DIFFERENTIATION BETWEEN DOGS WITH PRIMARY HYPOTHYROIDISM AND DOGS WITH NON-THYROIDAL ILLNESS BASED ON PLASMA CONCENTRATIONS OF GROWTH HORMONE AND THYROID STIMULATING HORMONE (TSH) WITH A TSH-RELEASING HORMONE STIMULATION TEST.

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MASTER THESIS

Title: Differentiation between dogs with primary hypothyroidism and dogs with non-thyroidal illness based on plasma concentrations of growth hormone and thyroid stimulating hormone (TSH) with a TSH-releasing hormone stimulation test.

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KEYWORDS

Primary hypothyroidism, Non-thyroidal illness, dogs, TRH-stimulation test

ABSTRACT

Primary hypothyroidism is one of the most common endocrine disorders in dogs. In most dogs with primary hypothyroidism total thyroxin (TT4) and total triiodothyronine (TT3) are below their respective reference range. However, these thyroid hormones may also be low in dogs with a normal thyroid function due to non-thyroid illness (NTI) or drugs. Additionally the plasma thyroid-stimulating hormone (TSH) concentration should be measured when TT4 is below its reference range. When the plasma TSH concentration is clearly above the upper limit of the reference range the dog has primary hypothyroidism. However when the TSH plasma concentration is within the reference range, the dog may still have primary hypothyroidism. When a low TT4 in combination with a normal cTSH has been found, the dog could undergo a biopsy of the thyroid gland or scintigraphy of the thyroid gland. However these tests are invasive for the dog and expensive for the owner. Thereby a thyroid scintigraphy can only be performed in a few specialized institutions. Previous studies showed that TSH producing cells in dogs with primary hypothyroidism become desensitized for TRH and that these dogs show signs mimicking acromegaly. The aim of this study is to investigate a different way to differentiate between dogs with primary hypothyroidism and dogs with NTI, using TRH stimulated plasma concentrations of GH and TSH. The hypothesis is that the plasma concentration of GH will increase and that of TSH will not increase in dogs with primary hypothyroidism after TRH has been injected. The other way around, it is hypothesized that in dogs with NTI, the plasma concentration of GH will not increase significantly and the plasma TSH concentration will increase after administration of TRH. If that is true, it is possible to differentiate between dogs with primary hypothyroidism and dogs with NTI. Plasma GH concentrations differed statistically significant both basal and 30 respectively 45 minutes after TRH administration ($p = 0.036$, $p = 0.017$, and $p = 0.036$, respectively). The TRH stimulated plasma TSH concentrations did also differ statically significant between both groups ($p = 0.011$ at 30 minutes after TRH administration and $p = 0.023$ at 45 minutes after TRH administration). A diagnostic test could be based on the plasma GH concentration in combination with an increase in TSH after TRH administration. The test could also be based on the GH/TSH ratio at 45 minutes after TRH administration.

INTRODUCTION

With regard to endocrine disorders in dogs, primary hypothyroidism is one of the most common disorders. Primary hypothyroidism is mostly caused by autoimmune destruction of thyroid gland tissue. It may also have a genetic cause.[1] In most dogs with hypothyroidism, the concentrations of total thyroxin (TT4) and total triiodothyronine, (TT3) are below their respective reference range. However these thyroid hormones may also be low in dogs with a normal thyroid function that suffer from non-thyroidal illness (NTI) or due to drugs.[2-4] Therefore, low concentrations of TT4 and TT3 in dogs are not very specific for

hypothyroidism. More specific than TT4 concentration is the concentration of free thyroxin (fT4), however this may also be low in dogs with NTI or even in some healthy dogs.[2,9] This makes the value of a low thyroid hormone concentration limited and therefore stimulation tests using either thyroid-stimulating hormone (TSH) or TSH-releasing hormone (TRH) have been advocated. Unfortunately the TRH-stimulation test, with measurement of plasma TT4, is not able to distinguish reliably between dogs with primary hypothyroidism and dogs with NTI. The TSH-stimulation test is rather expensive, however it is the best biochemical test to differentiate between NTI dogs and hypothyroid dogs.[6,7,10] The diagnosis of primary hypothyroidism is nowadays commonly based on a combination of a low circulating TT4 concentration and a high plasma endogenous TSH concentration, just like in human medicine.[11,12] However, in one third of the hypothyroid dogs the plasma TSH concentration is within the reference range.[6] Scintigraphy is a technique with the power to differentiate between dogs with primary hypothyroidism and dogs with NTI[6], however this can only be performed in a few specialized institutions. Another option is to take a biopsy for histological examination, a rather sensitive but invasive method. Finally, antibodies against thyroglobulin may help to differentiate between dogs with primary hypothyroidism and dogs with NTI. However, these antibodies may be also detected in dogs without hypothyroidism and may be absent in quite some dogs with primary hypothyroidism[13], which makes it an unreliable test. Consequently, there is a need for a reliable, inexpensive and practical test to differentiate between the two groups known as the dogs with primary hypothyroidism and those with NTI with a low thyroid hormone concentration and a plasma TSH concentration which is not clearly increased.

