Thyroid Function in Dogs with Spontaneous and Induced Congestive Heart Failure

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ABSTRACT

The effects of spontaneous and experimentally induced congestive heart failure on serum thyroxine (T4), 3,5,3′-triiodothyronine (T3), 3,3′5′-triiodothyronine (reverse T3), free T4, free T3 concentrations, and the serum T4 and T3 concentrations in response to administration of thyrotropin were studied. Serum thyroid hormone concentrations were not different between eight dogs with spontaneous congestive heart failure and normal age matched control dogs. Seven dogs with experimental heart failure were tested before and after induction of congestive heart failure by rapid ventricular pacing. Mean serum T4 and free T3 concentrations were decreased and mean serum reverse T3 concentration was increased following induction of heart failure. The serum T4 and T3 responses to thyrotropin were not altered. Thyroid gland morphology appeared normal in dogs with experimental heart failure. Experimental congestive heart failure, similar to some other nonthyroidal illnesses, alters thyroid hormone secretion and metabolism in dogs.

RÉSUMÉ

Dans la présente étude on a mesuré les effets d'une insuffisance cardiaque congestive spontanée et induite expérimentalement sur les concentrations de thyroxine sérique (T4), 3,5,3′-triiodothyronine (T3), 3,3′5′-triiodothyronine (T3 inverse), de T4 et T3 libres, ainsi que les concentrations de T4 et T3 sériques suite à l'administration de thyrotropine. Aucune différence n'a été notée dans les concentrations sériques d'hormone thyroïdienne de huit chiens avec une insuffisance cardiaque congestive spontanée lorsque comparées à celles de témoins normaux du même âge. Des données ont également été obtenues chez sept chiens avant et après induction expérimentale par tachycardie ventriculaire artificielle d'une insuffisance cardiaque congestive. Les concentrations moyennes de T4 sérique et de T3 libre étaient diminuées et la concentration sérique moyenne de T3 inverse était augmentée suite à l'induction de l'insuffisance cardiaque. L'administration de thyrotropine n'a pas modifié les concentrations de T4 et T3 sériques. La morphologie de la glande thyroïde apparaissait normale chez les chiens chez qui on a induit une insuffisance cardiaque. L'insuffisance cardiaque congestive expérimentale peut altérer la sécrétion d'hormone thyroïdienne et le métabolisme chez les chiens, de façon similaire à d'autres maladies d'origine non-thyroïdienne. ***(Traduit par Dr Serge Messier)***

INTRODUCTION

Systemic illness has the potential for a wide array of effects on thyroid function. Serum thyroid hormone concentrations are frequently decreased in man and animals with severe illness (1,2). A distinct pattern of altered thyroid function has emerged in human medicine, with reduction of serum total 3,5,3′-triiodothyronine (T3) and an elevation of 3,3′5′-triiodothyronine (reverse T3; rT3) occurring with mild to moderate illness, decreased total thyroxine (T4) in severe illness, and maintenance of nonprotein bound or free thyroxine (fT4) in most illnesses (1). Many factors interact to create these changes including decreased pituitary thyrotropin secretion, altered binding of thyroid hormones to plasma carrier proteins or cellular binding proteins, altered distribution and metabolism of hormones, and decreased thyroid hormone secretion (1). From the limited studies performed in the dog, it appears that serum T4 concentration is reduced at least as readily as T3 (2–8).

While severity of illness is an important consideration in euthyroid sick syndrome, the type of illness is a major factor in the pattern of serum thyroid hormone concentrations in man. Renal failure and acquired immunodeficiency syndrome, for example, are associated with a decrease in serum T3 concentration, but a normal or decreased serum reverse T3 concentration (1). In dogs, a wide variety of changes in serum thyroid hormone concentrations have been described. However, reports of specific diseases are few and often conflicting. This study was undertaken to determine the effect of congestive heart failure on thyroid function in dogs. Congestive heart failure was chosen because of the effects of thyroid hormones on cardiac function (9) and the high incidence of both

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hypothyroidism and cardiac disease in some breeds (10,11).

