiodine administration for treatment of hyperthyroidism has not been investigated.

CHAPTER 2: EVALUATION OF THYROID TO BACKGROUND RATIOS IN HYPERTHYROID CATS

A. Introduction

The ability of the thyroid gland to concentrate pertechnetate above plasma levels is the fundamental principle for this study. The T:B ratio on a scintigraphic image should be a better predictor of true thyroid function than the previously evaluated T:S ratio because any variation in salivary function is eliminated from the equation. Additionally, T:B ratio should be the most accurate representation of the ability of the thyroid gland to concentrate pertechnetate and therefore iodide above plasma levels. Previous work has looked at T:B ratio to characterize thyroid function but found that it was more variable than T:S ratio. (Beck, Hornof et al. 1985) However, the low number of patients in this study could have had an impact on the results. As previously mentioned a more recent study has utilized T:B ratio to predict radioiodine treatment response. However, to the author's knowledge the background location chosen for calculation of T:B ratio has not been validated.

The goals of this study were to identify and validate a background region of interest, which is representative of background activity on a scintigraphic image using plasma pertechnetate determined at the time of imaging as the gold standard. Additionally, we hypothesized that the T:B ratio would provide better correlation to pre-treatment serum T_4 than T:S ratio and % dose uptake, and therefore more indicative of the true functional ability of the thyroid lesion. A secondary goal was to evaluate the treatment outcomes of cats treated with the traditional 4 mCi fixed dose radioiodine therapy.

B. Materials and Methods

Experimental Procedure

Cats with hyperthyroidism that were presented to the Veterinary Teaching Hospital (VTH) at the Virginia-Maryland Regional College of Veterinary Medicine for radioiodine treatment were eligible for study. Client consent was obtained in all cases. The research was approved by the Virginia Tech Animal Care and Use Committee. One day prior to imaging, physical examination, serum chemistries, serum T_4 and TSH concentrations, CBC, urinalysis, and non-invasive blood pressure were obtained for each patient. Each cat underwent standard thyroid scintigraphy with left and right lateral and ventral static images including the head, neck, and thorax obtained approximately 30 minutes following intravenous administration of a target dose of 3 mCi (111 MBq) of ^{99m}Tc as pertechnetate. Static frame-mode acquisitions were obtained for 60 seconds using a low energy all purpose (LEAP) collimator and the images stored in a 256 x 256 x 16 digital matrix. (Mirage Acquisition Application (PCI) Version 5.715f7b Copyright© 1997-2008, Segami Corporation, Columbia, MD). Inclusion criteria for the study consisted of a serum T_4 concentration above the reference interval, a serum TSH concentration below the limit of the detection of the assay, and increased uptake of pertechnetate by the thyroid gland on scintigraphic images (Peterson and Becker 1984, Beck, Hornof et al. 1985, Daniel and Berry 1991, Mooney, Thoday et al. 1992, Nap, Pollak et al. 1994, Lambrecht, Jordaan et al. 1997, Nieckarz and Daniel 2001, Daniel, Sharp et al. 2002, Henrikson, Armbrust et al. 2005, Broome 2006, Daniel 2006, Peterson and Broome 2012).

Prior to imaging, the pertechnetate dose was measured in a dose calibrator and a 60 second static image of the dose syringe was acquired by the gamma camera immediately prior to and following injection. The injected dose was calculated by subtracting the post-injection value from the pre-injection value for each patient.

Immediately prior to imaging, a blood sample was collected for determination of plasma pertechnetate radioactivity for each patient. The blood samples were collected, placed in an EDTA tube, centrifuged at 5000 x g for 5 minutes to allow separation of plasma, and plasma was harvested. The plasma samples were placed in pre-weighed plastic counting tubes and the volume of plasma was then calculated based on the density (weight/volume) of plasma. (Trudnowski RJ 1974) Weights were used to minimize sources of error created with small volumetric measurements.

The samples were then placed in a NaI well-counter interfaced with a multichannel analyzer to determine plasma radioactivity in counts per minute (cpm)/ml of plasma. The window of the pulse height analyzer was centered over the 140 keV photopeak of ^{99m}Tc.

To express the radioactivity of the plasma sample in μCi, the efficiency of the well-counter, in terms of cpm per μCi was determined each day using a standard of known amount of radioactivity. The radioactive standard was prepared by placing a known quantity of ^{99m}Tc (range 100-150 μCi), measured accurately in the dose calibrator, into a 100 ml volumetric flask. An aliquot was removed from the volumetric flask and placed into a pre-weighed counting tube as described above. Assuming the density of water was 1.0 g/mL, the volume of the aliquot from volumetric flask was determined. Knowing the amount of radioactivity placed in the volumetric flask and taking a specific

volume aliquot from the flask created a standard of known quantity of radioactivity. The standard was placed into the well counter, and radioactivity in terms of cpm was recorded. Efficiency of the well counter was determined by dividing the cpm by the μCi in the standard.

Equation 1: Efficiency of well counter

$$Efficiency\ of\ well\ counter = Counts\ per\ minute\ cpm\ of\ the\ standard\ /\ \mu Ci\ of\ the\ standard$$

The radioactivity of the plasma, expressed as of uCi per ml, was then calculated by dividing the plasma activity (cpm) by the efficiency of the well counter.

Equation 2: Activity in plasma

$$Activity\ in\ Plasma\ \mu Ci = cmp\ plamsa / (cmp\ standard / \mu Ci\ Standard)$$

Times of all sample collections and measurements were recorded and decay corrected using the standard decay equation for ^{99m}Tc.

Image Analysis:

Image analysis was performed using NucLear Mac software (Scientific Imaging, Inc., Crested Butte, CO). Six different background regions of interest (ROIs) were drawn on the ventral image of the head, neck, and thorax and labeled based on location as an area just caudal to either salivary, just cranial to the thyroid lesion, immediately adjacent to the thyroid on either side, either axillary region, centrally over the heart, and the paw

utilizing the forelimb without the IV catheter. The ROI adjacent to the thyroid was placed on the left or right side depending on which area had more separation from the thyroid lesion(s). Additionally, an ROI was drawn over the heart on the left and right lateral views of the thorax for a total of 8 background ROIs (Appendix A, figure 1).

Count density for each ROI was determined by dividing the total number of counts in each ROI by the total number of pixels in each ROI to obtain the counts/pixel. The count density of each of the background ROIs was compared to the plasma radioactivity in uCi/gram of plasma as determined above for each patient.

ROIs were also drawn around the thyroid glands and/or ectopic thyroid tissue and salivary glands (Appendix A, figure 3). Count densities were determined in a similar fashion for the thyroid glands and/or ectopic tissue and salivary glands. The count density ratios were used to determine the thyroid to salivary ratio (T:S ratio) for each patient based on the ventral image including the head, neck, and thorax as described by others (Beck, Hornof et al. 1985) (Daniel, Sharp et al. 2002). Count densities were used to eliminate the influence of ROI sizes and all ROIs were drawn by the same individual. Thyroid ROIs were hand drawn after adjusting the pixel intensity to a value of 80% of the maximum pixel intensity with an inverse color palate in order to facilitate repeatability, standardization, and accurate detection of thyroid and salivary uptake margins. The average T:S ratio and the most intense T:S ratio were determined in a similar fashion as were the average T:B ratio and most intense T:B ratio as described above using count densities. The average ratios were determined using the average count density in all thyroid tissue identified; whereas the most intense, or hottest ratios were determined by using the count density in the hottest or most intense of the two thyroid glands.

The % dose uptake was determined as previously described.(Adams, Daniel et al. 1997, Nieckarz and Daniel 2001) The dose administered to the patient was determined from the images of the pre and post injection dose syringe and expressed in counts per minute (cpm)

Equation 3: Dose in cpm

$$Dose\ in\ cpm = \ pre\ injection\ syring(cpm) - \ post\ injection\ syringe\ (cpm)$$

Regions of interest were drawn over the thyroid glands and any ectopic thyroidal tissue. The gross thyroid counts were determined from this ROI. These gross counts were background corrected. The net thyroid counts were derived by subtracting the background count density multiplied by the number of pixels in the thyroid ROI from the gross thyroid ROI counts.

Equation 4: Net counts

$$\text{Net Counts} = \text{Gross counts} - \left(\left(\frac{\text{BKD ROI counts}}{\text{Number of Pixels in BKD ROI}}\right) x \text{ number of pixels in thyroid ROI}\right)$$

For this purpose, background count density was determined from the adjacent thyroid ROI. Depth correction was then performed using a standard depth of 1.2 cm for thyroidal tissue in each cat and a linear attenuation coefficient for pertechnetate in soft tissue of 0.153 cm^{-1} of soft tissue.(Daniel 2006) This was performed to determine the net depth corrected thyroid activity using the following equation(Nieckarz and Daniel 2001), (Adams, Daniel et al. 1997).

Equation 5: Depth corrected thyroid counts

$$\text{Depth Corrected Thyroid Counts} = \frac{\text{Thyroid Counts}}{e^{-0.153\,x}}$$

Where x = thyroid depth (cm).

Equation 6: Percent dose uptake

The following formula was used to determine percent dose uptake of pertechnetate by the thyroid gland at each time interval.

$$\text{Percent Dose Uptake} = \frac{\text{Corrected Counts (cpm) in Thyroid}}{\text{Radioisotope Dose in cpm}} \text{ x } 100$$

Treatment and Patient Follow-up

Three days following imaging, patients were administered 4 mCi of.^{131}I subcutaneously. Serum T_4 and TSH concentrations, serum chemistries, and urinalyses were assessed 1, 3, and 6 months following treatment. Analyses were performed at the VTH using the same methodology as the pre-treatment evaluation. Post-treatment hypothyroidism was defined as a T_4 below the reference range for our laboratory and an elevated TSH at 3 and 6 months. (Williams, Elliott et al. 2010, Peterson 2013, Peterson 2013) Additionally, cats requiring thyroid supplementation at 6 months following therapy were considered hypothyroid. TSH was not taken into consideration at 1-month post treatment for determination of thyroid status. At 1 month, hypothyroidism was defined as a T_4 below the reference range. Cats having a T_4 above the reference range were considered hyperthyroid following radioiodine treatment. Renal azotemia was defined as a serum creatinine above the reference range and a urine specific gravity less than 1.035.

Statistical Analysis:

Statistical analysis was performed using a commercial statistical software program (SAS version 9.3 Cary, NC, USA). Using linear regression analysis, a regression coefficient was obtained for each background ROI to determine which background ROI was most representative of plasma pertechnetate values. Linear regression analysis was also used to determine regression coefficient between the intensity ratios (average and most intense T:S and average and most intense T:B for each background ROI) and percent dose uptake compared to pre-treatment serum T_4. Significance for all tests was set at $p \leq 0.05$.

C. Results

Background ROI correlation to plasma pertechnetate

The owners of all cats presenting to the Veterinary Teaching Hospital for radioiodine therapy were offered enrollment into the study until 55 cats were enrolled that underwent thyroid scans. Fifty-six cats were enrolled and one cat was excluded because of an injection error during administration of pertechnetate at the time of scintigraphy and therefore could not undergo a thyroid scan. However, post treatment blood work was collected and reported for this cat. All cats had a serum T_4 concentration (mean +/- SD = 137.58 +/- 62.97) above the reference range (16-37.7 nmol/L) and TSH concentration below the detectable limit of the assay (0.03 ng/mL), as well as increased thyroid uptake on scintigraphic images. The correlation coefficients comparing the plasma pertechnetate activity to the various background ROIs were ranked from highest to lowest (Appendix B, table 1). The background ROI utilizing the heart count density on

the left lateral image provided the highest correlation coefficient (Appendix A, figure 1C, figure 5; Appendix B, table 1). The background ROIs utilizing the cranial thyroid and the axillary count densities provided the poorest correlation to plasma pertechnetate (Appendix A, figure 1B, figure 10, figure 11; Appendix B, table 1). The adjacent thyroid ROI was not statistically significant.

Scintigraphic correlations to serum T_4

Data from 39 cats was available to compare the correlation coefficient of pre-treatment T_4 with T:S (average and most intense), T:B (average and most intense), and percent dose uptake on the scintigraphic images. Four cats were excluded because the upper limit of their serum T_4 exceeded the measured range of the assay before the method was changed to extend the measurable concentration by dilution. One cat was excluded because the injected pertechnetate dose was not measured. Cats were also excluded from this portion of the data analysis due to treatment with methimazole within two weeks of imaging (n = 5), and/or feeding an iodine restricted diet (Hill's y/d) prior to scintigraphy (n = 6). One cat included in those groups was excluded for both reasons. As an example of the effect of an iodine restricted diet on serum T_4 concentration and scintigraphy, one of the cats on the y/d diet had a T_4 at the time of initial diagnosis of 160.9 nmol/L (reference range: 10.3-60.5 nmol/L) and a T_4 after y/d diet, and at the time imaging and radioiodine treatment of 58.8 nmol/L (reference range: 16-37.7 nmol/L). This is the most conspicuous case demonstrated in this study and illustrates how the y/d diet would alter the normal homeostatic mechanism of the thyroid: pituitary axis.