In previous studies the basal and TRH-stimulated release of TSH has been investigated in both healthy (euthyroid) dogs and dogs with primary hypothyroidism. As some dogs with primary hypothyroidism showed signs mimicking acromegaly, growth hormone (GH) levels, both basal and TRH-stimulated, were measured.[8] The basal plasma TSH levels were clearly elevated in dogs with primary hypothyroidism, however after prolonged periods of hypothyroidism the levels declined towards the reference range.[8] In contrast to the euthyroid dogs, the plasma concentration of TSH did not increase significantly after administration of TRH in dogs with primary hypothyroidism. This makes it likely that the continuously high hypothalamic TRH secretion may lead to desensitization of the pituitary thyrotropes, comparable to the desensitization of gonadotropes during continuous GnRH stimulation. Additionally it has been shown that primary hypothyroidism is associated with increased GH secretion, at least partly originating from bihormonal pituitary cells that produce both TSH and GH.[1] Due to these changes in the pituitary cells, GH release could be induced by intravenous administration of TRH, whereas no significant GH response is measured in euthyroid dogs.[8] The increase in basal plasma GH concentrations in dogs with primary hypothyroidism may be related to the high TRH release from the hypothalamus or a lower hypothalamic somatostatin secretion. Till now, no data are available on the effect of TRH stimulation in dogs with NTI. It is thought that these dogs will have normal or even lower basal plasma GH concentrations and that they will not have TRH-induced GH secretion.

AIM OF THE STUDY AND HYPOTHESIS

Hypothyroidism is a greatly "over-diagnosed" disease because it is hard to differentiate between dogs with primary hypothyroidism and dogs with NTI. The concentration of circulating TT4 is below the reference range in dogs with primary hypothyroidism but may also be low in euthyroid dogs due to drugs or illness, i.e. in dogs with NTI. A low plasma TT4 concentration is, consequently, a frequent finding in dogs. Using screening panels, without using proper diagnostic methods (these are expensive or may be very invasive for the animal) will even enhance the amount of dogs found with low T4 concentration. This may result in unnecessary treatment with thyroxin supplementation of dogs with NTI. Treatment of dogs with NTI with thyroxin is not only useless, it may also stop the search for the non-thyroidal illness the dog is actually suffering from and as a consequence a proper treatment is withheld. On the other hand dogs with primary hypothyroidism, which are not recognized because their plasma concentration of TSH is within the reference range, are lacking proper treatment with thyroxin supplementation that will solve their physical, dermatological and emotional problems associated with thyroid disease. Because both situations are unwanted and should be solved, it is important that differentiation between hypothyroid dogs and dogs with NTI can be made with a good, inexpensive and non-invasive test.

The aim of the study is to find out whether it is possible to differentiate between dogs with primary hypothyroidism and dogs with NTI based on the plasma concentrations of GH and TSH before and after intravenous administration of TRH.

The hypothesis is that the plasma concentration of GH will increase and that of TSH will not increase in dogs with primary hypothyroidism after TRH has been injected. The other way around, it is hypothesized that in dogs with NTI, the plasma concentration of GH will not increase significantly and the plasma TSH concentration will increase after administration of TRH. If that is true, it is possible to differentiate between dogs with primary hypothyroidism and dogs with NTI.

To test these hypothesis, in dogs suspected of hypothyroidism with a low plasma TT4concentration and a plasma TSH concentration within the reference range, scintigraphy and a TRH stimulation test were performed. The thyroid scintigraphy serves as the gold standard.

