

Efficacy and safety of iopanoic acid for treatment of experimentally-induced
hyperthyroidism in cats

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ABSTRACT

Objective: To determine the efficacy and safety of iopanoic acid for the treatment of experimentally-induced hyperthyroidism in cats.

Animals: 15 healthy adult domestic short hair cats

Procedures: Hyperthyroidism was induced by daily subcutaneous administration of levothyroxine for 42 days. On day 28, cats were randomized to a control group receiving a placebo PO every 12 hours, a low dose group receiving 50 mg iopanoic acid PO every 12 hours, and a high dose group receiving 100 mg iopanoic acid PO every 12 hours. Cats were treated for 14 days. Weight and heart rates were obtained on days -8, 0, 28, 35, and 42. Blood was collected for CBC and biochemical analysis and for T4, T3, and rT3 measurement on days -8, 28, 35, and 42.

Results: Two cats were removed prior to day 28 due to prolonged anorexia and another on day 36 because of heart failure. The low dose and high dose groups had significantly lower T3 concentrations on days 35 and 42 compared to the control group and to their own T3 concentrations on day 28. The T3 concentrations in cats administered iopanoic acid were not different from those obtained prior to induction of hyperthyroidism. Body weight and food consumption were not altered by iopanoic acid treatment, while heart rate was decreased in the low dose group on day 35 when compared to day 28.

Conclusions and clinical relevance: Iopanoic acid was effective in decreasing T3 concentrations, but its effect on clinical signs of hyperthyroidism was less apparent. Studies evaluating the long-term efficacy in cats with naturally-occurring hyperthyroidism are warranted.

DEDICATION

“Pigmaei gigantium humeris impositi plusquam ipsi gigantes vident”

If I have seen further it is by standing on the shoulders of giants - Sir Isaac Newton

“Our chief want in life is somebody who shall make us do what we can.”

- Ralph Waldo Emerson

I dedicate this work to my wife, Dr. Erica Marie Lacher. Without her love, support, and sacrifice, this fantastic journey would not have been possible. After four long years, I look forward to spending the rest of my life with you. To my mother, Jacqueline Gallagher, thank you for teaching me to achieve. And to my brother, Dr. David Gallagher, thank you for opening the doors to my career.

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DECLARATION OF WORK PERFORMED

I, Alexander E. Gallagher, declare that I performed all the work detailed in the materials and methods section of this manuscript with the exception of the following procedures.

Complete blood counts and biochemical profiles were performed by the Virginia-Maryland Regional College of Veterinary Medicine's Veterinary Teaching Hospital clinical pathology laboratory.

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LIST OF ABBREVIATIONS

ALKP	alkaline phosphatase
ALT	alanine aminotransferase
BUN	blood urea nitrogen
BW	body weight
cAMP	cyclic adenosine monophosphate
CBC	complete blood count
CV	coefficient of variation
D1	Type I 5'-deiodinase
D2	Type II 5'-deiodinase
D3	Type III 5'-deiodinase
DIT	diiodotyrosine
ER	endoplasmic reticulum
FH	feline hyperthyroidism
G _i	inhibitory G protein
H ₂ O ₂	hydrogen peroxide
HR	heart rate
I-	iodide
MER	maintenance energy requirement
MIT	monoiodotyrosine
NIS	Na ⁺ /I ⁻ symporter
OCA _s	oral cholecystographic agents
PBDE _s	polybrominated diphenyl ethers
PO	orally
rT3	3,3',5'-triiodothyronine, reverse T3
SEM	standard error of the mean
SSKI	saturated solution of potassium iodide
T0	iodine-free thyronine
T2	diiodothyronine
T3	3,5,3'-triiodothyronine
T4	thyroxine

TBG	thyroxine-binding globulin
Tg	thyroglobulin
TNG	toxic nodular goiter
TPO	thyroid peroxidase
TREs	thyroid response elements
TRH	thyrotropin-releasing hormone
TSH	thyroid stimulating hormone
TSHR	thyroid stimulating hormone receptor
TSIs	thyroid stimulating immunoglobulins

INTRODUCTION

Hyperthyroidism is the most common endocrine disorder of cats¹ and is caused by increased synthesis and release of thyroid hormones from the thyroid gland.² Most 3,5,3'-triiodothyronine (T3) in circulation is the result of extrathyroidal (peripheral) 5'-deiodination of thyroxine (T4) by the enzyme 5'-deiodinase.¹ T3 is three to five times more potent than T4, enters cells more rapidly, and has a more rapid onset of action.³ In effect, T4 acts as a prohormone for the metabolically active T3.

The three main treatment options for hyperthyroidism in cats are antithyroid medications, surgical thyroidectomy, and radioactive iodine. Antithyroid therapy results in a reversible euthyroid state whereas the latter two options usually result in a permanent euthyroid state and are thus considered definitive therapies. In most cases, antithyroid therapy is used prior to definitive therapy. Because of the cost and complications of thyroidectomy, and costs and low availability of radioiodine therapy, many owners elect to use antithyroid therapy long-term.

Although several antithyroid medications have been used in veterinary medicine, only methimazole is still used routinely because the other medications are unsuitable for chronic therapy or have unacceptable side effects.^{2,4,5} While effective in most cases, methimazole administration is associated with side effects in up to 18% of treated cats.⁶ The most common side effects are vomiting and anorexia, which appear to be dose dependent in most cases. More serious side effects necessitating discontinuation of the drug occur in up to 10% of cats and include self-induced excoriation, hepatotoxicity, thrombocytopenia, and agranulocytosis.⁶⁻⁹ In cats that cannot undergo thyroidectomy or radioiodine therapy, there are limited options.

In humans, oral cholecystographic agents (OCAs) such as ipodate and iopanoic acid have been used to treat hyperthyroidism due to their rapid action and excellent safety record.¹⁰⁻¹² Oral cholecystographic agents affect thyroid metabolism predominately by inhibition of deiodinases which are responsible for the peripheral conversion of T4 to T3.¹¹ Specifically, these agents inhibit outer ring deiodination of T4 to the more metabolically active T3, but do not affect inner ring deiodination of T4 to 3,3',5'-triiodothyronine (reverse T3, rT3), which is inactive.¹¹ Reverse T3 concentrations have been shown to rise with radiographic contrast agent administration¹³ and rT3 can inhibit monodeiodination of T4 to T3.¹⁴

Ipodate is the only oral cholecystographic agent that has been evaluated in cats. Ferguson et al¹⁵ induced hyperthyroidism in seven cats by daily subcutaneous administration of levothyroxine. Ipodate treated cats showed a significant reduction in T3 concentrations and increased body weight compared to the 4 control cats.¹⁵ Another study evaluated the effects of ipodate in 12 cats with naturally-occurring hyperthyroidism. Eight of 12 cats responded well with improvement in clinical signs and T3 concentrations, and no adverse effects were reported.¹⁶ Ipodate was discontinued by the manufacturer in 2001 making this agent unavailable. Iopanoic acid is a similar oral cholecystographic agent and has replaced ipodate for use in people.^{10,11} No studies evaluating the efficacy or safety of this agent in cats have been published.

The objective of this study was to evaluate the safety and efficacy of iopanoic acid for the treatment of cats with experimentally-induced hyperthyroidism.

CHAPTER I: LITERATURE REVIEW

A. The Thyroid Gland

Anatomy

The normal thyroid gland varies in shape, size, and location among different species as well as within species.¹⁷ In humans the thyroid gland consists of two lateral lobes connected by a narrow isthmus¹⁸ and is located immediately inferior to the larynx.¹⁹ The human thyroid is highly vascular with each lobe being supplied by a superior and inferior thyroid artery.¹⁸ In contrast, the feline thyroid gland consists of two lobes, usually without an isthmus, that are located on the ventrolateral aspect of the trachea just caudal to the larynx.²⁰ The major source of blood supply is the cranial thyroid artery and the caudal thyroid artery is absent in most cats.²⁰ Two parathyroid glands are associated with each thyroid lobe.^{20,21}

Microscopically, the thyroid glands of both species are similar. The major component is organized structures called follicles which are composed of a single layer of epithelial cells (follicular cells) that enclose a central lumen that is filled with colloid.¹⁸ The apex of the follicular cells faces toward the lumen and the base is directed toward the basement membrane which is in contact with a network of blood vessels.²¹ The thyroid also contains parafollicular cells (C cells) that produce calcitonin, a hormone that is important in calcium homeostasis.¹⁹

Synthesis and secretion of thyroid hormones

Thyroid hormone production is similar among species¹⁷ and consists of three main steps: intracellular iodine trapping, incorporation of iodine into thyroglobulin (Tg), and degradation of Tg to release T4 and T3.²²

Iodide (I⁻) is absorbed from the gastrointestinal tract and transported in the blood to the thyroid gland where it enters follicular cells through active transport. This is accomplished by a Na⁺/I⁻ symporter (NIS) located in the basement membrane driven by Na⁺,K⁺-ATPase. The result is influx of I⁻ into the cell against its electrical gradient resulting in a concentration 20-40 times that in the extracellular space.²² This is called iodide trapping. The degree of I⁻ transport depends in part on expression of the NIS gene which is regulated by cyclic adenosine monophosphate (cAMP) concentrations.²³ Increased cAMP, the production of which is stimulated by thyroid stimulating hormone (TSH), causes increased iodide trapping.²³

Thyroglobulin is a large glycoprotein that is synthesized in the endoplasmic reticulum (ER) of follicular cells. As it moves from the ER to the Golgi apparatus, further processing including glycosylation and phosphorylation occurs before Tg arrives at the apical membrane for iodination.^{19,22} Thyroglobulin contains approximately 70 tyrosine amino acids which are the major substrate for thyroid hormone production.¹⁹

At the apical membrane, two events occur. The first is oxidation of I⁻, catalyzed by thyroid peroxidase (TPO) and hydrogen peroxide (H₂O₂). Oxidation is necessary in order for I⁻ to attach to proteins such as Tg.²² The second event is iodination of Tg, a process that is termed organification. This process is enhanced by the presence of iodonases and TPO.^{19,22} In the initial steps, tyrosine is iodinated to monoiodotyrosine

(MIT) and diiodotyrosine (DIT). Then, over minutes to hours, iodothyronine residues undergo a coupling reaction catalyzed by TPO to form predominately T4 and small amounts of T3.^{19,22} During and after this process organified Tg moves from the follicular cell to the follicular lumen to be stored as colloid via exocytosis.²¹

The thyroid hormone is stored in the colloid until its release is stimulated, primarily by TSH.²² At that time Tg reuptake by the follicular cells occurs by endocytosis. This is accomplished by pseudopod extensions from the apical membrane that enclose small portions of colloid forming pinocytic vesicles.¹⁹ Lysosomes containing proteases fuse with these vesicles resulting in the digestion of the Tg molecule and release of T3 and T4.¹⁹ The T3 and T4 then diffuse through the basement membrane and into the blood, with T4 being the predominant hormone released. Approximately 70% of the iodine in Tg is in the form of MIT and DIT which remain trapped in the cell.²² Subsequently, MIT and DIT are degraded by deiodinases and the iodide is recycled.