A significant correlation with T_4 was identified utilizing the heart ROI on the ventral image for calculation of the average T:B ratio, and demonstrated the highest

regression coefficient of the 8 evaluated background ROIs (Appendix A, figure 2, figure 16; Appendix B, table 2). The heart on the left lateral view for calculation of the average T:B ratio provided a similar regression coefficient (Appendix A, figure 17; Appendix B, table 2). The average T:B ratio utilizing the paw ROI demonstrated the poorest correlation with T_4, of the background ROIs (Appendix A, figure 31; Appendix B, table 2). The average T:S ratio demonstrated a larger regression coefficient than any of the examined T:B ratios (Appendix A, figure 3, figure 14; Appendix B, table 2). Overall, the percent dose uptake provided the highest correlation with pre-treatment serum T_4 (Appendix A, figure 13; Appendix B, table 2). Therefore, our hypothesis that T:B would be a better predictor of pre-treatment serum T_4 is rejected.

Therapeutic Response

At one month following radioiodine therapy a serum T_4 value was received on 48 cats; 24/47 (51%) were hypothyroid, 16/47 (34%) were euthyroid, and 7/47 (15%) were persistently hyperthyroid (Appendix A, figure 32). Of the 24 cats that were hypothyroid based on T_4 alone, 5 had a low TSH, 8 had a normal TSH, and 11 had a high TSH. Of 40 with a chemistry and urinalysis 1 month following therapy, 7 (18%) had renal azotemia. Of these 7 cats with renal azotemia, 5 were hypothyroid and 2 were euthyroid. At 3 months following therapy 9/38 (24%) cats evaluated were hypothyroid, 23/38 (61%) were euthyroid, and 5/38 (13%) were hyperthyroid (Appendix A, figure 32). Twelve cats that were hypothyroid at 1 month were euthyroid at 3 months. Two cats that were hyperthyroid at 1 month were euthyroid and 4 were persistently hyperthyroid at 3 months. One cat that was euthyroid at 1 month was hyperthyroid at 3 months. All 9 cats

that were hypothyroid at 3 months were also hypothyroid at 1 month. Of these 9 cats, 8 had a high TSH, and TSH was not performed in 1. At 3 months 6/30 cats (20%) demonstrated renal azotemia, with 3 of those being hypothyroid, 2 were euthyroid, and 1 azotemic cat was persistently hyperthyroid. Six months after treatment, 11/31 (28%) cats evaluated were considered hypothyroid including one cat on thyroxine (1/31), 17/31 (55%) were euthyroid, and 2/31 (6%) were hyperthyroid (Appendix A, figure 32). Two cats went back on methimazole and another cat underwent repeat radioiodine treatment for persistent hyperthyroidism after 3 months. Of the 2 persistently hyperthyroid cats at 6 months, both were hyperthyroid at 3 months. Of the 11 hypothyroid cats, 7 were hypothyroid at 3 months, 3 were euthyroid at 1 month but not tested at 3 months, and one was euthyroid at 3 months but hypothyroid at 1 month following treatment. Also, of the 11 hypothyroid cats, 10 had a high TSH, and 1 was on thyroid supplementation and TSH was not available. Several cats had an elevated TSH at 3 and 6 months post treatment in the face of a normal T_4. One cat had a T_4 below the reference range and a normal TSH throughout the study period following radioiodine treatment. Renal data was recorded for 23 cats at 6 months with values for 4/23 (17%) being consistent with renal azotemia; 2 of these 4 cats were hypothyroid and 2 were euthyroid. One cat died 1 day following scintigraphy from acute anuric renal failure. One cat was euthanized at 5 months following therapy because of septic peritonitis secondary to suspected GI neoplasia. One cat was euthanized at 1 month following therapy for unknown reasons.

D. Discussion

This study identified a positive correlation between background activity on a scintigraphic image and pertechnetate activity in plasma collected at the time of the scan. This supports using background activity on an image to represent plasma activity for quantifying the thyroid's ability to concentrate pertechnetate or iodide above plasma levels or the T:B ratio. However, there is wide variation in this relationship depending on the location of the ROI. A region of interest placed over the heart on the left lateral image provided the best correlation to plasma pertechnetate activity. This ROI along with the heart ROI on the ventral and right lateral images were the areas that demonstrated better correlation with plasma pertechnetate than the other ROIs investigated. Background activity on an image is representative of equilibration of pertechnetate between the vascular and extracellular fluid spaces/interstitium, which occurs rapidly following injection. (Driver 2003) The heart contains a large blood volume and the heart activity seen on the scan is essentially the blood pool within its chambers. The heart is readily identifiable on an image with enough radioactivity to provide excellent count statistics when compared to other background areas. This should allow a reduction in indeterminate errors associated with the random nature of radioactive decay. (Daniel 2006) Additionally, with the patient placed on top of the gamma camera in left lateral recumbency, the heart tends to shift towards that side. This may be exaggerated with sedated or anesthetized patients as in this study, leading to atelectasis and further shifting of the cardiac silhouette towards the gamma camera. The cardiac silhouette does not tend to shift toward the dependent side as much in right lateral recumbency due to the restriction by the phrenicopericardial ligament. (Kealy 2011) Additionally, with the

patient in ventral recumbency the heart and thyroid glands are close to the gamma camera and fairly centrally located on the face of the camera where photon representation is optimal. These anatomic relationships may be contributing to the more optimal representation of background activity on the image as it correlates to plasma radioactivity for the heart ROIs when compared to the other background ROIs, as well as differences in correlation between left and right lateral, and ventral views.

The axillary/shoulder area, which has been utilized by others, (Beck, Hornof et al. 1985, Wallack, Metcalf et al. 2010) provided poorer correlation with plasma pertechnetate when compared to the heart ROIs. It is feasible that this poorer correlation could be associated with variations in forelimb placement and differences in body habitus. The background ROI cranial to the thyroid and the paw were also less correlative to plasma pertechnetate when compared to the other background ROIs. For the cranial thyroid ROI, variation in overlying tissue and superimposition of major blood vessels could contribute to error. The paw is a small area with less blood pool activity to generate counts when compared to the other background ROIs evaluated. Additionally, the paw is not routinely placed in the center of the gamma camera.

In the second portion of the study we evaluated the relationship between T:B ratios calculated from all of the investigated background ROIs and serum T_4 concentration. A positive correlation between T:B ratio and serum T_4 concentration was established. Again there was variation in the strength of this relationship depending on which background ROI was utilized. However, the effect of the location of background ROI for correlation with serum T_4 concentration was less than variation in the relationship between background activity and plasma pertechnetate activity.

When used with the average thyroid activity the heart on the ventral image provided the strongest T:B ratio correlation to T_4. This outcome was anticipated based on the finding of the heart background ROIs having the highest correlation with plasma pertechnetate activity. However, the T:B ratio utilizing several other background ROIs demonstrated only a slightly weaker relationship with T_4. Therefore, variation in background activity may have little influence on this measurement as it correlates to T_4. It appears that the other component of the ratio, the thyroid activity, may have greater influence in the overall measurement. Additionally, it is difficult to draw conclusions between correlation of plasma pertechnetate to background ROI count density and correlation of T:B ratio to T_4. Stated another way, the correlation of plasma pertechnetate and background activity on an image does not necessarily translate equally to the correlation between T_4 and T:B ratio, as these are two separate facets under multifactorial influences. The T:S ratio may be more correlative to T_4 when compared to T:B ratio because salivary uptake in the cat is more predictable or uniform among individuals than the physiologic and anatomic contributions of the various background ROIs. The discrete anatomic nature of the salivary gland may also contribute to more consistent ROI delineation. The basic premise of background activity representing blood pool was shown to be valid but radioactivity measured within an ROI is influenced by a variety of factors including vascularity of tissue, thickness of tissue, attenuation of overlying tissue, and the position of the animal. The purpose of the study was to evaluate the various locations of the background ROI to identify the best location for predicting the severity of the hyperthyroid state. As previously mentioned, others have identified a positive relationship between T:B ratio utilizing the axillary location for background ROI and

treatment response to radioiodine therapy, and more specifically, failure to respond to radioiodine therapy. (Wallack, Metcalf et al. 2010)

The current study did not have enough follow up on patients after therapy to draw any conclusions regarding the relationship between treatment outcome and T:B ratio. However, this area should be further investigated as others have found T:B ratio is higher in patients that fail standard fixed dose radioiodine therapy, whereas there is no significance difference in T:S ratio in these patients. (Wallack, Metcalf et al. 2010) Others have not been able to make predictions on radioiodine treatment response and pre-treatment serum T_4 alone, other than stating that higher serum T_4 before treatment is related to higher T_4 values post-treatment, with no correlation of lack of resolution of hyperthyroidism identified. (Chun, Garrett et al. 2002)It is likely that response to radioiodine treatment is dependent on multiple factors including tumor volume, delivery of the radionuclide to the tumor cells, and biologic half-life. As the beta particle most responsible for cell death with radioiodine therapy, depositing most of its energy in a 1 mm diameter area at the decay site. (Daniel 2006)

The study reported here identified % dose uptake to provide the best correlation to T_4 of the evaluated measurements. In the most recent and largest study to date evaluating T:S and T:B ratios and their correlation to T_4 in 2,096 hyperthyroid cats the authors demonstrated similar correlations between average T:S (R = 0.6) and average T:B (R = 0.54) (Peterson 2014)ratios with T_4 to that achieved in the current study. This study did not investigate other background ROIs for T:B ratio and utilized a background ROI similar to the axillary region evaluated in this study. A large scale study evaluating the heart background ROI has potential to provide better correlation to T_4 than T:S ratio. The

above-mentioned study also demonstrated greater sensitivity for detecting hyperthyroidism utilizing the T:S and T:B ratios when compared to T_4. That study did not evaluate % dose uptake. It is possible that T_4 should not be utilized as the gold standard for characterizing thyroid disease. Perhaps % dose uptake should be utilized as the gold standard when evaluating T:S and T:B ratios. An additional consideration would be to use a combination of tests, such as % dose uptake and T4, to result in improved accuracy. Further studies are needed in this area.

Studies have attempted to improve or predict radioiodine treatment response based on T:B ratio (Wallack, Metcalf et al. 2010), volumetric analysis from scintigraphy (Forrest, Baty et al. 1996), a scoring system including severity of clinical signs, size of thyroid gland, and magnitude of serum T_4 (Peterson and Becker 1995), and pattern of uptake (Nykamp, Dykes et al. 2005). Another study demonstrated that while greater T:S ratios are associated with higher serum thyroxine concentrations at 1 week post-treatment, by 1 month following therapy and beyond, scintigraphy did not correlate significantly (Chun, Garrett et al. 2002). The latter study established a significant correlation with pre-treatment serum T_4 and post treatment T_4 at several time intervals. However, this study also did not identify any relationship between pre-treatment T_4, T:S ratio, and treatment outcome. Therefore, it is possible that while establishing a relationship between scintigraphic measurements and pre-treatment serum T_4 is possible, this may not translate to a similar relationship between scintigraphic measurements and treatment response, more specifically, iatrogenic hypothyroidism.

In the first portion of the study, every enrolled cat was included in the data for comparing plasma pertechnetate activity to background ROI activity. Sixteen cats were

excluded from the second portion of the study comparing quantitative measurements on scintigraphy to pre-treatment T_4 for reasons that might influence either the T_4 level or the uptake of pertechnetate by the thyroid. All cats fed the y/d diet were excluded because the duration or magnitude of influence of the diet on pertechnetate uptake of normal thyroid tissue or T_4 levels, as well as withdrawl times, is unknown. Most patients on this diet were on the diet for various amounts of time, and/or had been off the diet for various amounts of time prior to presentation, or were not fed the diet exclusively. Y/d is an iodine-restricted diet that reduces thyroid hormone production. Iodine restricted diets are not likely to decrease the actual tumoral or hyperplastic tissue in the thyroid gland but instead deprives those cells of the iodine needed to synthesize thyroid hormone. Therefore, the autonomously functioning tissue would be expected to continue to grow over time in hyperthyroid cats. When an iodine analog such as pertechnetate is provided, it is expected that the hyperplastic thyroidal tissue would continue to concentrate the radionuclide as it did prior to initiating the diet. Since it is known that the T_4 level would drop after being fed y/d, the correlation of T_4 level to thyroid uptake would be invalid. Stated another way, scintigraphy likely reflects the true disease present; however, serum T_4 may be lower than it actually would be in the absence of the diet. Therefore, the two cannot be compared. Cases in which the scintigraphic image visually did not correspond with the T_4 value were observed during the study. Some cases in which this was observed were also on the y/d diet. Other cases were not on the diet. Imaging of the cat on the y/d diet (Appendix A, figure 4A) demonstrates a much more intense and larger lesion than the cat not on y/d diet (Appendix A, figure 4B). However, due to T_4 suppression as a result of the y/d diet, the T_4 values are similar. Moreover, this patient had a significant

drop in T_4 between the time of diagnosis and the time of enrollment in the study, suggestive of dietary iodine restriction leading to T_4 reduction. This represents the most conspicuous case of a cat on the y/d diet and demonstrates why the T_4 of cats on the y/d diet would not correlate very well to scintigraphic representation of disease. Consequences of iodine-restricted diets, in terms of thyroid health, duration of action of the y/d diet following its discontinuance, and this effect on serum T_4 levels have not been fully elucidated at this time. For these reasons, these cases were not included in the study population for comparing T_4 to scintigraphy.

