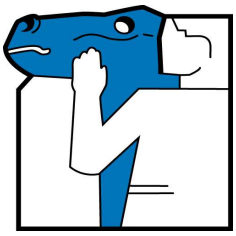

Evaluation of canine thyroid function in physiological and pathological conditions

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Dit werk draag ik op aan mijn overleden Papa

Alleen met je hart kun je goed zien. Het wezenlijke is onzichtbaar voor het oog.
De Kleine Prins, Antoine de Saint-Exupéry

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LIST OF ABBREVIATIONS

ATG	Anti-thyroglobuline antibodies
ASA	Acetylsalicylic acid
CRFA	Canine recurrent flank alopecia
FT4	Free thyroxine
FT3	Free triiodothyronine
KBr	Potassium bromide
Keto	Ketoprofen
NSAIDs	Non-steroidal anti-inflammatory drugs
T3	3,5,3'- triiodothyronine
TT3	Total triiodothyronine
T4	Thyroxine
TgAA	thyroglobulin autoantibodies
TBG	thyroxine-binding globulin
TSH	Thyroid-stimulating hormone/thyrotropin
TRH	Thyrotropin-releasing hormone
TT4	Total thyroxine
rT3	3,3',5'- triiodothyronine/reverse triiodothyronine

LIST OF ABBREVIATIONS

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Hypothyroidism is the most common endocrine disorder in dogs, but it is also the most over diagnosed. Indeed, evaluation of thyroid function in dogs is not always straightforward. The vague and non-specific clinical signs of hypothyroidism and the fact that numerous factors can influence thyroid function test results are major contributors to the difficulty in diagnosing this disease. Thyroid physiology, canine hypothyroidism, specific diagnostic tests and factors influencing thyroid homeostasis will be described in chapter 1.

Changes in thyroid hormone concentrations induced by drugs or disease can be confusing and lead to an erroneous diagnosis of hypothyroidism resulting in inappropriate life long treatment. Many drugs have been shown to alter thyroid function in humans and rats. These medications alter the synthesis, secretion, transport, or metabolism of thyroid hormones. Some drugs also directly inhibit the hypothalamic-pituitary-thyroid axis. Many of these drugs affect more than one aspect of thyroid hormone physiology. Species differences exist in all areas of the thyroid axis, therefore it is not surprising that drug-thyroid interactions will vary among species. In humans, several drugs may cause marked changes in the results of thyroid function tests, leading to difficulty in interpretation, but only rarely lead to clinical features of thyroid dysfunction. The effects of many of these drugs have not yet been studied in dogs. Therefore, chapters 2 and 3 will focus on the effects of some very commonly used drugs in canine practice on thyroid function test results.

To further assess the possible influence of some very common clinical situations on thyroid function test results, the possible effects of obesity and weight loss on canine thyroid function will be assessed in chapter 4. Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment, therefore it is important to clarify how obesity and weight loss can affect thyroid function test results in dogs.

Clinical manifestations of hypothyroidism are nonspecific; this further complicates the diagnosis of canine hypothyroidism. Several diseases have a clinical

presentation that can be similar to hypothyroidism. The most striking example is the symmetrical flank alopecia frequently observed in hypothyroidism but also a characteristic feature of a recently documented dermatological disease: canine recurrent flank alopecia (CRFA). During the last decades, many dogs have been erroneously diagnosed with hypothyroidism when they in fact were suffering from CRFA. Canine recurrent flank alopecia is a recently recognized skin disorder of unknown etiology, characterized by episodes of truncal hair loss that often occurs on a recurrent basis. Typically, this alopecia affects adult dogs on a yearly seasonal basis and resolves spontaneously without treatment within several months. The episodes do not always reoccur annually. In chapter 5 we shall evaluate thyroid function in dogs with CRFA.

SCIENTIFIC AIMS

SCIENTIFIC AIMS

Hypothyroidism is the most common endocrine disease in dogs, but also the most over diagnosed. Indeed, clinical evaluation of thyroid function in dogs is not always straightforward. First, numerous factors such as the presence of systemic disease or the administration of drugs can affect thyroid homeostasis and frequently used thyroid function tests. Many of those factors have not yet been well evaluated in dogs. Second, clinical manifestations of hypothyroidism, such as lethargy, alopecia and obesity, are nonspecific. Several diseases have a clinical presentation that can be similar to hypothyroidism. The most striking example is the symmetrical flank alopecia frequently observed in hypothyroidism but also a characteristic feature of canine recurrent flank alopecia (CRFA).

The aim of this study is to clarify, if and how, some commonly observed clinical situations could affect thyroid homeostasis, in order to avoid an erroneous diagnosis of hypothyroidism resulting in inappropriate treatment.

Specific aims:

1/ To evaluate the effects of some very commonly prescribed drugs on canine thyroid function test results.

1a. To evaluate the effects of prednisone and phenobarbital on canine thyroid function.

1b. To evaluate the effects of acetylsalicylic acid and ketoprofen on canine thyroid function test results.

2/ To investigate the influence of obesity and weight loss on thyroid homeostasis in dogs.

Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment, therefore it is important to clarify how obesity and weight loss can affect thyroid function test results in dogs.

3/ To examine thyroid function in dogs with canine recurrent flank alopecia.

The symmetrical alopecia described in canine recurrent flank alopecia can easily mislead one to a diagnosis of hypothyroidism. The etiology of this disease is still largely unknown.

CHAPTER 1

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REVIEW OF THE LITERATURE ON THYROID PHYSIOLOGY, CANINE HYPOTHYROIDISM AND THE INFLUENCE OF DRUGS ON THYROID FUNCTION IN DOGS

Adapted from:

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And

2. S. Daminet[1], D.C. Ferguson[2]. Influence of drugs on thyroid function in dogs. Journal of Veterinary Internal Medicine (2003). In press.
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CHAPTER 1

I. Physiology of the hypothalamic-pituitary-thyroid axis

An understanding of the basic physiology of the hypothalamic-pituitary-thyroid axis is critical to appreciating the various ways in which diseases or drugs can interact with this system and influence thyroid hormone concentrations. Figure 1 shows a schematic representation of the hypothalamic-pituitary-thyroid axis.

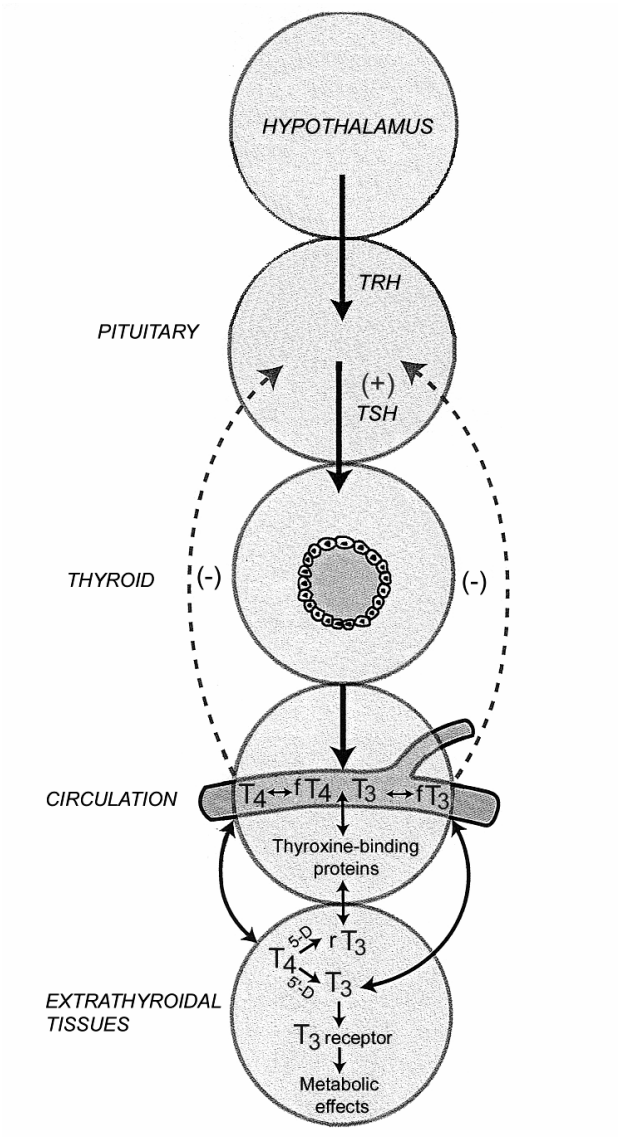


Figure 1: schematic representation of hypothalamic-pituitary-thyroid axis. T4: thyroxine, fT4: free thyroxine, T3: triiodothyronine, fT3: free triiodothyronine, rT3: reverse triiodothyronine, TSH: thyrotropin, TRH: thyrotropin-releasing hormone.

CHAPTER 1

Iodine metabolism

Thyroid hormones are the only iodinated organic compounds in the body with the 2 major secretory products of the thyroid gland, being thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3). Ingested iodide is absorbed from the gastrointestinal tract into the circulation. Dogs have plasma iodide concentrations of 5 to 10 μg/dl, which are 10 to 20 times concentrations in human plasma. In the thyroid gland, iodide is concentrated or "trapped" by active transport mechanisms of the thyroid follicular cell resulting in intracellular iodide concentrations, which are 10 to 200 times those of serum. This process is stimulated by the interaction of TSH with follicular cell surface receptors leading to stimulation of cyclic AMP.

Iodide trapping can be inhibited by other anions such as thiocyanate (SCN^-) and perchlorate (ClO_4^-). Thiocyanate is a metabolic product of some naturally occurring compounds in plants (e.g. cyanogenic glucosides found in sweet potatoes or lima beans) and may result in goitrogenic (antithyroid) activity of the plant. Another naturally occurring goitrinogen is goitrin found in plants of the genus Brassica (Ferguson, 2001). Oral administration of perchlorate after the administration of a tracer dose of radioiodine can be used to diagnose congenital defects in the thyroidal organification of iodide (Taurog, 1996).

Thyroid hormone synthesis

Thyroglobulin (Tg), an iodinated glycoprotein with a molecular weight of 660,000 daltons, serves as a synthesis and storage site for thyroid hormone and its precursors in the thyroid follicle. Once inside the thyroid cell, iodide diffuses down a concentration gradient to the apical surface of the cell where it is oxidized by the enzyme thyroid peroxidase (TPO) to iodine. It then is incorporated into tyrosine residues of Tg in a process called organification, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). Thyroxine then is formed by coupling 2 DIT molecules, and T_3 is formed by coupling 1 MIT molecule with 1 DIT molecule. When iodine intake is adequate, production of T_4 is favored. However, in iodine-deficient states and impending thyroid failure, the intrathyroidal synthesis of T_3 is preferred over that of T_4. By this autoregulation, the thyroid gland produces the most active thyroid hormone (T_3 is 3 to 10 times more potent than T_4) while using less iodide (Taurog, 1996).

The Wolff-Chaikoff effect, another intrathyroidal regulatory mechanism, is key to understanding the potential acute antithyroid effect of large amounts of ingested iodide. Mediated via inhibition of the thyroid peroxidase (TPO) enzyme, iodide decreases the rate of its own relative and absolute organification. In humans, this effect is transient and "escape" is seen within several weeks. This inhibitory effect may represent a mechanism by which the organism is protected from massive thyroid hormone release after a large dietary iodine load. In puppies, increasing the iodine content of the diet results in a fall in total and free T4 concentration (Castillo *et al.,* 2001).

Iodide derived from iodine-containing drugs (e.g. amiodarone, radiographic contrast agents) or nutrients may influence thyroid gland function by similar mechanisms (Wolff, 1989).

Thyroid hormone secretion

Thyroid hormone secretion is initiated as the epithelial follicular cells take up thyroglobulin in colloid droplets by a process called pinocytosis. Degradation of thyroglobulin by the lysosomal proteolytic enzymes produces both the iodotyrosines (MIT and DIT) and iodothyronines (T_4 and T_3). Little of the released MIT and DIT enters the circulation because the iodine is removed from these substances by a specific dehalogenase enzyme. Some of this iodine is recycled internally for iodination of new tyrosine residues in thyroglobulin, but in carnivores much iodine is released to the circulation. Enzymes present within the thyroid gland, however, can deiodinate T_4 to either T_3 or 3',5',3-T_3 (reverse T_3). Whereas T3 is the biologically active hormone, rT3 has no biological activity. As a result, although the T_4:T_3 ratio stored in the gland is 12:1 in the canine thyroid, the ratio of secreted products is 4:1. Production rates of the thyroid hormones in the dog have been estimated to be 8 μg/kg/day for T_4 and 0.8 to 1.5 μg/kg/day for T_3 (Kaptein *et al.,* 1990; 1993; 1994). The production rates observed in dogs, are more than twice those observed for TT4 and TT3 in humans (Kaptein *et al.*, 1993; 1994).

Hypothalamic-pituitary-thyroid axis

Thyrotropin, a glycoprotein produced in the thyrotropes of the pituitary pars distalis, has a stimulatory effect on thyroid hormone synthesis and secretion. In addition, TSH stimulates thyroid growth and function. The effects of TSH on the

thyroid gland are mediated by interaction with a specific TSH follicular cell surface receptor leading to stimulation of adenylate cyclase. Thyrotropin has a molecular weight of about 30,000 and consists of an α and β subunit. The α subunit is identical to the α subunit of the other glycoprotein pituitary hormones (e.g. luteinizing hormone [LH], follicle stimulating hormone [FSH]), whereas the β subunit is species specific and specific to the TSH molecule. The structure of the hormone-specific β subunit of TSH recently has been reported for the dog (Yang *et al.,* 2000).

The negative feedback effect of thyroid hormones (in the free or unbound form) is the principal mechanism regulating TSH secretion. Tonic stimulation by thyrotropin-releasing hormone (TRH) has a permissive role in TSH secretion. The pituitary thyrotrope cell completely deiodinates T_4 (derived from the plasma) to T_3 which subsequently inhibits TSH synthesis and secretion by alteration of nuclear receptor binding, mRNA transcription, and protein synthesis. Circulating T_4 taken up by the pituitary gland is the preferred source of T_3 in the pituitary gland, at least in the rat (Larsen *et al.,* 1981; Reichlin, 1986; Magner, 1990; Fish *et al.,* 1997).

Metabolism of thyroid hormone

The metabolically active thyroid hormones are the iodothyronines, T_4 and T_3. Thyroxine is the main secretory product of the normal thyroid gland, but, T_3, which is about 3 to 10 times more potent than T_4, and smaller amounts of inactive 3,3',5'-L-triiodothyronine (reverse T_3) and other deiodinated metabolites are secreted also. Although all T_4 is secreted by the thyroid gland, a considerable amount (40 to 60 % in the dog) of T_3 is derived from extrathyroidal enzymatic 5'-deiodination of T_4. Therefore, although it also has intrinsic metabolic activity, T_4 has been called a "prohormone" because of its conversion to the more potent T_3 in a step regulated by peripheral tissues (Belshaw *et al.,* 1974; Inada *et al.,* 1975; Laurberg, 1980; Ferguson, 1984; Kaptein *et al.*, 1993; 1994). Peripheral deiodination of T4 into T3 occurs intracellularly. The identification of 3 distinct types of 5'-deiodinase (5'-D) enzymes has underscored the importance of the regulation of T_3 production from T_4 in individual tissues. Type I 5'-D is found in most peripheral tissues, but has its highest activity in liver, kidney, muscle and thyroid gland. This enzyme is now known to be a selenoenzyme requiring trace quantities of selenium for optimal activity. Muscle, although it has low enzyme activity, may produce as much as

60% of the body's T_3 solely because of its large mass. The physiological role of Type I 5'-D is the provision of circulating T_3. This enzyme is capable of "outer ring" and "inner ring" deiodination and can deiodinate T_4 with a high capacity and is sensitive to propylthiouracil (PTU). Type II 5'-D is found in the central nervous system (CNS), pituitary gland, brown fat, placenta and skin (dog) and its physiological role is provision of intracellular T_3. This enzyme acts on T_4 and other compounds with outer ring iodine at concentrations within the physiological range and is resistant to PTU. Type III 5'-D is present in placenta, CNS, skin (not in the dog), and fetal liver and has as its physiologic role inactivation of T_4 and T_3 by inner ring (i.e. ring closest to the amino acid moiety) deiodination, with preference for T_3 as a substrate (Ferguson, 2001).

Plasma hormone binding of thyroid hormone and free hormone fraction

Thyroxine and T3 are water insoluble lipophilic compounds. Their ability to circulate in plasma is dependent upon binding by specific binding proteins, thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA; transthyretin), as well as by albumin itself. Thyroid hormone binding proteins provide a hormone reservoir in the plasma and "buffer" hormone delivery into tissue. These transport proteins act to provide a continuously available source of hormone while keeping the free or active fraction of the hormone within a tight range. Thyroxine-binding prealbumin, and possibly albumin, also may serve as intermediary carriers for specific uptake of the hormone by individual tissues. Dogs do have a high affinity thyroid hormone binding protein comparable to TBG in humans, but plasma concentrations of TBG in the dog are only 15 % of those observed in humans. In addition to TBG, TBPA, and albumin, circulating T_4 in canine plasma appears to bind to certain plasma lipoproteins. The cat does not appear to have a high affinity thyroid binding protein (such as TBG) but has only TBPA and albumin as serum thyroid hormone binding proteins. Partly as a result of weaker serum protein binding, total T_4 concentrations are lower, the unbound or free fraction of circulating T_4 is higher, and hormone metabolism is more rapid in most domestic animals than in humans (Bigler, 1976; Larsson *et al.,* 1985). This observation explains in part why dosages for thyroid hormone replacement therapy are higher in dogs than in humans.

The “free hormone hypothesis” states that it is the unbound fraction of hormone, which is available to tissues and therefore proportional to the action, metabolism, and

elimination of that hormone (Robbins & Rall, 1960; Mendel, 1989). Most evidence suggests that the free hormone fraction predicts the amount of hormone that is available to tissues at equilibrium. Plasma proteins buffer hormone delivery into tissue and provide a hormone reservoir. While normal variation in the free fraction of thyroxine (FFT4) is fairly small, ranging from 0.05-0.15%, this binding relationship may change in response to drugs or illness (Ferguson & Peterson, 1992). Partly as a result of weaker serum protein binding in the dog compared to humans, total T4 concentrations are lower, and the unbound or free fraction of circulating T4 is higher (0.1 vs. ~0.03% in humans) (Bigler, 1976; Larsson *et al.*, 1985; Kaptein *et al.*, 1994).

Evidence also exists that certain cell types actively transport or exchange thyroid hormone from the plasma into the cytosol, and that these transport and exchange systems may be targeted by some drugs. However, some investigators believe that serum-binding proteins, especially albumin and TBPA, may serve to distribute hormones to specific tissues. Most theories of thyroid hormone exchange have assigned a passive "reservoir" role to cytosolic thyroid hormone binding proteins, proteins that retain thyroid hormone in a predominantly bound state inside the cell (Pardridge, 1981; Mendel, 1989; Burrow *et al.,* 1989).

Most evidence suggests that thyroid hormone uptake by tissues is proportional to, but not limited to, the free or unbound fraction of circulating hormone. Approximately 50 to 60 % of the body's T_4 and 90 to 95 % of the body's T_3 is located in the intracellular compartment (Fish *et al.*, 1997). Certain organs, especially the liver and kidney, can concentrate thyroid hormones and exchange hormone rapidly with the plasma. In humans, about 60 % of the intracellular T_4 is in rapidly equilibrating tissues (e.g., liver, kidney) whereas only 6 % of the intracellular T_3 is in these tissues. About 80 % of all extrathyroidal T_3 is located in slowly equilibrating tissues (e.g., muscle, skin), whereas only 20 % of intracellular T_4 is in this compartment. As a result, most of the body's T_4 is located in plasma, interstitial fluid, liver and kidney. The majority of the body's extrathyroidal T_3 is in the cells of the muscles and skin and in a conjugated form in the intestinal tract.

Metabolic clearance rates

The plasma half-life of T4 in the dog has been estimated to be 8 hours (Kaptein *et al.*, 1993; 1994) or 10-16 hours (Fox & Nachreiner, 1981) compared to a plasma half-life of about 7 days in humans. Similarly, the plasma half-life of T3 in the dog has been estimated to be 5 to 6 hours, compared to 24 to 36 hours in humans. These figures reflect plasma disappearance rates and do not necessarily indicate extent or duration of biological action. The shorter half-life of T4 explains why when treating hypothyroidism a twice-daily administration of synthetic T4 will more consistently normalize serum T4 concentrations (Nachreiner *et al.,* 1993). In most hypothyroid dogs, however, once daily administration of thyroxine often leads to good clinical control of the disease (Ferguson, 2001).

II. Etiology of canine hypothyroidism

Although dysfunction anywhere in the hypothalamic-pituitary-thyroidal axis may result in thyroid hormone deficiency, more than 95 per cent of clinical cases of hypothyroidism in dogs result from destruction of the thyroid gland itself (i.e., primary hypothyroidism) (Scott-Moncrieff & Guptill-Yoran, 2000). The 2 most common causes of adult-onset primary hypothyroidism in dogs are lymphocytic thyroiditis and idiopathic atrophy of the thyroid gland, each accounting for about one-half of the cases of hypothyroidism (Gosselin *et al.,* 1981). Other rare forms of canine hypothyroidism include iatrogenic conditions, neoplastic destruction of thyroid tissue and congenital hypothyroidism. Hypothyroidism occasionally also may result from impaired ability of the pituitary gland to synthesize and secrete TSH, resulting in secondary thyroid follicular atrophy. Secondary hypothyroidism can be caused by pituitary tumors, congenital pituitary malformation, isolated TSH deficiency, or pituitary trauma or surgery and accounts for less than 5 % of clinical cases of hypothyroidism (Rijnberk, 1996; Scott-Moncrieff & Guptill-Yoran, 2000).

Congenital hypothyroidism is rare compared to acquired hypothyroidism. Possible etiologies include: dysgeneses of the thyroid gland, an enzymatic deficit in thyroid hormone synthesis or a central problem (most frequent) (Rijnberk, 1996). There

are some reports of congenital hypothyroidism in the literature; a young Boxer, a family of giant Schnauzers (central hypothyroidism) and young German Shepherds suffering from dwarfism (Greco *et al.,* 1991; Mooney & Anderson, 1993; Kooistra *et al.,* 2000b). German Shepherds with pituitary dwarfism, a recessive inheritable disease, suffer from a combined deficiency in growth hormone, TSH and prolactin (Kooistra *et al.*, 2000b).

The lymphocytic thyroiditis can occasionally be seen with other autoimmune diseases. Schmidt syndrome or polyglandular autoimmune disease is well described in humans, but is also described in dogs. This disease is characterized by the presence of 2 or more endocrine diseases, e.g. hypoadrenocorticisme, autoimmune thyroiditis and insuline-dependant diabetes mellitus. The association of several endocrine diseases can be a diagnostic and therapeutic challenge (Kintzer, 1992).

III. Clinical signs of hypothyroidism in dogs

Thyroid hormones have an effect on all levels of metabolism and their deficiency can lead to dysfunction of several organs. Clinical signs of hypothyroidism are vague and insidious in onset. Furthermore, many diseases, such as canine recurrent flank alopecia (CRFA) or any systemic condition can lead to clinical signs suggestive of hypothyroidism. This disease is most commonly observed in middle sized to large breed dogs and occurs preferably between 3 and 8 years of age. A few breeds, such as the Doberman, Golden Retriever, de Irish Setter, Airedale Terrier, Great Dane, Bobtail and Beagle seem predisposed (Feldman & Nelson, 1996). Primary acquired hypothyroidism is infrequent under the age of 2 years. Table 1 gives a summary of the clinical signs that can be observed with hypothyroidism.

Table 1: Summary of the most common clinical signs observed in dogs with hypothyroidism

Frequent	Less frequent	Doubtful relationship
Lethargy/weakness	Neuropathy, vestibular syndrome	Male infertility
Obesity	Female infertility	Dilated cardiomyopathy
Alopecia/hypotrichosis	Myxoedema	Larynx paralysis
Seborrhea	Lipid keratopathy	Megaoesofagus
Pyodermia or recurrent otitis		

Twenty to 76% of the hypothyroid dogs are presented to their veterinarian with complaints of weakness, lethargy or exercise intolerance (Panciera, 1997; Dixon *et al.,* 1999; Scott-Moncrieff & Guptill-Yoran, 2000). Sometimes the disease is so insidious in onset that the owners only note after initiating the therapy how lethargic their pet was.

Dermatological changes such as a dry skin, changes in coat quality or color, alopecia or seborrhoea are described in 60-80 % of hypothyroid dogs (Panciera, 1997; Dixon *et al.*, 1999; Scott-Moncrieff & Guptill-Yoran, 2000). The alopecia can be observed in areas undergoing friction (neck or tail) or have a bilateral symmetrical distribution (thorax or flank) sparing the limbs. Alopecia can occur because thyroid hormones are essential to initiate the anagen phase (growth) of the hair follicles. Hyperpigmentation can sometimes be observed. A predisposition for recurrent bacterial pyoderma or external otitis and Malassezia infections can be seen. Alopecia is a common clinical sign of hypothyroidism; 25% of hypothyroid dogs have been reported

to present with symmetrical bilateral endocrine alopecia (Feldman & Nelson, 1996). The symmetrical alopecia described in canine recurrent flank alopecia (CRFA) can easily mislead one to a diagnosis of hypothyroidism. Canine recurrent flank alopecia is a recently recognized skin disorder of unknown etiology, characterized by episodes of truncal hair loss that often occurs on a recurrent basis. It has been described under several synonyms: seasonal flank alopecia, canine idiopathic cyclic flank alopecia, cyclic follicular dysplasia, and follicular dysplasia. Typically, this alopecia affects adult dogs on a yearly seasonal basis and resolves spontaneously without treatment within several months. The episodes do not always reoccur annually. A breed predisposition seems to exist for CRFA, with boxers (may account for approximately half of all reported cases), Airedale terriers, schnauzers, and English bulldogs being the more frequently cited breeds (Scott, 1990; Miller & Dunstan, 1993; Curtis *et al.,* 1996). However, it has been reported in several other breeds (Fontaine *et al.,* 1998). Interestingly, the same breeds are suspected to be predisposed for hypothyroidism (Feldman & Nelson, 1996). Furthermore, the recognition of CRFA as a separate dermatological condition became clear only in the 1990s (Scott, 1990). Therefore, some dogs previously classified as hypothyroid based on the presence of flank alopecia resolving with thyroid supplementation might very well have been dogs suffering from CRFA. Another explanation is that the breeds cited earlier are predisposed to both hypothyroidism and CRFA. Both diseases are more frequent in young to middle-aged dogs. Several hypotheses, such as photoperiod and melatonin deficiency, have been proposed to explain the disease, but the etiology remains unclear (Scott, 1990; Curtis *et al.*, 1996; Fontaine *et al.*, 1998; Paradis, 2000). Thorough assessment of thyroid function in dogs with CRFA has not been reported to our knowledge and will be further explored in chapter 4.