EXPIRIMENTAL METHODS AND DESIGN

Dogs

Twelve dogs were included. All dogs were client owned and presented to the department of Clinical Sciences of Companion Animals, Utrecht University, The Netherlands in 2014 and 2015. The dogs were between two and ten years of age and from different breeds (Table 1). There were both male and female and castrated and uncastrated dogs in this experiment. All dogs recruited for this study had a combination of a low plasma TT4 (below the lower limit of the reference range, i.e. 19 nmol/L) and a plasma cTSH within the reference range (below 0.60 µg/l) and overt clinical signs which may be consistent with hypothyroidism. The owners were asked permission for diagnostic thyroid scintigraphy and TRH-stimulation test and collection of blood for additional investigation; they all signed the informed consent. The

diagnosis NTI was made based on the images of the thyroid glands obtained by scintigraphy using radioactive pertechnate ($^{99m}TcO_4^-$). NTI was diagnosed when the image of the thyroid gland was normal. Primary hypothyroidism was diagnosed when the image obtained by scintigraphy did show no or very limited uptake of $^{99m}TcO_4^-$ in the thyroid glands (Figure 1).

Dog	Breed	Gender	Age (years)	Body weight (kg)	TT4 (nmol/L)	TSH (µg/L)	Diagnosis based on Scintigraphy
1	Cross-bred	F	10	28.8	17	0.09	NTI
2	American Cocker Spaniel	F	8	14.2	<6	0.32	Hypothyroidism
3	Beagle	M	6	16.4	9	0.23	NTI
4	Cross-bred	M	7	28.8	7	0.21	NTI
5	Rhodesian Ridgeback	CF	9	48.0	9	0.34	NTI
6	Cross-bred	CF	9	36.9	9	0.14	NTI
7	English Cocker Spaniel	M	7	17.6	2	0.46	Hypothyroidism
8	Leonberger	CM	5	78.0	3	0.33	Hypothyroidism
9	Golden Retriever	M	7	38.0	<2	0.48	Hypothyroidism
10	German Wirehaired Pointer	M	4	34.5	4	0.14	NTI
11	Alaska Malamute	CF	1	34.0	13	0.06	NTI
12	Stabyhound	CF	11	25.4	7	0.39	NTI
Reference range					19-46	<0.60	

Table 1. Signalments and presenting plasma TT4 and TSH values in the twelve dogs. M: male, F: female, CM: castrated male, CF: castrated female.

TRH stimulation test and sample collection

The TRH-stimulation test was performed using intravenous injection of 10 µg TRH/kg body weight. Blood samples from the jugular vein were collected at -15, 0, 30 and 45 minutes and immediately transferred into chilled EDTA-coated or heparin-coated tubes for respectively GH and TSH measurement. Samples were centrifuged at 4ºC and plasma samples were stored at -20º until analysis.

Hormone determination

Plasma TSH concentrations were determined with the Immulite® canine assay (Siemens), whereas plasma GH were measured by the clinics unique canine GH assay as described previously[14] as no commercial assays are available.

Data analysis

All statistics have been done with the program IBM® SPSS statistics version 22. The test for normality was done with the Kolmogorov-Smirnov test for both hormones for the two groups: hypothyroid dogs and NTI dogs. H_0: The results do not differ from normal divided values. H_1: The results do differ from normal divided values. No group showed a significant outcome. Based on these findings the results can be seen as normally divided.

The basal level of both TSH and GH were measured twice at -15 and 0 minutes. The mean of these two measurements were used in the statistical analysis as being the basal plasma concentration of the hormones GH and TSH. The reason why two basal samples were taken is because both GH and TSH are hormones from the pituitary gland which releases these hormones in a pulsatile way.

To compare the mean plasma concentrations for the different hormones in the two groups, hypothyroid and NTI, at every time point, independent sample T-tests have been used. The variables were the different time points and the groups were divided by giving the hypothyroid dogs a 1 and the NTI dogs a 0. This test has been done for both GH and TSH plasma concentration levels. To compare the plasma concentrations of the different hormones between the different time points paired sample T-tests have been used. Every time point has been compared to all the other time points. The test has been used for GH concentrations in hypothyroid dogs, for GH concentrations in NTI dogs, for TSH concentrations in hypothyroid dogs and for TSH concentrations in NTI dogs.

The increment and percentage of increase in TSH and GH have been compared for both groups using the independent sample t-test, after the values had been tested for normality with the Kolmogorov-Smirnov. All values are normally divided.

GH/TSH ratios have been calculated and differences have been investigated using independent sample t-test.

Values are expressed as mean ± Standard Deviation. $P<0.05$ was considered significant.

RESULTS

Four of the twelve dogs were proven hypothyroid and the other eight dogs were diagnosed with NTI, using scintigraphy. Figure 1 shows images of a scintigraphy of a dog with primary hypothyroidism and a dog with normal thyroid function. The uptake of $^{99m}TcO_4^-$ in dogs with primary hypothyroidism is low or absent because the thyroid gland has been destructed by the dogs immune system.