Cats excluded for methimazole use within two weeks of imaging has been previously discussed in the literature. Others have demonstrated that methimazole use within two weeks of scintigraphy can result in alterations in appearance of the normal thyroid tissue. More specifically, normal thyroid tissue can be visualized on scintigraphy when under the influence of methimazole.(Nieckarz and Daniel 2001, Fischetti, DiBartola et al. 2005)Therefore, the thyroid disease may be over represented on scintigraphy and this along with the blockage thyroid hormone production result in inaccurate correlation of T_4 to scintigraphic uptake measures. Pre and post scintigraphy was not performed on any patient in this study receiving medical or dietary management or otherwise, as this was not a goal of the study.

Post-treatment evaluation revealed a similar number (20-30%) of biochemically hypothyroid cats by 3-6 months following radioiodine therapy to what is reported by others in the literature. (Slater, Komkov et al. 1994), (Peterson and Becker 1995, Chun, Garrett et al. 2002), (Nykamp, Dykes et al. 2005) The proportion of persistently hyperthyroid cats following radioiodine therapy in our study was higher than that

reported in the literature, which is widely accepted to be around 2-5%. Caution should be exercised when drawing conclusions from the small number of patients that participated in this part of the study. The number could be diluted in a larger study population. Additionally, the patients who chose to participate in the follow up may represent a population more biased towards those, which did not respond well to therapy. In this instance, the biochemically hypothyroid and hyperthyroid patients following therapy may be over represented.

The vast majority of hypothyroid cats had an elevated TSH at 3 and 6 months following treatment. However, there was one cat with a low T4 that had a persistently normal TSH at 1,3, and 6 months following therapy. It is possible that this cat has an illness unrelated to the thyroid, which could be contributing to a low serum T_4. Furthermore, several cats had a high TSH but normal T_4 at 3 and 6 months. It is possible that this could represent sub clinical hypothyroidism.

The proportion of cats with azotemia following radioiodine therapy was similar to what is reported in the literature. (Adams, Daniel et al. 1997), (Slater, Komkov et al. 1994, Williams, Peak et al. 2010, Daniel and Neelis 2014) A greater number of azotemic cats fell into the hypothyroid category following therapy and this association has been seen by others and has resulted in a significantly shorter survival time in cats following radioiodine therapy. (Williams, Elliott et al. 2010) While our study population was not large enough to make any statistical inferences, a similar trend regarding azotemia is identified in this study. Survival times were not evaluated.

There are several limitations associated with this study. Serum T_4 was not drawn at the time the images were made, but rather one day prior to imaging. Daily and hourly

fluctuations in serum T_4 values collected in hyperthyroid cats have been reported, (Peterson, Graves et al. 1987, Broome, Feldman et al. 1988) and may vary up to 21%. (Broome, Feldman et al. 1988)This may have some influence on the correlation with the physiologic process demonstrated on the image and the T_4 it is compared to. Additionally, myocardial changes associated with cardiac disease could have an influence on utilization of the heart ROIs in some patients and this was not evaluated for the purposes of this study. There was variation in volume of blood drawn for measuring of plasma pertechnetate levels. Although a target amount of 1.5 mls of blood was attempted in each patient, this was not always obtainable. A small plasma sample size could have an influence of count statistics and increased indeterminate errors. However, each sample was measured three consecutive times then averaged to minimize statistical variation in the random nature of radioactive decay. No attempts were made to standardize the sedation/anesthesia protocol utilized by the attending clinician. However, every cat did receive propofol titrated to effect during imaging. In addition several patients received ketamine and/or midazolam and/or acepromazine prior to imaging. It is reported that the T:S ratio for euthyroid cats receiving ketamine with midazolam is higher than when propofol alone is used. (Schaafsma, Pollak et al. 2006) Translation to hyperthyroid cats is not known. Additionally, some variation in ROI around thyroid and salivary tissue may contribute to these discrepancies. Attempts to minimize ROI variation in the current study were made by systematically adjusting the gray scale as described above to limit blooming artifact. The same individual was responsible for all ROIs. The number of cats included in the portion of the study comparing T:B ratio, T:S ratio, and % dose uptake to T_4 was reduced for the reasons discussed above and even fewer patients

participated in post-treatment follow up. More patients are needed to identify any correlations between T:B ratio and treatment outcome.

CHAPTER 3: CONCLUSIONS AND FURTHER RESEARCH

In summary, location of background ROI placement is important if it is intended to be representative of plasma concentration of pertechnetate. We found wide variation in the correlation of background activity on a scintigraphic image with plasma pertechnetate activity between the various background ROIs. Some background ROI locations may not accurately reflect the plasma pertechnetate activity. The background ROI utilizing the heart provides the most accurate representation of plasma pertechnetate or true background activity. Furthermore, the T:B ratio utilizing the heart ROI is a good predictor of serum T_4 . However, previously established methods utilizing % dose uptake and T:S ratio provide a stronger relationship with T_4. It is not known if these relationships can be applied to predicting treatment response. A substantial number of cats with hypothyroidism following ^{131}I therapy were identified in this study and by others. (Slater, Komkov et al. 1994) (Chun, Garrett et al. 2002) (Peterson and Becker 1995, Nykamp, Dykes et al. 2005)Previous work revealing decreased survival times when iatrogenic hypothyroidism occurs with renal azotemia(Williams, Elliott et al. 2010) suggests that methods to predict treatment response, more specifically iatrogenic hypothyroidism, should continue to be investigated. With increased numbers of cats reporting data on response to radioiodine therapy, conclusions may be drawn in the future regarding scintigraphic methods to predict treatment response and more specifically post treatment hypothyroidism. This information could support tailored radioiodine dosing protocols for prevention of iatrogenic hypothyroidism and improved survival times.

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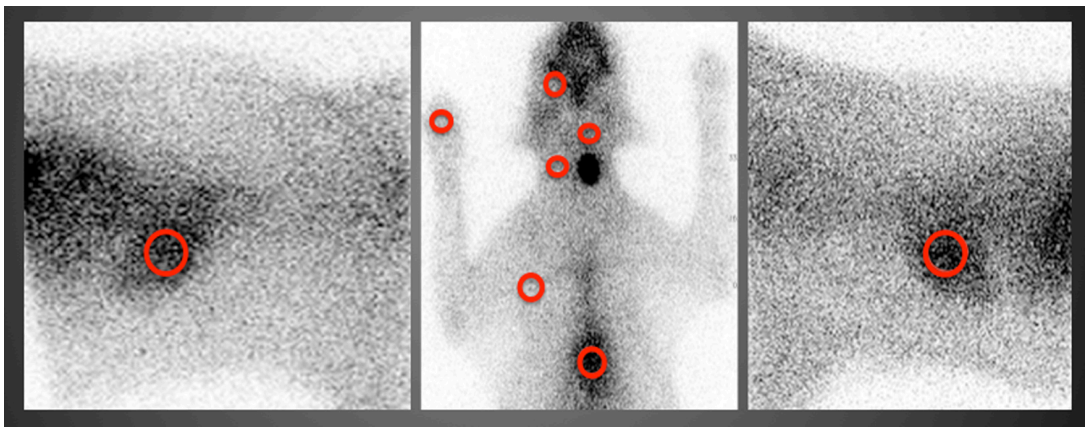
APPENDIX A: FIGURES

Figure 1: Background ROIs



A. Right Lateral Thorax B. Ventral Image C. Left Lateral Thorax

Regions of interest for background activity. (A) Right lateral image of the thorax, (B) ventral image of the head, neck, and thorax, and (C) left lateral image of the thorax; Red circles demonstrate each of the background ROIs evaluated including the heart on the right lateral image in A, the caudal salivary, cranial thyroid, adjacent thyroid, axillary, heart, and paw on the ventral image in B, and the heart on the left lateral image in C.

Figure 2: Heart ROI on the ventral image for T:B ratio

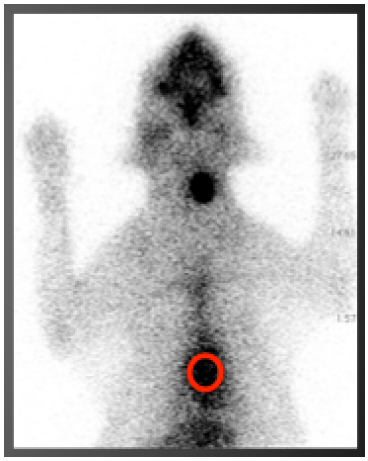


Figure 3: Salivary ROI for T:S ratio calculation

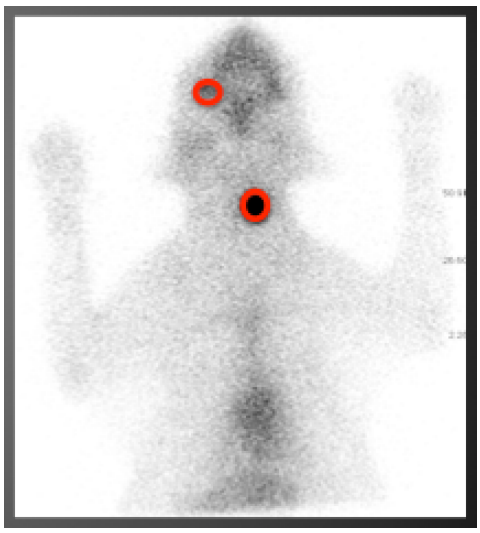
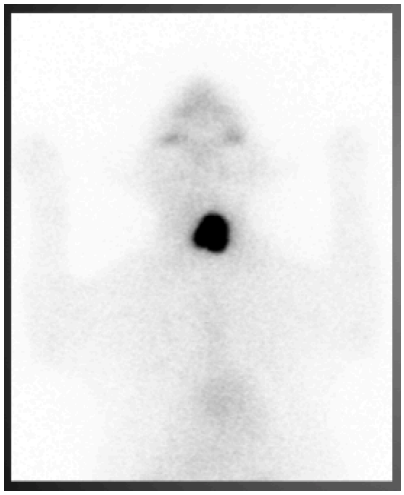
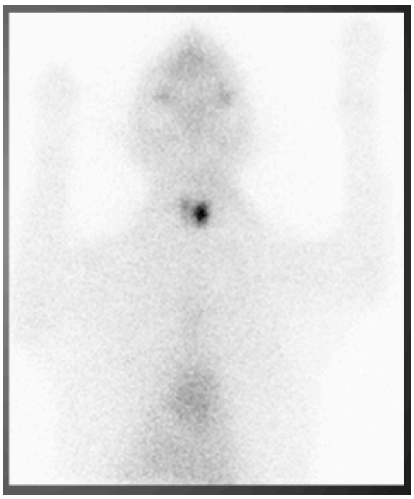


Figure 4: y/d diet comparison

A. 

B. 

The image on the left (A) is a cat on the y/d diet with a T_4 of 58.8 nmol/L at time of the scan. The cat on the right (B) is not on the y/d diet with a T_4 of 56 nmol/L (laboratory reference range is 16.0-37.7 nmol/L).