Obesity is observed in more than 40 % of dogs with hypothyroidism in several studies (Kaelin *et al.,* 1986; Panciera, 1994; Feldman & Nelson, 1996; Dixon *et al.*, 1999). Therefore, many dogs in practice are tested and/or treated for hypothyroidism because they are overweight. Obesity, however, is a common nutritionally related problem in dogs and many dogs with obesity are not hypothyroid. It is estimated in the general population that between 21 and 30 % of dogs seen in practice are obese

(Laflamme *et al.,* 1994). Many obese hypothyroid dogs are mildly to moderately obese but occasionally the obesity can be very outspoken. Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment, therefore it is important to clarify how obesity and weight loss can affect thyroid function test results in dogs. This will be further evaluated in chapter 5.

Some other complaints such as myxoedema, reproduction, neurological, cardiovascular or ophthalmologic problems can be present but less frequently.

Myxoedema gives the patient a typical 'tragic' expression and is caused by the accumulation of hyaluronic acid in the dermis around the face (Scott-Moncrieff & Guptill-Yoran, 2000). The most severe and extremely rare presentation for hypothyroidism is myxoedema coma. This leads to impairment of mental status, thermoregulation and cardiorespiration. Dobermans seem to be predisposed.

Thyroid hormones, through stimulation of the myocytes, have a positive chrono- and inotrop effect on the hart. This is partly due to increased transcription of the heavy myosin chains, an increase in calcium ATPase pumps and increased amount of β receptors (Panciera, 2000). The cardiovascular effects of thyroid hormone concentrations are most pronounced in cats with hyperthyroidism (excessive thyroid hormone concentrations), these animals can develop a hypertrophic cardiomyopathy. In cases of canine hypothyroidism, the cardiovascular consequences are subtler, but can include, decreased cardiac output, bradycardia, and a decrease in the R wave amplitude on ECG (Panciera, 2000). Hypothyroidism was observed more frequently in a group of dogs with atrial fibrillation (Gerritsen *et al.,* 1996). Hypothyroidism might predispose a dog, with an already existing cardiac disease, to develop atrial fibrillation. A causative effect between hypothyroidism and the development of dilated cardiomyopathy is still controversial.

The lipid keratopathies sometimes observed in dogs with hypothyroidism are most likely a consequence of the hyperlipidemia frequently observed in this condition. But the association between corneal lipid deposits and hypothyroidism has been poorly documented yet in dogs.

In some cases the patients are brought in with neurological signs. Most of these dogs also show classical signs of hypothyroidism. Generalised peripheral neuropathies or localised neuropathies (vestibular disease, facial paralysis, laryngeal paralysis, megaoesofagus) were described in hypothyroid dogs (Jaggy & Oliver, 1994; Jaggy, 2000). A direct causative link is controversial, especially concerning the megaoesofagus or the laryngeal paralysis. A decreased activity of the sodium/potassium pump, atherosclerose and myxoedema have been proposed as possible mechanisms (Jaggy, 2000).

Reproduction abnormalities have been considered as a classical feature for dogs with hypothyroidism in the past. A recent study has not shown a decrease in reproduction capacity in male dogs with hypothyroidism (Johnson *et al.,* 1999). With advanced hypothyroidism bitches could show infertility, shortened oestrus or prolonged anoestrus. A direct causative effect has however not been shown. Intact hypothyroid bitches, through stimulation of prolactin secretion by the increased TSH concentrations, can sometimes show galactorrhea (Cortese *et al.,* 1997).

Thyroid hormones are essential in the young age for the development of tissues and skeleton. In addition to the clinical signs seen with acquired hypothyroidism, dogs with congenital hypothyroidism can show lameness, macroglossia, delayed tooth eruption and disproportionate dwarfism. On radiographs a typical epiphyseal dysgenesis can be observed (Greco *et al.*, 1991; Mooney & Anderson, 1993). Young German shepperd dogs with pituitary dwarfism typically have a proportionate dwarfism, an abnormal coat with retention of secondary hairs, an absence of primary hairs and a symmetrical bilateral alopecia. Male dogs often have cryptorchidism and bitches can sometimes show a persistent oestrus (Kooistra *et al.*, 2000b).

IV. Non-specific laboratory changes observed in canine hypothyroidism

Finding a hypercholesterolemia and a non-regenerative anemia on a blood analysis can increase the suspicion for hypothyroidism. Hypercholesterolemia is observed in approximately 75% of the cases and would be due to a decreased clearance and hepatic use combined with an increased hepatic production of cholesterol (Feldman

& Nelson, 1996; Christopher, 1997; Scott-Moncrieff & Guptill-Yoran, 2000). The animal has to be fasted for at least 12 hours before taking the sample in order to avoid postprandial hypercholesterolemia. The hypercholesterolemia is often pronounced in hypothyroid dogs with some dogs showing values in excess of 10 mmol/L. Unfortunately, hypercholesterolemia is observed in many other diseases such as hyperadrenocorticism, diabetes mellitus, glomerulopathies and liver diseases.

Normocytic normochromic anemia is observed in 30% of dogs with hypothyroidism, but the packed cell volume typically remains above 25%. This anemia could be due to a decreased stimulation of erythropoiesis by erythropoietin and thyroid hormones. Occasionally, target cells can be seen on a blood smear. These are thought to be the result of cholesterol overload of the erythrocyte membrane (Feldman & Nelson, 1996).

Thrombocytosis can be observed and platelets are sometimes smaller (Sullivan *et al.,* 1993). A few years ago, it was thought that hypothyroidism could lead to a decrease in von Willebrandfactor and bleeding tendencies. Recent studies have however shown that this was not the case (Panciera & Johnson, 1994). It is interesting to note that some breeds, such as the Dobermans, which are predisposed for hypothyroidism, also are predisposed for von Willebrand's disease.

Frequently, the dermatological abnormalities observed suggest an endocrine origin but are seldom specific for hypothyroidism. Skin biopsies are unnecessary in most cases suspected of canine hypothyroidism. Cutaneous changes that would support a diagnosis of hypothyroidism, are hypertrophy and a vacuolisation of the musculi erectori around the hair follicle (controversial), dermal mucinosis and a thickening of the dermis with the presence of myxoedema (Miller & Dunstan, 1993; Scott *et al.,* 1995).

V. Specific thyroid tests used in dogs

Because of the vague clinical signs and the absence of specific abnormalities on a routine blood test, the diagnosis should be confirmed through a specific evaluation of the thyroid gland. These tests should always be run on a dog with a clinical suspicion

for hypothyroidism, and not as a screening test in dogs without any clinical signs suggestive of the disease. It is indeed important to mention that the positive predictive value of a diagnostic test increases when the prevalence of the disease increases. A thorough clinical examination of the patient, knowledge of the advantages and disadvantages of all available tests and knowledge of the factors that can influence the results, will allow the veterinarian to correctly diagnose the disease. Table 2 contains a summary of the advantages and disadvantages of the most commonly used thyroid hormone tests in dogs.

Table 2: Advantages and disadvantages of the most commonly used tests to evaluate thyroid function in dogs

Test	Advantages	Disadvantages
TT4	Easy Not expensive Readily available Normal values allow exclusion of hypothyroidism	Decreased with SNTD Decreased after administration of certain drugs A decreased T4 alone does not allow a reliable diagnosis of hypothyroidism (low specificity)
TSH	Easy Not expensive Available	1/4 of hypothyroid dogs have TSH values within the reference range (low sensitivity) Always use in combination with T4
FT4	Is less influenced by SNTD or through drug administration than TT4	The only reliable method is through equilibrium dialysis Not readily available in Europe
T3	Theoretically interesting	Few clinical interest because of overlapping values between hypothyroid, euthyroid and sick dogs
TSH stimulation test	Was and still is considered as the gold standard	Bovine TSH is not easily available anymore Expensive 4 to 6 hours lasting test Anaphylactical reactions were described
TRH stimulation test *with T4 determination *with TSH determination	Allows diagnosis of primary hypothyroidism Interesting for the diagnosis of secondary hypothyroidism	Not reliable No extra benefit compared to combination of TT4 and TSH determination Expensive Possibly vomiting, salivation and tachycardia

SNTD: systemic non-thyroid disease

CHAPTER 1

Until very recently, veterinarians have been required to choose from a variety of diagnostic tests of thyroid function, with no single test being optimal. Following development of canine-specific TSH assays and further experience with free thyroxine by equilibrium dialysis (FT4ED), the benefits of these assays have become apparent, but so also have their limitations. Experience with canine TSH assays, has not increased estimates of thyroid failure secondary to deficiency of pituitary TSH secretion; but, it has helped confirm the goitrogenic effect of some commonly used drugs (Campbell *et al.,* 1995; Scott-Moncrieff & Nelson, 1998). Specificity, sensitivity and accuracy of FT4 and TSH determinations for the diagnosis of canine hypothyroidism are summarized in table 3.

Table 3: Thyroid diagnostic test comparisons in dog (%)

Test	TT4	TT3	FT4D	TSH	TT4/TSH	FT4D/TSH
Sensitivity	89/100	10	98/80	76/86.7	67/86.7	74/80
Specificity	82/75.3	92	93/93.5	93/81.8	98/92.2	98/97.4
Accuracy	85	55	95	84	82	86

Data from Peterson et al (Peterson *et al.,* 1997) and Dixon et al (Dixon & Mooney, 1999b). If only 1 number listed, from Peterson et al..
Sensitivity is the percentage of hypothyroid dogs that had abnormal test results.
Specificity is the percentage of euthyroid dogs that had normal test results.
Accuracy is the percentage of cases that are neither falsely positive nor falsely negative.

Thyroid hormones kept in plastic tubes, remain stable for 5 days at room temperature, and can be kept frozen for years. Serum concentrations of total thyroxine (TT4) and free thyroxine (FT4) will increase if they are kept in glass tubes for 5 days at 37 ° C (Behrend *et al.,* 1998). Furthermore, glass tubes break easier during transport. Hemolysis and lipemia do not interfere with measurement of thyroid hormones measured by radioimmunoassay (RIA) (Behrend *et al.*, 1998; Scott-Moncrieff & Guptill-Yoran, 2000).

Total thyroxine

Canine thyroid function is most frequently evaluated in practice through measurement of a baseline serum TT4, which is easy and not expensive. But it is important to realise its limitations. Indeed, numerous factors such as systemic diseases or the administration of medications can influence the TT4 serum concentrations and will be discussed further. Furthermore there is an important overlap between the TT4 serum measurements observed in euthyroid and hypothyroid dogs. A study indicates that basal TT4 measurements observed in 62 normal dogs varied between 12,8 and 42,5 nmol/L and from not detectable to 19,3 in 51 hypothyroid dogs (Nelson *et al.,* 1991). The measurement of a baseline TT4 alone to evaluate thyroid function is insufficient, except when the TT4 measurement falls well within the reference limits, this allows exclusion of the diagnosis of hypothyroidism in the majority of cases (high sensitivity of the test). When TT4 serum concentrations are under the reference range, further testing is indicated.

The methods for detection used are also of importance. Canine TT4 serum concentrations are only 10 to 25% of those observed in humans. Therefore it is preferable to use canine kits to perform the measurements. Radioimmunoassay (RIA) is the most commonly used test. The presence of anti-thyroxine antibodies in some dogs with lymphocytic thyroiditis can interfere with the measurement of TT4 through RIA. Consequently, false low or more commonly falsely elevated TT4 measurements can be observed (Thacker *et al.,* 1992).

Endogenous thyrotropin

With primary hypothyroidism, an increase in TSH serum concentrations would be expected because of the lack of negative feed back mechanism of the thyroid hormones on the pituitary. In human medicine, measurement of serum TSH is recommended to evaluate thyroid function. An increase will confirm a diagnosis of primary hypothyroidism. It is important to note that in human medicine, third generation kits for TSH measurements are very sensitive and allow detection of small variations in TSH concentrations. This would not be the case yet for the kits used in dogs. The measurement of canine endogenous TSH has only been available for a few years now. Sometimes, a euthyroid dog can have increased TSH values (Scott-

Moncrieff *et al.,* 1998; Dixon & Mooney, 1999b). But, more problematic is the fact that approximately one fourth of the dogs with hypothyroidism show TSH serum concentrations within the reference range (Peterson *et al.*, 1997; Scott-Moncrieff *et al.*, 1998; Dixon & Mooney, 1999b). Several hypotheses have been suggested: fluctuation of the TSH serum concentration, the non detection of all isoforms of TSH, some dogs might suffer from secondary or tertiary hypothyroidism, or finally that with time TSH decreases in dogs with primary hypothyroidism (Ramsey *et al.,* 1997; Peterson *et al.*, 1997; Kooistra *et al.,* 2000a). In other words, because of the weak sensitivity of the TSH measurement for the diagnosis of hypothyroidism, this test cannot be recommended solely. To reliably evaluate canine thyroid function, a T4 measurement (FT4 or TT4) is always combined with a TSH measurement. A serum sample with decreased T4 and increased TSH serum concentrations (>0,6 ng/ml) will confirm with confidence the diagnosis of primary hypothyroidism (Scott-Moncrieff *et al*, 1998; Kantrowitz *et al.,* 1999). When the measurements of T4 and TSH give contradictory results, it is recommended to repeat measurements 4-8 weeks later.

Free thyroxine

Only the free fraction of T4 (not bound to transport proteins) can enter the cells, link to the receptors and lead to a biological effect. In theory, the measurement of FT4 should reflect more precisely thyroid function. But the low FT4 serum concentrations (<1% of TT4) render the measurement more difficult. The most reliable technique used to measure FT4 is equilibrium dialysis, only available in some laboratories and more expensive then measurement of a TT4. In this technique, a semi-permeable membrane allows passage of small FT4 molecules and not of transport proteins; this allows isolation and measurement of FT4. One-study reports a sensitivity of 98%, when the measurement of FT4 is used alone for the diagnosis of hypothyroidism (Peterson *et al.*, 1997). However, another study concludes that measurement of FT4 by equilibrium dialysis only added minimal supplementary information compared to measurement of TT4 serum concentrations (Dixon & Mooney, 1999b). Other methods used for determination of FT4 such as chemoluminescence and RIA seem less reliable and offer no advantage over the simple dosage of TT4 levels by RIA (Nelson *et al.*, 1991; Scott-Moncrieff & Guptill-Yoran, 2000). Free T4 serum concentrations seem less influenced

by non-thyroidal illnesses then TT4 (Peterson *et al.*, 1997). Measuring FT4 seems therefore more interesting to evaluate thyroid function, if measurement is performed through equilibrium dialysis.

Triiodothyronine

Determination of total or free triiodothyronine (TT3, FT3), the hormone active at the cellular level, would be interesting, at least in theory. However there is an important overlap between values observed in euthyroid dogs, hypothyroid dogs and dogs suffering from a systemic illness. Therefore, determination of TT3 concentration is not very interesting in the evaluation of thyroid function in practice (Nelson *et al.*, 1991; Miller *et al.,* 1992).

Thyrotropin stimulation test

Canine thyroid stimulation with bovine TSH is less affected by the presence of non-systemic thyroid diseases then is a baseline TT4 measurement. Therefore the bovine TSH stimulation test has long been considered as the gold standard for thyroid evaluation in dogs (Scott-Moncrieff & Guptill-Yoran, 2000). Today, the bovine TSH stimulation test is less used for several reasons: expense, 4-6 hour test, bovine TSH is difficult to obtain and endogenous TSH measurements are available. Measurement of TT4 and TSH are readily available, but in non-unfrequent cases with controversial results, performing a TSH stimulation test would still be very interesting. A recent study showed that recombinant human TSH would stimulate the thyroid gland of euthyroid beagle dogs (Sauvé & Paradis, 2000). Clinical studies to evaluate its clinical usefulness are underway.

Thyrotropin-releasing hormone stimulation test

Because bovine TSH was difficult to obtain, studies were performed to evaluate the use of TRH stimulation tests (thyrotropin-releasing hormone) as an alternative.
Thyrotropin-releasing hormone should stimulate the secretion of TSH by the pituitary; induce a stimulation of the thyroid gland and an increased release of T4. But the increase in TT4 or FT4 after injection of TRH is less pronounced than with a TSH stimulation test. Indeed, a study revealed that the TRH stimulation test is not useful in

the diagnosis of hypothyroidism (Frank, 1996). Determination of TSH levels, instead of TT4, after stimulation with TRH, allows differentiation between hypothyroid and euthyroid dogs (Meij *et al.,* 1996; Scott-Moncrieff & Nelson, 1998), but this test did not offer any advantage over the classical combination of TT4 and TSH measurement. The TRH-stimulation test can be useful in the diagnosis of secondary hypothyroidism (pituitary origin). The administration of TRH can lead to undesirable effects such as vomiting, diarrhea and tachycardia. For all those reasons, this test is seldom used in practice in the evaluation of thyroid function in dogs.

Antibodies against thyroid hormones

Antibodies against thyroid hormones can sometimes by found in dogs. Thyroid hormones are haptens that are minimally immunogenic. However it seems that thyroglobulin (TG), which is released with lymphocytic thyroiditis, provides the antigenic stimulus and presents T3 and T4 to the immune system (Gaschen *et al.,* 1993). Therefore, finding those antibodies suggests the presence of a lymphocytic thyroiditis. However, the presence of these antibodies does not reflect thyroid function, as 75% of the thyroid gland must be destroyed in order to lead to decreased T4 blood levels. Furthermore, anti-T3 and anti-T4 antibodies are only observed in approximately 30% of the hypothyroid dogs. Therefore their clinical use is limited (Thacker *et al.*, 1992). These antibodies can interfere with radioimmunoassay determination of T3 or T4, and lead to falsely increased values as a consequence. In this case, measurement of FT4 by equilibrium dialysis is recommended, as this test is not affected by the presence of anti-T4 antibodies.

The measurement of anti-thyroglobulin antibodies (ATG) was evaluated as a diagnostic tool but high percentages of false positive were found especially in dogs with systemic diseases (Thacker *et al.*, 1992). This limited their clinical use. More reliable measurement methods and interpretation criteria were recently published. However, these ATG are found only in 36 to 60% of hypothyroid dogs (Nachreiner *et al.,* 1998; Dixon & Mooney, 1999a; Daminet & Paradis, 2000). The presence of ATG in euthyroid dogs (fals positives) is now only observed in less then 5% of healthy dogs (Nachreiner *et al.*, 1998; Dixon & Mooney, 1999a). A diagnosis of hypothyroidism could never be based on the finding of those antibodies alone, as they do not reflect thyroid ability to

synthesise thyroid hormones. But if hypothyroidism was already diagnosed, then finding ATG can confirm the presence of a lymphocytic thyroiditis as a cause of the hypothyroidism. The presence of antibodies in combination with normal thyroid hormone values can be an indication that hypothyroidism might develop. Clinical studies evaluating the development of hypothyroidism in dogs positive for ATG are lacking. It is interesting to note that the measurement of ATG is part of the screening required by the OFA (Orthopedic Foundation for Animals) for reproduction animals.

Diagnostic therapy

Response to therapy as a sole method of diagnosing hypothyroidism is not recommended for several reasons. Even if the primary complaints improve with the administration of synthetic thyroid hormones, many intermittent disorders will improve with the administration of thyroid hormones (e.g. canine recurrent flank alopecia or just general stimulation of the animal). The consequence will be an unnecessary and expensive therapy, potential side effects or the delay in the diagnosis of the real problem.

Scintigraphy

Scintigraphy is a very useful method for evaluation of thyroid function. It is more commonly used in the evaluation of feline hyperthyroidism than in the evaluation of canine hypothyroidism. Unfortunately, because of the need for specialised equipment and the use of radioactive products, it is not widely available. Furthermore, very often the patient needs to be anaesthetized.

VI. Influence on thyroid function tests

Numerous diseases and medications can influence thyroid function. Besides this, many other physiological factors such as, age, breed and fluctuating serum concentrations can influence the results. Therefore the interpretation of the laboratory result alone will lead to overdiagnosis of hypothyroidism.

CHAPTER 1

A. Physiological influences

Thyroid hormone concentrations vary opposite to age. At birth, thyroid hormone concentrations observed are very close to adult values. Gradually after birth they increase. In puppies younger than 6 weeks of age, thyroid hormone serum concentrations are 2 to 5 times higher than in adult dogs. From 6 to 12 weeks of age, TT4 values are comparable to those observed in adult dogs (Reimers *et al.,* 1990). Half of the dogs older then 6 years of age have lower TT4 serum values. Thyrotropin stimulation test results are also less pronounced in older dogs. The exact cause for these differences in thyroid hormone levels with age is unknown, but highlights the importance of having specific reference values for each age category.

Small breed dogs have TT4 values that are somewhat higher then mid- or large breed dogs. Their average TT4 value remains well within the reference range (Reimers *et al.*, 1990). Therefore, from a clinical point of view these changes are not very relevant.

Greyhounds have TT4 values that are clearly lower (half) than in other breeds. Thyroid function test results in this breed should therefore be interpreted very cautiously (Gaughan & Bruyette, 2001). Newfoundland dogs are also sometimes mentioned as a breed with lower TT4 values, but in one study, TT4 and TSH serum concentrations were comparable to values observed in other breeds (Sauvé *et al.,* 1997). If some other breeds could have decreased thyroid hormone concentrations remains to be studied.

Globally, there are no differences in thyroid hormone concentrations between male and female dogs. But, pregnant bitches or bitches in dioestrus (progesterone influence) have TT4 serum concentrations that are higher then a non-selected population (Reimers *et al.*, 1990).

Daily random fluctuations in thyroid hormone concentrations have been described in euthyroid dogs (Miller *et al.*, 1992).

A pronounced protein deficit and fasting can decrease thyroid hormone values, but the influence of obesity has not yet been well documented in dogs and will be evaluated in chapter 5 (van Haasteren *et al.,* 1996).

B. Effects of drugs on canine thyroid function

Table 4 shows drugs affecting thyroid function in humans with some proposed mechanisms of action. Table 5 summarizes the effects of some studied drugs on thyroid function in dogs.

CHAPTER 1

Table 5: Summary of the effects of drugs on canine thyroid function test results. The symbols ↓, ↑, =, indicate that the measurements may be increased, decreased or unchanged, respectively.

Drugs	**TT4**	**FT4**	**TSH**	**TSH stimulation test**
Glucocorticoids (immunosuppressive dosage)	↓	Not studied	Not studied	Blunted at high doses and durations
Sulfonamides (30 mg/kg q12h)	↓	↓	↑	↓
Propranolol	=	=	=	=
Carprofen	↓	= (↓)	↓	Not studied
Potassium bromide	=	=	=	=

Glucocorticoids

Studies performed on humans and rats have demonstrated that endogenous or exogenous glucocorticoids directly inhibit the hypothalamic-pituitary-thyroid axis and also influence peripheral metabolism of thyroid hormones (Davies & Franklyn, 1991; Kaptein *et al.,* 1992; Wenzel, 1996). More precisely, in humans, glucocorticoids inhibit pituitary secretion of TSH, impair peripheral 5'-deiodination of T4, and decrease TBG serum concentrations. As a result, decreased serum concentrations of TT4, FT4, TT3 and TSH and increased concentrations of rT3 can be observed in humans receiving glucocorticoid therapy (Cavalieri, 1991; Rubello *et al.,* 1992; Samuels *et al.,* 1994; Surks & Stievert, 1995; Hangaard *et al.,* 1996). Mechanisms advocated to explain increased rT3 include reduced rT3 clearance and inhibition of 5'-deiodination (Davies & Franklyn, 1991; Moore *et al.,* 1993).

High endogenous cortisol concentrations in dogs with hyperadrenocorticism markedly lowered baseline serum TT4 concentration in 57 % of affected dogs and significantly blunted post-TSH T4 measurements (Peterson *et al.,* 1984). Total T4 concentrations normalized after treatment of the hyperadrenocorticism. Decreased TSH secretion, changes in thyroid hormone binding and alterations in peripheral thyroid hormone metabolism were suggested in this study to explain the decreased thyroid hormone concentrations observed.

As glucocorticoids are very commonly used in canine medicine, the potential effects of exogenous corticosteroid administration on canine thyroid function test results will be further evaluated in chapter 2.

Phenobarbital

In rats, phenobarbital markedly alters thyroid function by increasing the rate of clearance of T4. Increased hepatic deiodination of thyroid hormones, biliary clearance, and fecal excretion result in decreased concentrations of circulating thyroid hormones. Decreased concentrations of thyroid hormones, in turn, increase TSH secretion by the classical negative feedback pathway (McClain *et al.,* 1989; Curran & Degroot, 1991; Johnson *et al.,* 1993; Barter & Klaassen, 1994). Increased conversion of T4 to T3 in peripheral tissues also may play a role (Gieger *et al.,* 2000). Phenobarbital also could have additional central effects on the hypothalamus-pituitary-thyroid axis

(Theodoropoulos & Zolman, 1989; Liu *et al.,* 1995). Most studies in rats report decreased T4 with normal or, more often, increased TSH concentrations (Ohnhaus *et al.,* 1981; Theodoropoulos & Zolman, 1989; Attia & Aref, 1991; Curran & Degroot, 1991; DeSandro *et al.,* 1991; Deda *et al.,* 1992; Barter & Klaassen, 1994; Verma & Haidukewych, 1994).

In veterinary medicine, phenobarbital has long been listed as a drug that potentially can decrease serum TT4 concentrations in dogs, but this hypothesis has only been verified in the last few years and will be further discussed and evaluated in chapter 2.

Further complicating the clinical evaluation of a dog tested for hypothyroidism and administered phenobarbital, is that weight gain (due to polyphagia), lethargy and hypercholesterolemia are reported in dogs on phenobarbital treatment, but are also commonly observed in dogs with hypothyroidism (Chrisman, 1991; Scott-Moncrieff & Guptill-Yoran, 2000; Gieger *et al.*, 2000).

Potassium bromide

For years, phenobarbital has been considered the most effective initial treatment for dogs with epilepsy (Chrisman, 1991). Recently, many veterinary neurologists have started using potassium bromide (KBr) as the initial therapy of choice for dogs with epilepsy. Bromide is a halide chemically related to iodide and potentially could interact with iodine in the thyroid gland. In rats, bromide produces a relative iodine deficiency, thereby interfering with iodine uptake, iodine transport and iodination of tyrosine and tyrosyl residues on thyroglobulin (Velicky *et al.,* 1998). A dose-dependent decrease in total T4 concentration, and morphological changes of the thyroid gland have been shown after KBr administration to rats (Loeber *et al.,* 1983; van Leeuwen *et al.,* 1983; Velicky *et al.,* 1997; Velicky *et al.*, 1998). Some of these effects already were noticed in thyroid glands of rats with small dosages of bromide (i.e. dosages encountered in the environment), and questions have been raised regarding the potential deleterious effects of environmental bromide on the human thyroid gland. No major effects were noted however on thyroid hormone concentrations after oral KBr administration in healthy humans (Sangster *et al.,* 1983). A single case report of KBr overdose does however describe transient hypothyroidism in a human (Mizukami *et al.,* 1988).

In one study, epileptic dogs receiving KBr alone (n=8) had normal thyroid function test results (Kantrowitz *et al.*, 1999). Similar results were obtained in another study where thyroid function (TT4, TSH, FT4 and TRH stimulation) remained unchanged in healthy dogs receiving KBr over a 6-month period (Paull *et al.,* 2000). In summary, administration of KBr does not seem to significantly affect thyroid function in dogs when used at usual dosages.