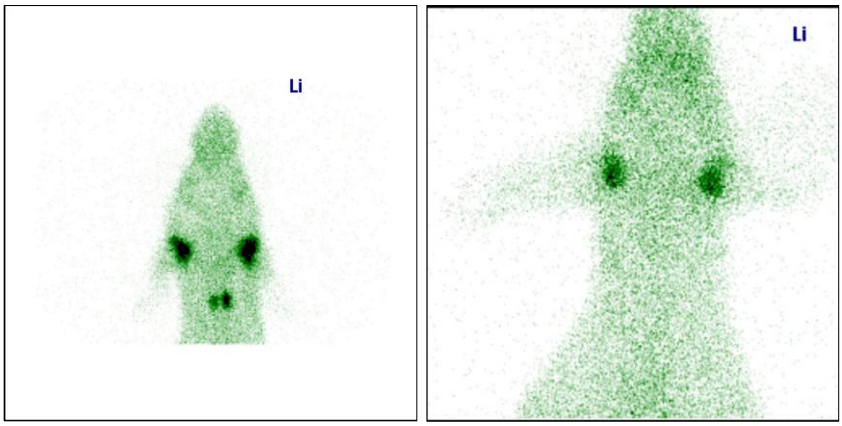

Figure 1. Left a dog with normal uptake of $^{99m}TcO_4$-. Right a dog with primary hypothyroidism.

Table 2 and 3 show the plasma concentrations of GH and TSH before and after the administration of TRH..

	GH plasma concentration			
Dog	**T-15**	**T0**	**T30**	**T45**
1	0.70	0.70	1.30	0.59
2	1.60	1.30	1.40	0.37
3	0.20	0.90	1.20	0.79
4	1.40	3.20	1.00	0.68
5	1.90	1.30	1.90	0.94
6	0.30	0.60	0.50	0.78
7	1.50	2.50	7.50	9.01
8	0.80	0.60	1.10	1.10
9	0.60	0.90	1.10	0.79
10	4.00	3.40	6.40	4.19
11	1.70	2.60	4.50	3.82
12	5.60	3.30	3.50	4.12

Table 2. GH plasma concentration

	TSH plasma concentration			
Dog	**T-15**	**T0**	**T30**	**T45**
1	0.03	0.03	0.14	0.16
2	0.22	0.25	0.27	0.24
3	0.07	0.07	1.09	0.83
4	0.14	0.15	0.63	0.4
5	0.05	0.05	0.31	0.25
6	0.45	0.36	1.18	1.1
7	0.27	0.32	0.92	0.72
8	0.03	0.03	0.44	0.38
9	0.52	0.49	0.99	0.82
10	0.26	0.25	0.28	0.25
11	0.35	0.32	0.34	0.35
12	0.1	0.07	0.08	0.08

Table 3. TSH plasma concentration

In the TRH-stimulation test the basal level of TSH in hypothyroid dogs and dogs with NTI did not differ significantly ($p = 0.675$). The basal plasma concentration of TSH in dog with primary hypothyroidism was 0.23 µg/l ± 0.10 µg/l. The basal plasma concentration of TSH in dogs with NTI was 0.19 µg/l ± 0.19 µg/l. Administration of TRH caused a rise of plasma TSH concentrations in the NTI dogs from 0.19 µg/l ± 0.19 µg/l to 0.71µg/l ± 0.39 µg/l 30 minutes after administration of TRH and to 0.58 µg/l ± 0.33 µg/l 45 minutes after administration of TRH. The increment in plasma concentration of TSH at 30 and 45 minutes after administration of TRH is significant ($p = 0.001$ and $p = 0.002$, respectively). It is to be

expected that the plasma TSH concentration will rise after administration of TRH in a physiological situation. However the plasma TSH concentration in hypothyroid dogs did not increase. The basal plasma concentration was 0.23 µg/l ± 0.10 µg/l. 30 minutes after TRH administration the mean plasma TSH concentration was 0.24 µg/l ± 0.11 µg/l. After 45 minutes this was 0.23 µg/l ± 0.11 µg/l. There is no significant difference in plasma TSH concentrations between basal concentration and 30 and 45 minutes after administration of TRH ($p = 0.199$ and $p = 0.638$ respectively) in dogs with primary hypothyroidism. The plasma TSH concentrations at 30 respectively 45 minutes after administration of TRH differ significantly between the hypothyroid dogs and the dogs with NTI ($p = 0.011$ and $p = 0.023$ respectively). Figure 2 shows the mean TSH plasma concentration at every time point of the TRH stimulation test for both groups. Additionally Figure 3 shows a boxplot where the boxes represent the TSH plasma concentration at the different time points for the different groups. The mean, minimum and maximum TSH plasma concentration are also given in this figure.