Figure 5: Heart ROI count density on left lateral image correlation with plasma activity (uCi/gm of blood)

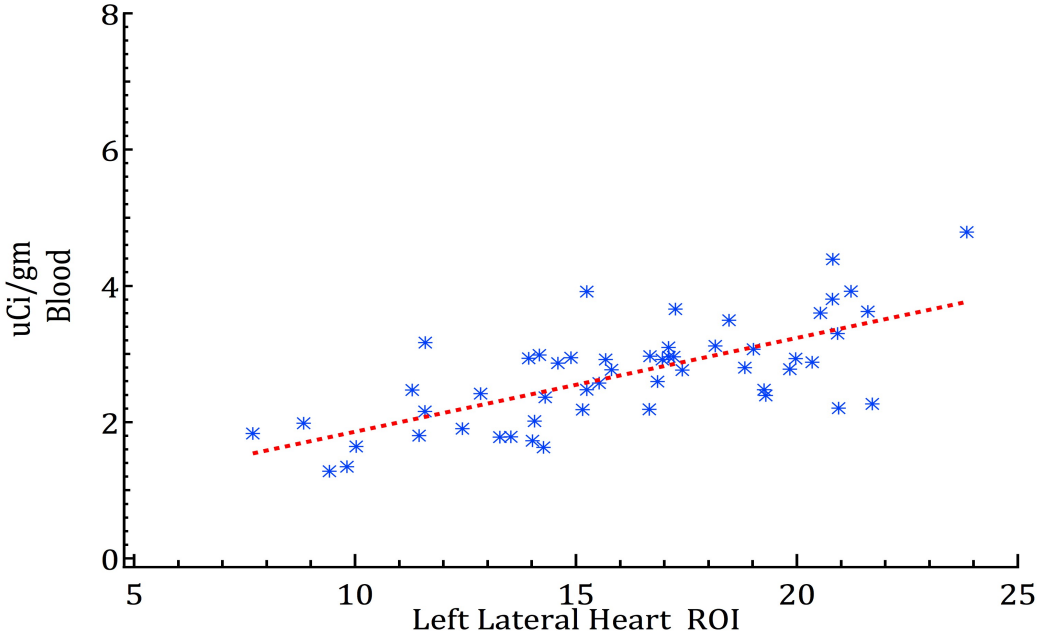


Figure 6: Heart ROI count density on right lateral image correlation with plasma activity (uCi/gm of blood)

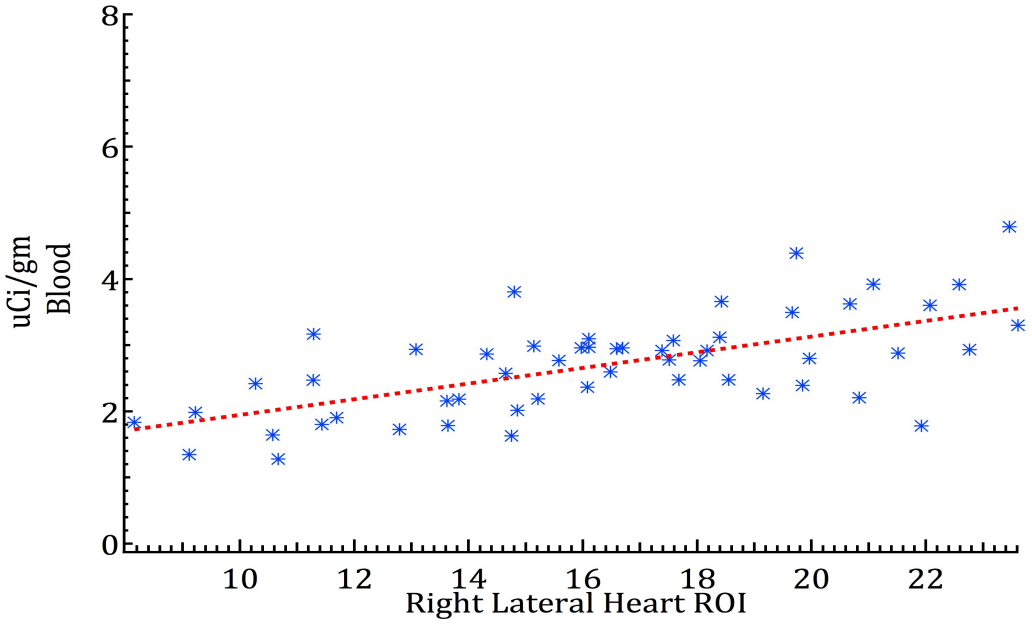


Figure 7: Heart ROI count density on ventral image correlation with plasma activity (uCi/gm of blood)

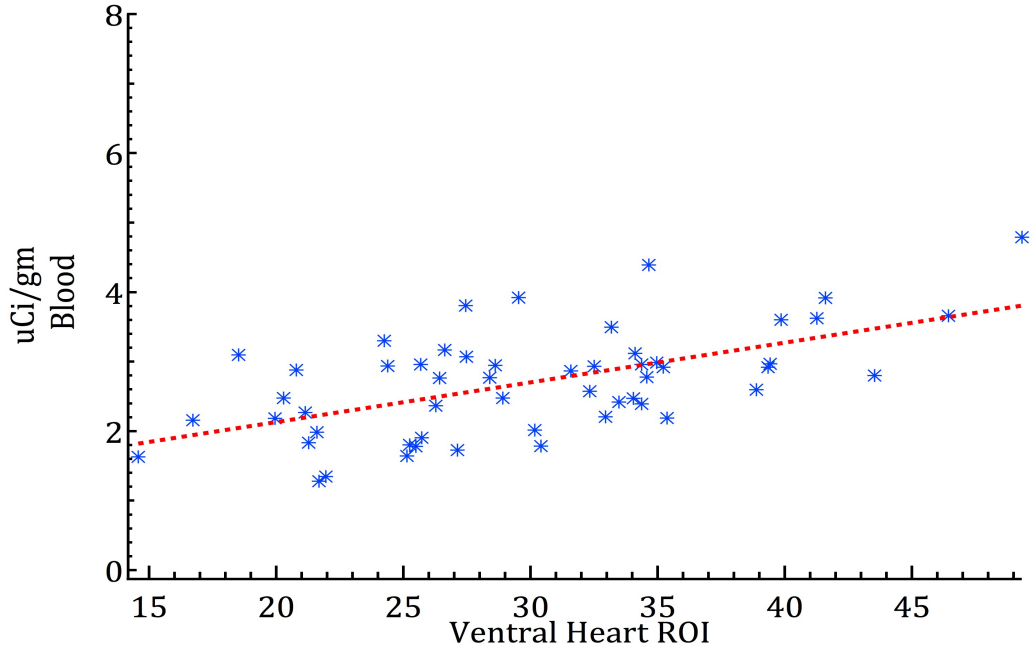


Figure 8: Caudal salivary ROI count density correlation with plasma activity (uCi/gm of blood)

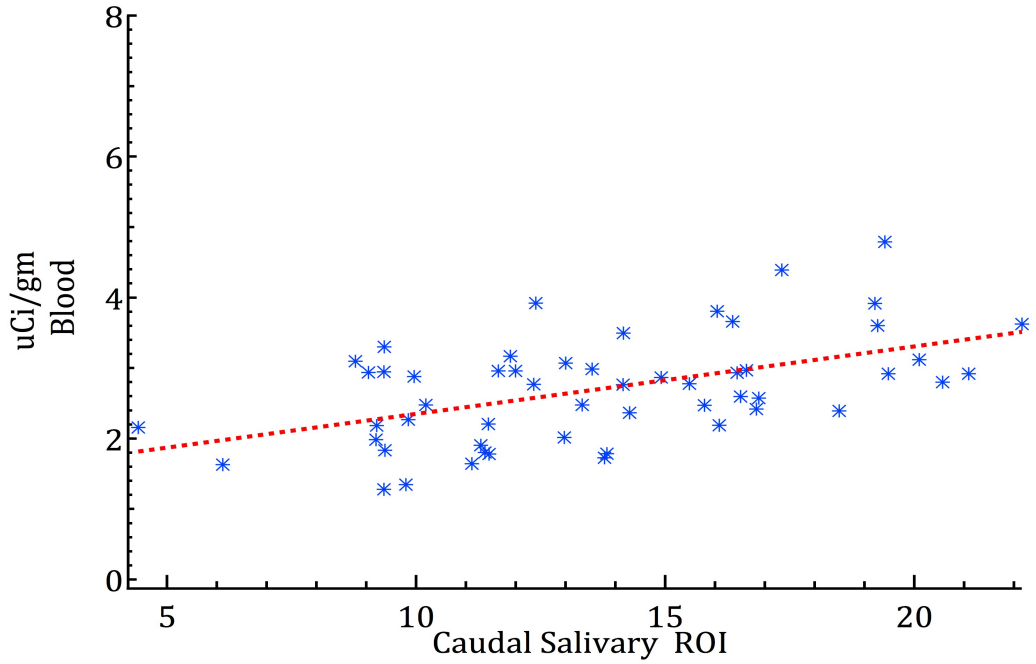


Figure 9: Paw ROI count density correlation with plasma activity (uCi/gm of blood)

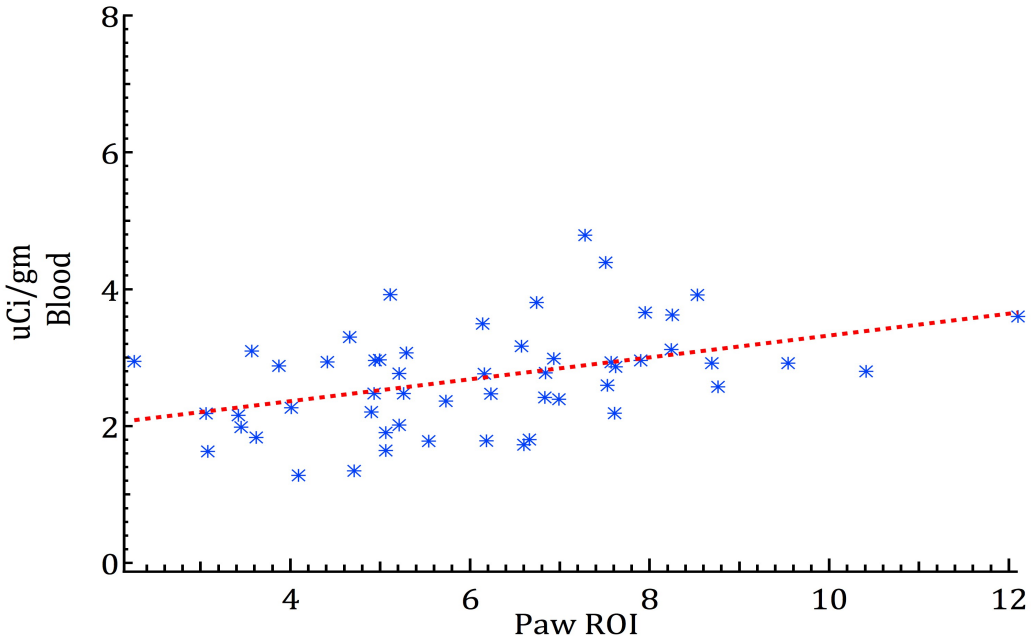


Figure 10: Cranial thyroid ROI count density correlation with plasma activity (uCi/gm of blood)

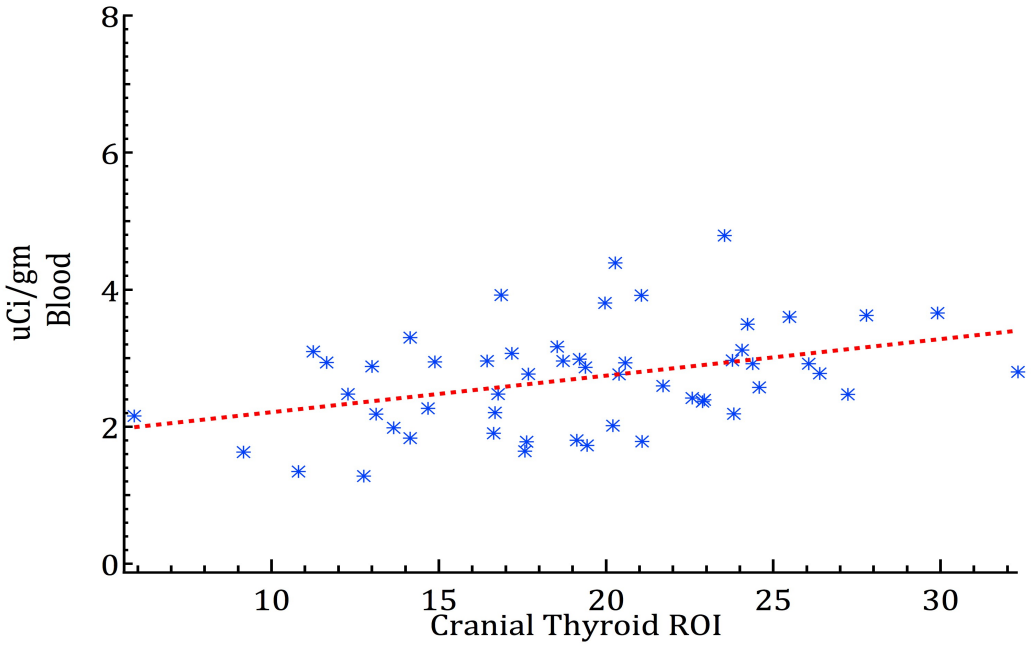


Figure 11: Axillary ROI count density correlation with plasma activity (uCi/gm of blood)