Sulfonamides

Sulfonamides can markedly interfere with thyroid hormone synthesis in rats by reversible inhibition of thyroid peroxidase, an enzyme essential for iodination (Comby *et al.,* 1993; Doerge & Decker, 1994). Sulfonamides therefore are goitrogens, leading secondarily to diminished thyroid hormone synthesis and secretion. By reduced negative feedback, an increase in pituitary secretion of TSH occurs which induces proliferative changes in the thyroid gland (Cohen *et al.,* 1981; Comby *et al.*, 1993; Capen, 1994). Long-term administration of sulfonamides and prolonged stimulation of the thyroid gland by TSH have been associated with thyroid neoplasia in rats (Doerge & Decker, 1994). Considerable interspecies variation however exists with respect to thyroid peroxidase inhibition by sulfonamides (Doerge & Decker, 1994; Capen, 1994). For example, only mild effects of sulfonamides are observed on human thyroid function (Cohen *et al.,* 1980). When a combination of sulfonamides and trimethoprim is used, it is the sulfonamide component which is responsible for thyroid inhibition, and trimethoprim alone failed to cause detectable alteration of thyroid function in rats (Cohen *et al.*, 1981) or dogs (Lagler *et al.,* 1976).

Several prospective studies have evaluated the effects of sulfonamide administration on thyroid function in dogs. Trimethoprim-sulfadiazine administered at a dosage of 15 mg/kg q12h to healthy dogs for 4 weeks had no effect on serum TT4, TT3, or FT4 concentrations or results of TSH stimulation tests (Panciera & Post, 1992). When trimethoprim-sulfadiazine was administered in the second half of gestation to pregnant bitches, no effects were noticed on thyroid hormone concentrations or thyroid gland morphology in the newborn puppies (Post *et al.,* 1993). However, when trimethoprim-sulfadimethoxazole was administered to dogs suffering from pyoderma for 6 weeks at a higher dosage of 30 mg/kg q12h (a dosage frequently recommended in

veterinary dermatology), serum TT4, TT3 and FT4 concentrations decreased (Hall *et al.,* 1993). Half of the dogs in this study had serum TT4 concentrations below the reference range at the end of the treatment period, which could have resulted in the inappropriate diagnosis of primary thyroid failure. Furthermore, 3 dogs had decreased responsiveness to bovine TSH stimulation at the end of the 6-week treatment. In one dog, TSH stimulation results normalized 8 weeks later, whereas in another dog normalization took 12 weeks. In 2 other studies, a similar dosage of trimethoprim-sulfadimethoxazole was given to healthy dogs for several weeks, and serum concentrations of thyroid hormones were markedly decreased and endogenous TSH increased as soon as 7 days after initiation of the treatment (Campbell *et al.,* 1996; Williamson *et al.,* 2002). Furthermore, radionuclide thyroid imaging showed increased uptake of pertechnetate, and thyroid gland biopsies disclosed hyperplasia of thyroid follicles and absence of colloid production (Campbell *et al.*, 1995). These findings further support the concept that sulfonamides act as goitrogens resulting in primary hypothyroidism and secondary changes associated with the proliferative effects of TSH.

Clearly, the effects of sulfonamides on thyroid function are species-specific, and dosage- and duration-dependent. Normalization of thyroid function test results in dogs after cessation of sulfonamide administration can take 8 to12 weeks. If the clinician is not aware of the effects of sulfonamides on thyroid function, an inappropriate diagnosis of hypothyroidism can easily be made in a dog receiving sulfonamides.

Not only can sulfonamides alter thyroid function test results, but they also can lead to clinical hypothyroidism in some dogs (i.e. sulfonamide-induced iatrogenic hypothyroidism) (Torres *et al.,* 1996; Gookin *et al.,* 1999). The dogs described in these 2 papers received a dosage of 24 mg/kg of trimethoprim-sulfadiazine for periods ranging from 30 to 126 days. Thyroid gland function tests in these dogs were indistinguishable from those seen in dogs with endogenous hypothyroidism in both situations decreased TT4 and FT4, increased TSH and decreased response to TSH stimulation test are observed. Thyroid scintigraphy was helpful in distinguishing endogenous from sulfonamide-induced hypothyroidism in one report (Gookin *et al.*, 1999). Uptake of pertechnetate by the thyroid gland should be normal or increased in dogs with sulfonamide-induced hypothyroidism, but should be minimal in dogs with

endogenous hypothyroidism. The hypothyroid state (i.e. clinical signs and tests results) resolves gradually (i.e., 7 days to several months) after the withdrawal of sulfonamides.

Non steroidal anti-inflammatory drugs

Many non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to alter thyroid function tests in humans, because circulating thyroid hormones are highly protein bound, and various NSAIDs can displace thyroid hormones from serum protein binding sites (Davies & Franklyn, 1991; Bishnoi *et al.,* 1994; Wenzel, 1996). These effects are most important in humans treated with salicylates, fenclofenac and phenylbutazone (Davies & Franklyn, 1991). Short-term administration of therapeutic dosages of salicylates led to a transient increase in unbound hormone concentrations and suppression of TSH concentrations in most studies (Davies & Franklyn, 1991; McConnell, 1999). For example, administration of a single dose of aspirin acutely increases free thyroid hormone concentrations by 2- to 3-fold (Larsen, 1972). During long-term treatment with salicylates, a new steady state is reached reflecting increased T4 turnover rate (Davies & Franklyn, 1991). Decreases in serum TT4 concentrations of 20 to 40 % have been reported (McConnell, 1992; Surks & Stievert, 1995). Free T4 concentrations can be unchanged or decreased (Surks & Stievert, 1995; McConnell, 1999). Thyrotropin concentrations return to the reference range within a few weeks of treatment (McConnell, 1992).

Salicylates alter thyroid function primarily by competition with low-affinity binding sites on serum thyroid hormone-binding proteins, but other mechanisms such as inhibition of hepatic 5'-monodeiodination and competition with plasma membrane, cytosolic or nuclear membrane binding also have been postulated (Chalmers *et al.,* 1993; Barlow *et al.,* 1996; Lim *et al.,* 1996). The contribution of these disturbances on peripheral thyroid hormone metabolism however is uncertain (McConnell, 1999).

Other NSAIDS have less pronounced effects on thyroid function in humans. Naproxen, diclofenac and ketoprofen induced a decrease in TT3 concentrations, but had no effect on TT4 and TSH concentrations (Bishnoi *et al.*, 1994; Carlson *et al.,* 1999). These results may be explained by the fact that, in humans, T3 is less avidly bound to transport proteins and is more easily displaced than T4 (Carlson *et al.*, 1999). Ibuprofen,

piroxicam and indomethacin had no effects on thyroid function test results in human patients (Bishnoi *et al.*, 1994).

Thyroid hormone binding to serum carrier proteins and hormone metabolic rates vary among species. Therefore, it seems reasonable to think that the effects of NSAIDS on thyroid hormones might be different between dogs and humans. Affinity of thyroid hormones for their serum proteins is lower in dogs than in humans (Larsson *et al.*, 1985; Larsson, 1987). Therefore it is possible that effects of different NSAIDS are more pronounced in dogs than in humans. Further studies on the effects of other NSAIDS on canine thyroid function are necessary and will be discussed and evaluated in chapter 3.

Propranolol

Propranolol may alter thyroid hormone metabolism in humans. Small decreases in T3 are observed, but TSH concentrations remain unchanged. In euthyroid beagle dogs, however, T4 and T3 measurements and TSH stimulation test results remained unchanged after 4 weeks of oral propranolol administration (TSH was not measured) (Center *et al.,* 1984). Propranolol does not seem to significantly affect serum thyroid hormone concentrations in dogs.

C. Systemic non-thyroid diseases

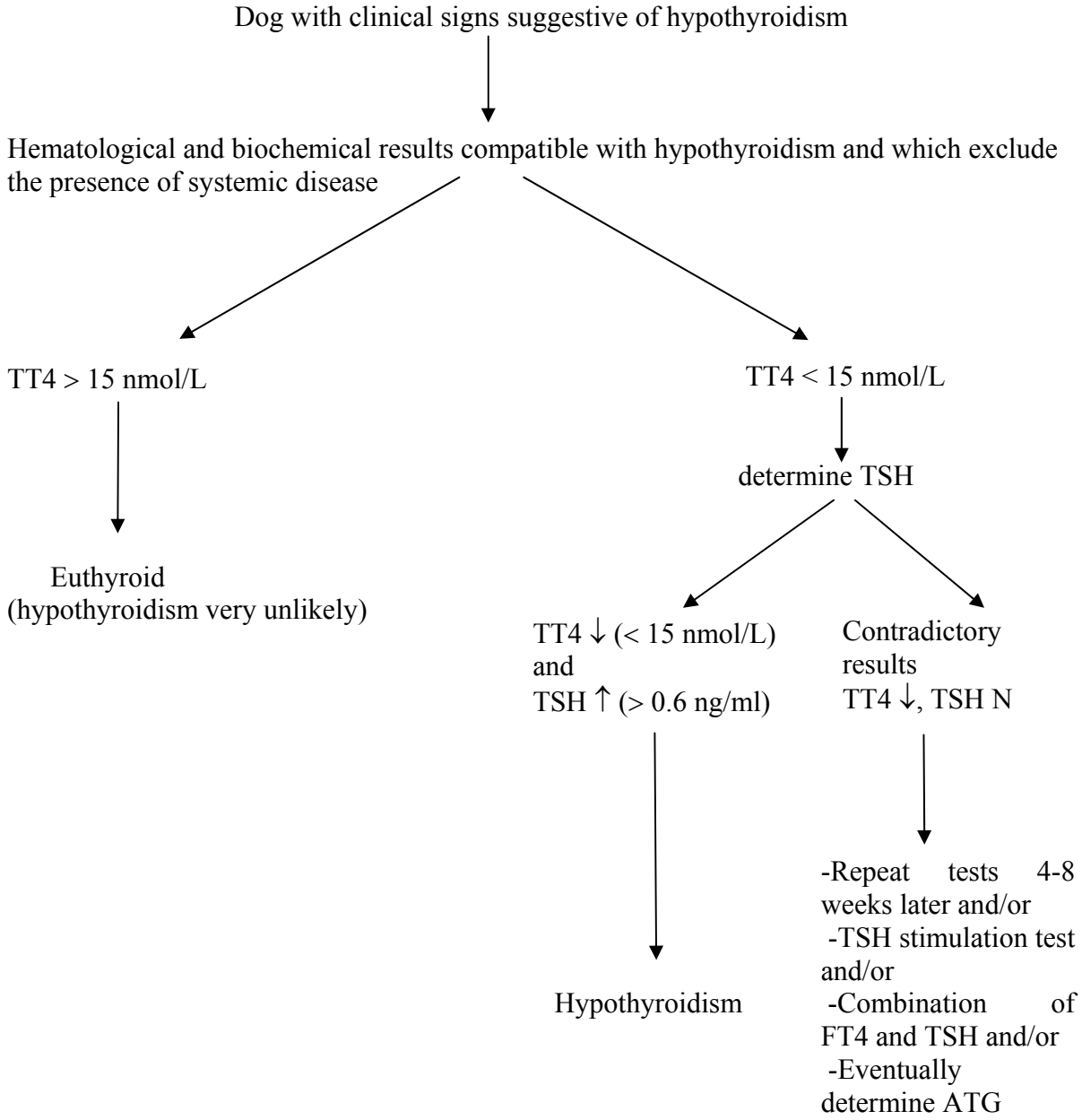
The presence of a systemic non-thyroidal disease (SNTD), such as diabetes mellitus, liver disease, hyperadrenocorticism and renal- or hart failure, is a frequent cause for decreased thyroid hormone concentrations. This phenomenon is referred to as the "euthyroïd sick syndrome" (Ferguson, 1997). These changes probably reflect a physiological adaptation of the organism leading to a decrease in tissue energy requirements. The administration of synthetic thyroid hormones to these patients is not recommended. The pathogenesis is complex but would include: change in the deiodinases activity, a decrease in thyroid hormone binding to their transport proteins, a decrease in TSH and thyroid hormone secretion (Nicoloff & LoPresti, 1995). Cytokines such as interleukine-1 (IL-1), IL-2, tumor necrosis factor-α and interferon, also seem to be involved. The administration of IL-2 to dogs has lead to a marked decrease in thyroid

hormone concentrations (Panciera *et al.,* 1995). In the presence of a SNTD, serum thyroid hormone concentrations can be decreased in a range compatible with hypothyroidism. For example, 57% of dogs with hyperadrenocorticism, showed a decrease in TT4 serum concentrations (Peterson *et al.*, 1984). Free T4 can also be decreased but often to a lesser extend (Scott-Moncrieff & Guptill-Yoran, 2000). The response to a TSH stimulation test can also be blunted, but usually is less affected then a baseline TT4. Therefore, it is indicated, whenever possible to treat the SNTD before testing thyroid function (Ferguson, 1997).

VII. Diagnostic approach

Diagram 1 gives a possible approach for a patient suspected of having hypothyroidism.

Diagram 1: Proposed algorithm for the evaluation of thyroid gland in practice



TT4: total thyroxine (reference range 15-45 nmol/L), FT4: free thyroxine (10-35 pmol/L), TSH: Thyrotropin (0-0.6 ng/ml), ATG: anti-thyroglobuline antibodies. Every laboratory should ideally determine its own reference values.

CHAPTER 1

VIII. Treatment of canine hypothyroidism

Treatment of hypothyroidism consists in life long administration of synthetic levothyroxine (L-T4) in order to: approximate the secretion of thyroid hormones by the thyroid gland, resolve clinical signs and avoid thyrotoxicosis. A normal thyroid gland secretes principally T4, which is thereafter transformed in T3 through peripheral deiodination. The administration of a medication containing T3 and T4 is contraindicated because both hormones have very different half-times and in consequence their frequency of administration is very different. Treatment with liothyronine T3 is also contraindicated because it should be administered 3 times a day and more easily lead to thyrotoxicosis, presumably because the organism cannot regulate deiodination of T4 into T3. The use of an original preparation ("brandname"), and a veterinary preparation should be prioritised, because biological availability of generic or human products varies in dogs (Nachreiner & Refsal, 1992). Dosages used to treat dogs are higher than in human medicine. This is explained by a difference in metabolism between the two species. In dogs, fecal excretion is higher and intestinal reabsorption less (10-50% compared to 50-80% in humans) (Ferguson, 1986). Therefore, half-time of T4 in dogs (18 hours) is shorter than in humans (7days). Initial treatment dosages varies from 11 to 22 μg/kg q 12 hour according to the author, with a maximum of 0,8 mg of L-thyroxine q 12 hour (Rijnberk, 1996; Greco, 2000; Scott-Moncrieff & Guptill-Yoran, 2000). The patient is revaluated 1 to 2 months after initiating therapy and dosage is adjusted based on clinical response and results of the T4 serum concentration. The pharmacokinetics of levothyroxine after oral administration varies from patient to patient, therefore an individual adjustment of the dosage is needed (Nachreiner *et al.*, 1993). After some time, in most dogs, 'once daily' administration will be sufficient. If the owner or veterinarian wishes to use generic products, revaluation of serum concentrations is recommended 1 to 2 months later. Successful therapy is first of all assessed by a good clinical examination. Lethargy should resolve within 2 weeks of initiating therapy. Hair regrowth will be more progressive, but an improvement should be noticed within 4-6 weeks. Blood abnormalities should be normalised within 4-6 weeks. The non-resolution of the clinical signs within 6-8 weeks can have several origins such as; poor owner's compliance with treatment, the dosage

used is not optimal, diagnosis could be wrong or an additional disease could be present. Most commonly treatment is evaluated through measurement of TT4. When interpreting the result of TT4 measurement, time of sampling compared to the administration of the medication, should be taken into consideration. When a blood sample is taken just before administration of the medication (pre-tablet test), nadir concentrations are measured. Most commonly blood is taken 4 to 6 hours after the last medication is administered (post-tablet test) and peak concentrations are measured. In this case, TT4 is expected to be within the reference range (upper half limit), but a TT4 value just above the reference range is accepted (Greco, 2000). Free T4 measurements can also be used to assess therapy. The recommendations concerning time of sampling and interpretation of results are similar as for TT4 (Scott-Moncrieff & Guptill-Yoran, 2000). In human medicine, a combination of TSH and FT4 determinations is used to assess optimal therapy. In humans and in dogs, the administration of an excess of levothyroxine can lead to thyrotoxicosis. In human medicine, a small overdosage (subclinical thyrotoxicosis) can already lead to more hidden, but serious complications such as osteoporosis, increased liver enzymes or an occult cardiac hypertrophy (Greco, 2000). This is accompanied by a decrease in TSH serum concentrations under the reference range. On the contrary, dogs only seldom suffer from clinical thyrotoxicosis (polyuria/polydipsia, polyphagia, weight loss, panting, hyperactivity). This is probably related to the more rapid elimination of thyroid hormones in dogs compared to humans. It is not clear whether the dog can, as observed in humans, suffer from subclinical thyrotoxicosis with side effect noted on the mid to long term. In theory, the best way to assess optimal therapy would be to asses whether the pituitary-thyroid axis is sufficiently inhibited by the thyroid hormones, this through showing normalisation of initially elevated TSH values. For the 18 to 38% hypothyroid dogs showing no initial increase in TSH values, normalisation of TSH serum concentrations cannot be used to assess therapy. Furthermore the tests available to measure TSH concentrations are very reliable to detect an increase in TSH values but not to detect decreases in the lower range. The use of endogenous TSH in the monitoring of therapy, allows detection of a dog which is insufficiently treated (TSH remains high). But it does not allow to differentiate an over treated dog from a well-controlled dog. Therefore, follow-up through TT4 serum concentrations (cheap and easy to perform) remains the most

commonly used parameter for the follow-up of a treated hypothyroid dog. However, additional TSH determinations can be valuable for the monitoring of therapy in some patients.

Conclusion

Treatment of hypothyroidism is relatively simple, but obtaining a reliable diagnosis can sometimes be more difficult. Numerous factors can influence thyroid homeostasis. Knowledge of these factors can contribute to decreasing the misdiagnosis of hypothyroidism. Non-thyroidal diseases and the administration of medications can lead to decreased thyroid hormone concentrations. The effects of several commonly used medications, obesity and weight loss on canine thyroid function will be studied in this thesis. Furthermore, thyroid function will also be evaluated in dogs with CRFA, a disease with a presentation suggesting hypothyroidism.

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CHAPTER 1

CHAPTER 2

CHAPTER 2

Introduction to chapter 2

Some drugs affect thyroid function. The list of drugs known to alter thyroid function or thyroid function test results in humans is much more extensive than those evaluated in dogs. The lack of information about possible influence of medications on canine thyroid function contributes to the over-diagnosis and over-interpretation of thyroid hormone results in dogs. This eventually can lead to misdiagnosis of hypothyroidism. Prednisone is widely used in veterinary medicine for its immunosuppressive or anti-inflammatory properties to treat a variety of disorders. Phenobarbital is the first line anticonvulsant of choice for treatment of canine epilepsy. Both drugs are extensively used in veterinary medicine. In this second chapter the short-term effects of prednisone and phenobarbital on canine thyroid function test results will be assessed.

CHAPTER 2

CHAPTER 2

SHORT-TERM INFLUENCE OF PREDNISONE AND PHENOBARBITAL ON THYROID FUNCTION IN EUTHYROID DOGS

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CHAPTER 2

Summary

The short-term effects of prednisone and phenobarbital on serum total thyroxine (TT4), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were evaluated in euthyroid dogs.

Twenty-six beagles were randomly divided into 3 groups, receiving respectively, a placebo, prednisone (1.2 to 2 mg/kg body weight, per os, every 12 hours) for 3 weeks or phenobarbital (1.8 to 3 mg/kg body weight for 1 week, then 2.7 to 4.5 mg/kg body weight, per os, every 12 hours for 2 weeks). Blood samples taken over a 6-week period were assayed for serum TT4, FT4, and TSH. Phenobarbital therapy in our study did not affect serum TT4, FT4, or TSH concentrations. Prednisone therapy, however, significantly decreased serum TT4 and FT4, but did not affect serum TSH concentrations.

CHAPTER 2

Introduction

The measurement of serum concentrations of total thyroxine (TT4) is frequently used to assess thyroid function in dogs. Serum TT4 concentrations are influenced by many factors, including daily fluctuations, severe non-thyroidal diseases, and certain drugs (1-3). Because of these influences, the measurement of a basal TT4 concentration is frequently unreliable as an indicator of thyroid function in dogs. Theoretically, free thyroxine (FT4) concentrations should be less subject to these variations (4). The most reliable assay for FT4 is by equilibrium dialysis, which is becoming more readily available from diagnostic laboratories (5). Until recently, the easiest and most reliable test to evaluate canine thyroid function was the thyroid-stimulating hormone (TSH) stimulation test. However, bovine TSH used to perform this test is no longer commercially available. Recently, methods to evaluate canine endogenous TSH have become available and seem promising for accurate assessment of canine thyroid function (6-9).

Several drugs have been shown to alter thyroid function in humans, rats, and dogs (2,10). Drugs frequently used in canine practice and known to alter thyroid function in man and rats include glucocorticoids and phenobarbital. Studies performed with humans and rats have demonstrated that endogenous or exogenous glucocorticoids directly inhibit the hypothalamic-pituitary-thyroid axis and also influence peripheral metabolism of thyroid hormones (11-14). Although several studies in dogs have reported the effects of exogenous or endogenous glucocorticoids on TT4, and triiodothyronine (T3), their effects on endogenous TSH are unknown (15-19).

In rats, phenobarbital alters thyroid function by affecting the peripheral elimination of T4 (10, 20). Indeed, increased hepatic deiodination of thyroid hormones, increased biliary clearance, and increased fecal excretion result in decreased concentrations of circulating thyroid hormone, which, in turn, increase TSH secretion through the classical negative feedback pathway (20-22). Phenobarbital could also have additional central effects on the hypothalamus-pituitary-thyroid axis (23, 24). Most studies in rats report decreased T4 with normal or, more often, increased TSH

concentrations (21, 23, 25). In humans, phenobarbital increases the metabolic clearance of T4 through increased deiodination and biliary clearance of thyroid hormones, as well as by an enhanced hepatocellular binding (26, 27). In veterinary medicine, phenobarbital is often listed as a drug that can potentially decrease canine TT4 levels (1, 2), but to our knowledge, there are no published studies available on the effects of phenobarbital on thyroid function in dogs.

The objective of this study was to evaluate the influence of short-term oral prednisone and phenobarbital on canine thyroid function.

Materials and methods

Dogs

Twenty-six, sexually intact, adult beagles (11 females, 15 males; 2 to 3 years old) were assessed as healthy based on results of physical examination, complete blood count and biochemical profile, and normal serum TT4, FT4, and TSH concentrations. The dogs were randomly divided into 3 groups. Group 1 consisted of 8 dogs, 3 females and 5 males. Group 2 contained 9 dogs, 4 females and 5 males. Group 3 consisted of 9 dogs, 4 females and 5 males. The mean body weights of these dogs were 11.07 kg +/- 0.59, 11.4 +/- 0.76, and 10.7 +/- 0.7 for groups 1, 2, and 3, respectively. All dogs were housed indoors, in 6 runs containing 4 to 5 dogs each. A one-week acclimatisation period was allowed before starting the experiment. Environmental conditions, such as photoperiod (12 hours on, 12 hours off), ventilation, and temperature were kept constant throughout the study. The dogs were fed a standard commercial maintenance pellet diet twice daily and water was available ad libitum.

Experimental protocol

Dogs in group 1 received a placebo (Lactose, Odan Ltee Laboratories, Montreal, Quebec) for 3 wk. Dogs in group 2 were given prednisone (Apotex, Weston, Ontario) at a dosage ranging from 1.2 to 2 mg/kg body weight (BW), PO, q12h for 3 wk. Dogs in group 3 were given phenobarbital (Parke-Davis, Scarborough, Ontario) at a dosage ranging from 1.8 to 3 mg/kg BW, PO, q12h for the first wk, then from 2.7 to 4.5 mg/kg BW for the next 2 wk. Monitoring was continued for 6 wk, with the treatments administered during the first 3 wk of the study.

Blood samples (18 mL) were taken by jugular venipuncture before initiating therapy (T0), at 48 h, and after 1, 3, 4, 5, and 6 wk. All blood samples were taken between 0800 and 0900. Blood samples were immediately centrifuged and serum was frozen in 4 aliquots at -20^0C, until assayed.

The protocol was approved by the Ethics Committee of the Faculté de Médecine Vétérinaire of the University of Montreal, and procedures were performed in accordance with the recommendations of the Canadian Council on Animal Care.

Assays for TT4, FT4 and TSH

Assays for TT4, FT4, and TSH were performed on all samples. Total thyroxine concentrations were determined by using a commercially available solid-phase radioimmunoassay (Coat-A-Count canine T4, Diagnostic Products, Los Angeles, California, USA) previously validated for canine serum (15). Assays for FT4 concentrations by equilibrium dialysis were performed using a commercial assay kit (Free T4 by equilibrium dialysis Nichols Institute Diagnostics, San Juan Capistrano, CA) at the Endocrine Section, Animal Health Diagnostic Laboratory, Michigan State University. Assay procedures were performed as per the manufacturer's instructions. The manufacturer of the kit reported negligible crossreactivity of other iodothyronines (range of 0.044% to 0.001%) in the assay. For estimates of assay repeatability, 3 pools of canine serum were established. In canine serum with a mean concentration of 15, 37, or 96 pmol/L, the interassay coefficient of variation (CV) was 18.6%, 14.2%, and 6.9%, respectively (n=10 assays). In the same serum pools, intraassay CV was 14.5%, 10.2%, and 6.6%, respectively (10 replicates). Canine serum with a concentration of FT4 of 111 pmol/L was diluted with "0" standard prior to placement in the dialysis chamber. In serum diluted with standard at rates of 1:1, 1:2, or 1:4, 100%, 74%, and 70% of expected concentrations of FT4 were measured in the assay. Thyroxine was added in varying amounts to aliquots of dialysate harvested after prior incubation with canine serum. The concentration of FT4 in the dialysate was 11 pmol/L after incubation with serum and before addition of exogenous T4. When T4 was added to dialysate at concentrations of 13, 26, 52, or 77 pmol/L, 92%, 95%, 99%, and 92% of added T4, was respectively measured in the assay. The sensitivity of the assay, defined as the concentration of FT4 at the point of 90% of total specific binding, was 1.8 pmol/L (mean of 10 assays). Serum TSH concentrations were determined by using a commercially available solid-phase radioimmunoassay (Coat-A-Count canine TSH IRMA, Diagnostic Products) recently validated in the dog (7, 9). The intra- assay CV was 7% for samples with a TSH concentration below 1 ng/mL, and 0.8% for samples with a TSH concentration above 1 ng/mL. The inter-assay CV was 7% for samples with a mean TSH concentration of 3 ng/mL, and 26% for samples with a mean TSH

concentration of 0.11 ng/mL. Dilutional parallelism, evaluated through assaying serum at 4 dilutions, was respected. The limit of detection was 0.03 ng/mL.