Thyroid hormone binding and metabolism

Greater than 99% of the T3 and T4 that enters the blood is immediately bound to proteins.¹⁹ The remainder circulates as free or unbound hormones. The major binding proteins include thyroxine-binding globulin (TBG), transthyretin (formerly called prealbumin), and albumin.¹⁹ Thyroxine-binding globulin serves as the main binding protein in humans.^{19,24} Thyroxine-binding globulin is absent in cats, but transthyretin and albumin are present.²⁵ The amount of binding to these two proteins in cats has not been elucidated. Bound thyroid hormones are inactive but serve as a circulating storage pool. Half-life of bound T3 and T4 is related to the binding affinity of the proteins. A high

affinity for T4 results in a half life of 168 hours in humans and 10.7 hours in cats²⁶ and low affinity for T3 results in a half-life of 24 hours in humans¹⁹ but is unknown in cats.

Free T3 and free T4 are the metabolically active forms of thyroid hormones. While T4 comes from the thyroid gland, 70-80% of the daily T3 production in humans is the result of peripheral intracellular deiodination of T4.²⁷ A majority of daily T3 production in the cat also likely comes from peripheral metabolism of T3.²⁸ These hormones had been thought to enter the cell passively, but newer evidence has shown that receptor mediated active transport is responsible for entry of these hormones into cells.²⁴ Intracellular deiodination of T4 is accomplished by multiple deiodinases that catalyze either 5'- or 5-deiodination, thus forming all the iodothyronines in the deiodination pathway from T4 to iodine-free thyronine (T0).²⁹

5'-deiodination results in removal of iodine from the 5'-position of the outer or phenolic ring of T4 resulting in the formation of T3.^{29,30} Because T3 is 2-4 times more metabolically active than T4 this is referred to as a bioactivating pathway.²⁹ Conversely, 5-deiodination results in the removal of iodine from the 5-position of the inner or tyrosyl ring of T4 resulting in the formation of rT3. Since rT3 is metabolically inactive, this is referred to as a bioinactivating pathway.²⁹ Further 5'- or 5-deiodination of T3 and rT3 results in the formation of diiodothyronines (T2), monoiodothyronines, and T0.

Three deiodinase isoforms have been found in humans and are identified as type I 5'-deiodinase, type II 5'-deiodinase, and 5-deiodinase or D1, D2, and D3, respectively.^{29,30} Type I 5'-deiodinase is capable of both 5'- and 5-deiodination and results in the production of both T3 and rT3 from T4. It is also important in the elimination of rT3.^{29,30} It is primarily located in the thyroid, pituitary, liver, and kidney

in humans.³⁰ Type II 5'-deiodinase is capable of only 5'-deiodination resulting in the conversion of T4 to T3 and is important for producing T3 for local use in the pituitary, brain, and brown fat.³⁰

The roles of D1 and D2 in thyroid hormone metabolism have recently been further characterized. The D2 enzyme has a higher catalytic efficiency for conversion of T4 to T3 compared to D1 when free T4 concentrations are in the hypothyroid and euthyroid range. In part, this is likely due to D2 producing one molecule of T3 for each T4 molecule while D1 produces one molecule of T3 (and one molecule of rT3) for every 2 T4 molecules, but also to the slower rate of D1-catalyzed conversion.³¹ However, at hyperthyroid free T4 concentrations, the efficiency of D2 is less than that of D1.³¹ This may be caused by increased ubiquitination of the D2 enzyme resulting in inactivation and decreased D2 gene expression due to increased T3 concentrations.³² In addition, T3 resulting from D2 activity appears to have a more potent effect on nuclear transcription than T3 resulting from D1 activity.³¹ In essence, D2 appears to be more important in T3 production and nuclear activity in hypothyroid and euthyroid subjects, while D1 plays a greater role in T3 production in hyperthyroid patients.

D3 is capable of only 5-deiodination resulting in the conversion of T4 and T3 to rT3 and T2, respectively. This is a major route of T3 degradation and T4 inactivation as this enzyme occurs in almost every tissue.³⁰ D1, D2, and D3 are present in the cat, but their substrate preferences and tissue locations may be different. For instance, D1 in the cat is not present in the thyroid and appears unable to act on rT3.²⁸

In addition to deiodination, other alternative pathways for thyroid hormone metabolism exist. Reactions affecting the alanine side chain include oxidative

deamination that forms acetic acid derivatives of T4 (tetrac) and T3 (triac), both of which have lower thyromimetic activity.²⁹ Transamination and decarboxylation of the alanine side chain may also occur, but have not been found *in vivo*. Sulfoconjugation of iodothyronines is an energy dependent metabolic pathway and results in rapid deiodination of iodothyronines, particularly by D1.^{29,33} Glucuronidation of iodothyronines results in a more hydrophilic molecule which facilitates excretion into urine, feces, and bile.²⁹

Molecular actions and functions of thyroid hormone

Most of the molecular actions of thyroid hormones are due to interaction with nuclear receptors.^{34,35} T3 is the predominant hormone representing more than 90% of the thyroid hormone that is bound to receptors.¹⁹ Hence, intracellular conversion of T4 to T3 plays a regulatory role in the action of thyroid hormones.

Thyroid hormone receptors (TRs) are located within the cell nucleus and are either attached to the DNA genetic strands or located close to them.¹⁹ They are part of a superfamily that includes receptors for estrogen, progesterone, androgen, and glucocorticoids.³⁵ The TRs have a 10- to 15-fold greater affinity for T3 than T4 which in part explains the greater biologic potency of T3.³⁵ After T3 binds with the TR, the TR binds to thyroid-response elements (TREs) on target genes within the nucleus.³⁴ This interaction can result in positive or negative regulation.

In positive regulation, the binding of TRs to TREs results in target gene transcription and formation of mRNA coding for proteins or enzymes.^{34,35} Examples of positive regulated genes include growth hormone, myosin heavy chain α , and type I 5'-

deiodinase (D1).³⁵ In negative regulation, the same binding results in repression of the target gene.^{34,35} Examples of negative regulated genes include TSH- α , thyrotropin-releasing hormone (TRH), and type II 5'-deiodinase (D2).³⁵ By regulating gene expression, thyroid hormones exert effects on cell differentiation, cell development, and metabolic pathways.³⁵ In the absence of T3, TRs bind to TREs and inhibit the basal rate of gene transcription.³⁴

Thyroid hormones exert effects on essentially all tissues and metabolic pathways in the body. Metabolic effects include stimulation of fat and carbohydrate metabolism, increased basal metabolic rate, and increased rate of secretion of hormones from other endocrine glands.¹⁹ Specific tissue effects include increased heart rate and strength, increased gastrointestinal motility, and excitation of the central nervous system.¹⁹

Regulation of thyroid hormone synthesis and secretion

Thyroid hormone synthesis and secretion is controlled by the hypothalamic-pituitary-thyroid axis. This control is mediated by the release of several hormones including TSH, TRH, and the thyroid hormones themselves.

Thyroid-stimulating hormone is a glycoprotein synthesized and released from thyrotroph cells of the anterior pituitary gland. It is similar to other glycoprotein hormones such as follicle-stimulating hormone and consists of a common alpha subunit and a unique TSH-beta subunit.³⁶ After secretion, TSH travels through the blood stream to the thyroid gland where it binds with G protein-coupled TSH receptors on the basal membrane surface of follicular cells.^{19,36} Binding to the receptor activates adenylyl

cyclase in the membrane resulting in the formation of intracellular cAMP, which acts as a second messenger to activate protein kinase.¹⁹

The activation of this cascade results in multiple specific effects within the follicular cells. First, reuptake and proteolysis of Tg stored in colloid results in release of thyroid hormones into the blood. The activity of the iodide pump is increased thus increasing the rate of iodide trapping. Subsequently, increased iodination of tyrosine occurs to increase formation of thyroid hormones. Lastly, there is an increase in size, number, and secretory activity of follicular cells¹⁹ and an increase in TPO activity.³⁷ The end result is an increase in circulating concentrations of T3 and T4.

Secretion of TSH is regulated by thyroid hormone concentrations through a classic feedback system and by TRH from the hypothalamus. Within the pituitary thyrotroph cells, T3 binds to nuclear receptors resulting in decreased transcription of the common alpha and TSH-beta subunits.³⁶ Thyrotroph cells contain a high activity of type II 5'-deiodinase (D2) accounting for 50% to 60% of the nuclear T3 content.³⁶ Thus, inhibition of TSH secretion is dependent on not only T3 concentrations, but also on T4 concentrations and local deiodinase activity.³⁶ T3 and T4 also decrease expression of the TRH-receptor gene.³⁸

Thyrotropin-releasing hormone is a tripeptide hormone produced by the neurons of the hypothalamus. It is transported along the axons to specialized nerve terminals in the median eminence where it is released into the hypophyseal portal blood.³⁸

Thyrotropin-releasing hormone travels directly to the anterior pituitary where it binds to plasma membrane receptors on thyrotroph cells.³⁶ Receptor binding causes activation of the phospholipase C second messenger system and results in activation of protein kinase

C and calcium-dependent protein kinases.³⁶ Protein kinase activation leads to increased transcription of the TSH subunit genes.³⁶ TRH gene translation is reduced by T3 and T4 but this negative feedback is less important than the effect of T3 and T4 on TSH secretion.³⁹