MATERIALS AND METHODS

ANIMALS

Eight dogs with spontaneous congestive heart failure, seven dogs with experimentally induced congestive heart failure, and eight normal age matched dogs (used as controls for the spontaneous heart failure group) were studied.

Spontaneous congestive heart failure — Dogs with congestive heart failure were identified from cases presented to the Veterinary Teaching Hospital, Western College of Veterinary Medicine, University of Saskatchewan. Congestive heart failure was diagnosed by demonstration of cardiac disease (mitral and tricuspid insufficiency in six, dilated cardiomyopathy in one, and patent ductus arteriosus in one), pulmonary edema (seven dogs), ascites (one dog), tachycardia (eight dogs), exercise intolerance (eight dogs), and cough (three dogs). The underlying cause of heart failure was determined by historical, physical examination, radiographic, and echocardiographic findings. All dogs were judged to be in New York Heart Association (NYHA) functional class III or IV heart failure. Functional class III heart failure was considered present if dogs had signs of heart failure during normal activity; functional class IV heart failure was recorded if a dog had signs of heart failure at rest or with minimal activity. The mean age was 10.5 ± 4.3 years. Six dogs were spayed females, while two were male and castrated male, respectively. Complete blood counts were normal or consistent with a stress leukogram in all cases. Serum biochemical profiles were performed in all dogs with abnormalities consisting of mild elevation of alanine aminotransferase and alkaline phosphatase activity in one dog and an elevated cholesterol concentration (7.95 nmol/L) in one dog.

Experimental congestive heart failure — Dogs were determined to be normal before study based on physical examination and normal thyroid function tests. Severe heart failure (NYHA class IV) was induced in all dogs in this group as evidenced by exercise intolerance and pulmonary edema. Seven conditioned, healthy, adult dogs weighing between 21 and 28 kg were used in the study. The ages of dogs were unknown, but were estimated to be between three and seven years. Four dogs were male and three were female. Dogs were housed in indoor runs at 25°C with a 12-hour light:dark cycle. Congestive heart failure was induced for purposes of studying the effect of heart failure on myocardial uptake of ^{131}I-metaiodobenzylguanidine (MIBG), a radiopharmaceutical which is concentrated in sympathoadrenal tissue. All dogs described in our study were used primarily for study of myocardial catecholamine uptake, and studies of thyroid function were designed to take advantage of this opportunity. This protocol was approved by the University Committee on Animal Care and Supply, University of Saskatchewan and the study followed the guidelines of the Guide to the Care and Use of Experimental Animals, the Canadian Council on Animal Care. Dogs underwent scintigraphy six hours after intravenous administration of 500 microcuries of ^{131}I-MIBG while under general anesthesia. Scintigraphy was performed twice, once after initial thyroid function testing and once following a second thyroid function test after induction of heart failure which was at least six weeks after initial ^{131}I-MIBG administration. One to two weeks after initial scintigraphy, a programmable pacemaker (Medtronics, Inc.) was implanted in each dog using a right lateral thoracotomy. The electrode was placed in the left ventricle and the pulse generator was implanted subcutaneously on the left flank. Dogs were allowed to recover for at least two weeks before the pacemaker was activated. The pacemaker was programmed at a rate of 250 beats per minute. The period of rapid ventricular pacing necessary for development of congestive heart failure ranged from two to four weeks. Congestive heart failure was confirmed using criteria similar to those described for spontaneous heart failure. Treatment of heart failure as well as discontinuation of rapid ventricular pacing was instituted in severe cases of congestive heart failure.

Control dogs — Healthy client-owned dogs were used as an age-matched control group for the spontaneous heart failure group. They were determined to be healthy on the basis of normal physical examination, complete blood count, and serum biochemical profile. The mean age was 9.7 ± 5 years. Two dogs were intact females, three were spayed females, one an intact male and two castrated males.