Serum phenobarbital concentrations

Serum concentrations of phenobarbital were determined for dogs in group 2, in samples from wk 1 and 3, by using an enzymatic technique (Cedia Phenobarbital, Boehringer Manheim, Indianapolis, Indiana, USA) at a commercial laboratory (Vita-Tech, Ontario).

Data analysis

Data were analyzed by two-way analysis of variance for repeated measures. When there were significant time by treatment interactions, a Dunnett's procedure was used to compare the groups at each time period. Data are presented as means +/- standard error of the mean (SX). Results were considered significant at $P < 0.05$.

Results

Total thyroxine concentrations

There was a significant effect of time on TT4 concentrations in the control and the prednisone groups (Figure 1, $P < 0.05$). There was a significant interaction between time and group; therefore, differences between groups were analyzed at each time period. After 1 and 3 wk of treatment, TT4 concentrations were significantly lower in the prednisone group compared with the control group (week 1; 12 +/- 2 nmol/L and 28.1 +/- 4 nmol/L, respectively; week 3; 15.8 +/- 2 nmol/L and 38.3 +/- 4.4, respectively). Phenobarbital therapy did not significantly affect TT4 values at any time (Figure 1).

Free thyroxine concentrations

There was a significant effect of time on FT4 concentrations in the control and the prednisone groups (Figure 2, $P < 0.05$), and a significant interaction between time and group. A significant decrease in FT4 concentrations was found at 3 wk in the prednisone group when compared with the control group (19.7 +/- 3.1 pmol/L and 31.4 +/- 3.5 pmol/L, respectively). Phenobarbital did not significantly affect the FT4 concentrations at any time (Figure 2).

Serum TSH concentrations

There was a significant effect of time on TSH concentrations in all 3 groups (Figure 3, $P < 0.05$). There was neither significant effect of group, nor an interaction between time and group. All concentrations for TSH levels were within the normal reference range throughout the study (< 0.7 ng/mL).

Serum phenobarbital concentrations

Measurement of serum phenobarbital concentrations after 1 wk of therapy revealed that the recommended therapeutic level of phenobarbital (65 to 150 μmol/L) had been obtained in 6 out of 9 dogs (28). Most dogs had serum concentrations towards the lower end of the therapeutic range (71.4 +/- 4.4 μmol/L). After the dosage of

phenobarbital had been increased by 50% in all dogs for the next 2 wk, the serum phenobarbital concentrations were again at the lower end of the therapeutic range (67.4 +/- 5.6 μmol/L). There was no correlation between serum phenobarbital levels and serum concentrations of TT4, FT4, or TSH. Side effects from phenobarbital administration, such as ataxia or lethargy, were not noticed.

Figure 1-Mean (+/- SX) TT4 concentrations in beagles during and after placebo, prednisone (1.2 to 2 mg/kg BW, PO q12h) or phenobarbital (1.8 to 3 mg/kg BW for 1 wk, then 2.7 to 4.5 mg/kg BW PO, q12h) administration. Medications were administered during the first 3 wk of the experiment. *=significantly different from control group ($P < 0.05$).

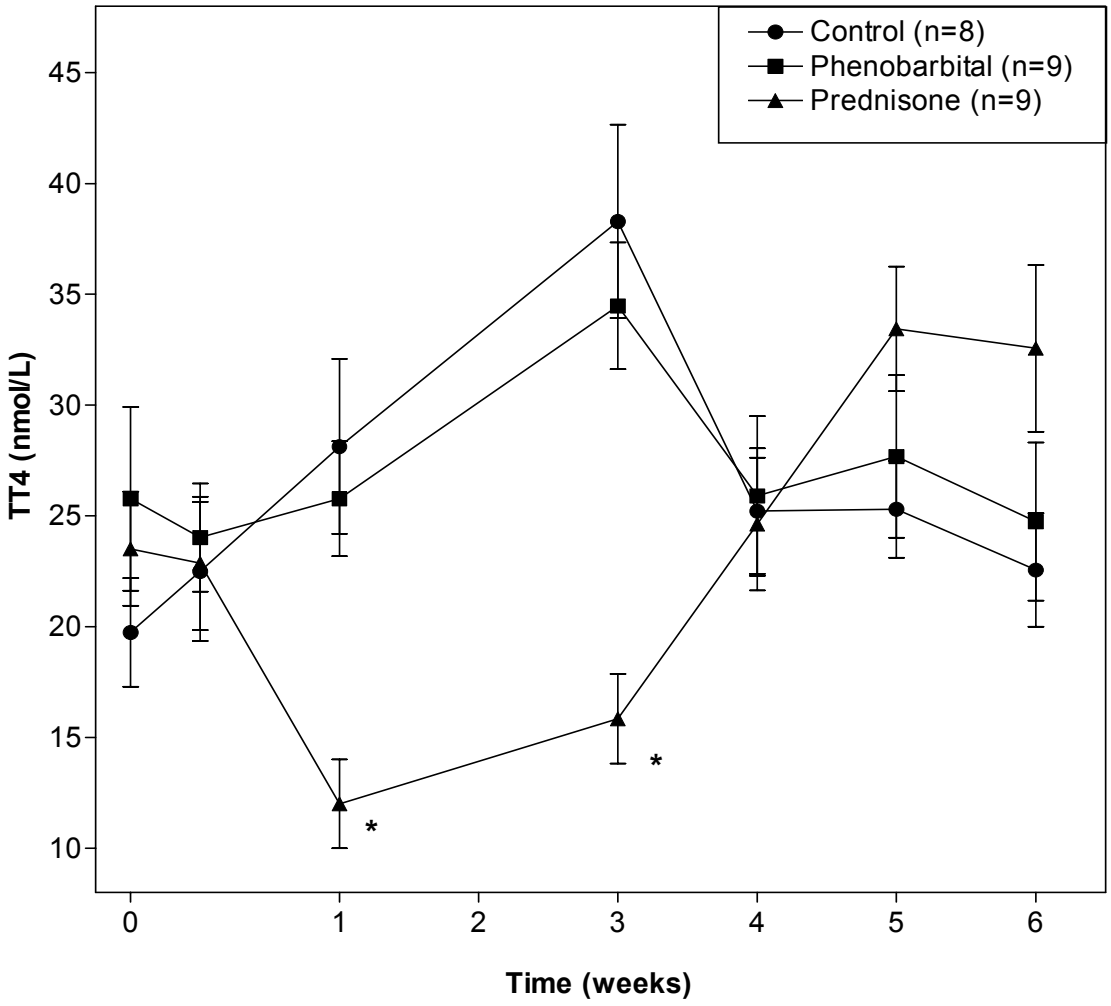


Figure 2-Mean (+/- SX) FT4 concentrations in beagles during and after placebo, prednisone (1.2 to 2 mg/kg BW PO, q12h) or phenobarbital (1.8 to 3 mg/kg BW for 1 wk, then 2.7 to 4.5 mg/kg BW PO, q12h) administration. Medications were administered during the first 3 wk of the experiment. *=significantly different from control group ($P < 0.05$).

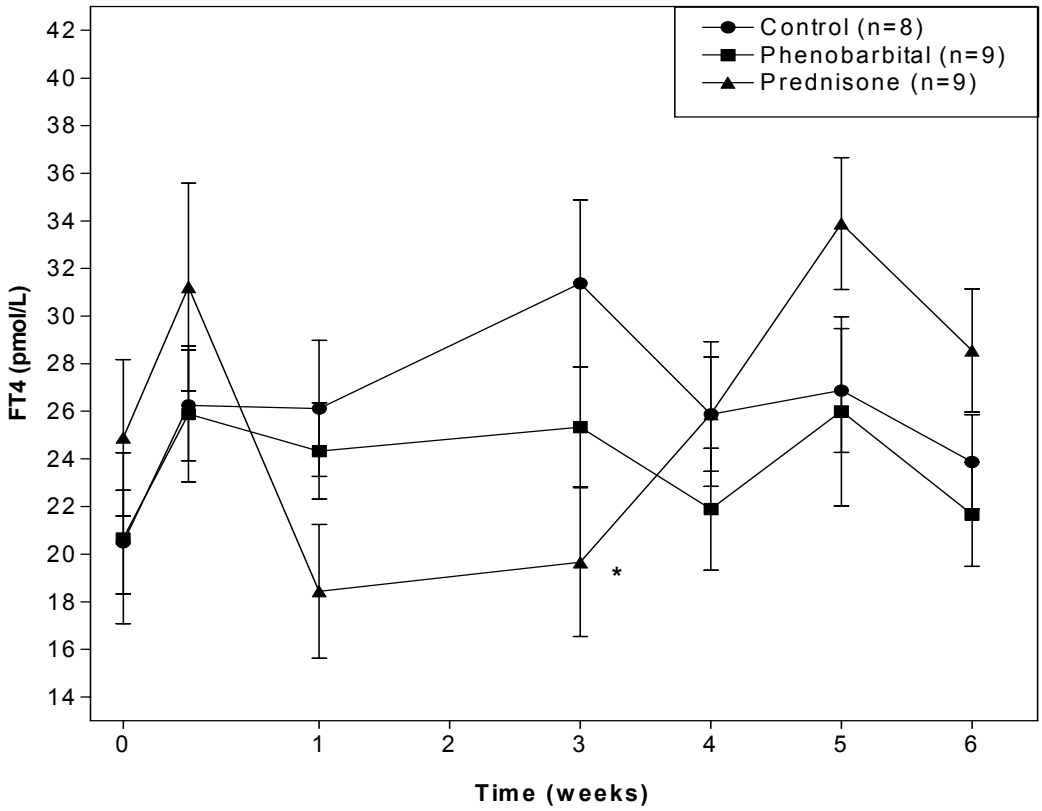
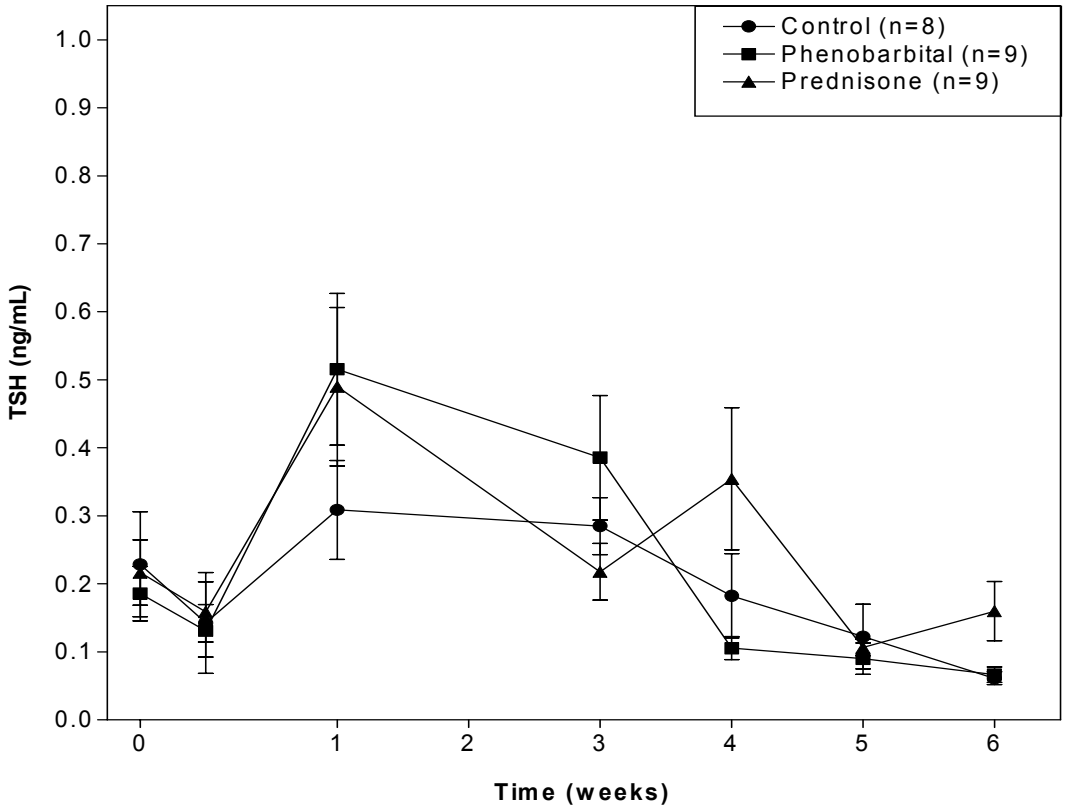


Figure 3-Mean (+/- SX) TSH concentrations in beagle dogs during and after placebo, prednisone (1.2 to 2 mg/kg BW PO, q12h) or phenobarbital (1.8 to 3 mg/kg BW for 1 wk, then 2.7 to 4.5 mg/kg BW PO, q12h) administration. Medications were administered during the first 3 wk of the experiment ($P < 0.05$).



Discussion

The effects of prednisone on canine endogenous TSH and the effects of phenobarbital on thyroid function in dogs are unknown. Our finding that, prednisone given at an immunosuppressive dosage significantly suppressed TT4 at 1 and 3 wk and FT4 levels after 3 wk of therapy is in agreement with a previous study by Torres et al (15) but contrasts with that by Moore et al (18). Torres et al, reported a decrease in canine TT4 and FT4 levels at 24 h and 3 wk after initiating oral prednisone therapy at an immunosuppressive dosage. However, serum thyrotropin concentrations were not determined and FT4 concentrations were not assayed by equilibrium dialysis, which is now considered the most reliable technique to determine FT4 levels (5, 29, 30). Moore et al later reported that 1 mo of oral prednisone did not affect basal TT4 levels in normal dogs, but a lower antiinflammatory dose of prednisone was used. The effects of glucocorticoids have previously been documented to be dependent on the dosage, route of administration, duration of treatment, and chemical form used (15, 31).

The fact that, in our study, serum TSH concentrations were not significantly affected by prednisone administered orally at an immunosuppressive dosage during a 3-week period, suggests that the measurement of TSH concentrations in euthyroid dogs receiving short-term prednisone therapy may be a more reliable tool than that of TT4 to evaluate thyroid function. However, this is in contrast to studies with humans, in which TSH concentrations were decreased by exogenous or endogenous glucocorticoids (11, 12, 32-35). It is possible that a suppression of TSH secretion is less easily detected in dogs compared with humans, because euthyroid dogs have TSH concentrations very close to the limit of detection of the current assay. It is also possible that there is a species difference between dogs and humans in the pituitary response to glucocorticoids. In the clinic, TSH measurement is frequently used in combination with TT4 or FT4 to evaluate thyroid function in dogs (8). Further studies to assess the effects of glucocorticoids on hypothyroid dogs with high TSH levels are needed.

The mechanism of action of glucocorticoids on thyroid function appears complex, with suppression of TSH release reported in humans (12-14, 32, 36-38) and

rats (37, 38), and disturbed T3 and T4 partition, clearance rates, and metabolism reported in humans (13, 39), rats, and dogs (15-17, 31). Our study was not designed to determine the mechanism of action of glucocorticoids on thyroid function.

Another commonly used drug that may affect thyroid function is phenobarbital. In rats, phenobarbital increases biliary thyroid hormone excretion, which decreases serum TT4 levels with normal or more often, increased TSH levels (20-25, 40). There are no published studies on the effects of phenobarbital on thyroid function in dogs, but, by extrapolation, phenobarbital is often listed as a drug that can potentially affect canine thyroid function (1, 2). Recently, 2 short communications have suggested that long-term administration of phenobarbital influences canine thyroid function (41, 42). Surprisingly, in our study, phenobarbital did not significantly affect TT4, FT4, or TSH concentrations. Interestingly, most studies in rats report decreased levels of TT4 and increased levels of TSH, but the dosages administered to these rats were extremely high (100 mg/kg BW/d) and serum levels of phenobarbital were not determined (20, 23, 25). Hepatic enzyme induction and decreased concentrations of TT4 in rats receiving phenobarbital are dose-related (24). Most studies in humans report no significant changes of TT4 and TSH levels in epileptic patients taking phenobarbital (10, 26, 27, 43). Dosages of phenobarbital administered to human patients range from 1 to 5 mg/kg BW/day, a dose similar to the recommended initial dosage for phenobarbital in dogs.

In the present study, serum phenobarbital concentrations after 1 wk of treatment were at the lower end of the therapeutic range in group 2 dogs. As the elimination half-life of phenobarbital in beagles is much shorter than in other breeds, serum steady-state concentrations should be achieved after 1 wk, instead of 2 to 3 wk (44-46). To reach higher therapeutic concentrations, the initial dosage of phenobarbital was increased by 50 % in all dogs of group 2 for the next 2 wk. Despite these higher doses of phenobarbital, serum concentrations of phenobarbital were somewhat lower than expected. This suggests that short-term administration of phenobarbital, at antiepileptic dosages, does not affect thyroid function in dogs.

There was a significant effect of time on TT4, FT4, and TSH concentrations in the control group. Several factors can influence basal TT4 and less severely, FT4

concentrations in dogs (3). Factors such as photoperiod, ambient temperature and body weight were constant throughout the experiment, making it unlikely that they were responsible for the variation of the hormone concentrations in the control group. Also, the dogs were allowed to acclimatize for a period of 1 wk. It has been reported that, in females, increased progesterone levels can increase TT4 levels (47), although another study suggests no difference in TT4 concentrations between male and female dogs (48). The females in our study were in anestrus, based on the absence of clinical signs compatible with proestrus and estrus throughout the study. Furthermore, the tendency toward increased concentrations of TT4 after 1 and 3 wk of treatment was noted in both the female and male dogs of the control group.

Conclusion

In conclusion, oral prednisone administered over a 3-week period at immunosuppressive dosages significantly suppressed TT4 and FT4 concentrations, but did not affect TSH concentrations. Short-term administration of phenobarbital does not significantly alter canine thyroid function.

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CHAPTER 3

CHAPTER 3

Introduction to chapter 3

In the previous chapter we demonstrated a significant effect of prednisone on TT4 serum concentrations. To further investigate the influence of drugs on canine thyroid function tests, we will, in the following chapter, evaluate the possible influence of some commonly used NSAIDs. Indeed, as the importance of recognising and treating pain in dogs grows, so does the use of NSAIDs.

CHAPTER 3

CHAPTER 3

INFLUENCE OF ACETYLSALICYLIC ACID AND KETOPROFEN ON CANINE THYROID FUNCTION TESTS

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CHAPTER 3

Summary

Many factors including drugs can influence thyroid function in humans, rats and dogs. Studies in humans report significant effects of non-steroidal anti-inflammatory agents (NSAIDs) on thyroid function test results, which can lead to misinterpretation of the results and inappropriate therapeutic decisions. As NSAIDs are used more and more frequently in dogs, it is important to know whether they affect thyroid function test results.

The objective of this study was to determine whether acetylsalicylic acid (ASA) or ketoprofen (Keto) influence thyroid function test results in dogs. Eighteen spayed female beagle dogs were randomly assigned to three treatment sequences in a 3x3 cross over study design with treatments consisting of ASA (25 mg/kg BW q 12 h), Keto (1 mg/kg BW q 24 h) or placebo administered for a one-week period with a three-week washout period between treatment periods. Blood samples for determination of total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3), thyrotropin (TSH), reverse triiodothyronine (rT3), Keto and ASA concentrations were taken during each treatment period on days 0, 1, 3 and 7. During the washout period samples were taken weekly.

A significant decrease in TT4 was observed as soon as 24 h after ASA administration, whereas the decrease in TT3 was less pronounced and differed significantly with placebo only after one week of the ASA administration. No significant effects were found for free T4 and TSH with ASA administration. No significant effects on thyroid results were found for Keto administration. The results indicate that TT4 can be markedly decreased by ASA therapy and until the results of further studies are available, thyroid function test results should be interpreted cautiously in dogs on NSAIDs therapy.

CHAPTER 3

Introduction

Hypothyroidism is the most diagnosed and probably also the most over-diagnosed endocrine disease in dogs. Evaluation of thyroid function in dogs is not always straightforward as clinical manifestations of hypothyroidism are nonspecific and no single thyroid function test is fully reliable to confirm the diagnosis (Peterson *et al.,* 1997; Scott-Moncrieff *et al.,* 1998; Dixon & Mooney, 1999). Furthermore, numerous nonthyroidal factors such as the presence of systemic disease (euthyroid sick syndrome) or the administration of drugs can affect thyroid function (Ferguson, 1988; Ferguson, 1997). Drugs frequently used in canine practice and known to alter thyroid function in dogs include glucocorticoids, phenobarbital and sulphonamides (Daminet & Ferguson, 2003). Changes in thyroid hormone concentrations induced by drugs can be confusing and lead to an erroneous diagnosis of hypothyroidism resulting in inappropriate treatment.

Currently, the combined use of measurement of thyroxine (T4) and thyrotropin (thyroid stimulating hormone; TSH) serum concentrations seems to be the easiest and most reliable way to assess canine thyroid function in a clinical setting (Scott-Moncrieff *et al.,* 1998). The measurement of free thyroxine (FT4), especially by equilibrium dialysis (FT4ED), is thought by many authors to be valuable because it is less influenced by non-thyroidal diseases than determination of a total thyroxine (TT4) (Peterson *et al.*, 1997; Scott-Moncrieff & Guptill-Yoran, 2000), although other studies have not confirmed this finding (Dixon & Mooney, 1999).

Non-steroidal anti-inflammatory agents (NSAIDs) are used increasingly frequently in dogs. The possible gastro-intestinal and renal side effects of NSAIDs have been extensively described in the literature (Mathews, 1996), but studies on the effects of NSAIDs on canine thyroid function have not been reported to our knowledge other than in 2 abstracts (Ferguson *et al.,* 1999; Sauvé *et al.,* 2002).

There are, however, reports of the effect of NSAIDs on thyroid function in humans and horses. Non-steroidal anti-inflammatory agents can alter thyroid function in humans, since circulating thyroid hormones are protein bound, and various NSAIDs can displace thyroid hormones from serum protein-binding sites (Davies & Franklyn,

1991; Bishnoi *et al.,* 1994; Wenzel, 1996). These effects are most important in humans treated with salicylates (e.g. salsalate, acetylsalicylic acid), or fenclofenac and phenylbutazone (Davies & Franklyn, 1991). Several studies in horses have shown that phenylbutazone markedly affects TT4 and to a lesser extend FT4 serum concentrations in this species (Sojka *et al.,* 1993; Ramirez *et al.,* 1997).

It is, however, important to study the effects of NSAIDs on thyroid function in the dog separately because the results found in humans cannot be extrapolated to dogs for the following reasons. Firstly, thyroid hormones are lipophilic compounds and, as such, need to be transported by specific binding proteins, thyroxine-binding protein (TBG), thyroxine-binding prealbumin (TBPA; transthyretin) and albumin. Plasma concentrations of TBG in the dog are only 15 % of those in man and the affinity of thyroid hormones for their transport proteins is also less than in humans (Larsson *et al.,* 1985; Kaptein *et al.,* 1994). Secondly, and partly as a result of this weaker serum protein binding, TT4 concentrations are lower, the unbound or free fraction of circulating T4 is higher (0.1 versus ~0.03% in man), and hormone metabolism is more rapid in most domestic animals than in humans (Bigler, 1976). It is the unbound (free) fraction of the hormone, which is available to tissues and therefore the concentration is proportional to the action, metabolism, and elimination of that hormone (Robbins & Rall, 1960; Mendel, 1989). While the normal variation of the free fraction of thyroxine (FFT4) is fairly small, ranging from 0.05-0.15%, this binding relationship may change in response to drugs or illness (Ferguson & Peterson, 1992). Because of reduced affinity of thyroid hormones for their carrier proteins in dogs compared to humans, the effects of different NSAIDs may be more pronounced in dogs than in humans.

In our study, the effects of two different NSAIDs on thyroid function was investigated. Acetylsalicylic acid (ASA), a salicylate, has been the cornerstone of analgesic therapy in humans and dogs for decades. Newer, more potent NSAIDs are now available but ASA's low cost and historical usage still make its use widespread in practice. Acetylsalicylic acid is highly protein bound (50-70%) to plasma transport proteins, especially albumin (Davis & Westfall, 1972; Boothe, 1995). Following several doses of ASA of 25 mg/kg q 12 h, Boothe (1995) reported a plasma elimination half-life of 7.5 h in dogs. Plasma salicylate concentrations < 50 µg/mL are considered

to be subtherapeutic in the dog and concentrations > 300 µg/mL are considered potentially toxic (Nap *et al.,* 1991).

Ketoprofen (Keto), a propionic acid derivate with well-established analgesic properties, is another NSAID increasingly used in dogs, horses and humans. The molecule is highly (99%) bound to transport proteins, principally albumin (Boothe, 1995). Peak plasma concentrations in dogs are reported to occur 0.8 +/- 0.6 h after oral administration and the drug has a plasma elimination half-life of 4 +/- 1 h (Schmitt & Guentert, 1990; Mathews, 1996). Maximal plasma concentration after oral administration has been reported to be 2.8 +/- 1.3 µg/mL (Schmitt & Guentert, 1990).

The objective of the present study was to assess the effect of ASA and Keto, on thyroid function tests in dogs through determination of TT4, FT4, TSH, TT3 and rT3 serum concentrations.

CHAPTER 3

Materials and methods

Dogs

Eighteen spayed female Beagles, weighing from 10 to 14 kg (median: 12) and aged between two and three years were used. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium. All dogs were assessed as healthy based on physical examination, normal complete blood count and biochemistry and a TT4 serum concentration above 15 nmol/L. Anti-canine thyroglobulin autoantibodies (TgAA) by ELISA were negative (ELISA, Oxford Biomedicals) (Nachreiner *et al.,* 1998) . The dogs were kept in the same conditions throughout the study and were fed twice a day with a standard maintenance diet (08:00 and 20:00 h).

Study design

A 3x3 crossover study design was used to evaluate differences between three treatments: placebo, acetylsalicylic acid and ketoprofen. Each dog received each of the treatments in one of the three treatment periods with a three-week washout period between treatment periods. Six dogs were randomly assigned to each of the three treatment sequences. A treatment period consisted of seven days with daily drug administration. The placebo (lactose monohydrate 200 Mesh) was administered orally q 24 h during one week. Acetylsalicylic acid powder, (Federa), was weighed, placed in hard gelatine capsules and administered at a dosage of 25 mg/kg BW PO q 12 h over the one week trial period. Ketoprofen (Ketofen, Merial) was given at a dosage of 1 mg/kg BW orally q 24 h for one week. Ketofen tablets were pulverised and to allow the accurate administration of 1 mg ketoprofen/kg BW, the exact amount of powder was calculated, weighed and placed in hard gelatine capsules.

During the treatment periods, all medications were administered at 08:00 h and for the ASA group also at 20:00 h, except on day zero of each *treatment period* where medications were given after blood sampling at 09:00 h (T0). Food was given twice a day to all dogs at 08:00 h and 20:00 h, except on the mornings of blood sampling, when food was given after blood sampling at 09:00 h. Capsules were administered with one

tablespoon of highly digestible canned food (Hill's, Prescription Diet, canine i/d) in order to reduce the risk of gastro-intestinal irritation and vomiting.
Dogs were examined daily and potential side effects noted. Faecal testing for occult blood (Hexagon OBScreen, Human) and weighing were both performed weekly throughout the study.