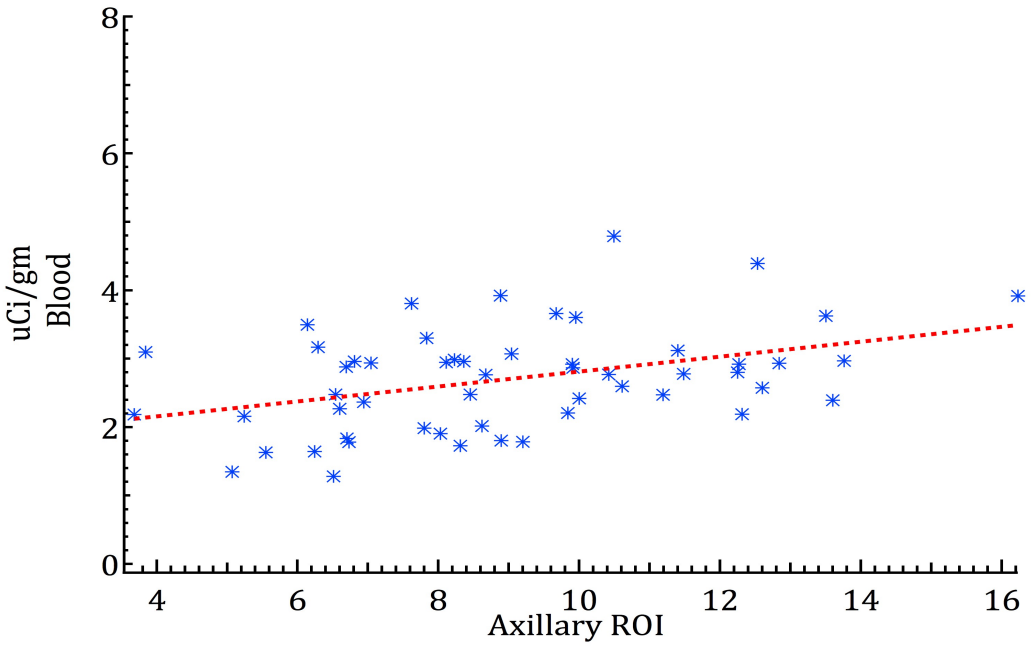


Figure 12: Adjacent thyroid ROI count density correlation with plasma activity (uCi/gm of blood)