STUDY DESIGN

No dog in any group had received medication for at least two weeks before being studied and none had been previously treated for congestive heart failure. The initial basal blood sample was taken before treatment for heart failure was initiated. A thyrotropin response test was performed in all dogs with experimentally induced heart failure one to six weeks before pacemaker implantation prior to administration of ^{131}I-MIBG. Dogs with experimentally induced heart failure were tested at the time that initial clinical signs of heart failure were noted, before discontinuation of rapid ventricular pacing. Following radionuclide imaging, dogs with experimentally induced heart failure were euthanized and thyroid glands were collected and fixed in 10% buffered formalin. Following fixation, thyroid glands were sectioned on the midline, perpendicular to the long axis and three 5 μm sections were stained with periodic acid-Schiff and examined by light microscopy.

Thyrotropin response tests were performed by obtaining blood samples before and six hours after intravenous administration of 0.15 IU/kg of bovine thyrotropin (Sigma Chemical Co., St. Louis, Missouri). Serum was obtained within two hours of blood collection and stored frozen at −20°C until assayed. Samples were analyzed for T4 (12; Magic T4 [^{125}I] Radioimmunoassay, CIBA Corning Diagnostics Corp., East Walpole, Massachusetts), T3 (13), and reverse T3 (rT3) (14; Reverse T3 PEG Method, Serono-Baker Diagnostics, Inc., Allentown, Pennsylvania) by previously validated radioimmunoassays. Free T4 (fT4) was determined using an equilibrium dialysis kit (Free T4 by Equilibrium Dialysis, Nichols Institute, Los Angeles, California)

TABLE I. Serum thyroid hormone concentrations in dogs with experimental congestive heart failure

	Preheart failure		Heart failure		
	mean ± sd	range	mean ± sd	range	Normal
T4 (nmol/L)	34 ± 10.7	16–45	21 ± 13.1[a]	6.5–44	22–54
T3 (nmol/L)	0.95 ± 0.57	0.6–1.6	0.57 ± 0.45	0.1–1.4	1.2–3.1
fT4 (pmol/L)	18.2 ± 10.7	6.9–27.6	11.9 ± 6.0	4.2–29.8	8.9–40.4
fT3 (pmol/L)	4.86 ± 1.01	3.5–6.0	2.71 ± 1.96[a]	0.8–5.4	3–6
rT3 (nmol/L)	0.65 ± 0.31	0.09–1.0	1.16 ± 0.54[a]	0.66–2.05	0.41–0.95
T3/T4	0.03 ± 0.02	0.01–0.07	0.03 ± 0.01	0.01–0.05	
rT3/T4	0.02 ± 0.01	0.002–0.034	0.10 ± 0.09	0.002–0.244	
T3/rT3	1.28 ± 0.77	0.53–2.32	0.55 ± 0.60	0.06–1.67	
fT3/rT3	6.93 ± 2.89	4.30–10.94	2.84 ± 3.02	0.47–8.18	
T4 post TSH	89 ± 17.2	69–116	73 ± 32.5	24–112	54–120
T4 post/T4 pre TSH	2.62 ± 0.75	1.67–4.79	3.06 ± 0.52	2.52–3.75	
T3 post TSH	1.93 ± 1.66	0.8–4.6	2.33 ± 1.16	0.5–3.6	
T3 post/T3 pre TSH	2.72 ± 1.88	1.58–5.11	3.42 ± 1.35	1.86–5.64	
Albumin (g/L)	33 ± 3.2	27–37	32 ± 5.4	25–37	27–36