Blood samples were taken by jugular venipuncture, serum samples allowed to clot, and, after centrifuging the serum or plasma aliquots were frozen at –20C in plastic tubes until assayed. Blood samples for determination of serum TT4, TT3, rT3, FT4, TSH and for plasma Keto and ASA concentrations were taken at 09:00 h during the treatment periods on days 0 (T0), 1 (T1), 3 (T3) and 7 (T7). During the washout periods sampling was performed once a week (T14, T21, T28). Before initiating the project, encapsulated ASA and Keto were administered each to two dogs and blood sampled every 15 min in order to evaluate the time to obtain drug peak plasma concentrations.

Thyroid hormone assays

Determinations of TT4 and TSH serum concentrations were performed using previously validated radioimmunoassays (Clinical Assays Gammacoat M Total T4 125I RIA Kit, Diasorin; Coat-A-Count canine TSH IRMA, Diagnostic Products Corp). Total T3 concentrations were measured through a validated in-house charcoal separation assay (Panciera *et al.,* 1990). Determinations of free T4 (FT4) serum concentrations by equilibrium dialysis (FT4ED) were performed using a validated commercial assay kit (Nichols Institute Diagnostics) (Daminet *et al.,* 1999). Reverse T3 serum concentrations were also measured using a validated radioimmunoassay method (Biodate S.p.A.) (Center *et al.,* 1984). Serum TgAA concentrations, were determined once in all dogs (ELISA, Oxford Biomedicals). A positive result was defined as at least twice (200 %) the optical density of the negative control sample (Nachreiner *et al.*, 1998). Analyses were performed at the Endocrine Section, Animal Health Diagnostic Laboratory, Michigan State University.

Salicylate and ketoprofen assays

Salicylate and ketoprofen plasma concentrations were quantified by high-performance liquid chromatography with ultraviolet detection (HPLC-UV method) at the Department of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Ghent University. In brief, the method involved the addition of internal standard (50 µL of a fenoprofen solution of 100 µg/mL) to 500 µL of dog plasma for the Keto analysis, or 25 µL of plasma for the salicylic acid analysis. Next, a protein precipitation step was performed by adding 100 µL of HCl 1 M. Thereafter, 3 mL of ethyl acetate was added and the sample was extracted for 20 min on a horizontal roller bank. The sample was centrifuged at ± 2500 rpm for 10 min and the organic layer was evaporated under N_2 at ± 40 °C. The dry residue was dissolved in 250 µL of HPLC water and vortex mixed for 15 s. Finally, the residue was transferred to an autosampler vial and 100 µL were injected into the HPLC-UV system. A reversed-phase C_{18} column (type Hypersil, 100 x 3 mm i.d., 5 µm, Varian) was used in combination with a reversed-phase guard column (10 x 2 mm i.d., Varian). The mobile phase contained 1% (v/v) glacial acetic acid in HPLC grade water (A) and methanol (B). A gradient solvent program with the following conditions was run: 0-4 min: 50% A, 50% B; 4.5-15 min: 30% A, 70% B; 15.5-25 min: 50% A, 50% B. The flow rate was set at 0.5 mL/min. The UV trace was monitored at λ=305 nm between 0-6 min to detect salicylic acid, at λ=259 nm between 6-10.5 min for ketoprofen and at λ=236 nm from 10.5 min onwards to detect fenoprofen.

By using this method it was possible to quantify Keto and salicylic acid concentrations in plasma at levels as low as 0.1 µg/mL ketoprofen and 2 µg/mL salicylic acid. Calibration curves were linear in the range of 0.1 to 10 µg/mL for ketoprofen ($r>0.9991$) and 2 to 200 µg/mL for salicylic acid ($r>0.9961$).

Quality control (QC) samples were included in each run to check the sample preparation and chromatographic procedure. Quality control samples were prepared from drug free dog plasma spiked at a level of 1.0 µg/mL of Keto and 20.0 µg/mL of salicylic acid.

Statistical analyses

A mixed model with DOG as random effect and TREATMENT and PERIOD as fixed effects was used to analyze the crossover trial (SAS, version 8). Ketoprofen and salicylic acid were compared with placebo separately on days 1, 3 and 7 of the treatment period using as response variable the difference between the hormone concentrations at the particular day with day 0. With a family confidence coefficient equal to 95%, each of the six individual comparisons (two drug comparisons at three time points) were tested at a significance level of 0.00833 (Bonferoni's multiple comparisons adjustment).

Results

Results for mean TT4, FT4, TT3, and TSH serum concentrations within the treatment period are shown in Fig. 1. A significant decrease in serum TT4 was observed as early as 24 h following ASA administration (P=0.0008) and persisted during the ASA treatment (Fig. 1a). Total T4 values then normalized within one week of ASA withdrawal (Fig. 2). Results for the TT3/TT4 ratio and the free fraction of T4 (FT4/TT4) are shown in Fig. 3 and for rT3 serum concentrations in Fig. 4. During Keto treatment, no statistically significant changes were observed compared to the control group (Fig. 1).

The number of samples with TT4 serum concentrations < 15 nmol/L, a range compatible with hypothyroidism, were counted during the treatment periods (T1, T3 and T7) for each group. During ASA treatment, 30 measurements of TT4 of 54 (55%) were lower than 15 nmol/L, 10 of 54 (18%) in the Keto group and five of 54 (9%) in the placebo group.

The decrease in TT3 serum concentrations was less pronounced than for TT4 but differed significantly with placebo one week after the ASA administration (P=0.0006). No significant differences between Keto and placebo groups were observed (Fig. 1b).

No significant changes were found for FT4 concentrations in the ASA (P=0.1998 at day 1, P=0.1794 at day 3, P=0.4033 at day 7) or the Keto group (P=0.9349 at day 1, P=0.9349 at day 3, P=0.9349 at day 7) (Fig. 1c). Neither drug significantly affected TSH serum concentrations (Fig. 1d).

The free fraction of T4 (FFT4) was significantly higher in the ASA group compared with the control group, at two time periods (P=0.0003 at day 1, P=0.0243 at day 3, P=0.0001 at day 7). Free fraction of T4 did not significantly differ between the Keto and control groups (Fig. 3a).

Total T3/TT4 ratio was significantly increased during ASA treatment (Fig. 3b). This was statistically significant at T1 in the ASA group (P=0.0035 at day 1, P=0.0449 at day 3, P= 0.04 at day 7).

Therapeutic plasma concentrations were obtained for both drugs in all animals (except for one measurement of ASA with a concentration of 36 µg/mL). During ASA

treatment, plasma salicylate concentrations ranged from 36 to 177 µg/mL (mean value at T1: 96.5; at T3: 105.3 and at T7: 76.2 µg/mL). During Keto treatment, plasma concentrations ranged from 1.2 to 7.5 µg/mL (mean value at T1: 3.9; at T3: 4.3 and at T7: 3.8 µg/mL). Mean values for plasma concentrations expressed in µmol/ml were for ASA: T1: 0.698, at T3: 0.762 and at T7: 0.552 µmol/mL; for Keto: T1: 0.0153; at T3: 0.0169 and at T7: 0.0149 µmol/mL. In the pilot study performed on 4 dogs before initiating the experiment, peak plasma concentrations of both drugs were obtained after approximately 1 hour.

Salicylic plasma levels and TT4 serum concentrations correlated negatively, with the Pearson correlation coefficient equal to -0.43 at day 1, -0.53 at day 3 and -0.059 at day 7.

All dogs remained generally healthy throughout the study, weight remained stable and no serious side effects were noted. Faecal testing for occult blood occurred weekly and three of 18 tested positive for occult blood one week after ASA administration, and four dogs of 18 tested positive one week after Keto administration. None of the placebo dogs tested positive for occult blood at any time during treatment periods. During the washout periods, all dogs tested negative except for one animal, which remained positive one week after discontinuing ASA administration. This dog had also tested positive after one week of treatment with ASA and had bloody diarrhoea during this treatment. Overall, bloody diarrhoea was observed in one dog during Keto treatment and in two dogs during ASA therapy.

Fig. 1. Mean total thyroxine (TT4) (a), total triiodothyronine (TT3) (b), free thyroxine (FT4) (c), and thyrotropin (TSH) (d) serum concentrations from dogs administered a placebo, acetylsalicylic acid (ASA) or ketoprofen (Keto) for seven days. * Indicates significant differences with placebo group. P < 0.00833.

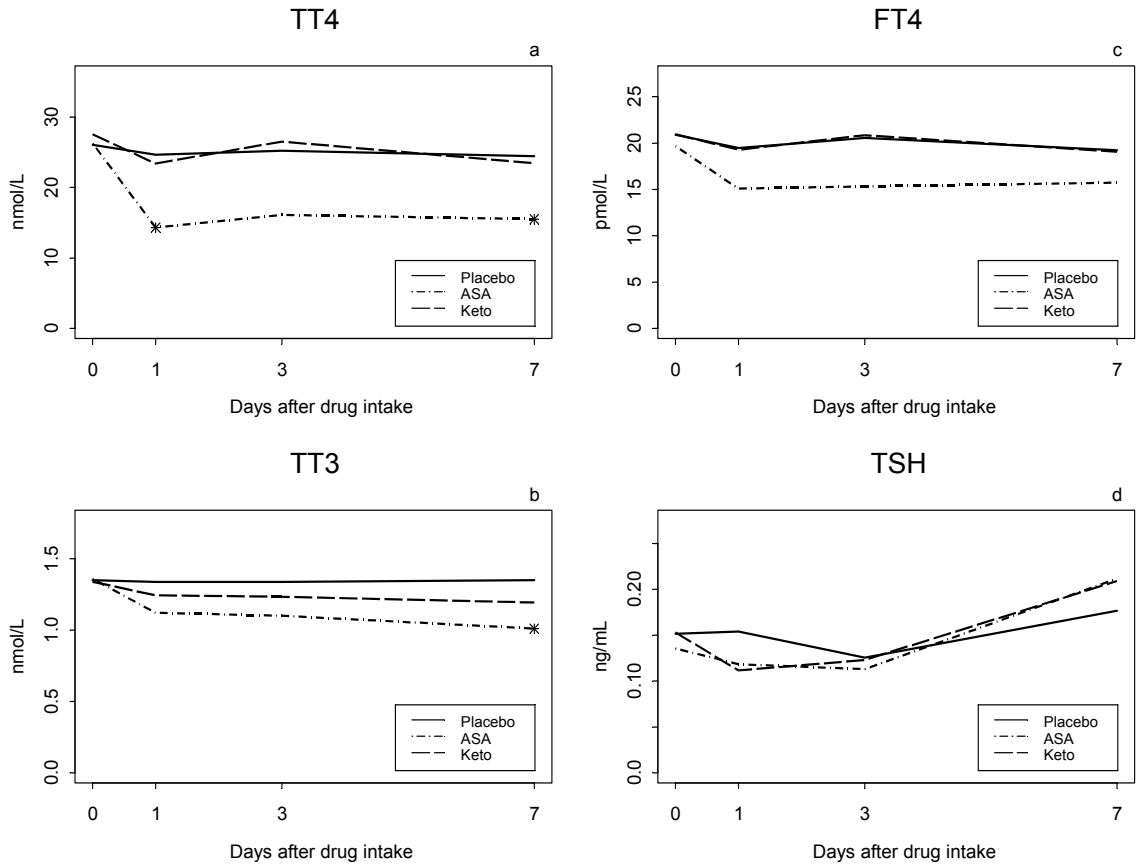


Fig. 2. Mean total thyroxine (TT4) serum concentrations from dogs administered a placebo, ASA or Keto shown over a four-week period. Treatments were administered for seven days. * Indicates a significant difference with control group. P < 0.00833.

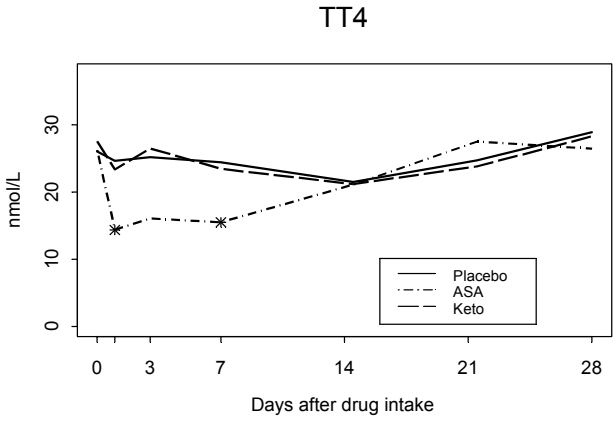


Fig. 3. Mean percentages for free fraction of T4 (FFT4) and TT3/TT4 ratio are represented. * Indicates a significant difference with control group. P < 0.00833.

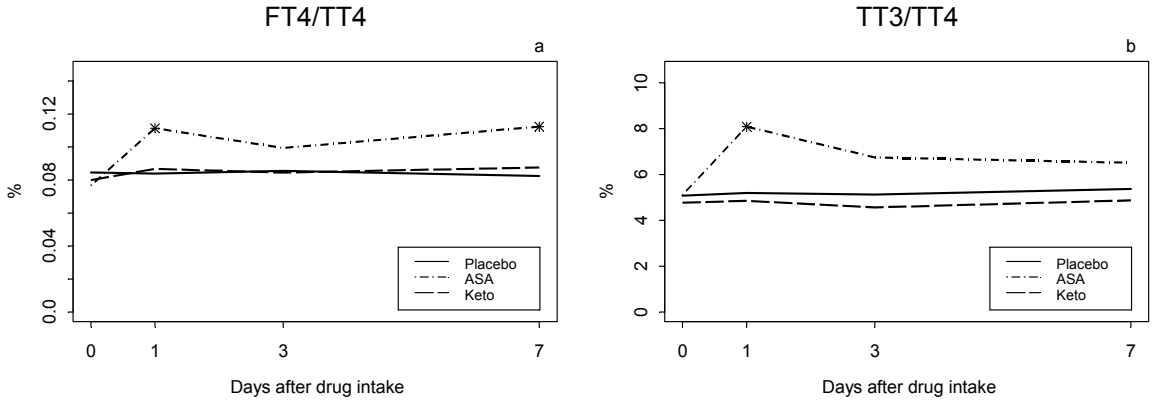
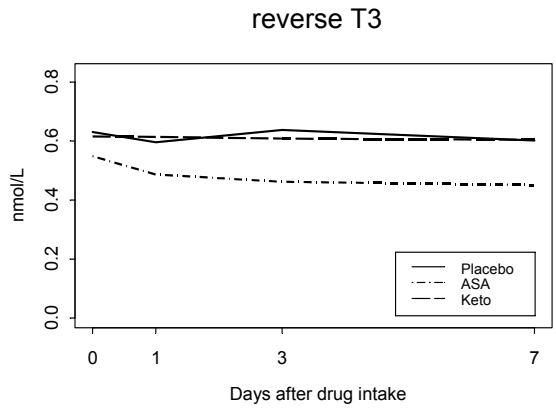


Fig. 4. Mean reverse triiodothyronine (rT3) serum concentrations from dogs administered a placebo, ASA or Keto for seven days. * Indicates significant differences with control group. P < 0.00833.



CHAPTER 3

Discussion

Our results support previous studies in humans and horses that have shown that treatment with certain NSAIDs can significantly affect thyroid function test results (Sojka *et al.*, 1993; Surks & Stievert, 1995; Ramirez *et al.*, 1997). In our study, a decrease in TT4 serum concentration was observed as soon as 24 h after initiating treatment with ASA and TT4 concentrations remained decreased throughout the one-week treatment period. Total T4 serum values normalized one week after discontinuing treatment. Free T4 serum concentrations, measured by equilibrium dialysis, were less affected by ASA treatment than TT4 concentrations.

With ASA treatment, TT4 serum concentrations decreased, FT4 concentration remained normal and the free fraction of T4 increased. These changes probably reflect displacement of thyroid hormones from their serum transport-binding site. The negative correlation we found between ASA plasma concentrations and TT4 levels also favors the competition for serum binding sites as the major mechanism accounting for our observations. An increase in FT4 values was not observed. It might be postulated that the unbound hormone was cleared more rapidly or that the pituitary responded quickly to an increase in FT4 resulting in a lowering of T4 secretion. Reverse T3 serum concentrations remained unchanged, and there was no net change of free T4 concentration and no significant effect of ASA on peripheral 5 mono-deiodination of T4. It may be postulated that ASA affects the clearance of thyroid hormones, suppresses TSH synthesis, affects thyroid hormone synthesis, or alters the sensitivity of the thyroid gland to TSH. In humans, salicylates alter thyroid function primarily through competition with low-affinity binding sites on serum thyroid hormone-binding proteins, but other mechanisms such as decreased uptake of iodine by the thyroid gland, inhibition of the hepatic 5'-monodeiodination, and competition with plasma membrane, cytosolic or nuclear membrane binding have also been postulated (Lim *et al.,* 1996; McConnell, 1999). However, the contribution of these disturbances on peripheral thyroid hormone metabolism in humans is uncertain (McConnell, 1999). Some drugs such as furosemide and fenclofenac are potent competitors for thyroid hormones and share some structural similarities with thyroid hormones (Munro *et al.,* 1989).

Total T3 and FT4, the hormones responsible for feedback regulation of TSH secretion, were not significantly affected by ASA or Keto administration; this may explain why dogs in this study had normal TSH concentrations. Therefore one could argue that T3 and TSH concentrations should be used to assess thyroid function in dogs on NSAIDs therapy. Unfortunately, serum T3 and TSH are less useful for the diagnosis of hypothyroidism. In one study, serum T3 and TSH concentrations remained within reference limits in 90 and 24 % of dogs with hypothyroidism, respectively (Peterson *et al.*, 1997). Therefore, measurement of T3, although less affected by drugs in our study and several other studies, is of limited benefit in the evaluation of thyroid function in clinics (Kantrowitz *et al.,* 1999; Müller *et al.,* 2000). No significant changes were observed in TSH serum concentrations in our study after ASA or Keto administration, supporting euthyroidism in the ASA-treated animals. However, most euthyroid dogs have TSH values in the lower end of the reference range (Kooistra *et al.,* 2000). Therefore, changes in TSH concentrations in this low reference range might be more difficult to detect. Furthermore the limit of detection of the canine TSH assay is 0.03 ng/mL with some dogs having undetectable TSH serum concentrations. Further studies are needed to confirm the mechanism by which ASA affects thyroid homeostasis in dogs.

Ketoprofen administered for one week did not significantly affect TT4, TT3, FT4, rT3, or TSH serum concentrations. A study in humans reports no effect of ketoprofen at therapeutic dosages on thyroid function (TT4 and TSH) except on TT3 (Bishnoi *et al.*, 1994; Carlson *et al.,* 1999). It is interesting to see from our data that the hormone most affected by ketoprofen is also serum TT3. Our findings might be explained by the fact that as well in humans as in dogs, T3 is less avidly bound to transport proteins and in consequence more easily displaced than T4 (Carlson *et al.*, 1999). This finding also raises the question as to why TT3 was not more affected than TT4 by ASA administration.

Our study revealed a marked influence of ASA but only a minor effect of Keto on TT4 serum concentrations despite the fact that ASA is somewhat less bound to serum transport proteins than Keto, and that affinity constant values (Ka) of Keto for albumin are somewhat higher then Ka values of ASA for albumin (Ka respectively 3.8 x

10^6 and 1.6 x 10^6 M^{-1}) (Carter & Ho, 1994; Sowell *et al.,* 2001). Our observations may reflect the much higher molar concentrations obtained with ASA treatment than with Keto treatment. Ketoprofen molar plasma concentrations represented approximately 2 % of ASA molar plasma concentrations. Displacement of T4 from its serum transport protein binding sites could also, at least in part, be favoured by the fact that ASA plasma concentrations (around 698 000 nmol/L) are much higher than TT4 concentrations (around 20 nmol/L).

Also, ASA binds to the two major drug-binding sites (site I of subdomain IIA and site II of subdomain IIIA) of albumin, whereas Keto only binds to albumin's site II (Rahman *et al.,* 1993; Carter & Ho, 1994; Chuang *et al.,* 1999). In humans, the high-affinity binding sites for T3 and T4 molecules are located in subdomain IIA of the albumin molecule (Robbins, 2000). Interestingly, Wang (1999) has shown that salicylates, although their principal serum binding site is albumin, also displace T4 from TBG, TTR and albumin in human serum (Wang *et al.*, 1999).

In our study, the effects of short-term administration of two NSAIDs are described, but potential changes in thyroid homeostasis after long-term administration of NSAIDs in dogs might be different. For example, in humans short-term administration of therapeutic dosages of salicylates leads to a decrease in TT4 serum concentration, a transient increase in unbound hormone levels and suppression of TSH concentrations in most studies (Davies & Franklyn, 1991; McConnel, 1992; McConnell, 1999) However, during long-term treatment with salicylates, a new steady state is reached reflecting an increased T4 turnover rate, mildly decreased serum TT4 concentrations and unchanged or decreased FT4 concentrations are observed (Davies & Franklyn, 1991; McConnell, 1992; Surks & Stievert, 1995; McConnell, 1999). Thyrotropin concentrations return to the reference range within a few weeks of treatment (McConnell, 1992).

Carprofen, another propionic acid derivate, is another commonly used NSAID in dogs and is highly protein bound (Isaacs, 1999). One abstract reports a mild decrease in TT4 and TSH measurements after administration of carprofen to dogs (2.2-3.3 mg/kg BW q 12 h for 5 weeks). Free T4 also decreased mildly but this was not statistically

significant (Ferguson *et al.*, 1999). The proposed mechanism includes displacement of T4 from serum binding sites; displacement of pituitary intracellular binding of thyroid hormone may explain the persistence of TSH suppression. As serum samples in this study were taken at 0, 2 and 5 weeks, it is possible that the more rapid increases in FT4, as seen in humans, may not have been observed.

Our protocol for administration of ASA and Keto was based on knowledge of the clinical use of these drugs as well as on our desire to obtain samples for thyroid hormone concentrations at drug maximal plasma concentration. Dosages used for both drugs in this study were common dosages used in practice and were likely to lead to therapeutic concentrations of these drugs (Mathews, 1996). As the mechanism traditionally believed to be involved to explain effects of NSAIDs on thyroid function in humans and horses was displacement of T4 from transport proteins, samples were taken at the maximal serum concentration of both drugs. Samples were not taken at other moments during the day to evaluate if this decrease in TT4 persisted throughout the day. Interestingly, in a study in horses, decreased TT4 concentrations were observed after phenylbutazone administration, and sampling did not occur at maximal serum concentration in this study (Ramirez *et al.*, 1997). But many protocols for ASA therapy in dogs will lead to stable plasma concentrations, therefore, our observations are likely to be valid throughout the day (Lipowitz *et al.,* 1986).

Few side effects were observed in our study following ASA and Keto administration. The mild gastro-intestinal side effects seen in a few dogs are not likely to have influenced our results. Despite the abnormal thyroid function test results observed in the dogs on ASA therapy, no clinical signs compatible with hypothyroidism were observed in any of the dogs during the study.

Conclusion

There are several clinical implications to this study. Firstly, as observed in human studies and corroborated in our study, the importance of the effects of NSAIDs on thyroid function can be marked, but varies from drug to drug. Therefore, until further studies on the potential effects of NSAIDs on thyroid function are published, caution is advised when interpreting thyroid function test results in a dog on NSAIDs therapy. Secondly, a seven-day treatment with ASA resulted in a decrease in TT4- potentially sufficient to lead to the misinterpretation of the thyroid profile and misdiagnosis of hypothyroidism in more than half of the dogs. Thirdly, FT4 measured by equilibrium dialysis and TSH serum concentrations were less affected by ASA and Keto administration than TT4. Therefore, a diagnosis of hypothyroidism, in a dog on NSAIDs therapy, should not be based on a TT4 measurement only. Fourthly, after short-term (one-week) administration of ASA, and maybe other NSAIDs as well, we advise a minimal seven-day period before thyroid function testing in dogs.

This study revealed that ASA can rapidly and significantly affect thyroid homeostasis in dogs. The effect was most pronounced on TT4 serum concentration and probably reflected the displacement of thyroid hormones from serum protein binding sites.

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CHAPTER 4

CHAPTER 4

Introduction to chapter 4

Some factors can influence thyroid hormone test results in humans and dogs, contributing to the difficulty in evaluation of thyroid function. In chapters 2 and 3 we investigated the effects of commonly used drugs on canine thyroid function. To further investigate the possible influence of common clinical situations on thyroid function tests, the effects of obesity and weight loss will be assessed in the following chapter. Indeed, in dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment, therefore it was important to clarify how obesity and weight loss can affect thyroid function test results in dogs.

CHAPTER 4

EVALUATION OF THYROID FUNCTION IN OBESE DOGS AND IN DOGS UNDERGOING A WEIGHT LOSS PROTOCOL

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CHAPTER 4

Summary

Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment. Therefore it is important to clarify how obesity and weight loss can affect thyroid function test results in that species. The objectives of this study were to compare thyroid function in obese dogs and in lean dogs and to explore the effects of caloric restriction and weight loss on thyroid hormone serum concentrations in obese dogs.

In the first experiment, 12 healthy lean beagles and 12 obese beagles were compared. Thyroid function was evaluated by measuring serum concentrations of total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT3), thyrotropin (TSH), and reverse triiodothyronine (rT3) as well as a TSH stimulation test using 75 μg IV of recombinant human TSH.

In the second experiment, eight obese beagles were fed an energy-restricted diet (average 63 % MER) until optimal weight was obtained. Blood samples for determination of TT4, FT4, TT3, TSH, and rT3, were taken at the start and then weekly during weight loss.

Only TT3 and TT4 serum concentrations were significantly higher in obese dogs as compared to lean dogs. In the second experiment, weight loss resulted in a significant decrease in TT3 and TSH serum concentrations. Thus obesity and energy restriction significantly alter thyroid homeostasis in dogs, but the observed changes are unlikely to affect interpretation of thyroid function test results in clinics.

CHAPTER 4

Introduction

Hypothyroidism is the most common, but probably also the most over diagnosed endocrine disease in dogs, partially because clinical manifestations of hypothyroidism are vague and insidious. Obesity is observed in more than 40 % of dogs with hypothyroidism in several studies (Dixon et al., 1999b; Feldman et al., 1996; Kaelin et al., 1986; Panciera,1994). Therefore, many dogs in practice are tested and/or treated for hypothyroidism because they are overweight. Obesity, however, is a common nutritionally related problem in dogs and many dogs with obesity are not hypothyroid. It is estimated in the general population that between 21 and 30 % of dogs seen in practice are obese (Laflamme et al., 1994). Gosselin et al. (1980) reported that thyroid hormone concentrations in obese beagle dogs were increased in comparison to euthyroid lean dogs, but this statement was based on only 6 dogs. Furthermore tests available then to assess canine thyroid function were less reliable than current ones. Nevertheless, the effect of obesity on canine thyroid function is frequently cited in the literature (Peterson et al., 1989; Scott-Moncrieff et al., 2000) and for the reasons mentioned above (small sample size and reliability of diagnostic tests) it is worthwhile to further investigate this issue.