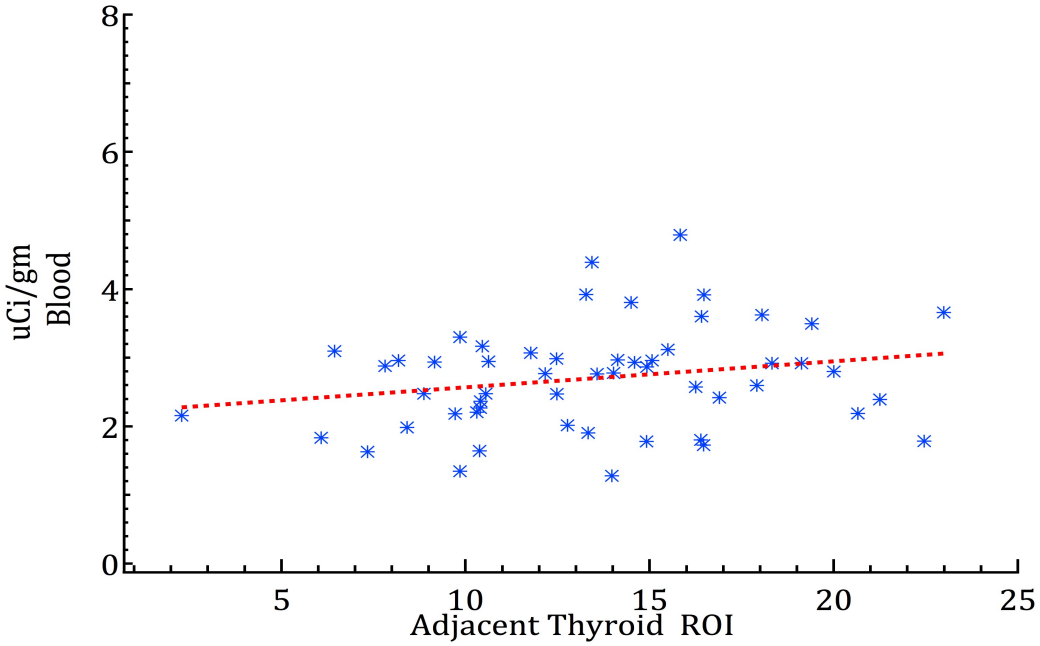


Figure 13: % dose uptake correlation with T_4

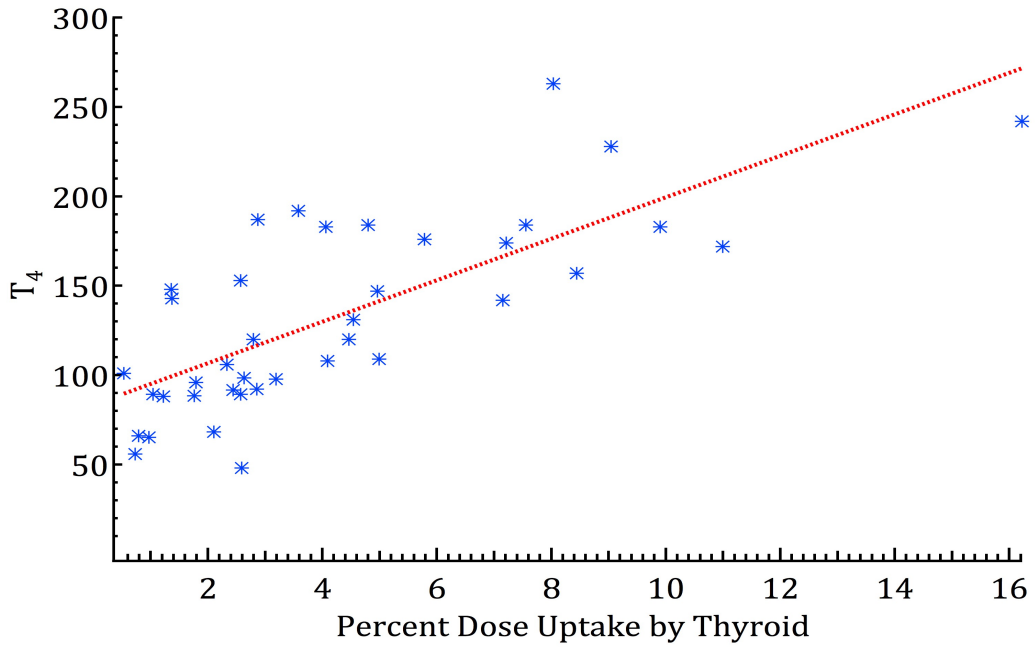


Figure 14: Average T:S correlation with T_4

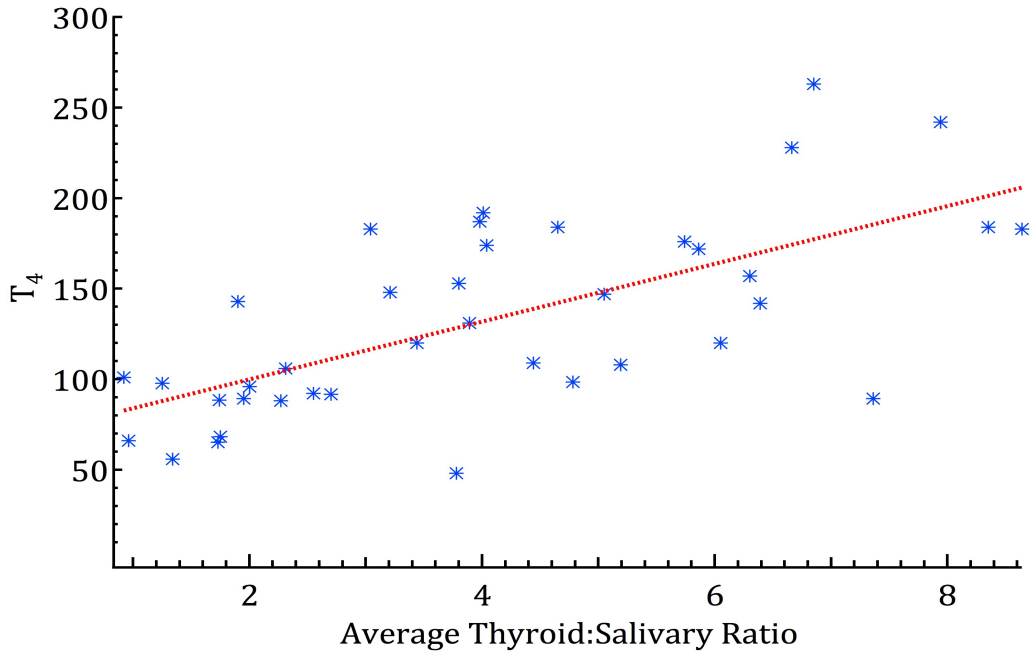


Figure 15: Hottest T:S correlation with T_4

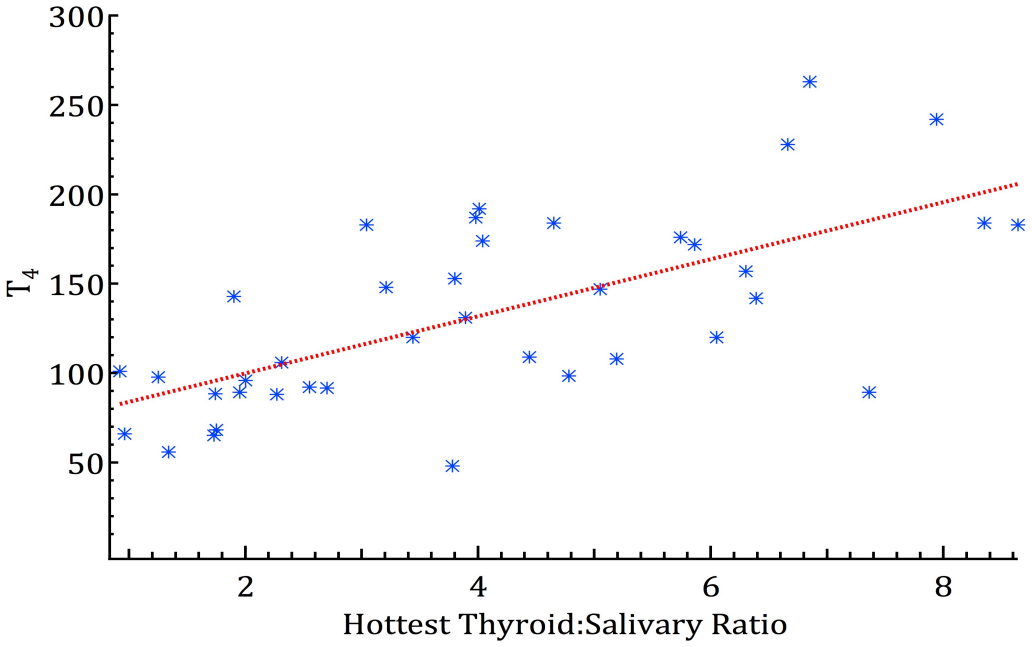


Figure 16: Average T:B heart ROI ventral image correlation with T_4

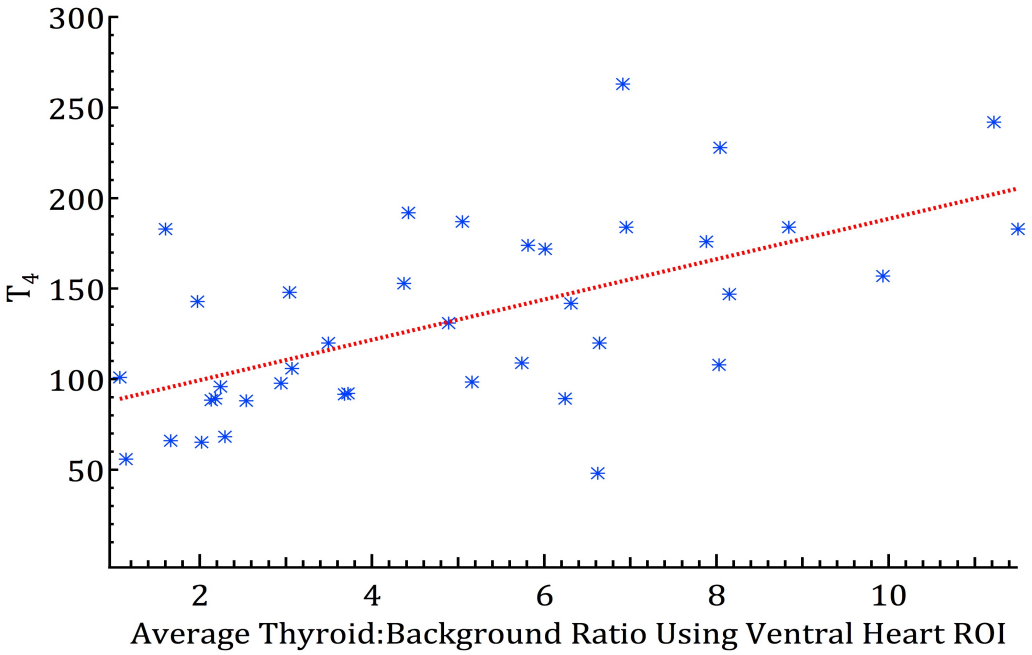


Figure 17: Average T:B heart ROI left lateral image correlation with T_4

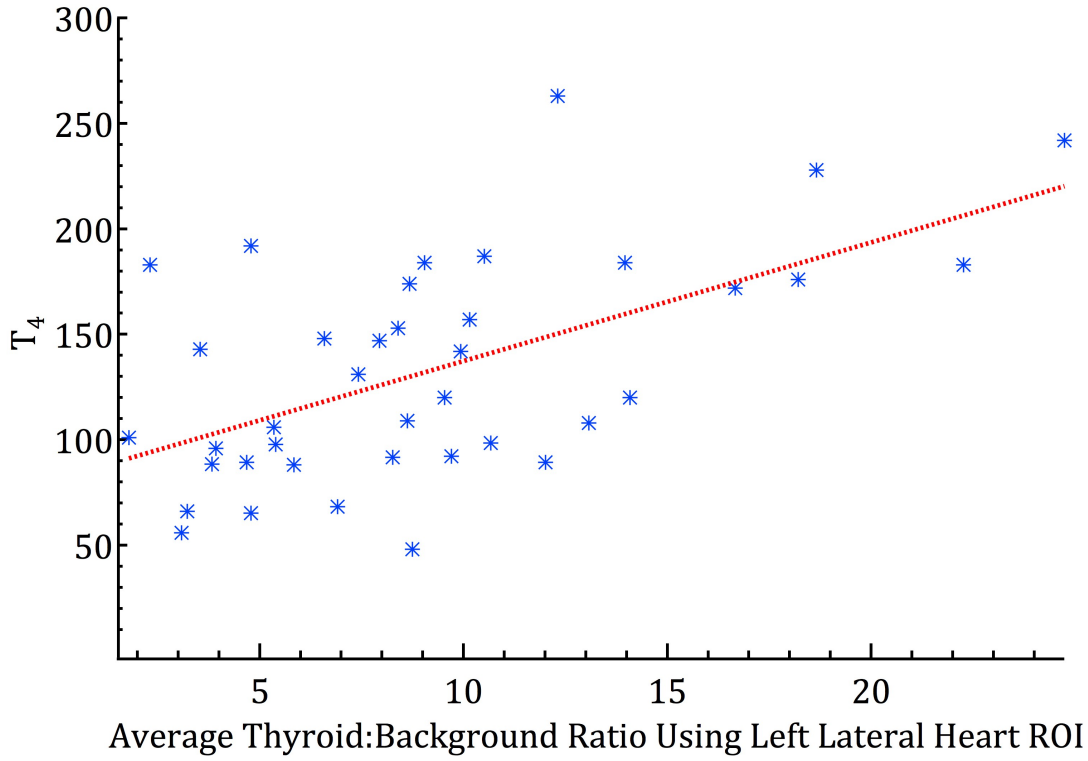


Figure 18: Hottest T:B caudal salivary ROI correlation with T_4

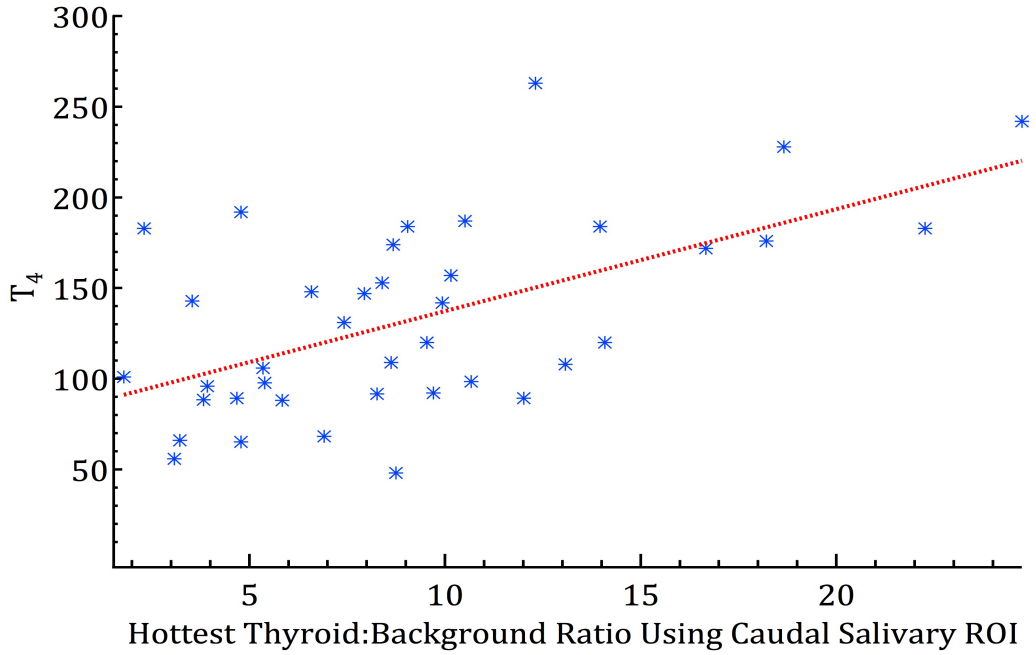


Figure 19: Hottest T:B heart ROI ventral image correlation with T_4

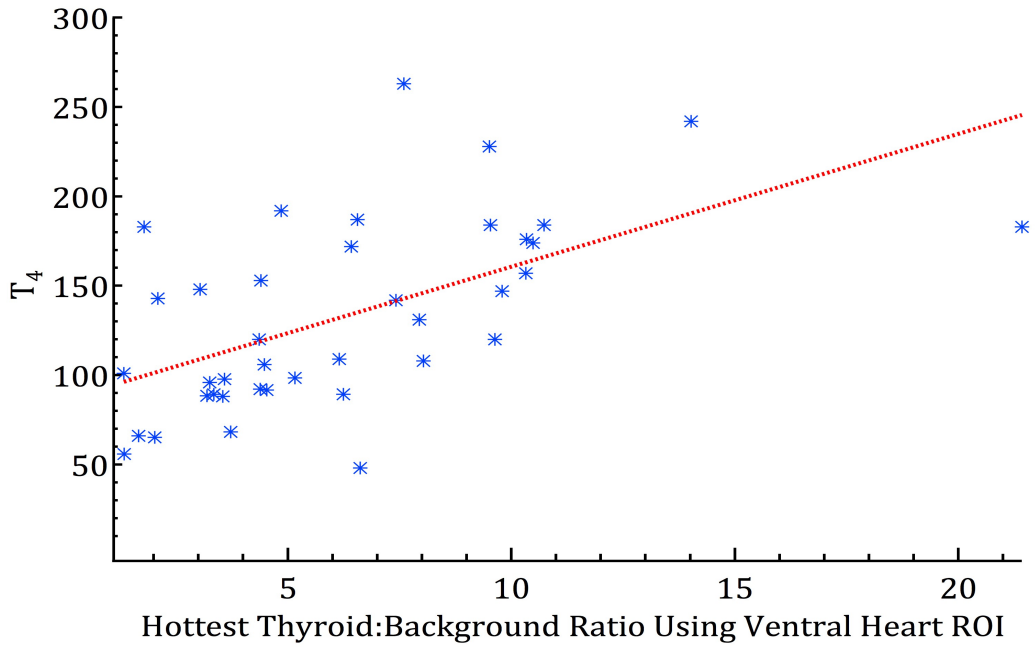


Figure 20: Hottest T:B cranial thyroid ROI correlation with T_4

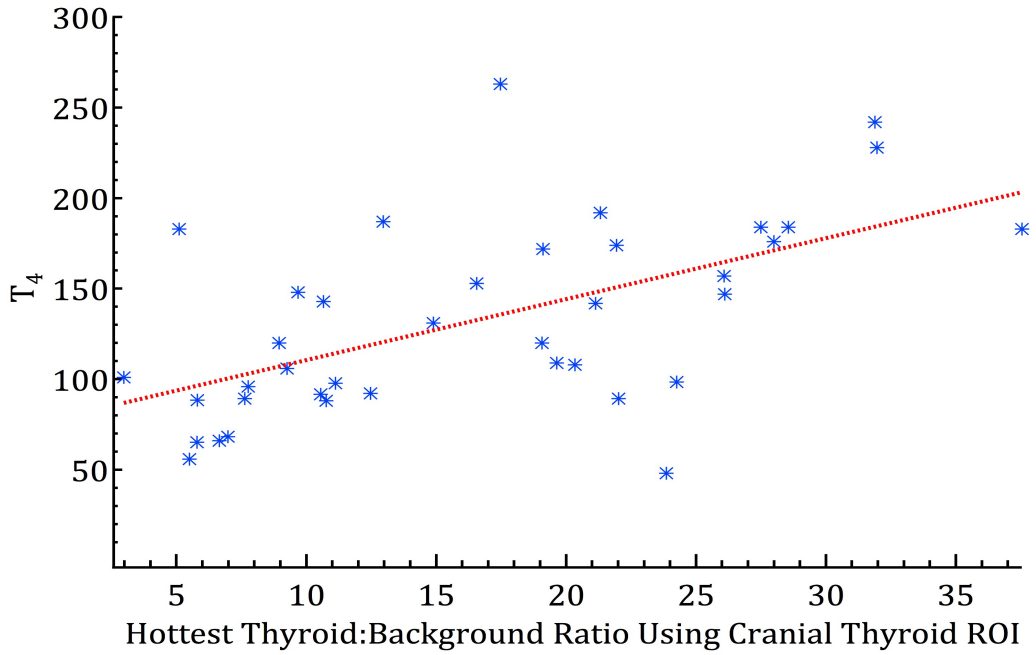


Figure 21: Average T:B cranial thyroid ROI correlation with T_4

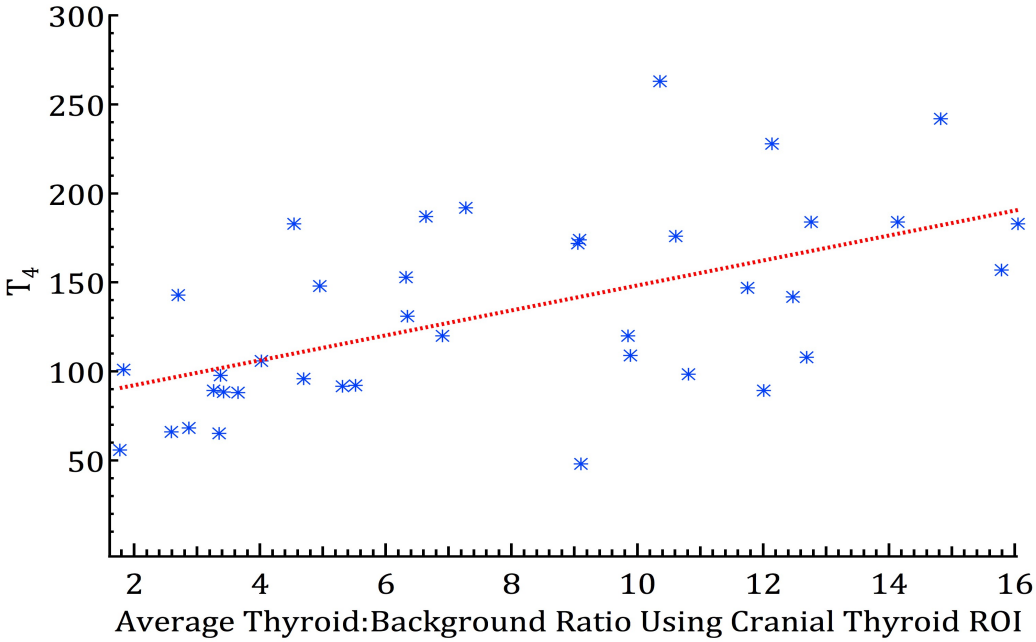


Figure 22: Average T:B caudal salivary ROI correlation with T_4

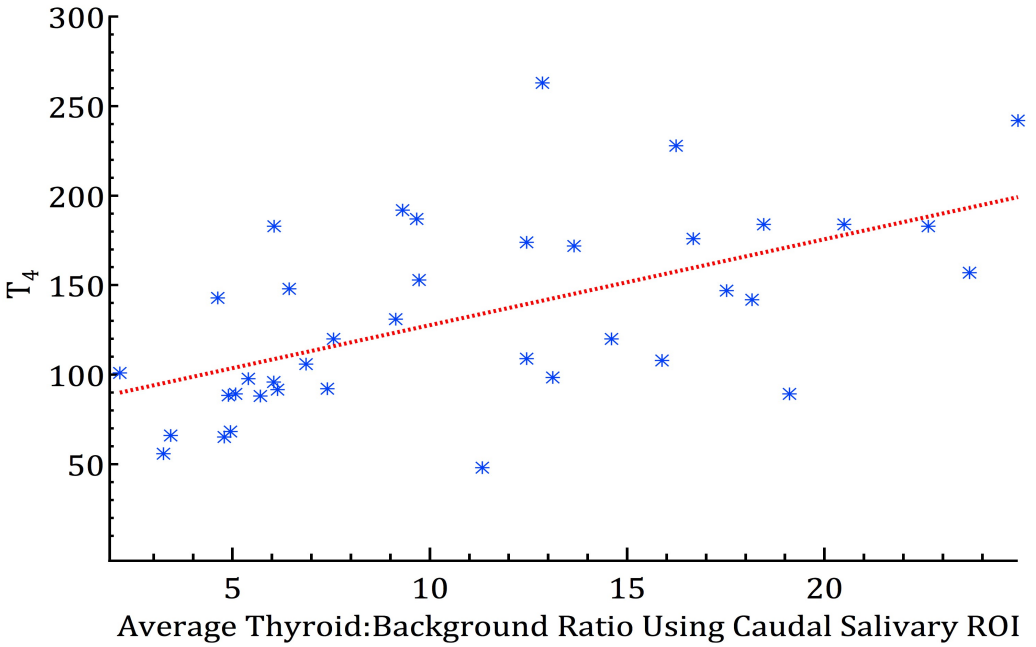