B. Feline Hyperthyroidism

History and Incidence

Feline hyperthyroidism (FH) was first reported as a clinical diagnosis by Peterson in 1979⁴⁰ and Holzworth in 1980.⁴¹ Since that time the prevalence of this disease has continued to rise^{42,43} and it is now the most common endocrine disorder of cats with a prevalence of 2%.⁴³ The increase in prevalence may be due to better clinician recognition of the disease, better diagnostic testing, or an increased incidence of disease. Hyperthyroidism is mostly a disease of middle-aged to older cats with a mean age of 12-13 years and range of 4-22 years.^{42,44-46} Juvenile hyperthyroidism was reported in an 8-month old cat, but the histopathological changes were different than those seen in adult cats suggesting this may have been a different disease entity.⁴⁷ No sex predilection has been observed.^{42,44-46} Siamese and Himalayan, two related breeds, as well as purebred cats appear to be at decreased risk of developing hyperthyroidism.⁴⁸⁻⁵⁰

Pathophysiology and relation to hyperthyroidism in humans

Feline hyperthyroidism is the result of increased concentrations of circulating T3 and T4. This most commonly is caused by benign adenomas or adenomatous hyperplasia of the thyroid gland accounting for approximately 98% of cases.^{41,51} Histologically this is represented by multifocal areas of hyperplastic tissue, in some cases forming nodules of 1 mm to 3 cm in size.⁵² Bilateral thyroid involvement occurs in approximately 70% of cases with unilateral involvement in the other 30%.^{53,54} Ectopic thyroid tissue in the neck or cranial mediastinum may occur in up to 10% of cases.⁵⁵ Thyroid carcinomas are uncommon, causing hyperthyroidism in only 2-3% of cases.^{55,56}

The exact cause of FH has not been elucidated. Due to the large proportion of cats suffering from bilateral disease, an immune-mediated cause similar to Graves' disease in people was initially suspected. Graves' disease is an autoimmune disorder that results from the production of antibodies against TSH receptors called thyroid-stimulating immunoglobulins (TSIs). The TSIs bind to TSH receptors and mimic the actions of TSH.⁵⁷ However, studies have failed to identify TSIs as a cause for FH.⁵⁸⁻⁶¹ In addition, autonomous (TSH independent) growth of hyperplastic thyroid tissue has been shown both in cell culture and by transplantation into TSH deficient nude mice.^{52,62}

The autonomous thyroid growth seen in FH is most similar to toxic nodular goiter (TNG), or Plummer's disease, in people which has also been shown to have autonomous growth in TSH deficient nude mice.⁶³ The condition most commonly affects people in the fifth decade or later of life with an insidious onset of clinical signs^{64,65} which is similar to findings in FH. The pathogenesis of TNG appears to be related to gain-of-function mutations in the genes coding for the TSH receptor (TSHR) and to a lesser

extent G proteins.^{66,67} Recently, similar mutations have been identified in cats with hyperthyroidism. Watson et al⁶⁸ evaluated genomic DNA from hyperplastic nodules in 50 hyperthyroid cats. Twenty-eight of the 50 cats had at least one mis-sense mutation in the TSHR gene. However, functional studies were not performed to evaluate the effect of these mutations.

Two studies have documented decreased expression of inhibitory G (G_i) proteins in adenomatous thyroid glands from hyperthyroid cats.^{69,70} The authors concluded that this decrease in G_i results in increased cAMP production and thus autonomous growth of the thyroid follicles. Lastly, Merryman et al⁷¹ demonstrated overexpression of *c-ras* within hyperplastic and adenomatous regions of the thyroid gland from hyperthyroid cats. Gain-of-function mutations in *c-ras* have been associated with the development of follicular adenomas in human thyroid glands.⁷² A specific cause for these mutations has not been identified.

Numerous epidemiological studies have evaluated risk factors that may be involved in the pathogenesis of FH. A large number of studies have identified an increase risk of developing hyperthyroidism with increasing amounts of canned food in the diet.^{43,48-50,73} One of these studies found an association with pop-top cans⁴³ while another found an association with particular flavors of canned food including fish or liver and giblets.⁷³

Due to the association with feeding canned food, further studies have evaluated iodine levels in pet food. Iodine content is quite variable in commercial diets ranging from low to very high.^{74,75} Although thyroid hormone concentrations are acutely sensitive to changes in iodine intake⁷⁶, cats fed diets high or low in iodine long term had

normal free T4 concentrations.⁷⁷ Thus, iodine content of diets is unlikely to be a cause of feline hyperthyroidism. Soy is a common ingredient in cat foods⁷⁸ and increases T4 concentrations during short-term administration in a diet.⁷⁹ However, a correlation between soy and development of hyperthyroidism has not been made.

Two studies have made an association between exposure to pesticides and flea products and development of FH.^{49,50} Neither study was able to identify a specific agent. In a third study, results were equivocal in determining an association.⁴⁸ In another study, an association between pesticides and flea products was not found.⁷³ Kass et al⁴⁸ found an increased risk of hyperthyroidism associated with use of cat litter. The authors concluded that litter use was a marker for indoor cats and this finding complemented that of Scarlett et al⁵⁰ that predominately indoor cats were at greater risk of hyperthyroidism than predominately outdoor. However, in the Kass study the percentage of time cats spent indoors versus outdoors was not found to be a risk factor. This suggests that litter may contain goitrogenic compounds.

A number of environmental chemicals are known to affect thyroid function including the group of flame retardants which contain chemicals such as polybrominated diphenyl ethers (PBDEs).⁸⁰ In a recent study⁸¹, elevated levels of PBDEs were found in both cats with hyperthyroidism and sick non-hyperthyroid cats. Because the distribution and timing of increased levels of PBDEs in the environment have paralleled the increased distribution and increased prevalence of FH, the authors suggest that increased PBDEs may be related to the pathogenesis of FH. Studies in rats have shown a decrease in free T4 and T4 and normal TSH concentrations with exposure to PBDEs.^{82,83} In one study⁸², histopathological evaluation of the thyroid glands showed increased activity in the PBDE

group compared to the control group despite normal TSH concentrations. This suggests that PBDEs may be able to induce TSH independent growth in the thyroid gland.

Based on the current knowledge, there does not appear to be a singular factor that results in FH. It is likely that multiple genetic and environmental factors are required in the development of FH.

Clinical manifestations

Clinical signs of FH are due to the effects of excess thyroid hormones on target organs. Because thyroid hormones have effects throughout the body, almost any organ can be affected. The most common clinical signs include weight loss (87-98% of cats), polyphagia (49-81%), hyperactivity (31-76%), tachycardia (42-66%), polyuria/polydipsia (36-71%), vomiting (30-55%), diarrhea (15-51%), and cardiac murmurs (34-53%).^{42,44,46} Other less common manifestations of FH include tachypnea/dyspnea, increased fecal volume, anorexia, lethargy, and alopecia or other skin lesions.^{42,44,46}

Treatments

Therapy for FH can be divided into three categories: surgical thyroidectomy, radioactive iodine treatment, and antithyroid medications. The former two are considered definitive treatments and usually result in permanent resolution of hyperthyroidism. Antithyroid medication is recommended prior to definitive therapy and long-term in cases with concurrent kidney disease. In addition, many owners elect to use antithyroid medications long-term due to cost, risks, or availability of definitive therapy.

The thioureylene drug methimazole is the most commonly used antithyroid medication. This drug inhibits TPO which inhibits oxidation of iodide to iodine, incorporation of iodine into thyroglobulin, and coupling of tyrosine residues to form T4 and T3.⁸⁴ Thus, thyroid hormone synthesis is inhibited. Methimazole does not block release of previously formed thyroid hormones which results in a delay of 2-4 weeks before T4 concentrations return to normal in treated cats.⁶ While effective in most cases, methimazole administration is associated with side effects in up to 18% of treated cats.⁶⁻⁹ The most common side effects are vomiting and anorexia, but more serious side effects necessitating discontinuation of the drug occur in up to 10% of cats.⁶⁻⁹ These include self-induced excoriation, hepatotoxicity, thrombocytopenia, and agranulocytosis.

Other thioureylene drugs including carbimazole and propylthiouracil have also been used in treatment of FH. Carbimazole is largely converted to methimazole in vivo⁸⁵ and has similar side effects as methimazole.⁸⁶ Propylthiouracil was the initial drug used in the treatment of FH in the early 1980's.⁸⁷ However, this drug was associated with severe adverse reactions including hemolytic anemia, thrombocytopenia, and antinuclear antibody formation in 8% of cats.⁴ Propylthiouracil is no longer recommended for treatment of FH.

Stable iodine can also be used for the treatment of FH. Large doses of iodine reduce peroxidase-catalyzed organification of iodide and thus decrease thyroid hormone synthesis, coined the Wolf-Chaikoff effect.⁸⁸ In one report, potassium iodate was combined with propranolol for presurgical management of FH and was effective in most cats.⁵ However, approximately 50% had side effects including vomiting and anorexia which led to the development of hepatic lipidosis in some of the cats. In addition, iodine

therapy in humans has shown a rapid escape from inhibitory control.⁸⁹ Therefore this therapy is not routinely used in the management of FH.

C. Oral cholecystographic agents

Structure

Oral cholecystographic agents are triiodobenzene ring compounds with a high degree of lipid solubility. The phenolic ring provides binding sites for iodine atoms and an ethyl group attached to the second carbon makes the compound more hydrophilic. The OCAs differ from each other at the 4 position of the benzene ring and substitutions on the ethyl side chain.¹¹

Modes of action

The effects of OCAs on thyroid hormones were first described by Burgi et al¹³ in 1976. In this study, euthyroid patients that received an oral cholecystographic agent (sodium iopanoate) had significant increases in T4, free T4, and rT3, but decreases in T3. These changes were not seen in patients receiving an IV cholecystographic agent (sodium ioglycamate) or an IV urographic agent (sodium diatrizoate). Numerous studies have since been conducted to determine the mechanism of action of OCAs.

The principle action of OCAs is inhibition of predominately D2 but also D1 which are responsible for the outer ring deiodination of T4 to the more active T3 in many tissues including the liver, kidney, thyroid, and pituitary.^{11,13,90-92} Inner ring deiodination of T4 to the inactive rT3 is not affected. These enzymes are also responsible for the

deiodination of rT3 and thus inhibition of rT3 degradation also occurs.^{90,91} Subsequent elevations of rT3 may further inhibit monodeiodination of T4 to T3^{14,93,94} and may have an “anti-T4” effect due to reversible competitive inhibition at receptor sites.⁹⁵ The end result is reduced T3 concentrations and thus reduced effects of thyroid hormones on metabolism.