[a] Significantly different ($p < 0.05$) than before induction of congestive heart failure

validated for use in the dog (15). Measurement of free T3 (fT3) was accomplished by a labelled analogue radioimmunoassay (Magic [^{125}I] Radioimmunoassay, CIBA Corning Diagnostics Corp.). The specificity of this assay as provided by the manufacturer was given as percent cross-reactivity with related compounds: tetraiodothyronine, 0.64%; D-thyroxine, 0.17%; L-thyroxine, 0.13%; all other iodothyronines <0.01%. The sensitivity of the fT3 assay was 0.3 pmol/L, determined as the point of 90% of total binding on the standard curve. Intraassay coefficients of variation were 1.1% and 1.7%, as determined in 30 samples of each of two serum pools with fT3 concentrations of 4.3 ± 0.4 and 7.9 ± 1.0 pmol/L, respectively. Interassay coefficients of variation on the same two serum pools were 9.3% and 12.7%, respectively. Accuracy (addition and recovery) and dilutional parallelism were not determined as they are not applicable for this type of assay. Serum albumin concentration was assessed by a bromcresol green method using an automated chemistry analyzer (Ektachem 500, Eastman Kodak Co., Rochester, New York).

STATISTICAL ANALYSIS

Results of thyroid hormone analyses and serum albumin obtained after induction of congestive heart failure were compared to those before heart failure using the paired Student's t-test. Dogs with spontaneous heart failure were compared to control dogs using a Student's unpaired t-test.

RESULTS

Results of thyroid hormone tests are reported in Tables I and II. Significant differences were not present in any serum iodothyronine measured when dogs with spontaneous congestive heart failure were compared with controls. Decreases in basal serum T4 ($p = 0.048$) and fT3 ($p = 0.037$) were noted following experimental induction of heart failure. Serum rT3 concentration was higher after induction of heart failure ($p = 0.041$). Serum T4 and T3 concentrations following thyrotropin administration were not different in dogs with congestive heart failure occurring spontaneously or experimentally. Because of the relationship between production of T3 and rT3 from T4, ratios of T3:rT3, fT3:rT3, rT3:T4, and T3:T4 were calculated. The fT3:rT3 and rT3:T4 ratios approached significance ($p = 0.057$ and 0.06 respectively) in dogs with experimentally induced heart failure.

Serum albumin concentration was not significantly different before and after induction of heart failure experimentally or between control dogs and those with spontaneous heart failure. The age and sex of controls and dogs with spontaneous heart failure were not significantly different. Histological examination of thyroid glands failed to reveal any abnormalities.

DISCUSSION

The pattern of serum thyroid hormone changes noted in dogs with experimentally induced congestive heart failure in the present study is similar in most respects to that found in dogs with a variety of illnesses (2–8,10,16). Serum T4 concentration has been shown to be decreased in some dogs with hyperadrenocorticism (4,5), hypoadrenocorticism (2), diabetic ketoacidosis (2), chronic renal failure (2), peripheral neuropathy (6), cachexia from a variety of illnesses (3), and congestive heart failure (10,16). In two studies of Doberman pinschers with congestive heart failure, the mean baseline serum T4 concentrations were reduced, while single baseline T4 concentrations were below the reference range in 5 of 21 dogs (10,16). Measurement of free hormones theoretically represents a more accurate assessment of the functional thyroid hormone status than total hormone concentrations because it accounts for any alteration in protein binding of hormones. In some instances, such as diseases associated with cachexia, fT4 seems to most accurately reflect the euthyroid state since T4 and T3 are decreased while fT4 measured by equilibrium dialysis is normal (3). The decrease in T4 without a significant decrease in fT4 in experimental heart failure suggests that binding of T4 to carrier proteins in the plasma is reduced.

The increased rT3 in dogs with experimental heart failure is indicative of either increased production or decreased metabolism and/or excretion of rT3. This finding combined with the reduction in fT3, the trend toward a decrease in fT3:rT3 ratio and

TABLE II. Serum thyroid hormone concentrations in dogs with spontaneous congestive heart failure