Figure 23: Average T:B axillary ROI correlation with T_4

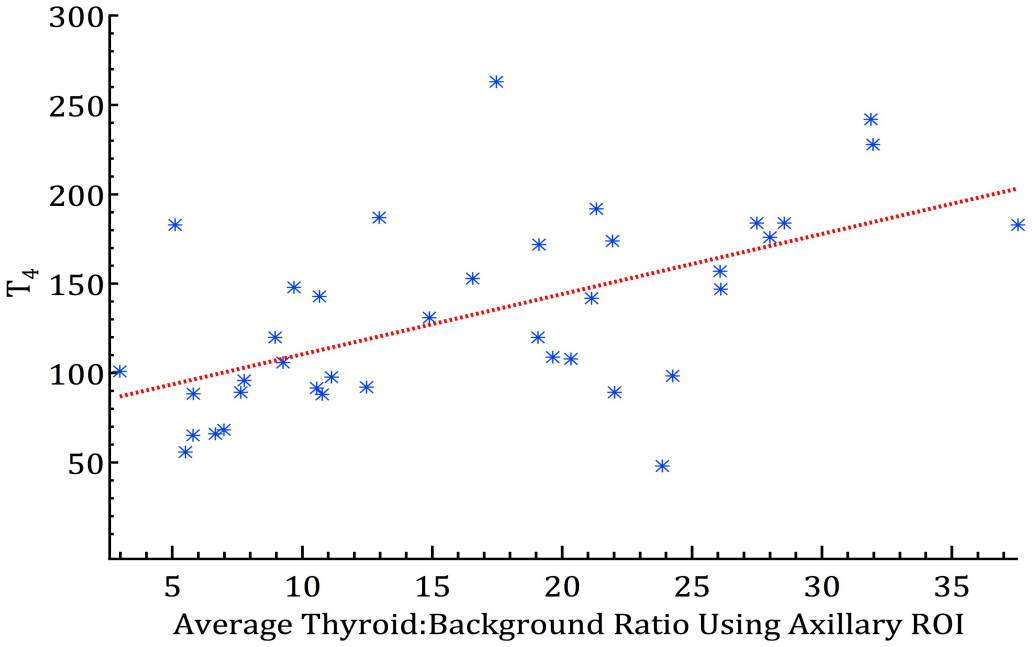


Figure 24: Hottest T:B adjacent thyroid ROI correlation with T_4

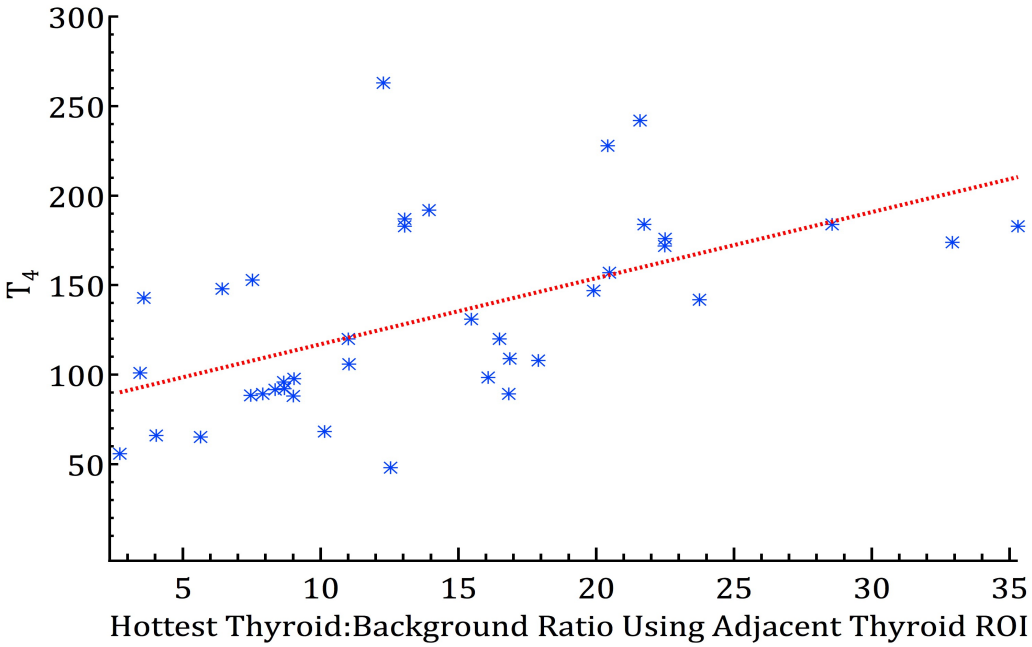


Figure 25: Hottest T:B axillary ROI correlation with T_4

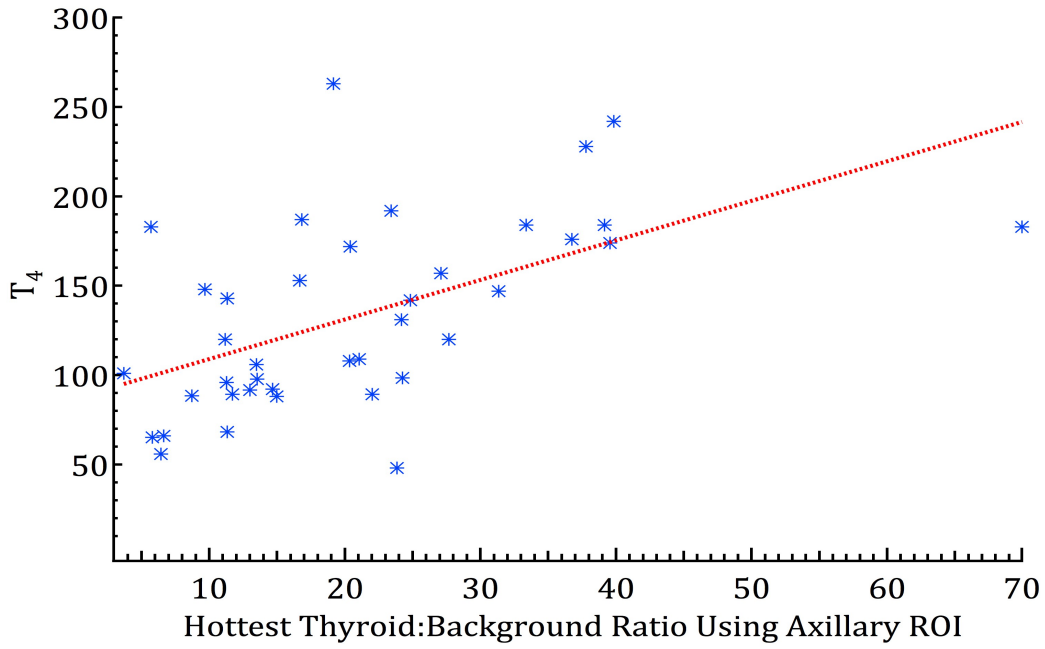


Figure 26: Average T:B adjacent thyroid ROI correlation with T_4

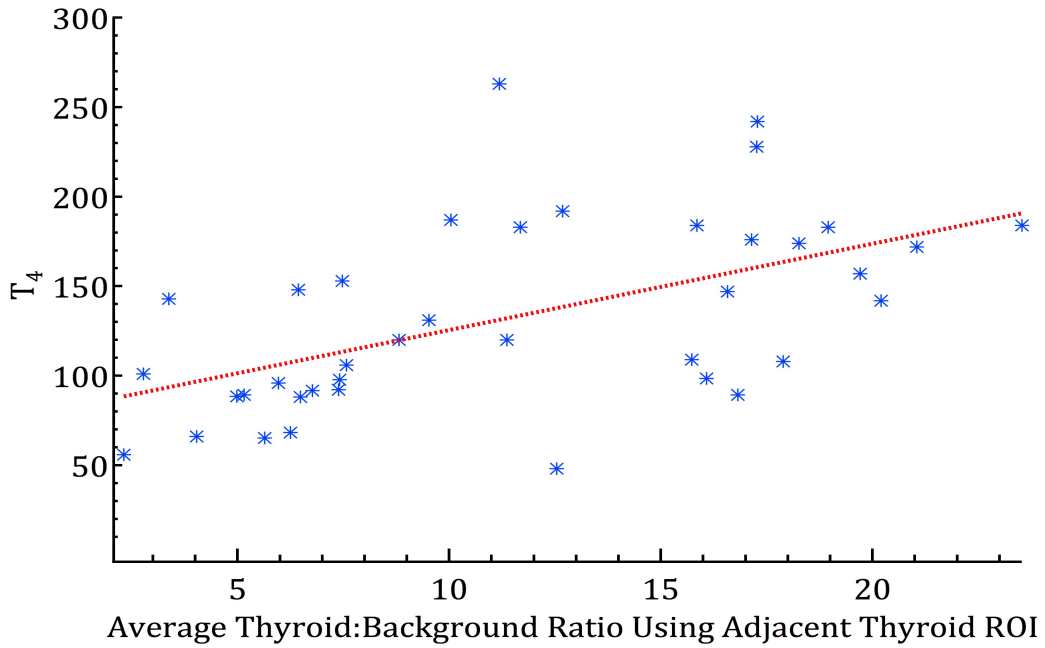


Figure 27: Hottest T:B heart ROI left lateral image correlation with T_4

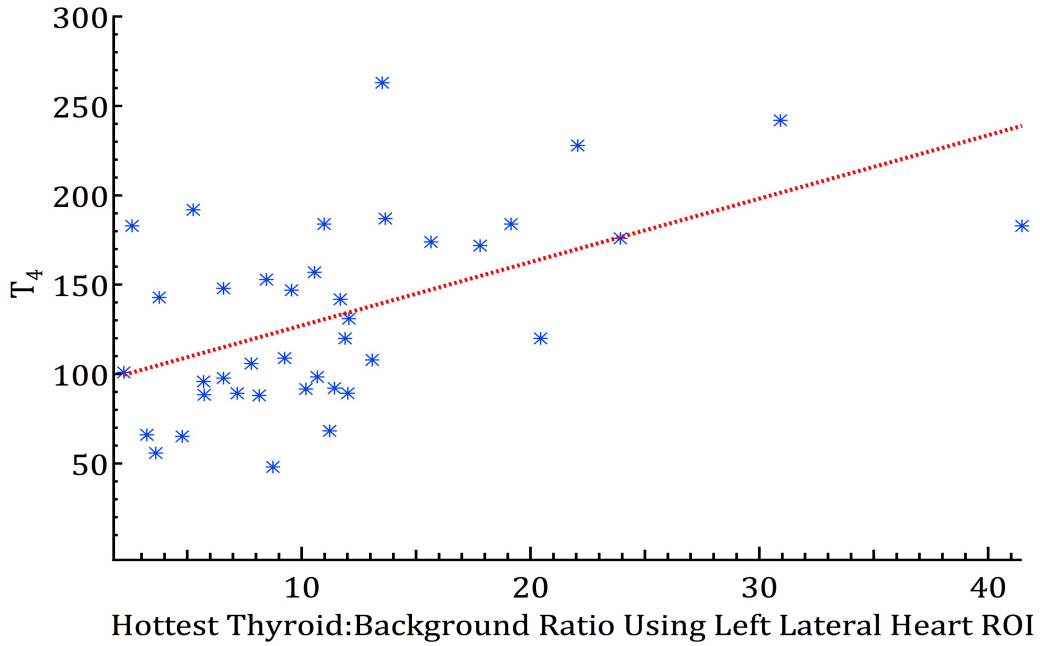


Figure 28: Average T:B heart ROI right lateral image correlation with T_4

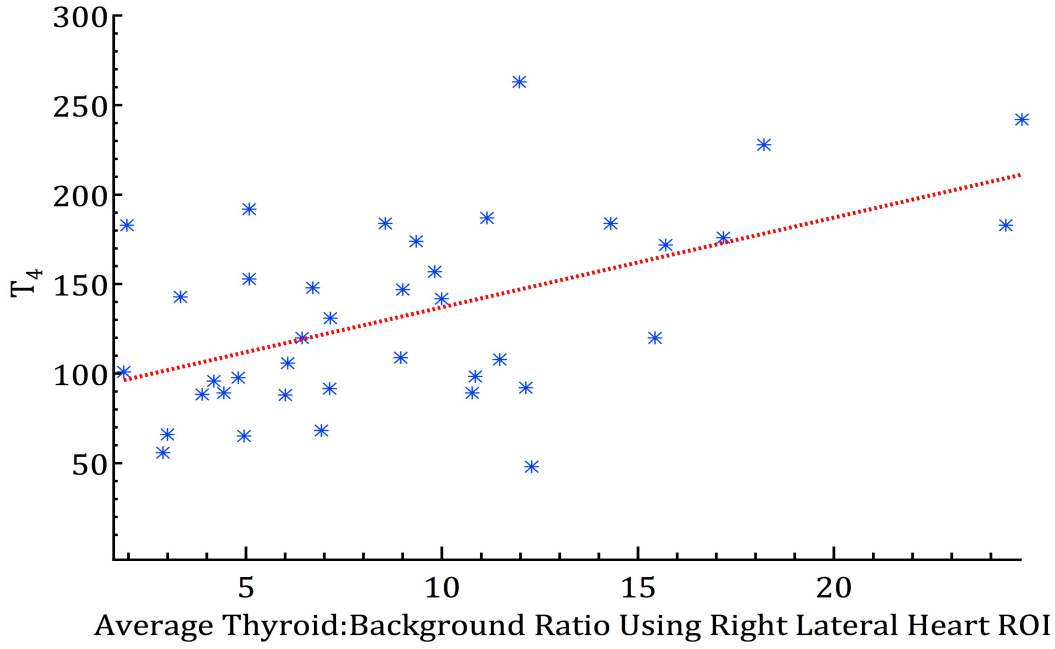


Figure 29: Hottest T:B heart ROI right lateral image correlation with T_4

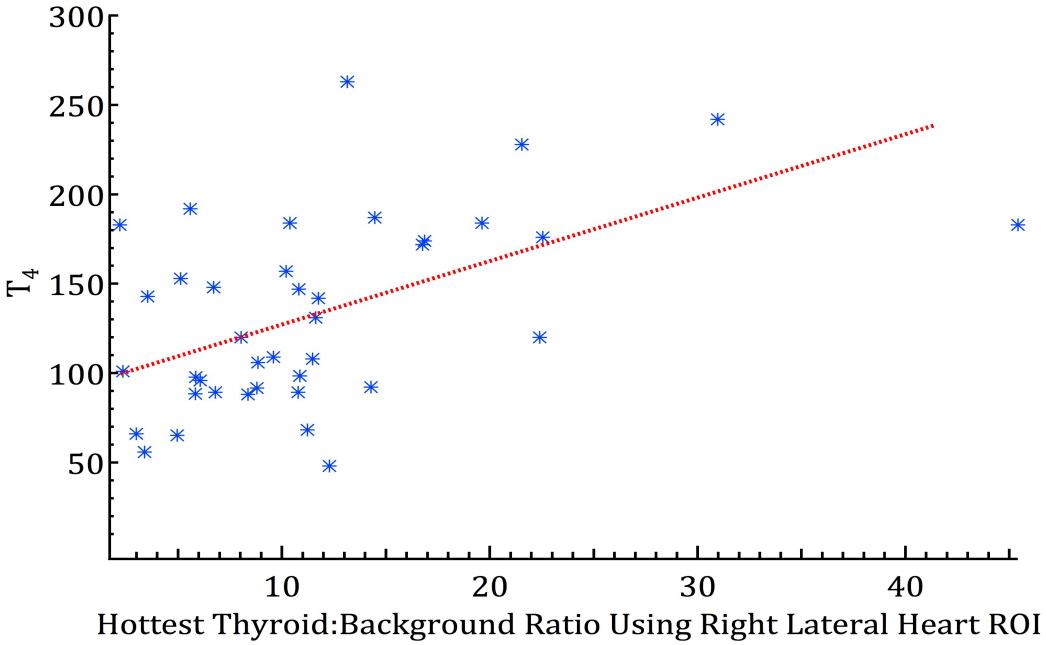


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Figure 30: Hottest T:B paw ROI correlation with T_4

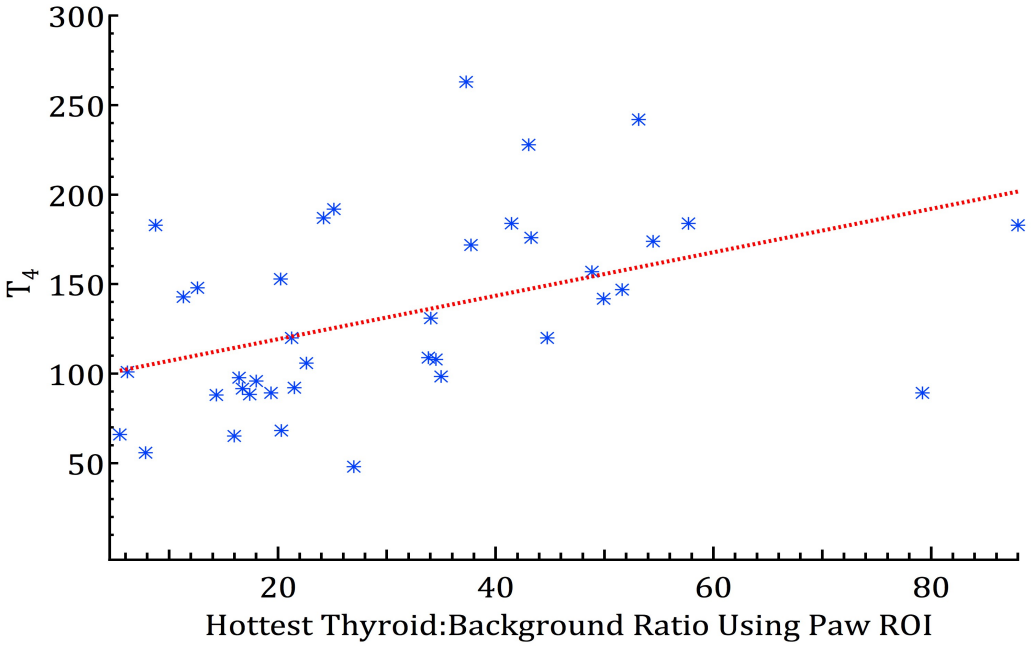


Figure 31: Average T:B paw ROI correlation with T_4

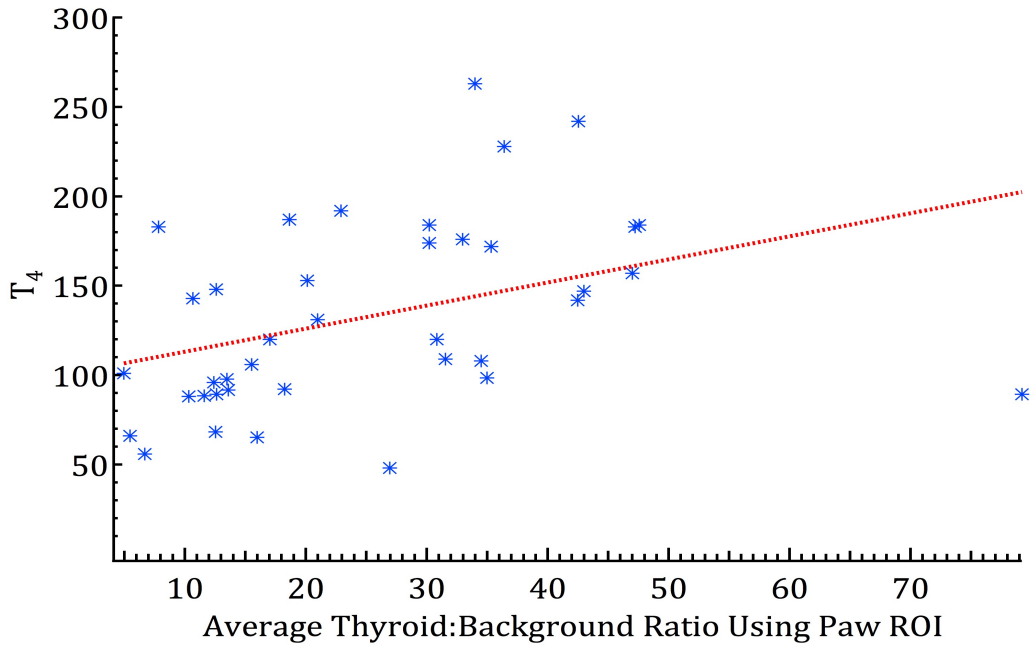
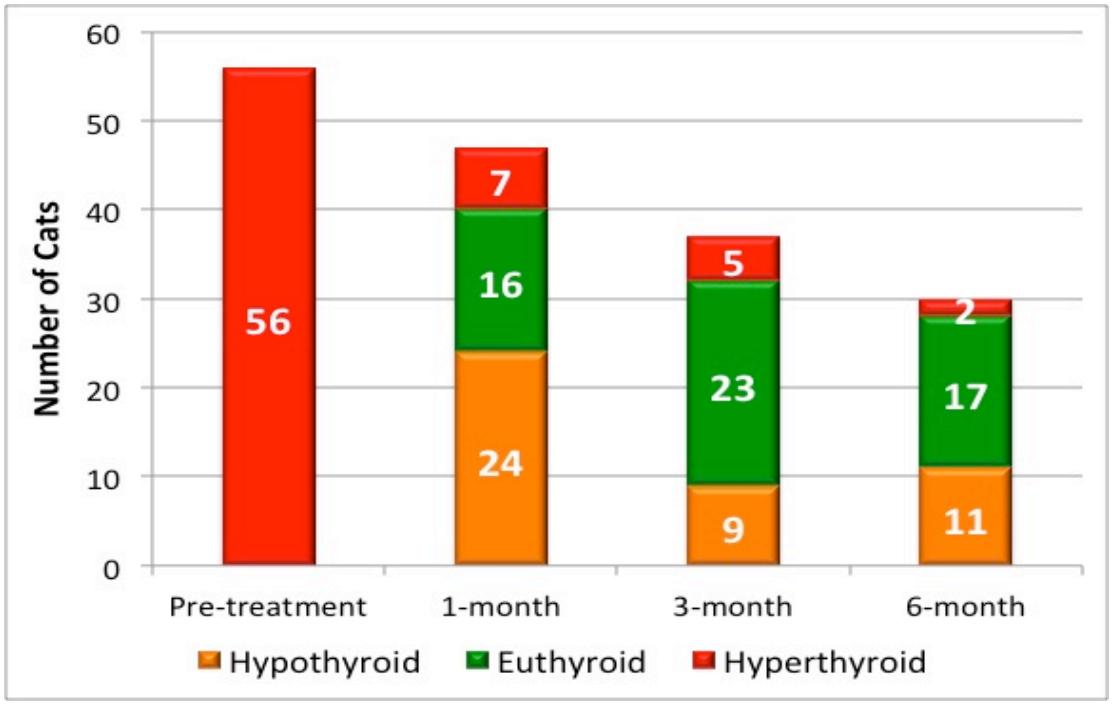


Figure 32: Treatment Outcomes