In addition to deiodinase inhibition, OCAs have other effects on thyroid metabolism. Felicetta et al⁹⁶ demonstrated that two different OCAs, tyropanoate and ipodate, both displaced T4 from hepatic binding sites which suggest that they can inhibit thyroid hormone effects on hepatic metabolism. DeGroot et al⁹⁷ demonstrated that several OCAs including ipodate and iopanoic acid inhibited nuclear binding of T3 in rat livers *in vitro* but were unable to demonstrate the effect *in vivo*. However, in another study ipodate was shown to inhibit T3 nuclear binding both *in vitro* and *in vivo* in rat livers.⁹⁸ This effect is likely due to the similar structure between these OCAs and iodothyronines resulting in competitive binding to the nuclear receptors.

In the pituitary gland, TSH secretion is modulated by serum T4 concentrations through conversion of T4 to T3 within the pituitary. Several studies have evaluated the effect of OCAs on TSH concentrations both before and after TRH stimulation in euthyroid people and hypothyroid rats.^{92,99,100} Suzuki et al¹⁰⁰ showed that OCAs resulted in a decrease in T3 and increases in T4, rT3, and TSH in euthyroid subjects. The administration of TRH resulted in significant increases in TSH. In another study, Kleinmann et al⁹⁹ showed similar findings in euthyroid subjects, but also demonstrated a greater response of TSH to TRH in patients treated with iopanoic acid. This response was prevented by the administration of T3 to the subjects prior to TRH stimulation.

Lastly, Larsen et al⁹² showed that TSH concentrations in hypothyroid rats were not effected by T4 administration after pre-treatment with iopanoic acid whereas T3 administration resulted in decreases in TSH. The effects of OCAs on TSH in hyperthyroid patients have not been evaluated.

Studies evaluating the effects of OCAs on perfused canine thyroid glands have shown multiple effects. In one study, Laurberg et al¹⁰¹ showed that ipodate and iopanoic acid at 10^{-5} M inhibited TSH-stimulated secretion of T3 and rT3, but had no effect on T4. However, at a 10^{-3} M concentration ipodate resulted in a rapid and reversible inhibition of T4, T3, and rT3 secretion. In a subsequent study by the same group, a perfused canine thyroid gland model was used to elucidate the mechanisms by which this inhibition occurs.¹⁰² In this study, infusion of ipodate at 1 mM concentration resulted in similar inhibition of T4, T3, and rT3 secretion. This effect could not be reproduced with infusions of 3 mM iodide. When ipodate is used for cholecystography in people, serum concentrations approach 10^{-3} M.¹⁰² However, OCAs are highly protein bound and the concentration of biologically active substance in serum is likely lower.¹⁰² Further evaluation revealed that 1 mM ipodate administration inhibited TSH-induced increases in cAMP, cAMP-induced generation of intracellular colloid droplets, and liberation of T4 and T3 from thyroglobulin. The authors speculated that the latter process was the cause for the rapid decline in iodothyronine secretion after ipodate administration.

Another study evaluated the effects of iopanoic acid on perfused rat thyroids. This study showed similar inhibition of TSH-stimulated release of cAMP and T3 with 0.1 mg/ml and 0.01 mg/ml concentrations of iopanoic acid. Interestingly, administration of 10^{-5} M iodide resulted in similar inhibition in contrast to the findings by Laurberg et al.¹⁰²

This may represent species differences between dogs and rats, but also may indicate that the inhibitory effect in the thyroid is in part due to the iodine content of OCAs.

An additional study by Cardoso et al¹⁰³ evaluated TPO and thyroid H₂O₂ generating activities in human patients with toxic diffuse goiters after administration of either iopanoic acid or a saturated solution of potassium iodide (SSKI). Results showed that TPO activity was significantly lower in thyroid samples from patients treated with SSKI but not with iopanoic acid. However, both iopanoic acid and SSKI inhibited thyroid H₂O₂ generation. The authors suggest that this inhibition is due to the iodine content of iopanoic acid. However, if this is the case one would have expected a decline in TPO activity as well with iopanoic acid treatment.

In conclusion, OCAs have multiple mechanisms of action which result in decreases in serum T3 concentrations, inhibition of thyroid hormone binding, and decreased iodothyronine secretion by the thyroid gland. Future studies may elucidate other mechanisms of action.

Clinical use in humans

Since Burgi et al first reported the effects of OCAs on thyroid metabolism, numerous studies have evaluated their efficacy in the treatment of various hyperthyroid conditions in people. When used to treat Graves' disease, OCAs including ipodate and iopanoic acid have been effective in reducing T3 concentrations and resolving clinical signs of disease during short term therapy.^{10,104,105}

However, long-term therapy of Graves' disease with OCAs has had variable results. In one study of 40 patients, 6 (15%) had excellent response to iopanoic acid and

12 (30%) showed considerable improvement. However, 22 (55%) of patients had a poor response. Eighteen of the 22 patients initially showed improvement followed by marked exacerbation of signs within 6 months of starting therapy.¹⁰⁶ In another study, 5 of 12 patients (42%) treated with ipodate had prolonged remission (22 months) of Graves' disease while 7 (58%) had recurrence of hyperthyroidism.¹⁰⁷ Shen et al¹⁰⁸ reported successful treatment of all 5 patients with ipodate during a 23-31 week period.

Oral cholecystographic agents have been used for rapid preparation of patients prior to thyroidectomy. Conventional preparation with other medical therapies usually requires 4-6 weeks.¹¹ Wu et al⁹⁰ showed that ipodate causes a significant decrease in T3 concentration within 8 hours of administration to hyperthyroid subjects with the nadir occurring at 2 days. Baeza et al¹⁰⁹ reported on a rapid preparation protocol using iopanoic acid in 14 patients. A significant decrease in T3 concentrations was noted as early as 24 hours, reaching near euthyroid concentrations by day 3. By day 5 of therapy, all patients were considered euthyroid and surgery occurred without complications. In another study, 17 patients were treated with a similar protocol using iopanoic acid. All 17 showed rapid improvement in T3 concentrations with near euthyroid concentrations 4 days after starting therapy, the first recheck, and had surgery a mean of 7 days after start of therapy without complications.¹²

Oral cholecystographic agents have also been used to treat other causes of thyrotoxicosis. Brown et al¹¹⁰ reported on the successful treatment with iopanoic acid of a 2-year old infant who accidentally ingested levothyroxine. Chopra et al¹¹¹ reported successful treatment of 5 patients with subacute thyroiditis using ipodate. Neonatal

Graves' disease has been successfully treated using either ipodate^{112,113} or iopanoic acid.¹¹⁴

Clinical use in cats

To date, only two studies have been published evaluating the use of OCAs in cats, both using ipodate. In the first study, Ferguson et al¹⁵ induced hyperthyroidism in seven cats by daily subcutaneous administration of T4 for one month prior to treatment and continued during the treatment period. Ipodate was administered to three cats at a dose of 15 mg/kg orally twice a day for one month. Results of the study showed significant reductions in T3 concentrations and increases in body weight in the ipodate treated cats compared to the controls.

In the second study, Murray et al¹⁶ evaluated ipodate in 12 cats with spontaneous hyperthyroidism. Cats were started at a dose of 50 mg orally twice a day with 50 mg increases in dose every 2 weeks up to 100 mg orally twice a day if the T3 concentration did not decrease into the reference range. During the 14 week study, 8 of 12 cats (66%) were considered responders. However, by the end of the study, 2 of the responders (25%) had return of clinical signs. Only one of the responders required dose escalation to 150 mg/day. Dose escalations were used in all non-responders. No adverse clinical signs or hematologic abnormalities were noted during the study.

Ipodate production was discontinued by the manufacturer in 2001. Iopanoic acid is a similar oral cholecystographic agent and has replaced ipodate for use in people.^{10,11} The use of iopanoic acid in cats has been reported anecdotally with a dose of 50 mg

orally twice a day. However, no studies have been published evaluating the efficacy or safety of iopanoic acid in cats.

CHAPTER 2: EFFICACY AND SAFETY OF IOPANOIC ACID FOR TREATMENT OF EXPERIMENTALLY-INDUCED HYPERTHYROIDISM IN CATS

A. Introduction

Methimazole is the mainstay of medical treatment for FH. While effective in most cases, methimazole administration is associated with side effects in up to 18% of treated cats.⁶⁻⁹ The most common side effects are vomiting and anorexia, but more serious side effects necessitating discontinuation of the drug occur in up to 10% of cats.⁶⁻⁹ These include self-induced excoriation, hepatotoxicity, thrombocytopenia, and agranulocytosis. In cats experiencing these side effects that cannot undergo surgical thyroidectomy or radioiodine therapy, there are limited options.

In humans, OCAs such as ipodate and iopanoic acid have been used to treat hyperthyroidism because of their rapid action and excellent safety record.¹⁰⁻¹² The principal effect of OCAs is inhibition of deiodinases that are responsible for the peripheral conversion of T4 to T3.¹¹ These effects are likely related to the chemical structure of the OCAs as these actions cannot be accounted for solely by iodine release.¹¹

Ipodate is the only OCA that has been evaluated for treatment of FH. Administration of ipodate to cats with experimentally-induced hyperthyroidism caused a significant reduction in serum T3 concentration and an increase in body weight.¹⁵ Ipodate treatment of cats with spontaneous hyperthyroidism resulted in effective control of the disease in 66% of cases.¹⁶ Ipodate production was discontinued by the manufacturer in 2001. Iopanoic acid is a similar oral cholecystographic agent and has replaced ipodate for use in people.^{10,11}

The authors are unaware of published studies evaluating this agent in cats. The purpose of the pilot study reported here was to evaluate the safety and effect of two dosages of iopanoic acid in cats administered levothyroxine to induce a hyperthyroid state.

B. Materials and Methods

Animals – Fifteen healthy, adult, female spayed, domestic short hair cats were used in the study. The cats were part of an established research colony and were determined to be healthy based on physical examination, complete blood count (CBC), biochemistry, and serum T4 concentration. The study was approved by the Virginia Tech Animal Care and Use Committee. All cats were housed in individual cages.

Experimental design - All cats were fed a commercially available dry adult maintenance diet^a. Maintenance energy requirement (MER) was calculated for each cat using the following formula: $MER = 1.2 \times 70 (W_{kg}^{0.75})$. Each cat was fed its MER for one week prior to the study and during the first week of the study. For weeks 2-6 of the study, each cat was fed twice its initial MER to provide ample food for intake monitoring in anticipation of polyphagia induced by hyperthyroidism. Food was weighed prior to feeding and 24 hours later to determine daily and weekly food intake. Cats were fasted for 12 hours prior to blood collection.