Fasting or weight loss has been reported to decrease thyroid hormone concentrations in humans and rats (Drent and van der Veen, 1995; Glass and Kushner, 1996). However, information on possible effects of energy restriction on canine thyroid function test results is scarce. Energy restriction is the cornerstone for the treatment of obesity. Obese dogs are frequently tested for hypothyroidism in practice when they have been fed a calorie restricted diet and the veterinarian or owner feel that weight loss is not sufficient. Therefore, studies clarifying the possible influence of weight loss or energy restriction on thyroid hormone concentrations in dogs are warranted.

Currently, the combined use of serum thyroxine (T4) and TSH concentrations seems to be the easiest and most reliable way to assess canine thyroid function (Scott-Moncrieff et al., 1996). The measurement of FT4, especially by equilibrium dialysis (FT4ED) is thought by many authors to be superior because it is less influenced by non-

thyroidal diseases than is determination of TT4 (Peterson et al., 1997; Scott-Moncrieff et al., 2000). Results of TSH stimulation tests using bovine TSH (bTSH) are less influenced by the presence of non-thyroidal systemic diseases than is the measurement of baseline T4. Therefore this dynamic test is still considered the gold standard to assess thyroid function in dogs (Scott-Moncrieff et al., 2000). Since the withdrawal of pharmaceutical grade of bTSH from the market, TSH stimulation tests have been infrequently used in dogs. A recent study has shown that recombinant human TSH (rhTSH) did significantly increase thyroid hormone concentrations in euthyroid beagle dogs (Sauvé and Paradis, 2000). Therefore, it could be an interesting alternative to the use of bTSH.

The objectives of this study were to compare thyroid function in obese and lean control dogs and to evaluate the effects of calorie restriction and weight loss on thyroid hormone concentrations in obese dogs. Thyroid function was evaluated by the determination of TT4, TT3, FT4ED, rT3, TSH serum concentrations and through performing a TSH stimulation test.

Materials and methods

First experiment: Effect of obesity on serum thyroid hormone concentrations

Twenty-four Beagle dogs (control and obese groups) aged between 1 and 9 years were used for the first experiment. All dogs were assessed as healthy (except for obesity) based on physical examination, normal complete blood count (CBC) and serum biochemistry profile. Body condition score was assessed according to a validated nine-point body condition scoring system (Laflamme et al., 1994). All dogs were kept in similar conditions throughout the study and fed once a day (9 AM) with a standard maintenance diet (Royal Canin Premium Croc adult) for at least one month before entering the study. All dogs had access to outside runs for several hours each day. Metabolic energy requirements were individually adjusted if needed in order to ensure stable weight, and ranged between 480 kJ kg $^{(0.75)}$ and 522 kJ kg $^{(0.75)}$. Weight in both groups was stable for at least 1 month before the experiment.

The lean **control group** consisted of 12 beagle dogs, 4 females (2 spayed and 2 in anoestrus) and 8 neutered males, weighing between 10.8 and 17.55 kg (mean 13.15 kg, median 12.1 kg) and aged between 1 and 9 years. Body condition score was between 4 and 5 on the nine-point scale at the time of evaluation.

The **obese group** consisted of 12 obese beagle dogs, 4 females (2 spayed and 2 in anoestrus) and 8 neutered males. Age ranged from 3 to 9 years and weight from 17.8 to 25.9 kg (mean 21.5 kg, median 20.7 kg). At the time of evaluation, all dogs in this group had a body condition score between 6 and 8 on the nine-point scale. Obesity had been induced through allowing *ad libitum* food intake over a period of 10-15 months. The dogs were already obese for 6 months at the time of evaluation.

Blood samples for determination of TT4, TSH, TT3, rT3, thyroglobulin auto antibodies (TgAA) and FT4ED were taken from all dogs at 9 AM after a 12-hour fast. Samples were taken through jugular venipuncture and allowed to remain at room temperature for approximately 20 minutes prior to centrifugation. Serum aliquots were frozen at –20°C in plastic tubes until assayed. A TSH stimulation test using 75 μg of

human recombinant TSH (Thyrogen®, Genzyme Corporation) was performed in all dogs. One vial of rhTSH (0.9 mg) was reconstituted with 5 ml of sterile injection water. Serum samples for determination of TT4 were taken before (T0) intravenous injection (i.v.) of rhTSH and 4 (T4) and 6 (T6) hours later.

Second experiment: Effect of weight loss due to caloric restriction on thyroid function

In the second experiment, the effect of weight loss on thyroid function was evaluated in eight chronically obese dogs following a protocol established by Diez et al.. These dogs were different from the obese dogs in the first experiment and consisted of 4 neutered males and 4 intact females, aged from 4 to 7 years. Before inclusion in the study, dogs were determined to be healthy based on physical examination, CBC, serum biochemistry profile and urinalysis. All dogs tested negative for TgAA. At the beginning of the experiment, weight ranged from 17.3 to 23.45 kg and all dogs had a body condition score of at least 6 on a nine-point scale (Laflamme et al., 1997). Dogs were at least 30% (30-72%) overweight for a minimum period of one year before entering the study. Initial energy requirements for weight loss were based on the individual previous food consumption of the dog. An average of 72% of maintenance energy for optimal body weight was required to induce weight loss and was decreased as needed by increments of 10% as needed to ensure a weekly weight loss of around 2 %. Dogs were fed with either a high-protein diet (%DM: crude protein, 47.5%; starch, 5.3%; total dietary fiber, 30.8%; ash, 7% and metabolizable energy, 11.6 kJ/g as fed) or with a commercially available weight reduction diet (%DM: crude protein, 23.8%; starch, 23.9%; total dietary fiber, 38.6%; ash, 5.2% and metabolizable energy, 9.8 kJ/g as fed). Each dog received one diet throughout the experiment (n=4 in each group). Water was provided *ad libitum*. Blood samples to assess TT4, TSH, FTED, TT3 and rT3 were taken every 4 weeks as described above. Dogs were examined daily and body weight was recorded weekly. The protocol was terminated when dog's optimal body weight was obtained. Optimal and thus target body weight was defined as a body condition score of 5/9.

The experiments were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium and/or by the Animal Use and Care Advisory Committee of the University of Liège, Belgium.

Assays

Serum TT4 and TSH serum concentrations were determined using radioimmunoassays previously validated for the dog (Clinical Assays Gammacoat M Total T4 125I RIA Kit, Diasorin, Stillwater, MN; and Coat-A-Count canine TSH IRMA, Diagnostic Products Corp, Los Angeles, CA, USA, respectively) (Sauvé et al., 2003). Reference range for TT4 and TSH serum concentrations was 15-67 nmol/L and 0-0.68 ng/ml respectively. Determination of FT4 serum concentrations by equilibrium dialysis (FT4ED) was performed using a commercial assay kit (Nichols Institute Diagnostics, San Juan Capistrano,CA) (Daminet et al., 1999). The reference range for FT4ED serum measurements was 6-42 pmol/L. Total T3 concentrations were measured through a validated in-house charcoal separation assay (Panciera et al., 1990). The reference range for TT3 serum concentrations was 1.0-2.4 nmol/L. Reverse T3 serum concentrations were measured through a validated radioimmunoassay method (Biodate S.p.A., Rome, Italy) (Center et al., 1984). Reference range for rT3 was 0.41-0.95 nmol/L. Thyroglobulin AA were determined through a previously validated technique (Nachreiner et al., 1998). All thyroid hormone analyses were performed at the Endocrine Section, Animal Health Diagnostic Laboratory, Michigan State University.

Statistical analysis

For the first experiment, the obese group was compared with the control group for the different hormones by analysis of variance with α=0.05.

Furthermore, a mixed model was fitted to TT4 concentrations with dog as random effect and time after TSH stimulation (0, 4 and 6 hours) as a categorical fixed effect. At each of the three timepoints, the obese group was compared with the normal group at the 0.017 significance level in order to ensure an overall 5% type I error rate (Bonferroni's multiple comparisons technique with comparisons at three different timepoints).

For the second experiment, a mixed model was fitted to each of the different hormone concentrations with dog as random effect and the fraction overweight ((starting weight-normal weight)/normal weight), as a continuous fixed effect. The diet group and its interaction with fraction overweight was also added to the model, but in case of non significance of these terms, they were dropped from the model. Based on these mixed models, the hypothesis was tested whether the slope, describing the linear effect of the weight fraction on the hormone concentrations, was equal to zero or not.

Finally, the influence of the TT4 and TT3 concentrations at the start and the end of the diet on the time needed to return to normal weight was studied by linear regression, with the hypothesis test based again on the slope being equal to zero or not.

Results

First experiment: Effect of obesity on serum thyroid hormone concentrations

Results for mean basal TT4, TSH, FT4ED, TT3 and rT3 serum concentrations (+/- SEM) are shown in table 1. Total T4 and TT3 serum concentrations were significantly higher in the obese group compared with the lean control group ($P<0.0001$) although basal TT4 serum values always remained within the reference range for euthyroid dogs (range from 15 to 67 nmol/L). No significant differences between groups were found for TSH, FT4ED and rT3.

Results for the TSH stimulation test using rhTSH are shown in figure 1. Total T4 serum concentrations increased markedly after the injection of rhTSH in both groups. Increases in TT4 concentrations were more pronounced 6 hours after TSH injection then after 4 hours in both groups. Total T4 serum concentrations were not statistically different between the two groups at 4 and 6 hours after TSH stimulation. No adverse reactions were noted after the i.v. injection of rhTSH in any of the dogs.

All dogs tested negative (< 200 % optical density) for TgAA.

Second experiment: Effect of weight loss due to caloric restriction on thyroid function

All 8 dogs remained healthy during the weight loss study. Time required to reach ideal body weight varied greatly between dogs and ranged from 12 to 26 weeks. Average percent body weight loss per week was 2.15 % +/- 0.14 (Mean +/- SEM).
Initial energy allowance was 675.6 +/- 13 kJ kg $^{(0.75)}$/day and to reach optimal body weight, energy requirement had to be decreased to mean levels of 63 % of maintenance energy requirement (MER=550 kJ kg $^{(0.75)}$).

No differences were found between the 2 diets groups and the interaction between time and diet group was also nonsignificant, so that diet could be removed from the model.

Serum TT3 and TSH concentrations decreased significantly over time during weight loss ($P<0.0001$) (Table 2, Figure 2). Total T3 serum concentration decreased significantly over time during weight loss, each 10% decrease in overweight corresponding to a decrease of 0.059 nmol/L. The mean TT3 concentration was equal to 1.65 at the start and 0.95 nmol/L at the end of the experiment. Total TSH serum concentration also decreased significantly over time during weight loss, each 10% decrease in overweight resulting in a decrease of 0.025 ng/ml. The mean TSH concentration was equal to 0.45 at the start and 0.15 ng/ml at the end of the experiment. Although TT4 concentrations decreased also as a function of fraction overweight, this effect was not statistically significant ($P=0.26$). Total T3 concentrations at the start of the diet influenced the time to return to normal weight significantly ($P=0.013$) but no such relationship was found for TT4 (Table 3). Dogs having a higher initial serum TT3 concentration required more time to lose weight. Total T4 and TT3 concentrations at the end of the experiment did not correlate with the time required to return to normal weight (Table 3).

Table 1. Experiment 1: Mean basal serum hormone concentrations (+/- SEM) in the obese (n=12) and lean control (n=12) groups. Values denoted by an asterix, *, indicate a significant difference between obese and control group (α=0.05).

Hormone	Obese	Control	P-value
TT4 (nmol/L)	47.00 +/- 2.38	31.00 +/- 2.38	<0.0001*
FT4 (pmol/L)	18.75 +/- 1.34	17.34 +/- 1.34	0.46
TT3 (nmol/L)	1.62 +/- 0.07	1.09 +/- 0.07	<0.0001*
TSH (ng/ml)	0.27 +/- 0.03	0.23 +/- 0.03	0.41
rT3 (nmol/L)	0.67 +/- 0.05	0.63 +/- 0.05	0.61

Figure 1: Experiment 1: Results of the TSH stimulation test using 75 µg of rhTSH in obese dogs (n=12) and in control dogs (n=12). Box plots for TT4 measurements are shown on T0 and 4 and 6 hours after rhTSH administration.

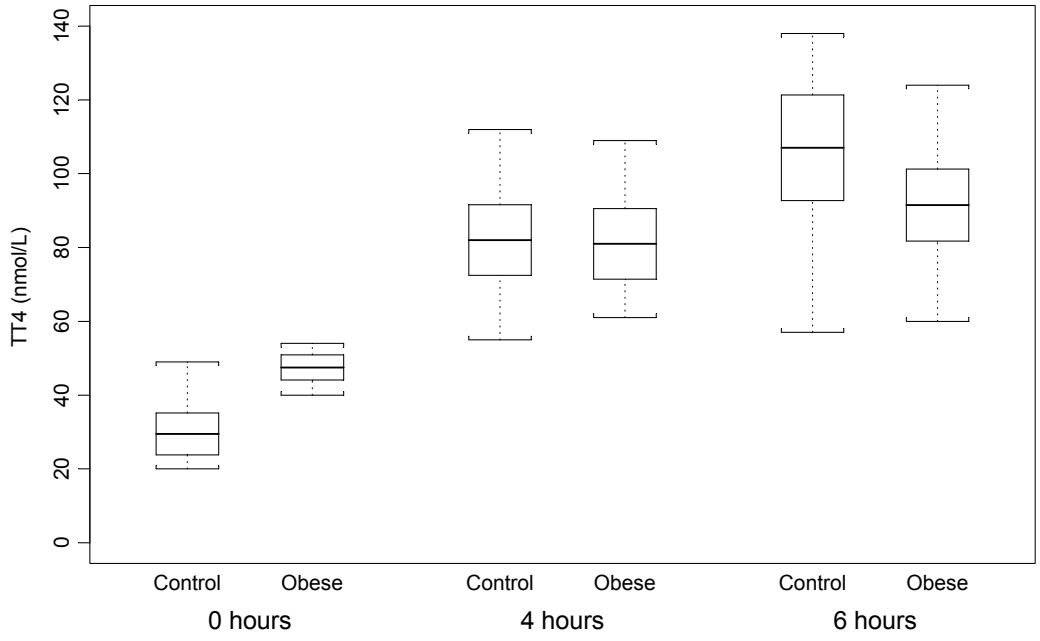

Table 2. Experiment 2: Evolution of different serum thyroid hormone concentrations as a function of the fraction overweight. Slope denotes the linear change of the hormone concentration when overweight goes from 100 to 0%. *Indicates a significant linear effect of fraction overweight on hormone concentration (α=0.05).

Hormone	Mean value at start	Mean value at end	Difference (SEM)	Slope (SEM)	P-value
TT4 (nmol/L)	32.38	27.26	-4.75 (4.52)	-5.22(4.62)	0.26
TT3 (nmol/L)	1.65	0.95	-0.7 (0.11)	-0.59 (0.12)	<0.0001*
FT4 (pmol/L)	20.50	22.25	+1.75 (4.21)	+1.12 (3.03)	0.71
TSH (ng/ml)	0.45	0.15	-0.30 (0.08)	-0.25 (0.05)	<0.0001*
rT3 (nmol/L)	0.55	0.59	+0.04 (0.05)	+0.03 (0.06)	0.62

Figure 2: Experiment 2: Evolution of serum TT4 (a), TSH (b), TT3 (c), FT4 (d) and rT3 (e) concentrations over time during weight loss. The thick line corresponds to the overall relationship; the thin lines correspond to the individual dogs.

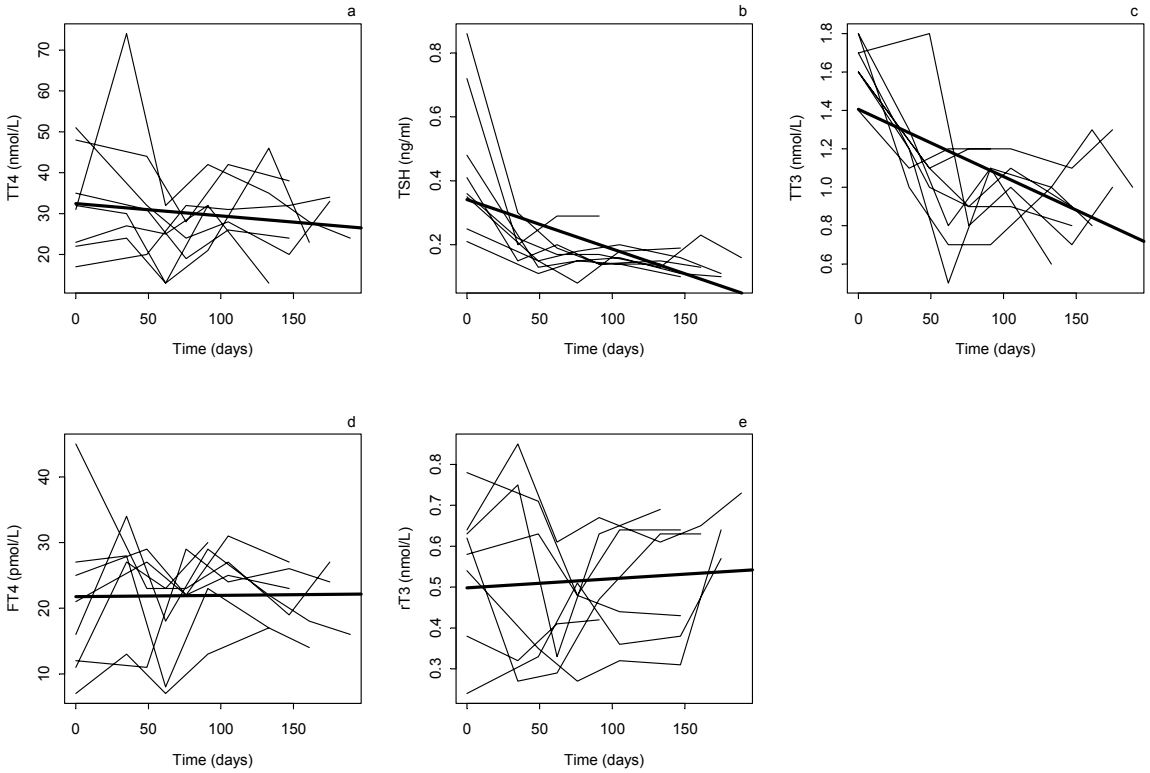

Table 3. Experiment 2: Effect of TT4 and TT3 serum concentrations at the start and the end of the experiment on the time to normalization. Slope denotes the linear change on time to normalization for a 1-unit change (TT4) or 0.1-unit change (TT3) in hormone concentration. The second column shows the mean hormone concentrations of the 8 dogs for which 152 days are required for normalization. * Indicates a statistically significant difference.

Hormone	Mean value	Slope (SEM)	P-value
TT4 at start (nmol/L)	32.4	0.405 (1.02)	0.71
TT3 at start (nmol/L)	1.65	19.25 (5.52)	0.013*
TT4 at end (nmol/L)	27.6	0.16 (1.55)	0.92
TT3 at end (nmol/L)	9.5	0.58 (5.54)	0.92

Discussion

Serum TT3 and TT4 concentrations were significantly higher in obese dogs. However the magnitude of the observed elevation in TT4 and TT3 serum concentrations was not clinically significant as both hormones always remained well within the reference range.

In obese humans, thyroid function generally remains normal, with most studies reporting normal TT4, TT3, rT3 and TSH serum concentrations (Drent and van der Veen, 1995; Glass and Kushner, 1996; Roti et al., 2000). However one-study reported increased TT4 levels (Scriba et al., 1979). The exact cause for a similar observation in our study is not known. Some studies in humans have documented increased serum thyroid binding globulin concentrations which could be responsible for the increased TT4 serum concentrations (Scriba et al., 1979).

Some studies also reported increased TT3 levels in obese humans (Bray et al., 1976; Scriba et al., 1979; Stichel et al., 2000). The exact cause of this observation is controversial but hypotheses include increased conversion of T4 into T3, decreased numbers of T3 receptors in obese patients or caloric overfeeding rather than obesity itself (Glass and van der Veen, 1996; Stichel et al., 2000). However in the first experiment the obese dogs were fed their maintenance energy requirements for one month before the time of evaluation, therefore, overfeeding should not be responsible for the increased serum TT3 concentrations observed in our study. In the second experiment however, initial energy allowance was higher (675.6 +/- 13 kJ kg $^{(0.75)}$/day), therefore, this initial overfeeding could have contributed to the higher serum TT3 concentrations observed at the start of the second experiment.

In veterinary practice, finding a baseline serum TT4 concentration within the reference range is commonly used to exclude hypothyroidism in dogs (Scott-Moncrieff et al., 2000). Many factors, especially illnesses and some drugs, can significantly decrease thyroid hormone concentrations and lead to misdiagnosis of hypothyroidism

(Scott-Moncrieff et al., 2000). Our study showed that obese dogs are euthyroid and have slightly higher TT4 values than lean dogs. Therefore exclusion of hypothyroidism in an (euthyroid) obese but otherwise healthy dog, based on a baseline TT4 measurement should be straightforward.

Whereas TT4 values were significantly increased in obese dogs, FT4 values measured through equilibrium dialysis were similar in obese and lean dogs. Therefore, FT4ED is reliable to evaluate thyroid function in obese dogs, which is in accordance with several studies showing that FT4ED is less influenced by non-thyroidal factors than TT4 measurements (Peterson et al., 1997; Scott-Moncrieff et al., 2000). The free or unbound fraction of thyroid hormones is biologically active. If the increased TT4 concentrations observed in our study were due to increased levels of thyroid binding globulin, normal FT4 and increased TT4 levels would be expected. Thyroglobulin AA are observed in 36 to 60 % of dogs with hypothyroidism and support a diagnosis of autoimmune thyroid disease (Daminet et al., 2000; Dixon et al., 1999a; Nachreiner et al., 1998). All our dogs, including the 20 dogs with obesity, tested negative for the presence of TgAA.

One intact female beagle had high serum TT4 (74 nmol/L) concentration one month after initiating weight loss (visible in figure 2). Serum progesterone concentration at that time was 43 nmol/L, which is compatible with dioestrus and could be responsible for this increased TT4 serum concentration (Reimers et al., 1990).

Reverse T3, produced by peripheral 5-deiodination of T4, is the biologically inactive form of T3. In humans with obesity rT3 levels remain unchanged in most studies (Roti et al., 2000), which corresponds to our findings.

As T3 and T4 are major regulators of energy metabolism, a defect in the thyroid function is frequently associated with changes in body weight. None of the 20 obese beagles (12 from the first experiment and 8 from the second experiment before weight loss) used in this study had results compatible with thyroid dysfunction. Interestingly, 2

obese dogs from the second experiment had high TSH serum concentrations before caloric restriction (0.86 and 0.72 ng/ml) in combination with normal TT4 serum concentrations. These results could suggest subclinical hypothyroidism where an early thyroid failure is compensated by increased stimulation of TSH secretion. Moreover, approximately 10% of euthyroid dogs have an increased TSH into the hypothyroid range. Further follow-up of these 2 dogs during a one-year period excluded the possibility of hypothyroidism based on the absence of clinical signs, normal TT4 and TSH measurements, negative TgAA evaluation and a normal TSH stimulation test (one dog). Most studies in obese humans report normal basal serum TSH concentrations, although a recent study reported high TSH levels in some obese children and adults (Pinkney et al., 1998; Stichel et al., 2000). High leptin levels observed in obese humans might contribute to this finding through stimulation of thyrotropin-releasing hormone (TRH) release (Pinkney et al., 1998; Stichel et al., 2000).

The study of Sauvé and Paradis in 2000 was the first to report the use of rhTS to perform a TSH stimulation test in euthyroid beagle dogs. These authors concluded that the intravenous use of 50 µg of rhTSH was clinically useful and that sampling for serum TT4 determination 4 and 6 hours after rhTSH injection was adequate. Based on further discussion with these authors and personal clinical experience with this molecule, a slightly higher dose (75 µg) was used to perform the TSH stimulation tests in our study. The use of 75 µg of rhTSH for dogs weighing between 10.8 and 25.9 kg (but obese) in our study allowed optimal stimulation of the thyroid gland. All dogs (lean and obese) met the criteria suggested by Sauvé&Paradis to confirm euthyroidism (post TSH TT4 value greater than 45 nmol/L or an increase in TT4 of at least 24 nmol/L) (Sauvé and Paradis, 2000). Furthermore with the aforementioned dose of rhTSH, TT4 results at 6 hours were higher than at 4 hours, suggesting that, with 75 µg of rhTSH, sampling 6 hours after the injection of rhTSH is more optimal than the 4-hour sample. It is interesting to mention that the reference values for the assay used to measure TT4 measurements in our study were higher than the reference range for the assay used in the study of Sauvé and Paradis.

CHAPTER 4

Diet changes are the mainstay in the treatment of obesity. Therefore, it is important to recognize how changes in dietary intake affect thyroid hormone physiology and results of thyroid hormone testing. One study in dogs reported a decrease in serum TT3 concentrations in dogs undergoing several weight loss protocols, but FT4, TSH and rT3 serum concentrations were not evaluated in that study (Laflamme et al., 1997). Percentage of maintenance energy requirement (MER) used in our study was similar to % MER used in the study of Laflamme in 1997. Our study showed that feeding an energy restricted diet (average 63 % of MER) to obese dogs led to a significant decrease of serum TT3 concentrations but that TT4, FT4ED, and rT3 remain normal. Such levels of restriction are routinely used in clinics to promote weight loss. In clinical practice, TT4 measurements are more routinely used to evaluate thyroid function than are TT3 serum concentrations because the latter show much greater overlapping results between normal, hypothyroid and sick dogs (Miller et al., 1992). Our results are in agreement with the observations in obese humans where decreased levels of TT3 are observed after caloric restriction in most studies; this is referred to as the 'low T3 state of undernutrition' (Cavallo et al., 1990; Glass and van der Veen, 1996). A decreased peripheral conversion of T4 into T3, through inhibition of a type I deiodinase, is the proposed mechanism (Glass and van der Veen, 1996; Roti et al., 2000; Vagenakis et al., 1977). The decreased concentration of T3 is believed to protect the organism during periods of fasting or calorie restriction by lowering the metabolic rate (Cavallo et al., 1990; Drent and Kushner, 1995; Shetty,1990).

A decrease in serum TT3 concentrations is also the hallmark of the 'euthyroid sick syndrome' observed in humans or dogs suffering from a systemic nonthyroid illness (Ferguson,1997). Anorexia and decreased energy intake frequently seen in association with systemic illness might play a role.

In humans losing weight parallel to the decrease in TT3 serum concentrations, an increase in rT3 levels due to diminished clearance is reported (Glass and van der Veen, 1996; Roti et al., 2000). Reverse T3 serum concentrations did not change significantly in our study.

Thyrotropin levels in our study decreased during caloric restriction, but remained within the reference range. Decreased or normal baseline serum TSH levels have also been documented in humans undergoing fasting or calorie restriction (Beer et al., 1989; Glass and van der Veen, 1996). Whether calorie-restriction could suppress the high TSH levels of a hypothyroid dog into the reference range and therefore leading to a diagnostic challenge remains a question.