APPENDIX B: TABLES

Table 1. Background ROI correlation with plasma pertechnetate

Background Region	p value	r value
Heart (Left Lateral)	<0.01	0.70
Heart (Right Lateral)	<0.01	0.63
Heart (Ventral)	<0.01	0.57
Caudal Salivary	<0.01	0.51
Paw	<0.01	0.42
Axillary	<0.01	0.39
Cranial Thyroid	<0.01	0.39
Adjacent Thyroid	0.1	

Table 2: Scintigraphic measurement correlation to pre-treatment serum T_4

Scintigraphic Measure	p value	r value
% dose uptake	<0.01	0.74
Average T:S	<0.01	0.66
Hottest T:S	<0.01	0.66
Average T:B Ventral Heart	<0.01	0.59
Average T:B Heart Left Lateral	<0.01	0.58
Hottest T:B Caudal Salivary	<0.01	0.58
Hottest T:B Ventral Heart	<0.01	0.57
Hottest T:B Cranial Thyroid	<0.01	0.57
Average T:B Cranial Thyroid	<0.01	0.57
Average T:B Caudal Salivary	<0.01	0.57
Average T:B Axillary	<0.01	0.57
Hottest T:B Adjacent Thyroid	<0.01	0.56
Hottest T:B Axillary	<0.01	0.55
Average T:B Adjacent Thyroid	<0.01	0.54
Hottest T:B Left Lateral	<0.01	0.53
Average T:B Right Lateral	<0.01	0.53
Hottest T:B Right Lateral	<0.01	0.49
Hottest T:B Paw	<0.01	0.44
Average T:B Paw	0.02	0.38