All cats were treated with levothyroxine for 42 days starting on day 0 in order to induce a hyperthyroid state. Initially levothyroxine^b (diluted to 500 µg/ml with 1 mM NaOH) was administered at a dose of 50 µg/kg once a day subcutaneously based on

previous studies.^{15,115,116} Severe hyperthyroidism manifested by complete anorexia developed in one cat on day 10 (T4 761 nmol/L, reference range 26-49 nmol/L). Subsequently, levothyroxine was discontinued in all cats on day 11 and administered at 25 µg/kg once a day from day 12 to day 42.

On day 28, 13 cats were randomly assigned to a control group (n = 4), a low dose group (n = 5), or a high dose group (n = 4). Two cats, including the cat with severe hyperthyroidism, were removed from the study prior to randomization due to anorexia of greater than 3 days duration. The low dose group received a capsule containing 50 mg of iopanoic acid^c and 70 mg of lactose orally (PO) every 12 hours, the high dose group received a capsule containing 100 mg of iopanoic acid and 20 mg of lactose PO every 12 hours, and the control group received a capsule containing 120 mg of lactose PO every 12 hours. Treatment was administered for 14 days. All capsules were the same size, shape, and color. All capsules were administered by one of the investigators (AEG) and both investigators were blinded to the treatment groups.

On days -8 (heart rate (HR) only), 0 (body weight (BW) only), 28, 35, and 42 of the study, HR and BW were recorded. Blood was collected by jugular venipuncture on days -8, 28, 35, and 42 for CBC, biochemistry, and serum T4, T3, and rT3 concentrations. All samples were collected 24 hours after levothyroxine administration and 12 hours after iopanoic acid or placebo administration. Additionally, on day 42 blood samples were collected four and eight hours after administration of levothyroxine and iopanoic acid or placebo for measurement of T4, T3, and rT3 concentrations. Samples were analyzed the day of collection for CBC and biochemistries. Samples for T4, T3, and rT3 concentrations were allowed to clot for 30 minutes before centrifugation

at 1,000 x g for 20 minutes. The serum was then harvested and frozen at -70° C until assayed.

Sample analysis - Complete blood counts^d and plasma biochemistry^e were performed by the Virginia-Maryland Regional College of Veterinary Medicine Veterinary Teaching Hospital clinical pathology laboratory. Biochemical analyses included concentrations of glucose, blood urea nitrogen (BUN), creatinine, phosphorus, calcium, total protein, albumin, globulin, total bilirubin, cholesterol, sodium, potassium, chloride, total CO₂, and activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALKP).

Serum T4^f, T3^g, and rT3^h concentrations were determined using commercially available radioimmunoassay kits. All assays were performed in duplicate. Any result that initially exceeded the highest standard was diluted using the “0” standard and reanalyzed. The T4 assay had been previously validated for use with feline serum.¹¹⁷ Assay sensitivity was estimated at 5.0 nmol/L, the point of 95% of total binding on the standard curve. Intra-assay coefficients of variation (CVs) for 2 feline pooled sera were 7.8% (mean, 29.8 nmol/L, n=10) and 14.4% (mean, 136.2 nmol/L, n=10). Dilutional parallelism was evaluated in the T3 assay by determining recovery of expected concentration when aliquots of one serum sample (3.15 nmol/L) were serially diluted with the “0” standard. When serum was diluted 75%, 50%, 25%, and 12.5% to final volume before addition to the assay, 103%, 96%, 96%, and 92%, respectively, of expected concentrations were recovered. Assay sensitivity was estimated at 0.15 nmol/L, the point of 95% of total binding on the standard curve. Intra-assay CVs for 2 feline pooled sera were 16.9% (mean, 0.65 nmol/L, n=8) and 5.2% (mean, 3.05 nmol/L, n=10).

Dilutional parallelism was evaluated in the rT3 assay by determining recovery of expected concentration when aliquots of one serum sample (0.39 ng/mL) were serially diluted with the “0” standard. When serum was diluted 75%, 50%, 25%, and 12.5% to final volume before addition to the assay, 100%, 96%, 101%, and 95%, respectively, of expected concentrations were recovered. Assay sensitivity was estimated at 0.04 ng/mL, the point of 95% of total binding on the standard curve. Intra-assay CVs for 2 feline pooled sera were 5.1% (mean, 0.16 ng/mL, n=4) and 6.8% (mean, 0.71 ng/mL, n=4).

Statistical analysis – Data are reported as mean \pm standard error of the mean (SEM). Serum T3, T4, and rT3 concentrations and HR and BW measurements between baseline values (day -8 or 0) and day 28 for all cats were compared using the Student’s t-test. Differences in these parameters and weekly food intake between groups and between time periods were compared using the GLIMMIX procedure for repeated measures ANOVA with a Tukey-Kramer adjustment using commercial statistical softwareⁱ. For all measurements, group means and SEMs for each time period were determined using separate spreadsheet and analysis software^j. Using the same software, differences in activities of ALT and ALKP, and concentrations of creatinine and phosphorus between groups and between time periods were compared using the Student’s t-test. For all comparisons, $P < 0.05$ was considered significant.

C. Results

Animals – The mean age of the 13 cats in the study was 5.4 ± 0.7 years. There was no difference in mean age between groups. Twelve cats completed the study. One cat in the control group was removed on day 36 due to development of heart failure. This cat was included in the analysis through day 35. In addition, one cat in the low dose group developed fever and inappetance on day 36 and was not included in the food intake analysis, but was able to complete the study. The cause of the cat's illness was not determined despite extensive diagnostic testing that included multiple physical examinations, CBC, plasma biochemistry, thoracic radiographs, and abdominal ultrasound and treatment with clindamycin. The illness persisted for 7 days after cessation of iopanoic acid administration until prednisolone was administered beginning day 49. No recurrence of the illness was noted 12 weeks after discontinuation of the 2 week course of prednisolone treatment.

Thyroid hormone concentrations – There was no difference between groups at day -8 or at day 28 for mean T4, T3, or rT3 concentrations. Compared to day -8, there was a significant increase at day 28 for T4 ($P < 0.001$), T3 ($P < 0.001$), and rT3 ($P < 0.001$) concentrations in all groups.

Compared to the control group, mean T4 concentrations were higher at day 35 for the low and high dose groups ($P = 0.008$ and $P = 0.012$, respectively), and at day 42 for the high dose group ($P = 0.0011$), but not the low dose group ($P = 0.053$) (Figure 1).

There was no difference in mean T4 concentrations between the low dose and high dose

groups at any time period. For the control group there was no difference in mean T4 concentrations between days 28, 35, and 42.

Compared to the control group, mean T3 concentrations were lower in both the low dose and high dose groups on days 35 ($P < 0.001$; $P = 0.003$, respectively) and 42 ($P < 0.001$; $P < 0.001$, respectively) (Figure 2). Compared to day -8, there was no difference for mean T3 concentrations in the low dose and high dose groups at days 35 and 42. There was no difference in mean T3 concentrations between the low and high dose groups at any time period. There was no difference in mean rT3 concentrations between groups at any time period (Figure 3).

On day 42, T3, T4, and rT3 concentrations were measured prior to T4 injection and iopanoic acid or placebo dosing (0 hour), and at 4 and 8 hours after dosing. When compared to 0 hour, mean T3 concentrations in the control group were increased at 4 hours ($P = 0.009$) but not at 8 hours while no difference was noted in either treatment group (Figure 4). When compared to 0 hour, mean T4 concentrations were increased in the control and high dose groups at 4 hours ($P = 0.0009$; $P = 0.040$, respectively) and in the low dose group at 4 and 8 hours ($P = 0.002$; $P = 0.011$, respectively). When compared to 0 hour, mean rT3 concentrations in the control group were increased at 8 hours ($P = 0.006$).

CBC and biochemistry analyses – There were no clinically relevant changes in CBCs for any group at any time period. In addition there were no clinically relevant changes in concentrations of BUN, glucose, calcium, albumin, globulins, total bilirubin, cholesterol, sodium, potassium, chloride, or total CO₂.

There was no difference in mean ALT activity between groups at days -8 or 28 (Table 1). On day 28, one cat in the control group had an ALT of 474 U/L, but on day 35 it was 250 U/L. Mean ALT activity of the control group at day 28 excluding this cat was 127.0 ± 20.8 U/L while it was 213.8 ± 88.0 U/L when the cat was included. For days 35 and 42 mean ALT activity in the control group was higher compared to the low dose ($P < 0.01$; $P = 0.03$, respectively) and high dose ($P < 0.01$; $P = 0.03$, respectively) groups.

The mean ALKP activity in the high dose group at day -8 was higher compared to the low dose group ($P = 0.03$), but was not different from the control group (Table 2). There was no difference between groups at days 28, 35, or 42. Mean creatinine concentrations were decreased ($P < 0.05$) and mean phosphorus concentrations were increased ($P < 0.05$) in all groups at days 28, 35, and 42 compared to day -8. There was no difference between groups at any time period for either variable.

Body weight, food intake, and heart rate – There was no significant difference in mean BW (Table 3) or mean weekly food intake between groups or between time periods. Compared to day -8, there was a significant increase in mean HR at day 28 for the low (204.4 ± 5.3 beats/minute vs. 250.8 ± 6.8 , $P = 0.002$) and high dose groups (207 ± 2.4 vs. 257 ± 3.3 , $P = 0.001$) (Table 4). There was a decrease in mean HR in the low dose group on day 35 (210.8 ± 11) compared with days 28 ($P = 0.012$) and 42 ($P = 0.044$), but there were no other differences in mean HR between groups or time periods.