	Control		Heart failure	
	mean ± sd	range	mean ± sd	range
T4 (nmol/L)	31 ± 7.1	18–41	27 ± 12.8	8–47
T3 (nmol/L)	1.20 ± 0.17	0.9–1.4	1.18 ± 0.55	0.4–2.2
fT4 (pmol/L)	15.3 ± 4.1	9.0–21.1	20.4 ± 8.2	1 2.6–28.9
fT3 (pmol/L)	5.4 ± 0.75	3.8–6.0	4.9 ± 2.49	1 .7–8.5
rT3 (nmol/L)	0.68 ± 0.29	0.09–0.97	0.56 ± 0.40	0.09–1.1
T3/T4	0.04 ± 0.01	0.03–0.07	0.05 ± 0.03	0.03–0.12
rT3/T4	0.05 ± 0.01	0.0–0.044	0.03 ± 0.03	0.002–0.044
T3/rT3	1.53 ± 0.28	1.11–2.0	1.64 ± 0.84	0.06–0.48-2.65
fT3/rT3	6.84 ± 1.05	5.76–8.92	7.16 ± 4.17	2.02–12.84
T4 post TSH	80 ± 19.2	61–122	66 ± 19.6	34–91
T4 post/T4 pre TSH	2.73 ± 0.80	1.79–4.26	2.80 ± 1.19	1.75–5.09
T3 post TSH	1.95 ± 0.70	1.1–2.7	2.38 ± 1.21	1.1–4.8
T3 post/T3 pre TSH	1.67 ± 0.66	1.00–2.70	2.09 ± 0.61	1.22–3.00
Albumin (g/L)	33 ± 4.4	29–39	30 ± 4.5	23–40

an increase in the rT3:T4 ratio suggest that decreased activity of 5′-deiodinase may be responsible for these changes. This enzyme is responsible for the deiodination of T4 to T3 and rT3 to diiodothyronine (20). Any decrease in 5′-deiodinase activity would result in an increase in rT3 as well as a decrease in T3. With the exception of hyperadrenocorticism (5), rT3 has been found to be elevated, often markedly in dogs with nonthyroidal illness. (2,8).

As found in dogs with experimental heart failure, serum T3 concentration is less consistently decreased in nonthyroidal disease in the dog (2–8). The finding of reduced serum fT3 concentration without a significant reduction in serum T3 in dogs with heart failure may simply be a reflection of the small number of dogs included in this study since one would expect a decrease in T3 concurrent with a decrease in fT3 unless plasma protein binding is increased. Increased protein binding, if prolonged, should result in an increase in T3, since less fT3 will be available to enter the pituitary to suppress thyrotropin secretion. The statistically insignificant tendency for a decrease in T3 could account for some of the decrease in fT3. Alternatively, the decrease in fT3 may reflect a flaw in the radioimmunoassay for fT3. The fT3 assay was a single step, solid phase assay which relies on a labeled T3 analog competing for the antibody. A T3 analog theoretically has no affinity for thyroxine-binding globulin and chemical blockers to prevent attachment to albumin are used to eliminate analog binding to albumin (17). This minimizes the effects that alterations in albumin concentrations may have on measured fT3 concentrations. However, circulating inhibitors of binding of thyroid hormones to carrier plasma proteins have been identified in humans with nonthyroidal illness (18) which may have important effects on free hormone radioimmunoassays. Some radioimmunoassays of free hormones have significant flaws when used in certain situations (17,19). Free T3 concentrations were found to be low in humans with nonthyroidal illness using several radioimmunoassay techniques, but it is controversial whether this accurately reflects the circulating concentrations or is merely artifactual (1). Comparison of the radioimmunoassay used in the present study with equilibrium dialysis or ultrafiltration techniques in normal and ill dogs will ultimately be necessary to determine the utility of the assay.

The finding of decreased T4 and fT3 and increased rT3 in dogs with experimental heart failure and not in those with spontaneous heart failure may be explained by the fact that heart failure developed more rapidly in the experimental group and that dogs in the experimental group acted as their own controls. While the healthy control dogs were matched with the spontaneous heart failure group for age, sex (21) and breed (22) differences could contribute to the failure to demonstrate a difference in thyroid hormone concentrations in this study. The decrease in serum T4 found in previous studies of doberman pinschers with spontaneous heart failure supports the validity of findings in dogs with experimental heart failure in the present study.