The decline in TT3 that accompanies caloric-restriction would theoretically be expected to be associated with an increase in serum TSH concentrations. However this is not the case. One possible explanation is that intracellular pituitary concentrations remain constant, because they are maintained by intracellular deiodination of the unchanged TT4 levels. Altered sensitivity of the hypothalamic-pituitary-thyroid axis or inhibition through inhibitors of TSH secretion might also play a role. But clearly, despite their low serum TT3 levels, dogs fed a calorie-restricted diet are not hypothyroid based on the absence of clinical signs of hypothyroidism and serum TSH and FT4 concentrations within the reference range.

Some studies have shown a correlation between TT3 values and resting metabolic requirements (Kiortsis et al., 1999). This could at least partially explain why the dogs with higher TT3 concentrations required more time to lose weight in our study. We found no correlation between body weight and serum TT3 concentrations.

Conclusion

The present study demonstrates that thyroid homeostasis is influenced by obesity and weight loss. Although always within their reference ranges, serum TT3 and TT4 concentrations were somewhat higher in obese dogs when compared to lean dogs. Furthermore, caloric-restriction led to a decline in serum TT4, TT3 and TSH concentrations, but only statistically significant for TT3 and TSH. Free T4ED and rT3 remained unchanged. Therefore, although significant changes in thyroid homeostasis

were observed in our study in obese dogs and in calorie-restricted dogs, these situations are unlikely to affect interpretation of usual thyroid function test results in practice.

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CHAPTER 5

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CHAPTER 5

Introduction to chapter 5

In chapters 2, 3 and 4, we focussed on the influence of drugs, obesity and weight loss on canine thyroid function test results. The awareness and knowledge of these influences should help the clinician to make a more reliable diagnosis of hypothyroidism in dogs. Another aspect of the difficulty in the diagnosis of hypothyroidism in dogs is that the clinical signs are vague and non-specific. Several diseases have a clinical presentation that can be similar to hypothyroidism. The most striking example is the symmetrical flank alopecia frequently observed in hypothyroidism but also a characteristic feature of a recently documented dermatological disease: canine recurrent flank alopecia. In this last chapter we shall evaluate thyroid function in dogs with CRFA.

CHAPTER 5

CHAPTER 5

EVALUATION OF THYROID FUNCTION IN DOGS SUFFERING FROM RECURRENT FLANK ALOPECIA

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CHAPTER 5

Summary

Thyroid function was assessed in euthyroid dogs (n=20), dogs suffering from recurrent flank alopecia (CRFA, n=18), and hypothyroid dogs (n=21). Blood samples obtained from all dogs in each group were assayed for total thyroxine (TT4), thyrotropin (TSH), and thyroglobulin autoantibodies (TgAA) serum concentrations. Total T4 and TSH serum concentrations were significantly decreased and increased, respectively, in the hypothyroid group compared with the other 2 groups. No significant differences in TT4 and TSH serum values were found between the euthyroid and CRFA groups. Thyroglobulin autoantibodies were detected in 10, 11.1, and 61.9 % of euthyroid dogs, dogs with CRFA, and hypothyroid dogs, respectively. In conclusion, dogs suffering from CRFA have a normal thyroid function, and the determination of TT4 and TSH serum concentrations allows differentiation of these dogs from dogs with hypothyroidism, in most cases. Occasionally, both diseases can be concomitant.

CHAPTER 5

Introduction

The diagnosis of canine hypothyroidism can be challenging for 2 main reasons: first, several other diseases can present with similar clinical signs, and second, there is no ideal test to assess canine thyroid function.

Alopecia is a common clinical sign of hypothyroidism; 25% of hypothyroid dogs have been reported to present with symmetrical bilateral endocrine alopecia (1). The symmetrical alopecia described in canine recurrent flank alopecia (CRFA) can easily mislead one to a diagnosis of hypothyroidism. Canine recurrent flank alopecia is a recently recognized skin disorder of unknown etiology, characterized by episodes of truncal hair loss that often occurs on a recurrent basis. It has been described under several synonyms*: seasonal flank alopecia, seasonal growth hormone deficiency, canine idiopathic cyclic flank alopecia, cyclic follicular dysplasia, and follicular dysplasia.* Typically, this alopecia affects adult dogs on a yearly seasonal basis and resolves spontaneously without treatment within several months. The episodes do not always reoccur annually. Several hypotheses, such as photoperiod and melatonin deficiency, have been proposed, but the etiology remains unclear (2-5). Assessment of thyroid function in dogs with CRFA through determination of endogenous thyrotropin (TSH) concentrations or serum thyroglobulin autoantibodies (TgAA) has not been reported, to our knowledge.

Overall, the assessment of canine thyroid function is also difficult because of the lack of one reliable test. Since the unavailability of bovine TSH, previously used to perform thyroid stimulation testing, much emphasis has been placed in the recent years on determination of endogenous TSH concentrations. Currently, the combined use of thyroxine (T4) and TSH serum concentrations seems to be the easiest and most reliable way to assess canine thyroid function (6,7). Most cases of canine hypothyroidism are primary and the result of a lymphocytic thyroiditis. The presence of circulating TgAA seems to correlate with this thyroiditis (8-11).

CHAPTER 5

The aim of our study was to assess thyroid function of dogs affected by CRFA. The objectives of our study were to determine serum total thyroxine (TT4), TSH, and TgAA concentrations in euthyroid dogs, dogs with CRFA, and hypothyroid dogs.

Materials and methods

The control group consisted of 20 dogs aged from 1.5 to 14 y (median 2 y). Body weights ranged from 8.5 to 54 kg (median 16 kg). Ten different breeds were represented in this group. These dogs were privately owned, or were part of a colony belonging to the Faculté de médecine vétérinaire, Université de Montréal, and were assessed as healthy, based on an unremarkable history and on physical examination findings. None of them received medication. A TSH stimulation test was not performed, as bovine TSH was not available. To be included in this group, TT4 serum concentration had to be ≥ 15 nmol/L.

A second group consisted of 18 dogs with signs compatible with CRFA. Ages ranged from 2 to 12 y (median 4.5 y) and weights from 20 to 51 kg (median 27.5 kg). Clinical diagnosis of CRFA was based on history and clinical signs, such as breed predisposition, time of the year of onset of the alopecia, spontaneous regrowth in the alopecic area, and absence of other clinical signs suggestive of hypothyroidism or hyperadrenocorticism. Seven different breeds were represented (boxer n=7, giant schnauzer n=2, Airedale n=5, Great Dane n=1, Dalmatian n=1, Labrador retriever n=1 and American pit bull terrier n=1). Of the 18 dogs in this group, 8 were spayed females, 2 were intact males, 4 were intact females, and 4 were castrated males. A third group consisted of 21 hypothyroid dogs. Diagnosis was based on clinical history, signs consistent with hypothyroidism, a TT4 concentration below 15 nmol/L, and/or a serum TSH concentration above 0.6 ng/mL and complete resolution of clinical signs after thyroid supplementation in all dogs. Ages ranged from 2 to 15 y (median 5 y), and weights from 5.5 to 68 kg (median 24 kg). This group was composed of cocker spaniels n=5, golden retrievers n=3, Shetland sheepdogs n=3, giant schnauzers n=2, and one of each of the following breeds: bloodhound, Dalmatian, St-Bernard, boxer, mixed breed dog, Akita, weimaraner and fox terrier. Five of these dogs were spayed females, 5 were intact males, 2 were intact females, and 9 were neutered males. Blood samples were collected by jugular venipuncture and centrifuged; each serum sample frozen in 3 aliquots at -20° C until assayed. Total T4 concentrations were determined using a

validated solid-phase radioimmunoassay (Coat-a-Count canine T4, Diagnostic Products Corporation, Los Angeles, California) (12). Serum TSH concentrations were determined by using a validated, commercially available solid-phase radioimmunoassay (Coat-a-Count canine TSH IRMA, Diagnostic Products Corporation) (12,13). Serum TgAA concentrations were measured at the Endocrinology Animal Health Diagnostic Laboratory of Michigan State University using a previously validated technique. A positive result was defined as at least twice (200 %) the optical density of the negative control sample (11). Data were analyzed by using a factorial ANOVA test and post hoc Bonferroni test, when appropriate. Results are presented as means ± standard deviation (means, s =) and were considered significant at $P<0.05$.

Results

Total thyroxine

Mean basal serum TT4 concentrations were 27.5, s = 8.86 nmol/L (range 15 to 43.32 nmol/L), 25.54, s = 11.95 nmol/L (range 7.21 to 48.5 nmol/L) and 4.16, s = 4.53 nmol/L (range 0.05 to 14.92 nmol/L) in euthyroid, CRFA, and hypothyroid dogs, respectively. There was a significant difference in serum TT4 concentrations between the hypothyroid group and the other 2 groups ($P<0.05$). No differences in TT4 concentrations were found between the CRFA and control groups (Figure 1).

Thyrotropin

Mean values for TSH concentrations in the euthyroid, CRFA, and hypothyroid groups were 0.16, s = 0.1 ng/mL (range 0.03 to 0.39 ng/ml), 0.34, s = 0.28 ng/mL (range 0.03 to 1.17 ng/mL), and 3.14, s = 2.5 ng/mL (range 0.46 to 8.1 ng/mL), respectively. A significant difference in serum TSH concentrations was found between the hypothyroid group and the other 2 groups ($P<0.05$). No differences were found between the concentrations of TSH in the CRFA and control groups (Figure 2). Three dogs with CRFA had serum TSH values above 0.6 ng/mL at one point in time; the results for their serum TT4, TSH, and TgAA concentrations are given in Table 1. None of these 3 dogs received any medication. The first boxer was presented in May 1996 with bilateral symmetrical flank alopecia. His complete blood cell count and biochemical profile (including cholesterol values) were normal, and the dog had no other clinical signs suggestive of hypothyroidism. The alopecia resolved completely within a few months. The dog suffered another episode of flank alopecia 1 y later, which again resolved spontaneously, confirming the CRFA. Despite the author's insistence, it was not possible to get a follow-up of thyroid function in this dog. The second dog, a giant schnauzer, had episodes of flank alopecia in January 1996 and in May 1999, both episodes of alopecia resolved spontaneously without any treatment. A follow-up in August 1999 revealed a normal physical examination and a healthy coat. According to the owner, the dog remains clinically normal. The third dog, a Dalmatian,

suffered 2 episodes of CRFA; initial results of thyroid function tests suggested hypothyroidism, although follow-up tests revealed normal thyroid function.

Thyroglobulin autoantibodies

Two of the 20 (10 %) control dogs were positive for serum TgAA. Two of 18 (11.1 %) dogs with CRFA tested positive for TgAA. Thirteen of the 21 (61.9 %) hypothyroid dogs tested positive for TgAA.

Figure 1: Mean serum TT4 concentrations in control dogs, hypothyroid dogs, and dogs with CRFA. Bars represent a 95 % confidence interval.

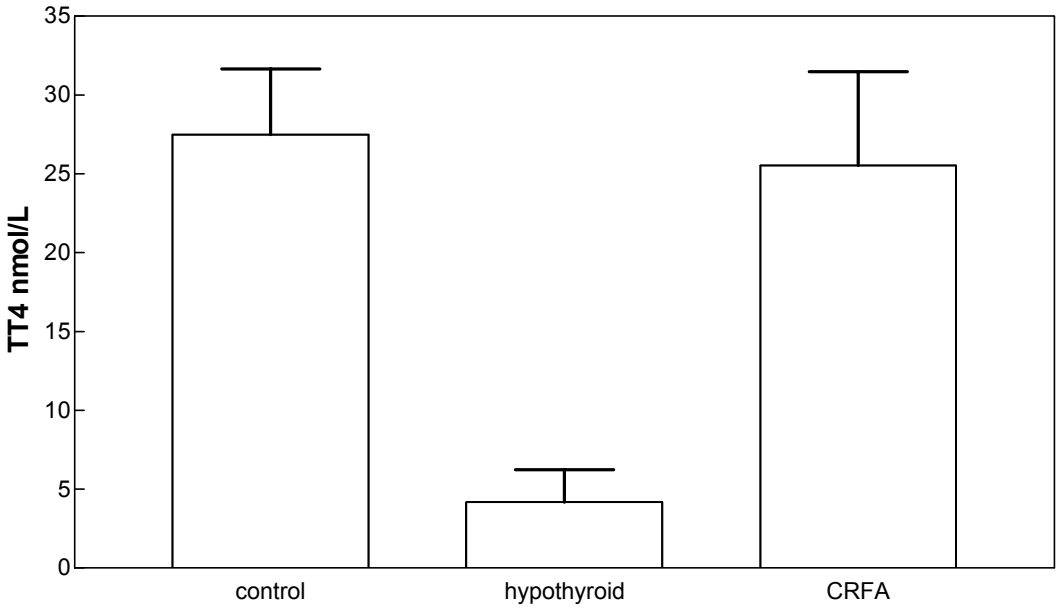
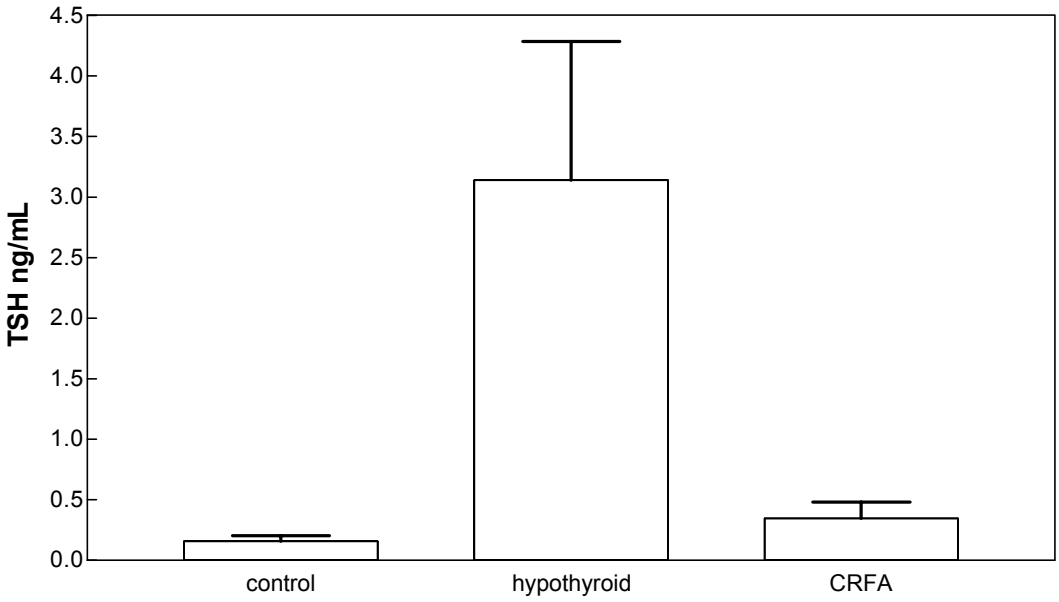


Figure 2: Mean TSH serum concentrations for the control group, hypothyroid dogs, and dogs with CRFA. Bars represent a 95 % confidence interval.



CHAPTER 5

Discussion

As expected, TT4 serum concentrations were decreased and TSH concentrations were increased in most hypothyroid dogs, as compared with the control group dogs. Nineteen of the 21 hypothyroid dogs (90.5 %) had a TSH concentration above 0.6 ng/mL. Two dogs (9.5 %) with a TSH concentration of 0.46 and 0.50 ng/mL were included in the hypothyroid group, because their clinical signs suggested hypothyroidism; their serum TT4 were 1.1 and 1.57 nmol/L, respectively, in the absence of any concomitant disease; and all their clinical signs resolved with thyroid supplementation. This finding is not surprising, as it has been reported in several studies that 24 % to 38 % of hypothyroid dogs have TSH values within the reference range (6,7).

Results from previous studies have suggested a correlation between serum TgAA and the presence of a lymphocytic thyroiditis (8-11). Until recently, the methods used to measure serum TgAA and/or the diagnostic cut-off values used, easily yielded false positive results, especially in dogs with systemic disease. This limited their clinical usefulness (8-10). Nachreiner et al (11) recently published the validation of a reliable TgAA assay with a useful diagnostic threshold, which should increase the clinical utility of determining serum TgAA in some dogs. This method for TgAA measurement was used in our study. We observed that 2 dogs (10 %) of the control group dogs tested positive for serum TgAA, whereas Nachreiner et al reported that 3.3 % of a random population of dogs had positive results for TgAA. One of our positive control dogs had only a mild-positive result with an optical density of 208 %, when the cut-off value for a positive sample is 200 % of the negative control result. It is, however, still unclear whether the presence of circulating TgAA precedes the clinical development of hypothyroidism, and further studies that follow dogs with circulating TgAA over a longer period of time are warranted. Thirteen of 21 (61.9 %) hypothyroid dogs tested positive for serum TgAA, which is similar to the prevalence of 42% to 59% for TgAA in hypothyroid dogs reported in the literature (8,10). Therefore, our findings also support a correlation between lymphocytic thyroiditis and hypothyroidism.

Dogs with CRFA present with dermatological signs that can be suggestive of hypothyroidism. Dogs of either sex and of all reproductive status can be affected. Canine recurrent flank alopecia is characterized by a nonscarring alopecia, most often confined to the thoracolumbar region. Lesions are usually bilaterally symmetric, but in occasional dogs (or episodes) only one side of the body is affected, or one side is more affected than the other (4). Typically, the alopecic lesions are "geographic" in shape with well-demarcated borders and the alopecic skin is often markedly hyperpigmented (2,5,14). Mean age at the onset of the first episode around 4 y, but can be quite variable (range: 8 mo to 11 y). The onset of alopecia is variable, but most commonly occurs between November and March in the northern hemisphere (4). Spontaneous regrowth of hair occurs in 3 to 8 mo in most cases and usually consists of normal pelage density. Although, approximately 20 % of dogs will only have one isolated episode of flank alopecia in their life span, most dogs will develop recurrent alopecic episodes for years (4). Other dogs have an occasional year when the alopecia does not recur. The degree of alopecia is variable, with some dogs developing a virtually identical hair loss (size and duration) year after year, and other dogs developing larger areas and/or longer episodes of hair loss as years go by. Occasionally, after several consecutive episodes of alopecia, some affected dogs will not experience complete hair regrowth before the onset of the next episode (4).

The cause of CRFA remains obscure. The higher incidence of CRFA at higher latitudes and its seasonal nature of recurrence suggest that photoperiod may be involved in the pathogenesis, with melatonin and/or prolactin, 2 photo-dependent hormones, being potentially implicated in the process.

For most cases of CRFA, the diagnosis is based on history, clinical signs, and rule-out of hypothyroidism (evaluation of thyroid function in dogs > 2 years of age). Histopathologic findings observed on skin biopsies such as atrophic, dilated, and troncated hair follicles with hyperkeratosis extending to secondary hair follicles (hence the colloquial name «witch's feet» or «octopus like hair follicles»), are suggestive of, but not pathognomonic, for CRFA (4,5,14). Moreover, these findings are not always

present in cases of CRFA and they may be found with low frequency in other alopecic diseases.

Hypothyroidism is an important differential diagnosis for CRFA. Moreover, hypothyroidism and CRFA could both occur in the same animal. The clinician must, therefore, remain vigilant, as this can represent a real diagnostic and therapeutic challenge. A breed predisposition seems to exist for CRFA, with boxers (may account for approximately half of all reported cases), Airedale terriers, schnauzers, and English bulldogs being the more frequently cited breeds (2,3,14). However, it has been reported in several other breeds (5). Interestingly, the same breeds are suspected to be predisposed for hypothyroidism (1). Furthermore, the recognition of CRFA or seasonal flank alopecia as a separate dermatological condition became clear only in the 1990s (3). Therefore, some dogs previously classified as hypothyroid based on the presence of flank alopecia resolving with thyroid supplementation might very well have been dogs suffering from CRFA. Another explanation is that the breeds cited earlier are predisposed to both hypothyroidism and CRFA. Both diseases are more frequent in young to middle-aged dogs. As most dogs with hypothyroidism have a lymphocytic thyroiditis, believed to be of autoimmune origin, the question arises whether CRFA could have an immune mediated origin as well? Results of the TSH response test were reported in 5 dogs with CRFA and were normal, but serum TSH values and TgAA were not available (3).

Results of T4 and TSH tests in our study were not significantly different between euthyroid dogs and dogs with CRFA. However 3 out of 18 dogs with CRFA were found to have results that could suggest concomitant hypothyroidism. All episodes of flank alopecia resolved completely without any treatment in all 3 dogs. Their history, clinical presentation, and follow-up confirmed CRFA. The increased TSH and positive TgAA suggested concomitant hypothyroidism due to a lymphocytic thyroiditis in dogs 1 and 2. None of these 3 dogs had a history or clinical signs suggestive of hypothyroidism, except for the flank alopecia, and they did not develop clinical hypothyroidism. The reported specificity of serum TSH concentrations in the diagnosis of hypothyroidism ranges from 0.88 to 0.93 (6,7). Total T4 and TSH serum

concentrations were repeated a year later in the second dog, and the TSH value was in the reference range. One possible explanation could be that TSH is secreted in a pulsatile fashion, as it is in humans (15,16). One study reported an absence of significant diurnal variation of the TSH concentrations in healthy dogs and dogs with iodine^{131}- induced hypothyroidism; however, dogs with naturally developing hypothyroidism had TSH values fluctuating in and out the reference range (17). This could, at least in part, explain the low sensitivity of determination of TSH concentrations in the diagnosis of hypothyroidism. The TT4 serum values in dogs 1 and 2, with CRFA and increased TSH values, were slightly below the reference range but higher than expected in most dogs with hypothyroidism. The human medical literature refers to subclinical hypothyroidism where an increase in TSH values maintains adequate thyroid hormone concentrations and few or no clinical signs of hypothyroidism are present (18). Progression towards overt hypothyroidism is slow in humans (18). Whether subclinical hypothyroidism exists in dogs is not known.

Our results emphasize that flank alopecia can be misleading and appropriate testing through a combination of T4 and endogenous TSH determinations or through a TSH stimulation test when available, are important to differentiate these conditions in some dogs. In addition, these disorders could be concomitant in some dogs. When a dog is presented with a typical presentation of CRFA a justifiable option is to wait a few months and see if the condition resolves spontaneously. Results of thyroid testing should be interpreted in light of the history and clinical findings to avoid overtreating euthyroid dogs with thyroid supplementation. Monitoring of dogs with slightly abnormal or equivocal results of thyroid function tests is recommended. The etiology of CRFA is still unclear, but thyroid dysfunction does not seem to be a predisposing factor for CRFA.

CHAPTER 5

Conclusion

In conclusion, dogs suffering from CRFA have a normal thyroid function. Determination of TT4 and TSH serum concentrations allows dogs with CRFA to be differentiated from dogs with hypothyroidism in most cases. Hypothyroidism and CRFA could be concomitant in some dogs.

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CHAPTER 5

General discussion

Adult onset primary hypothyroidism is a common endocrine disorder in dogs. However, many dogs are prescribed a life long unnecessary supplementation of thyroid hormones based on erroneous diagnoses of this disease. The vague and non-specific clinical signs of hypothyroidism and factors influencing thyroid function test results are greatly contributing to this problem. The possible effects of many factors have not been studied yet in dogs. Thyroid physiology, canine hypothyroidism, specific diagnostic tests and factors influencing thyroid homeostasis are described in chapter 1.

The aim of this study was to clarify the influence of some common clinical situations on canine thyroid homeostasis. In chapters 2, 3 and 4, we evaluated the effects of administration of commonly prescribed drugs, obesity and weight loss on canine thyroid function. In chapter 5 we thoroughly evaluated thyroid function in dogs with canine recurrent flank alopecia (CRFA), a dermatological disease of unknown origin that has been largely confused with canine hypothyroidism in the past.

The short-term effects of oral prednisone and phenobarbital were evaluated on canine thyroid function tests (Chapter 2). Prednisone therapy significantly decreased serum TT4 and to a lesser extend FT4 serum concentrations. Prednisone administration did not have a substantial effect on serum TSH concentrations in euthyroid dogs. However decreased TT4 levels should have resulted in increased TSH because of reduced negative feed back of these hormones on the pituitary gland, suggesting a central inhibition of the hypothalamus-pituitary axis. We administered prednisone at a moderate immunosuppressive dosage for 3 weeks. Normalisation of TT4 serum concentrations occurred within 1 week of cessation of the therapy. Corticoids are sometimes used for longer periods of time in practice. Longer treatment may result in a longer duration of suppressed thyroid function. Furthermore, previous studies have shown that the administration of prednisone at an anti-inflammatory dosage had no effects on thyroid hormone levels. These findings emphasize that duration of treatment and dosage of glucocorticoids used are important.

Short-term phenobarbital therapy in our study did not affect serum TT4, FT4, or TSH concentrations. Therapeutic levels were somewhat low in our study, therefore it cannot be excluded that higher dosages of phenobarbital might have an influence on thyroid function tests even after short-term administration. It should be mentioned that after the publication of our study, several other studies have investigated the effects of phenobarbital on canine thyroid function but after long-term administration. These studies revealed a significant decrease in TT4 serum values. Furthermore, endogenous TSH concentrations remained within reference range (Kantrowitz *et al.,* 1999) or were slightly increased (Gaskill *et al.,* 1999; Müller *et al.,* 2000; Gieger *et al.,* 2000) but remained < 0.6 ng/ml in most dogs (the upper reference range for TSH varied between 0.36 and 0.7 ng/ml in these studies). Complicating clinical evaluation, weight gain (due to polyphagia), lethargy and hypercholesterolemia are reported in dogs on phenobarbital treatment, but are also commonly observed in dogs with hypothyroidism. Confirming a diagnosis of primary hypothyroidism in a phenobarbital treated dog can for all these reasons be a real challenge, especially when TSH concentrations are increased. In situations in which it is not clear whether the observed effects on thyroid function are the effect of phenobarbital therapy or due to actual hypothyroidism, potassium bromide (KBr) therapy could be a therapeutic option. However, given the long duration to achieve steady-state (3 to 5 months) with KBr therapy, it would seem imprudent to switch from phenobarbital to KBr to determine whether an animal is hypothyroid or not.

NSAIDs are used more and more frequently in dogs. We evaluated whether acetylsalicylic acid (ASA) or ketoprofen (Keto) influence thyroid function test results in dogs (Chapter 3). A significant decrease in TT4 was observed as soon as 24 h after ASA administration, but no significant effects were found for FT4 and TSH. No significant effects on thyroid results were found with Keto administration. These results indicated that TT4 can be markedly decreased by ASA therapy and probably reflected the displacement of thyroid hormones from serum protein binding sites.

A few other studies have evaluated the effects of other NSAIDs on canine thyroid function tests. A mild decrease in TT4 serum concentrations was observed after caprofen administration, but FT4 remained unaltered (Ferguson *et al.,* 1999). Very

recently it was shown that the administration of meloxicam and etodolac had no effect on thyroid function test results in dogs (Sauvé *et al.,* 2002; Panciera & Johnston, 2002). This is in contrast with our study where ASA had a pronounced effect on thyroid function test results. This discrepancy could, at least in part, be due to the high molar concentrations obtained with ASA. Comparisons of these studies clearly point out that effect of various NSAIDs on canine thyroid function can be very different from drug to drug.