D. Discussion

The decrease in T3 noted in the present study was consistent with the primary mechanism of action of iopanoic acid, namely inhibition of conversion of T4 to T3. Because T4 is essentially a prohormone for T3, as thyroid hormone receptors have 10 times higher affinity for T3 than T4, the reduction in T3 would be expected to alleviate the hyperthyroid state despite continued elevation of serum T4 concentration. Serum T3 concentrations during treatment with iopanoic acid were not different from concentrations measured prior to the induction of hyperthyroidism. Therefore it is expected that the reduction in T3 would result in improvement or resolution of clinical signs of hyperthyroidism in cats with spontaneous disease if the response were similar. The magnitude of the decrease in T3 noted during iopanoic acid administration in this study was similar to that found in cats treated with calcium ipodate using a similar model of hyperthyroidism.¹⁵ Because ipodate is effective in treatment of some cats with spontaneous hyperthyroidism, it seems likely that iopanoic acid would have a similar efficacy.¹⁶

Iopanoic acid lowers T3 concentrations primarily by inhibition of 5'-deiodinases (D1 and D2), thus decreasing conversion of T4 to T3. Inhibition of D1 also reduces degradation of rT3, resulting in an increase in serum rT3, although it is unknown if this occurs in the cat.^{28,90} The increase in rT3 may further inhibit conversion of T4 to T3.¹⁴ In addition, oral cholecystographic agents inhibit cellular uptake of T4 and T3, thus reducing T4 available for conversion to T3 and T3 available to bind to thyroid hormone receptors.^{96,97,118} In the present study T3 concentrations were reduced within one week of

initiating treatment which is consistent with the rapid decrease seen in human patients treated with OCAs.⁹⁰

The increase in serum T4 concentration during iopanoic acid administration was most likely due to decreased degradation of T4 by inhibition of 5'-deiodinase⁹⁰ and reduced transport of T4 into cells.^{96,97,118} Increases in T4 concentrations have also been noted in human patients treated with oral cholecystographic agents. For this reason serum T3 rather than T4 concentration should be measured in cats treated with iopanoic acid to determine the effect of treatment.

An increase in serum rT3 would be expected in response to decreased type I iodothyronine deiodinase (D1) activity as this enzyme is the major catalyst for deiodination of rT3 in most species. In the cat, however, type I 5'-deiodinase (D1) in the liver and kidney have extremely low activity utilizing rT3 as a substrate; thus, rT3 concentrations would be minimally affected by iopanoic acid as noted in the present study.²⁸ In addition, enhanced excretion by alternative metabolic pathways for the degradation of rT3, such as sulfation, deamination, or decarboxylation that are not inhibited by oral cholecystographic agents may be present. Wu et al¹¹⁹ demonstrated that treatment with ipodate in humans resulted in increases in rT3 sulfate. In addition, iopanoic acid administration in humans has been shown to increase serum and urine concentrations of triiodothyroacetic acid.¹²⁰ If the increase in triiodothyroacetic acid is a result of increased production rather than decreased degradation, it is possible that this alternate pathway of T4 metabolism accounts in part for the decrease in T3 and lack of increase in rT3 noted in cats treated with iopanoic acid.

While the mean serum T3 concentration decreased significantly in cats treated with both dosages of iopanoic acid, clinical signs of hyperthyroidism induced by levothyroxine administration were not as convincingly affected. Of the three clinical parameters evaluated in the current study, heart rate was the only measurement in which improvement was noted during iopanoic acid administration, and that effect was inconsistent. Failure to note consistent and significant differences in heart rate, body weight, and food intake during the study in any group may have been a result of the model used, short duration of the study, or the small number of animals studied. Cats were fed twice MER because they consumed all food when fed MER prior to instituting levothyroxine administration. The investigators felt that offering all cats more food would allow for the opportunity to assess any increase in food intake anticipated after inducing hyperthyroidism, but this may have masked any change in body weight that would have occurred secondary to hyperthyroidism. Treatment duration was only 2 weeks, but a decrease in T3 after ipodate or iopanoic acid administration has been documented after a single dose in humans.^{13,90} Hence, a clinical response would have been expected after 2 weeks of treatment. Alternatively, the lack of consistent response to treatment may have been due to persistence of the hyperthyroid state regardless of treatment. However, ipodate administration to cats using a similar model for 4 weeks resulted in a proportionately smaller reduction in T3 yet body weight and heart weight to body weight ratios were lower in treated cats, indicating reduction of thyrotoxicosis.¹⁵

The biochemical abnormalities noted in this study are typical of those described in cats with spontaneous hyperthyroidism. The mean ALKP activity was increased above the reference range in all groups after the induction of hyperthyroidism and remained

elevated even in iopanoic acid treatment groups. The increased ALKP activity in hyperthyroid cats is predominately the result of increased bone isoenzyme activity.¹²¹⁻¹²³ The persistence of the increase in ALKP activity in cats administered iopanoic acid likely indicates that the hyperthyroid state was not completely controlled. Previous studies in rat models have shown that either T4 or T3 can affect bone metabolism resulting in increased bone ALKP isoenzyme activity and bone turnover.^{124,125} Therefore, the persistently increased ALKP activity seen in this study may be due to the increased T4 concentrations as T3 concentrations returned to normal in the low and high dose treatment groups. Because a concurrent increase in ALT activity was noted during the hyperthyroid state, some of the ALKP activity may have originated from the liver. The mean ALT activity was significantly lower in the high and low dose groups at days 35 and 42 compared to the control group. This suggests that iopanoic acid was partially effective in reversing the clinicopathologic abnormalities associated with hyperthyroidism.

One cat in the low dose group developed a fever and inappetance on day 36. The fever continued despite cessation of the iopanoic acid on day 42. A cause of the fever was not determined despite a thorough investigation. Although a reaction to the iopanoic acid cannot be excluded, the lack of other clinical signs and continuation of the fever for one week after stopping therapy would suggest that this is unlikely. No other cats developed fever or any other signs of illness unrelated to hyperthyroidism. When used at higher doses for cholecystography in people, adverse effects of iopanoic acid have included GI complaints, renal disease, and rarely thrombocytopenia.^{11,126} However, adverse effects have not been noted when iopanoic acid has been used for the treatment

of hyperthyroidism in people.^{11,12,106,109} The results of this study support the safety of iopanoic acid administration in hyperthyroid cats. However, the small number of cats evaluated and the short duration of treatment necessitates evaluation in a larger population of cats with spontaneous hyperthyroidism to confirm this finding.

The model of hyperthyroidism used in the present study has been used effectively by others to study the effects of hyperthyroidism on glucose homeostasis and renal function as well as the efficacy of ipodate for treatment of hyperthyroidism.^{15,115,116} However, spontaneous hyperthyroidism probably develops gradually over a period of many months and results in secretion of excessive amounts of both T4 and T3. The resultant clinical signs of this chronic disorder, including polyphagia, vomiting, diarrhea, weight loss, and tachycardia, were largely absent in the cats of the current study.^{44,46} This indicates that although our model was sufficient to induce hyperthyroidism based on elevated T3, T4, and rT3 concentrations and increased heart rate, the duration of thyroid hormone elevation was insufficient to allow other clinical signs to develop. The model was chosen because the primary mechanism of action of oral cholecystographic agents is inhibition of peripheral conversion of T4 to T3. However, the model does not account for other actions that these agents may have in spontaneous hyperthyroidism such as inhibition of thyroid hormone release from the thyroid gland.

In conclusion, this pilot study demonstrates that iopanoic acid administration markedly reduces T3 concentrations in cats made thyrotoxic by administration of levothyroxine, and that it appears to be safe. A clinical effect was not evident in this study, but this may have been due to the short duration of therapy or the experimental model. Based on the results of this study and previous studies with ipodate, further work

to evaluate the long-term efficacy and safety of iopanoic in cats with spontaneous hyperthyroidism is warranted.

CHAPTER 3: CONCLUSIONS

In conclusion, iopanoic acid at both 50-mg and 100-mg every 12 hours was effective in reducing T3 concentrations to pre-hyperthyroid concentrations in cats. This finding is consistent with previous studies of ipodate in cats and OCAs in people. However, a clinical effect on signs of hyperthyroidism was not evident. This may have been due to the short duration of therapy, the small number of cats in each group, or the experimental model used.

One objective of this study was to determine the safety of iopanoic acid in cats. Previous studies of ipodate in cats and OCAs used for thyroid disease in people have not reported any major adverse reactions. In this study, one cat receiving iopanoic acid developed a fever during the second week of therapy. The fever persisted despite discontinuation of the iopanoic acid, suggesting that it was not due to a drug reaction. The fever did respond to administration of prednisolone making an immune-mediated reaction to iopanoic acid a possibility. However, the pills also contained lactose, and a reaction to this component rather than the iopanoic acid cannot be excluded. In general, iopanoic acid appears to be safe, but further studies in larger populations of cats will be needed to truly assess safety.

Almost all patients with FH should at least initially be treated with antithyroid medication. Currently, methimazole is the only reasonable option. Due to the large proportion of hyperthyroid cats that are unable to tolerate methimazole therapy, an alternative medical therapy is needed. Based on the effect of iopanoic acid on T3 concentrations in this study and previous studies of ipodate in cats, it would be warranted to pursue a clinical trial to evaluate the effects of iopanoic acid in cats with spontaneous

hyperthyroidism. In addition to providing alternative therapy, iopanoic acid may also be useful for rapid preparation of patients prior to surgery or for rapid control of thyrotoxicosis during feline thyroid storm.

FOOTNOTES

- a – Science Diet Feline Adult Original dry food, Hill’s Pet Nutrition, Topeka, KS.
- b – L-thyroxine Na salt, Sigma Aldrich, St. Louis, MO
- c – Island Pharmacy Services, Woodruff, WI.
- d– Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, IL.
- e – Olympus AU400 automated chemistry analyzer, Olympus America, Melville, NY.
- f – Coat-A-Count Total T4, Diagnostic Products Corporation, Los Angeles, CA.
- g – Coat-A-Count Total T3, Diagnostic Products Corporation, Los Angeles, CA.
- h – Reverse T3, Adaltis Italia S.p.A., Bologna, Italy.
- i – SAS statistical software, version 9.1.3, SAS Institute Cary, NC.
- j – Microsoft Excel 2002, Microsoft Corporation, Redmond, WA.