There is some evidence that nutritional status plays an important role in alteration of serum thyroid hormones. Weight loss is correlated with decreases of serum T4, T3 and fT3 concentrations in dogs with cachexia resulting from a variety of illnesses (3). Nutritional status was assessed in the current study by measurement of serum albumin concentration. Evidence of chronic malnutrition was not noted in either group of dogs with congestive heart failure as assessed by serum albumin. Poor nutritional status, however, could be an important factor in dogs of this study without a decrease in serum albumin concentration since chronicity of disease and alterations in fluid balance might influence serum albumin.

Dynamic testing of thyroid function has been reported rarely in dogs with nonthyroidal illness. Dogs with hyperadrenocorticism (4,7), hypoadrenocorticism (7), and diabetes mellitus (7) have a reduced serum T4 response to thyrotropin administration. The serum T4 concentration in response to thyrotropin administration in 13 Doberman pinschers with congestive heart failure and dilated cardiomyopathy was 48 nmol/L, with a range of 6–103 nmol/L. Of five dogs with baseline T4 below normal, four had a normal response to thyrotropin administration, where normal was considered to be at least a twofold increase in baseline T4 if that value was at least 13 nmol/L (10). While that mean postthyrotropin T4 concentration suggests that there may be some reduction of responsiveness of the thyroid gland to thyrotropin in heart failure, our study indicates that thyroid reserve is unaffected in both spontaneous and experimentally induced congestive heart failure. This finding provides evidence that the mechanism resulting in depression of serum T4 and fT3 is not the result of direct effects on thyroidal secretion, but rather on the peripheral distribution, metabolism and excretion of these hormones. However, the thyrotropin response test utilizes a supra-

physiological dose and may not accurately reflect the sensitivity of the thyroid gland to physiological control by thyrotropin. In addition, one dog with experimentally induced heart failure had a subnormal T4 response (pre = 7; post = 24 nmol/L) to thyrotropin administration despite normal histological appearance of the thyroid gland.

Similar to dogs with experimental heart failure, people with advanced heart failure have been shown to have a low fT3 index, elevated rT3 and normal fT4 index (23). A decrease in the fT3 index/rT3 ratio was found in people with the most severe disease and was inversely correlated with survival. Dogs with heart failure also had a decrease in serum T4 concentration, a finding which appears to occur more frequently and with less severe illness in the dog than in man (2,3,8). In diseases other than heart failure, decreased serum T4 concentration is most closely correlated with a poor prognosis in people with most severe illnesses (23,24). The finding of a normal serum fT4 concentration in dogs in the present study suggests that a euthyroid state is present. Because thyroid hormones are integral to normal cardiac function, any decrease in activity of thyroid hormones at the tissue level could result in significant detrimental effects in patients with cardiovascular disease (25,26).

The mechanisms inducing changes in thyroid hormone concentrations in congestive heart failure are unknown. It is becoming apparent that cytokines including interleukins, interferon and tumor necrosis factor-alpha (TNF) may be responsible for many of the changes (27). Tumor necrosis factor-alpha has been shown to decrease thyrotropin releasing hormone and thyrotropin secretion and inhibits plasma thyroid hormone binding (28). Infusion of TNF in normal people results in alterations in thyroid function similar to those present with nonthyroidal illness, including decreased serum T3 and thyrotropin as well as increased serum rT3 (29). Tumor necrosis factor is elevated in many people with severe chronic heart failure (30) and could be responsible for the alterations in serum thyroid hormone concentrations found in the present study.

In conclusion, experimentally induced congestive heart failure resulted in decreased serum T4 and fT3 concentrations concurrent with increased serum rT3 concentrations. Because responsiveness to thyrotropin administration was minimally affected, thyroid function should be assessed using the thyrotropin response test, when indicated, in a dog with congestive heart failure.

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