Dogs in our study did not develop any clinical signs suggestive of hypothyroidism when administered ASA. Furthermore, FT4 serum concentrations were not significantly altered following ASA and carprofen administration. Therefore, these dogs, despite decreased TT4 concentrations, were not suffering from clinical hypothyroidism.

Thyroid function test results should be interpreted cautiously in dogs on NSAIDs therapy. A seven-day treatment with ASA resulted in a decrease in TT4-potentially sufficient to lead to the misinterpretation of the thyroid profile and misdiagnosis of hypothyroidism in more than half of the dogs. Therefore, a diagnosis of hypothyroidism, in a dog on NSAIDs therapy, should not be based on a TT4 measurement only. After short-term (one-week) administration of ASA, and maybe other NSAIDs as well, we advise a minimal seven-day period of drug withdrawal before thyroid function testing in dogs.

In our studies, prednisone and ASA were the drugs most likely to affect thyroid function test results and potentially lead to misdiagnosis of hypothyroidism. It is important to emphasize that abnormal test results were observed, but that none of these dogs clinically suffered from clinical thyroid dysfunction. This is also strengthened by the normal FT4 measurements in several of our experiments.

Our results emphasize that the diagnosis of hypothyroidism in dogs should not be based on a TT4 measurement solely.

Furthermore, patients being tested for possible hypothyroidism should be carefully scrutinised for any history of recent medication administration.

GENERAL DISCUSSION

Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment. Obesity is also a common observation in hypothyroid dogs, and therefore, thyroid function is frequently evaluated in practice because dogs are overweight. In chapter 4, we compared thyroid function in obese dogs and in lean dogs and explored the effects of calorie restriction and weight loss in obese dogs on thyroid hormone serum concentrations. Only TT3 and TT4 serum concentrations were significantly higher in obese dogs as compared to lean dogs. It is generally accepted that finding a normal TT4 serum concentration in a dog, allows exclusion of hypothyroidism in most cases. Therefore, exclusion of hypothyroidism in an obese dog based on finding a normal TT4 concentration should be straightforward. Weight loss resulted in a significant decrease in TT3 and TSH serum concentrations, but TT4 remained unaffected. In veterinary medicine, TT4 measurements are more routinely used to evaluate thyroid function then TT3. Therefore, evaluating an obese dog fed a calorie-restricted diet to promote weight loss, should not affect interpretation of thyroid function test results.

Clinical manifestations of hypothyroidism are nonspecific; this further complicates the diagnosis of canine hypothyroidism. Several diseases have a clinical presentation that can be similar to hypothyroidism. The most striking example is the symmetrical flank alopecia which is frequently observed in hypothyroidism but also is a characteristic feature of CRFA. During the last decades, many dogs have been erroneously diagnosed with hypothyroidism when they were suffering from CRFA. As the etiology of CRFA remains largely unknown, we decided to investigate the possible role of a thyroid dysfunction as a cause for CRFA. Thyroid function was assessed in dogs suffering from recurrent flank alopecia in chapter 5. The etiology of CRFA remains unknown but this study allowed ruling out thyroid dysfunction as a possible cause for this disease. Practitioners should be aware that the clinical presentation of CRFA can be misleading for hypothyroidism. Particularly in cases suspected for CRFA, hypothyroidism should be excluded by finding a TT4 within reference range or confirmed by finding a decreased TT4 combined with an increased TSH.

Here follows a discussion about the different thyroid hormones measured in our studies.

Total thyroxine serum concentrations were markedly decreased in more than half of the dogs after ASA or prednisone administration in our studies. This alteration in thyroid hormone concentrations can easily lead erroneously to a diagnosis of hypothyroidism when only a TT4 is determined. Measuring TT4 and TSH in combination should avoid an erroneous diagnosis of hypothyroidism in these cases, because TSH serum concentrations remain within the reference range during prednisone and ASA administration. On the other hand, with long term administration of phenobarbital and especially with sulfonamides, increased TSH serum concentrations can easily lead to an erroneous diagnosis of primary hypothyroidism.

Free T4 measurements were less affected than TT4 serum concentrations in several of our studies by the administration of different drugs, or with obesity and weight loss. This is in accordance with most findings in the literature. Unfortunately, at the present moment, FT4 measurement by equilibrium dialysis is not readily available in most European laboratories.

Canine-specific TSH assays were developed and are available only since 1996. The measurement of canine TSH levels has allowed gaining further insights in canine thyroid physiology, improved accuracy in the diagnosis of canine hypothyroidism and also helped to confirm the goitrogenic effects of some drugs. In our studies, TSH levels allowed a better exploration of the hypothalamo-pituitary-thyroid axis and contributed to the understanding of some mechanisms involved. Despite all these advantages, the limitations of TSH measurement have also become apparent in the last years. Poor sensitivity of the endogenous TSH for the diagnosis of hypothyroidism contributes to the difficulty in confirming hypothyroidism in clinics.

Reverse T3 (rT3) and T3 serum concentration measurements were used in several of our studies, although rT3 and TT3 are not routinely used to evaluate canine

thyroid gland function in practice. These tests allowed to better explore peripheral mechanisms possibly involved.

Thyroglobulin autoantibodies were detected in 10, 11.1, and 61.9 % of euthyroid dogs, dogs with CRFA, and hypothyroid dogs, respectively (Chapter 5). In the other studies we performed, euthyroid dogs were all negative. These findings are similar to literature reports. Measurement of TgAA can be of value in the early detection of dogs predisposed to hypothyroidism (breeding dogs) or in a research setting, especially because hypothyroidism can be a familial condition in beagle dogs.

Bovine TSH stimulation test has long been considered as the gold standard for thyroid function evaluation in dogs, but bovine TSH is no longer commercially available. Human recombinant TSH has only been used very recently in veterinary medicine in the evaluation of thyroid function in dogs. It proved to be a useful adjunct to the evaluation of thyroid function in our study (Chapter 4). No adverse reactions were observed and a dosage of 75 μg proved to be a useful dosage for optimal stimulation of the thyroid gland. Sampling after 6 hours seemed optimal. This protocol was practical for use in a clinical research setting and was not prohibitively expensive.

Our studies also allow making several recommendations to practicing veterinarians.

1. To scrutinise patients to be tested for hypothyroidism for any history of drug administration is crucial. Ideally, dogs should not be tested for hypothyroidism when administered drugs, and thyroid testing should be postponed to after cessation of drug therapy. When this is not an option clinically (e.g. with phenobarbital administration), then at least the clinical picture and the results of thyroid function tests should be interpreted with a healthy dose of scepticism.
If decreased TT4 serum levels are obtained in a dog administered ASA or prednisone, then measuring TSH serum levels can be a very useful adjunct. With phenobarbital administration, even combining TT4 and TSH can be misleading in some cases.

2. Although we showed that some alterations occur in thyroid hormone concentrations with obesity and with weight loss, these changes are unlikely to mislead the interpretation of thyroid function test results.

3. Veterinarians should be aware that CRFA exists and that its clinical presentation is particularly misleading for hypothyroidism. Particularly in cases suspected for CRFA, hypothyroidism should be excluded by finding TT4 levels within reference range or confirmed by finding a decreased TT4 combined with an increased TSH.

4. The findings in our work emphasize that, as with any clinicopathological test result, thyroid function tests should be interpreted in conjunction with a complete history and compatible physical examination findings.

Further studies on the influence of drugs and other factors on canine thyroid function tests are needed. Many questions regarding the mechanisms through which drugs alter canine thyroid function remain open. Techniques for TSH measurements should be improved. Research trying to clarify why some dogs with primary hypothyroidism have TSH serum concentrations within the reference range is essential. Medical imaging of the canine thyroid is still in its first steps and could lead to better insights into this complex but fascinating disease.

Conclusion

The present study contributed to the knowledge of various aspects of canine thyroid function. It identified additional factors that can influence thyroid function test results in dogs and clearly points out that TT4 serum concentration can rapidly and markedly be decreased by some commonly used drugs. The effect of weight loss was also significant on thyroid hormones, but the observed changes were clinically less relevant. The etiology of CRFA remains unknown but this study allowed ruling out thyroid dysfunction as a major cause for this disease.

GENERAL DISCUSSION

Awareness of the medications and factors that alter thyroid hormone concentrations should facilitate a more accurate interpretation of thyroid function test results, hopefully avoiding the erroneous diagnosis of hypothyroidism and subsequent unwarranted treatment.

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SUMMARY

SUMMARY

Hypothyroidism is the most common endocrine disorder in dogs, however it is also the most over diagnosed. Evaluation of thyroid function in dogs is not always straightforward. The vague and non-specific clinical signs of hypothyroidism and the numerous factors which influence thyroid function test results, are major contributors to the difficulty in diagnosing this disease. The introduction to this thesis first gives a detailed review of thyroid physiology and the general features of canine hypothyroidism. Thereafter, specific thyroid diagnostic tests currently used in dogs are discussed. Subsequently, current knowledge on influence of physiological factors, drugs and systemic diseases on thyroid homeostasis are reviewed (chapter 1).

In this thesis the influence of some commonly observed clinical situations, such as the administration of medications and obesity/weight loss, on thyroid homeostasis was studied. Furthermore, thyroid function was evaluated in dogs with canine recurrent flank alopecia, a disease commonly mistaken for hypothyroidism.

Chapters 2 and 3 evaluated the effects of some commonly used drugs in canine practice on thyroid function test results. The administration of medications in rats and humans can alter the synthesis, secretion, transport, or metabolism of thyroid hormones. Some drugs also directly inhibit the hypothalamic-pituitary-thyroid axis. Species differences exist in all areas of the thyroid axis, therefore it is not surprising that drug-thyroid interactions will vary among species. In humans, several drugs may cause marked changes in the results of thyroid function tests, leading to difficulty in interpretation of the results, but only rarely causing clinical features of thyroid dysfunction. The effects of many of these drugs have not yet been studied in dogs.

The effects of short-term (3 weeks) administration of oral prednisone and phenobarbital were evaluated on canine thyroid function tests (Chapter 2). Phenobarbital therapy in our study did not affect serum TT4, FT4, or TSH concentrations. Phenobarbital serum concentrations obtained in our study were slightly under usual levels aimed for in clinics. Prednisone administered at an immunosuppressive dosage, significantly decreased serum TT4 and, to a lesser extent,

FT4, but it did not affect serum TSH concentrations. One week after the administration of prednisone, 8 out of 9 dogs had a TT4 serum concentration below 15 nmol/L, a value observed in hypothyroidism. Serum concentrations of TT4 and FT4 normalised within 1 week of cessation of prednisone administration. Free T4 serum concentrations were less affected than TT4 serum concentrations by the 3-week administration of an immunosuppressive dosage of prednisone. In conclusion, an immunosuppressive dosage of prednisone can markedly affect thyroid homeostasis in dogs and can lead to misinterpretation of thyroid function test results.

As NSAIDs are used more and more frequently in dogs, it was important to know whether they affect thyroid function test results. Therefore, we evaluated whether ASA or Keto influence thyroid function test results in dogs (Chapter 3). A significant decrease in TT4 was observed as soon as 24 h after ASA administration whereas the decrease in TT3 was less pronounced. No significant effects were found for FT4 and TSH with ASA administration. No significant effects on thyroid results were found for Keto administration. The results indicate that TT4 can be markedly decreased by ASA therapy and probably reflected the displacement of thyroid hormones from serum protein binding sites. Until the results of further studies are available, thyroid function test results should be interpreted cautiously in dogs on NSAIDs therapy. A seven-day treatment with ASA resulted in a decrease in TT4, potentially sufficient to lead to the misinterpretation of the thyroid profile and misdiagnosis of hypothyroidism in more than half of the dogs. Free T4 measured by equilibrium dialysis and TSH serum concentrations were less affected by ASA and Keto administration than TT4. Therefore, a diagnosis of hypothyroidism in a dog on NSAIDs therapy, should not be based on a TT4 measurement only. After short-term (one-week) administration of ASA, and maybe other NSAIDs as well, we advise a minimal seven-day period before thyroid function testing in dogs.

To further assess the possible influence of common clinical situations on thyroid function test results, the effects of obesity and weight loss on canine thyroid function were assessed in chapter 4. In dogs, obesity is the most common nutritional problem

encountered and weight loss is the cornerstone of its treatment. Therefore it was important to clarify how obesity and weight loss can affect thyroid function test results in dogs. The objectives of this study were to compare thyroid function in obese dogs and in lean dogs and to explore the effects of calorie restriction and weight loss in obese dogs on thyroid hormone serum concentrations. Total T3 and TT4 serum concentrations were significantly higher in obese dogs as compared to lean dogs. Weight loss resulted in a significant decrease in TT3 and TSH serum concentrations. In this study we also used recombinant human TSH to perform TSH stimulation tests. The use of 75 µg of rhTSH in our study for dogs weighing between 10.8 and 25.9 kg (but obese) allowed optimal stimulation of the thyroid gland. All dogs (lean and obese) met the criteria suggested by Sauvé and Paradis (2000) to confirm euthyroidism. Furthermore sampling 6 hours after the injection of rhTSH seemed optimal and the 4-hour sample seemed not really necessary. Our findings showed that the rhTSH stimulation test could be a useful tool to evaluate thyroid function in dogs or in research setting to better explore thyroid physiology.

In conclusion, obesity and energy restriction significantly altered thyroid homeostasis in dogs, but the observed changes were unlikely to affect interpretation of thyroid function test results in clinics.

Clinical manifestations of hypothyroidism are nonspecific; this complicates the diagnosis of canine hypothyroidism. Several diseases have a clinical presentation that can be similar to hypothyroidism. The most striking example is the symmetrical flank alopecia frequently observed in hypothyroidism but also a characteristic feature of a recently documented dermatological disease; i.e. canine recurrent flank alopecia (CRFA). During the last decades, many dogs have been erroneously diagnosed with hypothyroidism when in fact they were suffering from CRFA. The etiology of CRFA remains largely unknown. In chapter 5 we evaluated thyroid function in dogs with CRFA. Total T4 and TSH serum concentrations were significantly decreased and increased, respectively, in the hypothyroid group compared with the control and CRFA groups. No significant differences in TT4 and TSH serum values were found between

the euthyroid and CRFA groups. Thyroglobulin autoantibodies were detected in 10, 11.1, and 61.9 % of euthyroid dogs, dogs with CRFA, and hypothyroid dogs, respectively. In conclusion, dogs suffering from CRFA had a normal thyroid function, and the determination of TT4 and TSH serum concentrations allowed differentiation of these dogs from dogs with hypothyroidism, in most cases.

The present study contributes to the knowledge of various aspects of canine thyroid function. It identified additional factors that can influence thyroid function test results in dogs and clearly points out that TT4 serum concentration can rapidly and markedly be decreased by some commonly used drugs. The effect of weight loss was also significant on thyroid hormones, but the observed changes were clinically less relevant. The etiology of CRFA remains unknown but this study allowed ruling out thyroid dysfunction as a major cause for this disease.

Awareness of the medications and factors that alter thyroid hormone concentrations should facilitate a more accurate interpretation of thyroid function test results, hopefully avoiding the erroneous diagnosis of hypothyroidism and subsequent unwarranted treatment.

SAMENVATTING

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Hypothyroïdie is de meest voorkomende endocrinologische aandoening bij de hond, maar ook de meest overgediagnosticeerde. Evaluatie van de schildklierfunctie bij de hond is niet altijd eenvoudig. De vage en niet-specifieke klinische symptomen van hypothyroïdie en de vele factoren die de testresultaten kunnen beïnvloeden, bemoeilijken de diagnose van deze ziekte. De inleiding van dit proefschrift geeft eerst een gedetailleerd overzicht weer van de schildklierfysiologie en de algemene kenmerken van hypothyroïdie bij de hond. Daarna worden de specifieke diagnostische schildkliertesten die tegenwoordig gebruikt worden besproken. Vervolgens worden de beschikbare literatuurgegevens over beïnvloeding van schildklierfunctietesten door fysiologische factoren, geneesmiddelen en systemische ziekten samengevat (hoofdstuk 1).

In het onderzoek van dit proefschrift werd de invloed van veel voorkomende klinische omstandigheden, zoals het toedienen van geneesmiddelen en obesitas/gewichtsverlies op de schildklierhomeostase bestudeerd. Verder werd de schildklierfunctie geëvalueerd bij honden met recidiverende flankalopecie (CRFA), een dermatologische aandoening vaak verkeerdelijk gediagnosticeerd als alopecie ten gevolge van hypothyroïdie.

Hoofdstukken 2 en 3 beschrijven de resultaten van eigen studies. De effecten van vaak gebruikte geneesmiddelen in de kleine huisdierenpraktijk op resultaten van schildklierfunctietesten werden onderzocht. De toediening van geneesmiddelen bij ratten en mens kan de synthese, de secretie, het transport of het metabolisme van schildklierhormonen beïnvloeden. Sommige geneesmiddelen kunnen ook rechtstreeks de hypothalamus-hypophyse-schildklieras inhiberen. Speciesverschillen bestaan op alle niveaus van de schildklieras. Daarom is het niet verwonderlijk dat de geneesmiddel-schildklierinteractie interspeciesvariatie zal vertonen. Verschillende geneesmiddelen kunnen bij de mens uitgesproken veranderingen teweeg brengen in de resultaten van schildklierfunctietesten, zodat de interpretatie van deze resultaten soms moeilijk is. Ze geven eerder zelden aanleiding tot klinische tekens van schildklierdysfunctie. De

mogelijke effecten van veel gebruikte geneesmiddelen op de schildklierfunctietesten bij de hond werden nog niet nagegaan.

De effecten van kortetermijntoediening (3 weken) van orale prednisone en phenobarbital op de schildklierfunctietesten werden bestudeerd (hoofdstuk 2). Phenobarbitalbehandeling in onze studie had geen invloed op TT4 (totale thyroxine), FT4 (vrije thyroxine), en TSH (thyrotropine) serumconcentraties. Phenobarbital serumconcentraties verkregen in onze studie lagen echter iets onder de therapeutische plasmaspiegels. Prednisone toegediend aan een immunosuppressieve dosering daarentegen verminderde significant de serum TT4 en in een mindere mate FT4, maar serum TSH concentraties bleven onveranderd. Een week na toediening van prednisone, hadden 8 van de 9 honden een TT4 onder 15 nmol/L, een waarde die gezien wordt bij hypothyroïdie. TT4 en FT4 serumconcentraties normaliseerden binnen de week na stopzetten van de prednisonetoediening. Vrije T4 serumwaarden werden minder beïnvloed dan TT4 serum concentraties door de toediening van een immunosuppressieve dosis prednisone gedurende drie weken. Men kan besluiten dat de toediening van een immunosuppressieve prednisonedosis een uitgesproken effect heeft op de schildklierhomeostase en kan leiden tot vals-positieve diagnoses van hypothyroïdie.

Niet-steroïdale ontstekingsremmers (NSAIDs) worden meer en meer gebruikt bij de hond. Daarom is het belangrijk om de mogelijke invloed van NSAIDs na te gaan op de resultaten van schildklierfunctietesten. De invloed van acetylsalicylzuur (ASA) en ketoprofen (Keto) op de schildklierfunctietesten bij de hond werd nagegaan (hoofdstuk 3). Een significante daling van TT4 serumconcentraties werd vastgesteld, reeds 24 uren na de ASA toediening, terwijl de daling van TT3 minder uitgesproken was. Er werd geen significante invloed gevonden op FT4 en TSH serumconcentraties na ASA toediening. Ketoprofen toediening had geen significante invloed op de schildklierfunctietesten. De resultaten tonen aan dat TT4 uitgesproken verlaagd kan zijn door ASA therapie en dit weerspiegelt hoogst waarschijnlijk de verdringing van de schildklierhormonen van hun bindingsplaatsen op de serumeiwitten. Tot er meer studies

gepubliceerd zijn, moeten schildkliertesten met de nodige voorzichtigheid geïnterpreteerd worden bij honden onderworpen aan een NSAIDs behandeling. Een 7-daagse behandeling resulteerde in een daling van TT4, die potentieel kon leiden tot overdiagnose van hypothyroïdie bij meer dan de helft van de honden. Vrije T4, bepaald door evenwichtsdialyse, en TSH serumconcentraties werden minder beïnvloed door ASA en Keto toediening dan TT4. Daarom zou de diagnose van hypothyroïdie bij een hond op NSAIDs therapie niet gebaseerd mogen zijn op een bepaling van TT4 alleen. Na een 1-week toediening van ASA, en misschien ook van andere NSAIDs, is het aan te bevelen een zevental dagen te wachten alvorens schildklierfunctietesten uit te voeren bij de hond.

Om verder de mogelijke invloed van vaak voorkomende klinische omstandigheden op de schildklierfunctietesten na te gaan, werd de invloed van obesitas en gewichtsverlies geëvalueerd in hoofdstuk 4. Bij de hond is obesitas de meest voorkomende nutritionele aandoening en gewichtsverlies is de hoeksteen van de behandeling. Daarom is het belangrijk na te gaan hoe obesitas en gewichtsverlies schildklierfunctietesten kunnen beïnvloeden bij de hond. De doelstellingen van deze studies waren eerst de schildklierfunctie te vergelijken tussen honden met obesitas en slanke honden. Verder werden de effecten van calorierestrictie en gewichtsverlies op schildklierhormoonconcentraties nagegaan bij honden met obesitas. Totale T3 en TT4 serum concentraties waren significant hoger bij honden met obesitas in vergelijking met deze bij slanke honden. Gewichtsverlies resulteerde in een significante daling van TT3 en TSH serumconcentraties. In deze studie werd ook recombinante humane TSH (rhTSH) gebruikt om TSH stimulatietesten uit te voeren. Het gebruik van 75 µg rhTSH in onze studie voor honden met een lichaamsgewicht tussen 10,8 en 25,9 kg (maar met obesitas) liet een optimale stimulatie van de schildklier toe. Alle honden (slank en met obesitas) voldeden aan de criteria gesuggereerd door Sauvé en Paradis om euthyroïdie te bevestigen. Bovendien bleek een staalname 6 uren na het injecteren van rhTSH optimaal en het staal genomen na 4 uren staal niet echt noodzakelijk. Onze bevindingen tonen aan dat de rhTSH stimulatietest een interessant middel is om schildklierfunctie te

evalueren bij honden in de kliniek of in een experimentele opzet om schildklierfysiologie te bestuderen.

Er kan besloten worden dat obesitas en gewichtsverlies tot een significante verandering van de schildklierhomeostase bij de hond leiden, maar het is weinig waarschijnlijk dat deze veranderingen relevant zijn voor de interpretatie van schildklierfunctietesten in de kliniek.

Klinische tekens van hypothyroïdie zijn niet specifiek; dit bemoeilijkt de diagnose van hypothyroïdie bij de hond. Verscheidene ziekten hebben een klinisch beeld dat lijkt op hypothyroïdie. Het meest opvallend voorbeeld is de symmetrische flankalopecie, frequent gezien bij hypothyroïdie maar ook een typisch kenmerk van een recent erkende dermatologische aandoening, namelijk recidiverende flankalopecie bij de hond. De laatste decennia werden veel honden foutief gediagnosticeerd als hypothyroïd wanneer ze eigenlijk aan CRFA leden. De etiologie van CRFA is grotendeels onbekend. In hoofdstuk 5 worden de resultaten beschreven van het onderzoek naar de schildklierfunctie bij honden met CRFA. Totale T4 en TSH serumconcentraties waren respectievelijk significant verminderd en verhoogd in de hypothyroïdiegroep in vergelijking met controle en CRFA groepen. Geen significante verschillen in TT4 en TSH serumwaarden werden gevonden tussen de euthyroïde en CRFA groepen. Thyroglobuline-antilichamen (TgAA) werden gedetecteerd in respectievelijk 10, 11.1, en 61.9 % van de euthyroïde honden, honden met CRFA, en hypothyroïde honden. Er kan besloten worden dat honden met CRFA een normale schildklierfunctie hebben, en dat, de bepaling van TT4 en TSH serumconcentraties in de meeste gevallen differentiatie toelaat tussen CRFA honden en honden met hypothyroïdie.

De huidige studie draagt bij tot de kennis van verscheidene aspekten van de schildklierfunctie bij de hond. Er werd vastgesteld dat verscheidene faktoren de schildklierfunctietesten kunnen beïnvloeden bij de hond en dat TT4 serumconcentraties snel en duidelijk verlaagd kunnen zijn door toediening van sommige vaak gebruikte

geneesmiddelen. Het effect van gewichtsverlies op schildklierhormoonconcentraties was ook significant, maar deze veranderingen zijn klinisch weinig relevant. De oorzaak van caniene recidiverende flank alopecie blijft onbekend maar in deze studie kon schildklierdysfunctie als voornaamste oorzaak van deze ziekte worden uitgesloten.

Door rekening te houden met de invloed van bepaalde geneesmiddelen en omstandigheden op de schildklierfunctietesten, worden er hopelijk minder vals-positieve diagnose van hypothyroïdie gesteld en bijgevolg ook minder onnodige behandelingen uitgevoerd.

SAMENVATTING

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Sylvie

DANKWOORD

AUTHOR'S CURRICULUM

Sylvie Daminet werd op 21 juli 1968 geboren te Genk, België. Zij begon in 1986 met de studie Diergeneeskunde aan de Universiteit Luik en behaalde het diploma van Doctor in de Diergeneeskunde in 1992 met grote onderscheiding.

Onmiddellijk daarna voltooide ze een internship gevolgd door een residency in de Interne Geneeskunde van de Kleine Huisdieren aan de Universiteit van Montreal in Canada. In 1996 werd zij diplomate van het Amerikaanse en het Europese College van Interne Geneeskunde (ACVIM&ECVIM-ca). In 1998 behaalde zij een Masters degree over de evaluatie van de schildklierfunctie bij de hond. Ze doceerde daarna aan de Faculteiten van Montreal en Prince Edward Island in Canada en was Lecturer aan de Royal Veterinary College van London in Engeland. Dr Daminet is nu reeds 2 jaren gastprofessor aan de Vakgroep Geneeskunde en Klinische Biologie van de Kleine Huisdieren en is er verantwoordelijk voor de Interne Geneeskunde van de Kleine Huisdieren. Binnen haar algemene activiteiten als internist heeft ze een bijzondere interesse voor de endocrinologie en de urologie/nefrologie van de kleine huisdieren.

Haar interesse in de 'schildklierproblematiek' bij de hond ontstond vanuit de klinische ervaring met vele honden die onterecht behandeld werden voor hypothyroïdie. Haar onderzoek in samenwerking met Prof. Paradis over deze topic begon in 1996. Uit deze eerste studies vloeiden verdere studies voort aan de Faculteit Diergeneeskunde van Gent onder promotorschap van Prof. De Rick.

Sylvie Daminet is auteur of mede-auteur van 31 publicaties in nationale en internationale tijdschriften. Zij gaf vele postuniversitaire bijscholingen voor practici en was 8 keren uitgenodigde spreker op internationale congressen. Ze is secretaresse van de European Society of Veterinary Urology and Nefrology en is lid van het examencomité van de ECVIM-ca.

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