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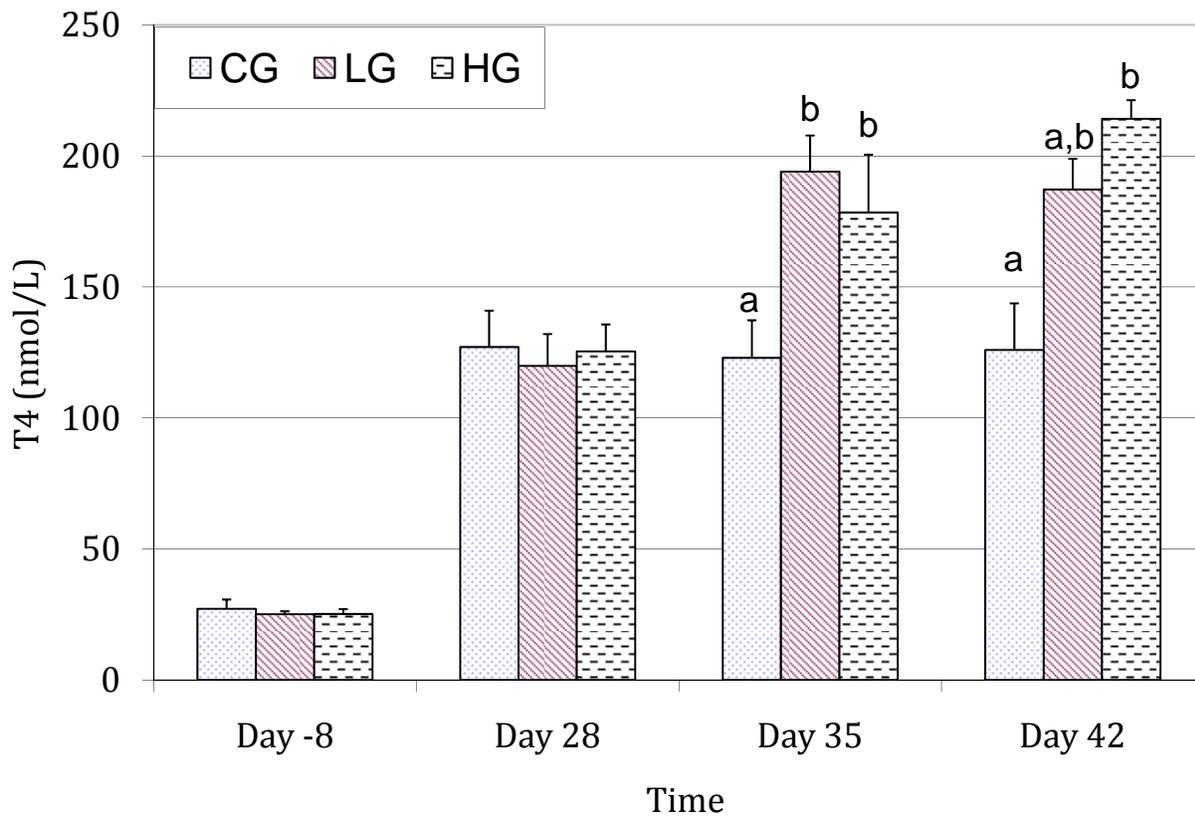
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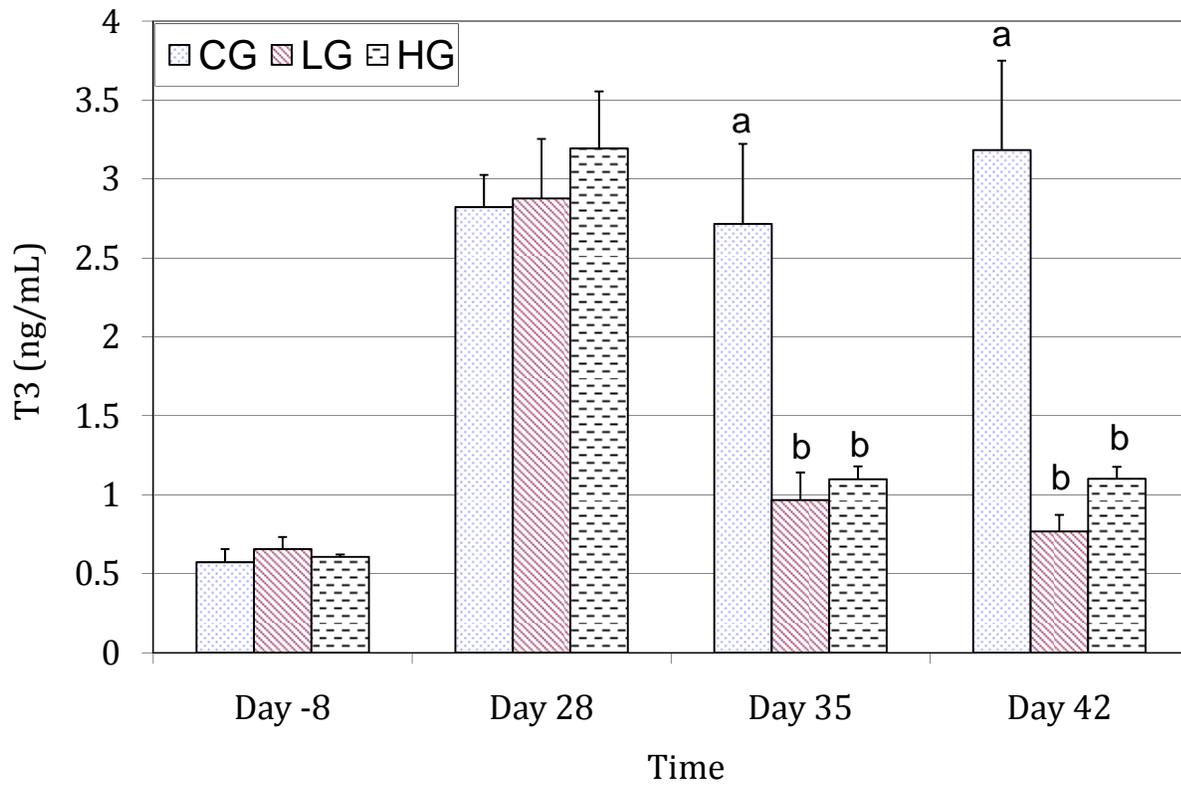
APPENDIX I: Figures

Figure 1 - Mean T4 concentrations for control group (CG), low dose group (LG), and high dose group (HG) at days -8, 28, 35, and 42.



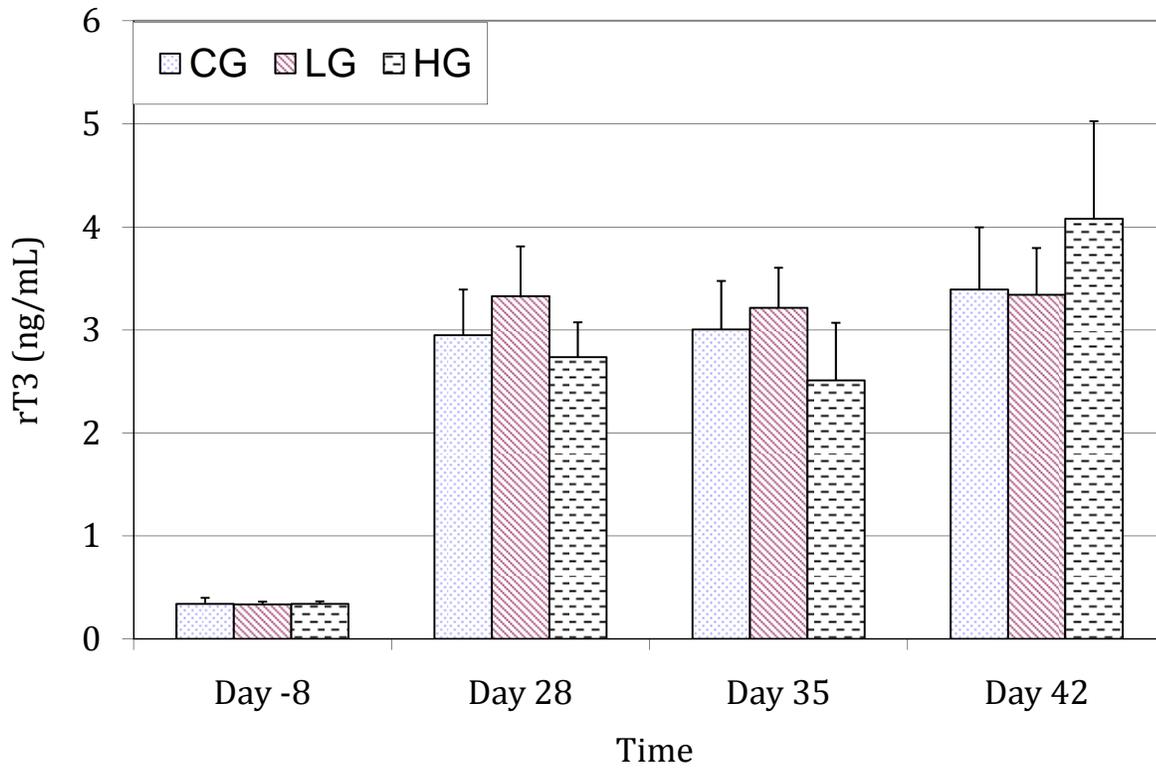
For each time period bars with different letters are significantly different ($P < 0.05$). Error bars denote SEM.

Figure 2. Mean T3 concentrations for control group (CG), low dose group (LG), and high dose group (HG) at days -8, 28, 35, and 42.



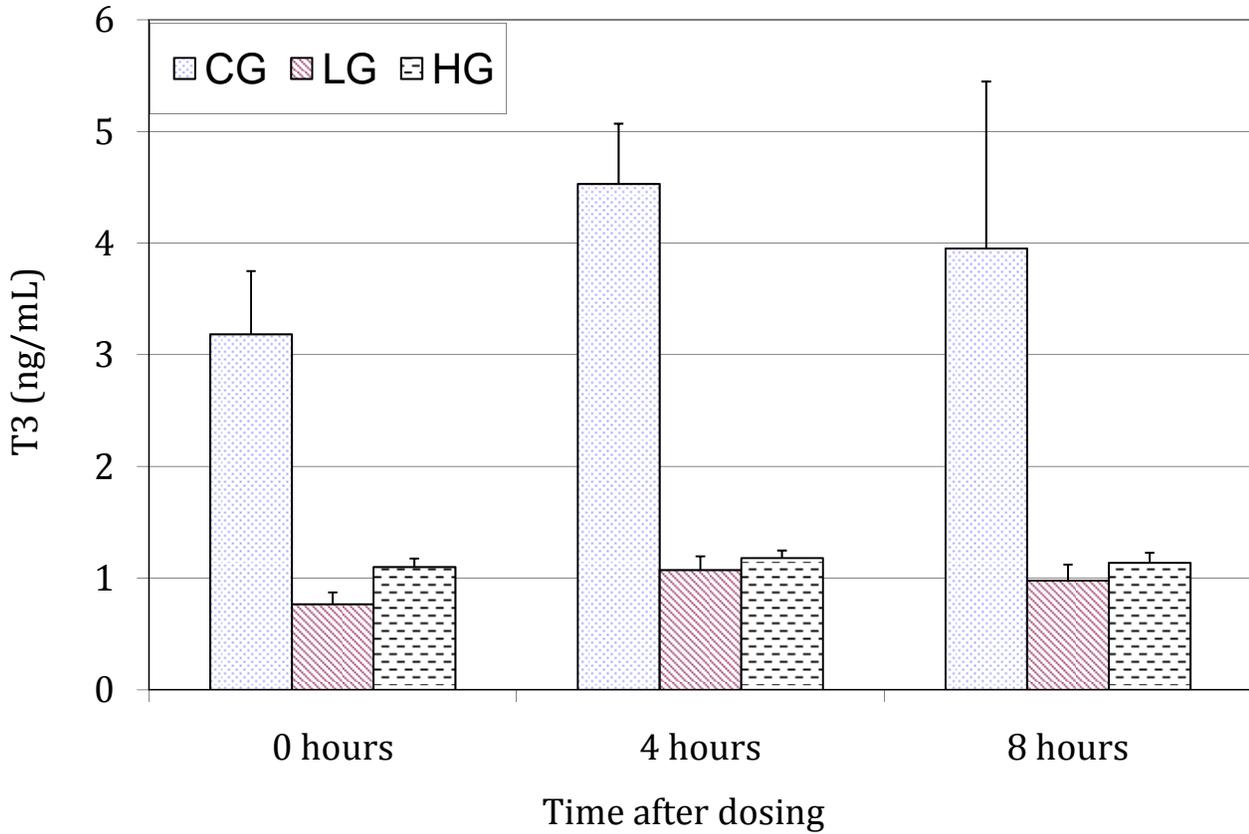
For each time period bars with different letters are significantly different ($P < 0.05$). Error bars denote SEM.

Figure 3. Mean rT3 concentrations for control group (CG), low dose group (LG), and high dose group (HG) at days -8, 28, 35, and 42.



Error bars denote SEM.

Figure 4. Mean T3 concentrations for control group (CG), low-dose group (LG), and high-dose group (HG) on day 42 prior to administration of T4, iopanoic acid, or placebo (0 hours) and at 4 and 8 hours after administration.



Error bars denote SEM.

APPENDIX II: Tables

Table 1. Mean ALT activities by group*

	Control	Low dose	High dose
Day -8	48.3 ± 4.6	46.6 ± 4.3	53.5 ± 10.1
Day 28	213.8 ± 88.0	82.4 ± 20.5	95.5 ± 10.3
Day 35	200.5 ± 22.7 ^a	62.6 ± 17.0 ^b	80.0 ± 9.4 ^b
Day 42	220.3 ± 37.3 ^a	57.2 ± 9.5 ^b	78.8 ± 7.0 ^b

* ALT in U/L as mean ± SEM. Reference interval 25-114 U/L.

a-b Means with different letters are significantly different (P < 0.05) within each row.

Table 2. Mean ALKP activity by group*

	Control	Low dose	High dose
Day -8	34.0 ± 9.4 ^{a,b}	39.6 ± 3.9 ^a	52.0 ± 4.0 ^b
Day 28	91.0 ± 14.2	76.0 ± 8.5	87.8 ± 12.6
Day 35	87.0 ± 9.4	70.8 ± 11.3	91.0 ± 13.7
Day 42	98.7 ± 19.8	70.0 ± 15.8	100.3 ± 14.2

* ALKP in U/L as mean ± SEM. Reference interval 11-45 U/L.

a-b Means with different letters are significantly different (P < 0.05) within each row.

Table 3. Mean body weights by group*

	Control	Low dose	High dose
Day -8	4.67 ± 0.60	4.85 ± 0.20	4.86 ± 0.22
Day 28	4.59 ± 0.57	5.00 ± 0.26	4.95 ± 0.32
Day 35	4.64 ± 0.54	5.07 ± 0.29	5.09 ± 0.32
Day 42	4.45 ± 0.69	5.07 ± 0.34	5.20 ± 0.37

* Weights in kilograms as mean ± SEM.

Table 4. Mean HR by group*

	Control	Low dose	High dose
Day -8	205 ± 9.0	204.4 ± 5.3	207 ± 2.4
Day 28	228 ± 9.9	250.8 ± 6.8	257 ± 3.3
Day 35	209 ± 5.3	210.8 ± 11.0 [†]	228 ± 14.8
Day 42	226.7 ± 17.6	243.2 ± 10.4	229 ± 12.3

* Heart rates in beats per minute as mean ± SEM.

† Significantly different compared to day 28 (P = 0.012) and day 42 (P = 0.044) in the low dose group.

APPENDIX III: Thyroid Hormone Data

T3 concentrations (nmol/L) in individual cats							
Cat	Group*	Day -8	Day 28	Day 35	Day 42	Day 42.4#	Day 42.8#
AKD7	0	0.62802	2.7701	3.6471	2.0481	3.8256	2.2486
IIP3	0	0.78342	2.3787	3.4039	3.708	4.1668	2.6717
AAX3	0	0.45581	2.7655	1.4363	ND	ND	ND
ALI5	0	0.41708	3.3717	2.3764	3.791	5.5944	6.9346
AKE7	1	0.75286	2.7916	1.0002	0.44133	0.65016	0.44265
XCZ1	1	0.44398	2.6287	1.0726	0.80773	1.0617	1.2146
RLO5	1	0.73951	2.2337	0.60808	0.62106	1.1886	0.98251
ITM3	1	0.49369	2.3977	0.59085	1.0188	1.0429	1.0161
AVN6	1	0.84224	4.3326	1.5526	0.9439	1.4133	1.235
ITM5	2	0.62751	2.4226	0.91564	0.92554	0.99144	0.96948
ITL3	2	0.62751	3.1251	1.3059	1.2021	1.3194	1.3975
QAF5	2	0.60945	4.1698	1.124	1.2544	1.211	1.0898
ATQ6	2	0.55666	3.0559	1.0443	1.0176	1.1977	1.0935

ND = not determined

* 0 = control group; 1 = low dose group; 2 = high dose group

Day 42.4 and 42.8 are 4 hours and 8 hours after levothyroxine and iopanoic acid or placebo administration

T4 concentrations (nmol/L) in individual cats							
Cat	Group*	Day -8	Day 28	Day 35	Day 42	Day 42.4#	Day 42.8#
AKD7	0	22.922	126.805	165.39	97.043	196.49	161.24
IIP3	0	37.157	99.006	90.962	122.55	244.37	225.76
AAX3	0	26.817	117.615	91.51	ND	ND	ND
ALI5	0	21.936	164.775	158.62	158.24	422.82	307.38
AKE7	1	24.851	97.485	178.63	158.24	254.78	230.18
XCZ1	1	28.025	127.51	181.25	188.1	272.18	379.76
RLO5	1	22.826	85.481	159.52	173.65	300.08	220.58
ITM3	1	23.229	147.475	236.45	188.14	330.52	331.24
AVN6	1	26.979	141.227	214.09	227.98	366.94	326.06
ITM5	2	29.63	107.445	192.61	221.84	261.22	310.5
ITL3	2	27.04	129.465	177.4	217.39	235.07	331.48
QAF5	2	22.764	152.735	224.48	223.92	348.84	317.7
ATQ6	2	21.342	111.913	119.17	193.28	351.78	251.25

ND = not determined

* 0 = control group; 1 = low dose group; 2 = high dose group

Day 42.4 and 42.8 are 4 hours and 8 hours after levothyroxine and iopanoic acid or placebo administration

rT3 concentrations (ng/mL) in individual cats							
Cat	Group*	Day -8	Day28	Day 35	Day 42	Day 42.4#	Day 42.8#
AKD7	0	ND	3.1473	4.0008	2.9888	4.068	6.8564
IIP3	0	0.43208	2.0507	2.0994	2.6074	4.1668	6.0304
AAX3	0	0.36201	2.5021	2.3091	ND	ND	ND
ALI5	0	0.22735	4.0922	3.6053	4.578	6.7072	6.8476
AKE7	1	0.40177	3.488	3.0616	3.887	4.1804	5.4188
XCZ1	1	0.27345	2.7037	2.3634	2.6643	5.2236	2.5
RLO5	1	0.39492	2.1286	2.4301	2.2243	2.6735	3.599
ITM3	1	0.31712	3.3129	3.9101	3.1396	3.8537	5.2264
AVN6	1	0.29041	4.9984	4.2996	4.7836	5.5164	4.6124
ITM5	2	0.36133	2.1888	2.1512	6.8564	5.4368	7.082
ITL3	2	0.36952	3.315	2.744	3.1154	3.1179	5.4128
QAF5	2	0.3625	3.3223	3.903	3.6562	3.9442	4.7304
ATQ6	2	0.26565	2.1125	1.242	2.6786	3.4886	3.9154

ND = not determined

* 0 = control group; 1 = low dose group; 2 = high dose group

Day 42.4 and 42.8 are 4 hours and 8 hours after levothyroxine and iopanoic acid or placebo administration

Vita

Alexander E. Gallagher was born in Tampa, Florida and grew up in the suburb of Brandon, Florida. He attended the Georgia Institute of Technology in Atlanta, Georgia, before transferring to the University of Florida in Gainesville, Florida, in 1994 to pursue a Bachelor's of Science in Animal Sciences. Alex was accepted into the College of Veterinary Medicine at the University of Florida in 1997 and earned his Doctor of Veterinary Medicine degree in May 2001.

After graduation, Alex completed a one year rotating internship in medicine and surgery at the Virginia-Maryland Regional College of Veterinary Medicine (VMRCVM), Virginia Tech, Blacksburg, Virginia, and then practiced for two years as an associate veterinarian at All Cats HealthCare Clinic in Gainesville, Florida. He then completed a one year internship in internal medicine at Affiliated Veterinary Specialists in Orlando, Florida, prior to returning to the VMRCVM for a residency in small animal internal medicine and pursuing a Master's degree in Biomedical and Veterinary Sciences. Alex defended his thesis on March 7th, 2008.