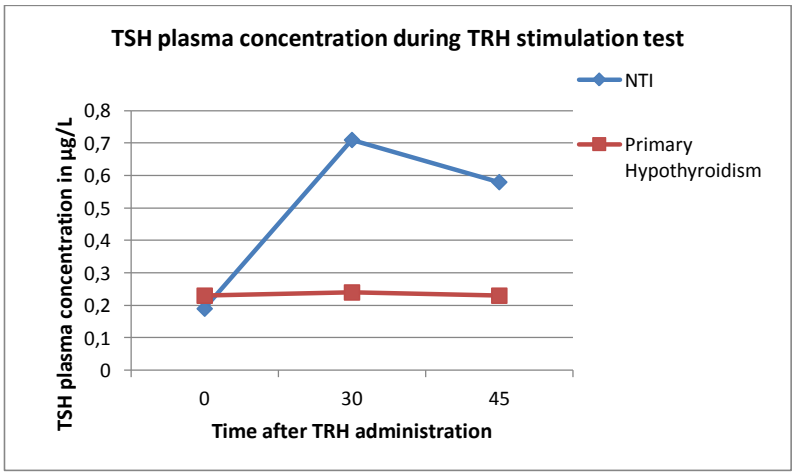

Figure 2. Mean plasma TSH concentration (µg/L) during TRH stimulation test.

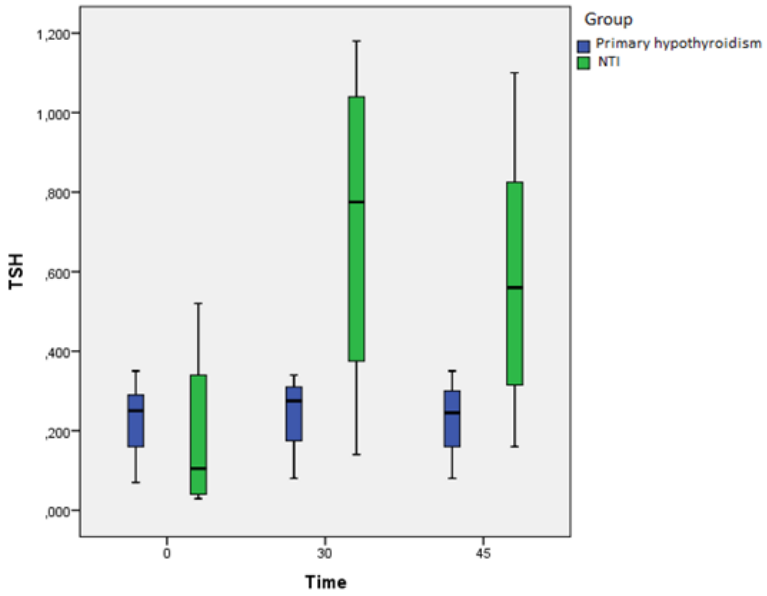

Figure 3. Boxplot with plasma TSH concentrations in µg/L

The basal plasma GH concentration was higher in dogs with hypothyroidism than in dogs with NTI (3.08 ± 1.19 µg/l versus 1.06 ± 0.65 µg/l), the difference is statistically significant (p = 0.036).

In dogs with NTI, plasma GH concentration did not increase significantly 30 and 45 minutes after TRH administration (p = 0.589, respectively p = 0.277)

The GH plasma concentration increased in dogs with hypothyroidism 30 and 45 minutes after administration of TRH (5.48 ± 1.81µg/l respectively 5.29 ± 2.49 µg/l), however the increase did not reach significance (p = 0.167 and p = 0.273). However the mean values of GH plasma concentration did differ statistically significant between the two groups 30 repetitively 45 minutes after TRH administration (p = 0.017 respectively p = 0.036). Figure 4 shows the mean plasma concentrations of GH during the TRH stimulation test for both groups. Additionally Figure 5 shows a boxplot where the boxes represent the plasma GH concentration at the different time points for both groups. This figure demontrates the mean, minimun and maximun GH plasma concentration.

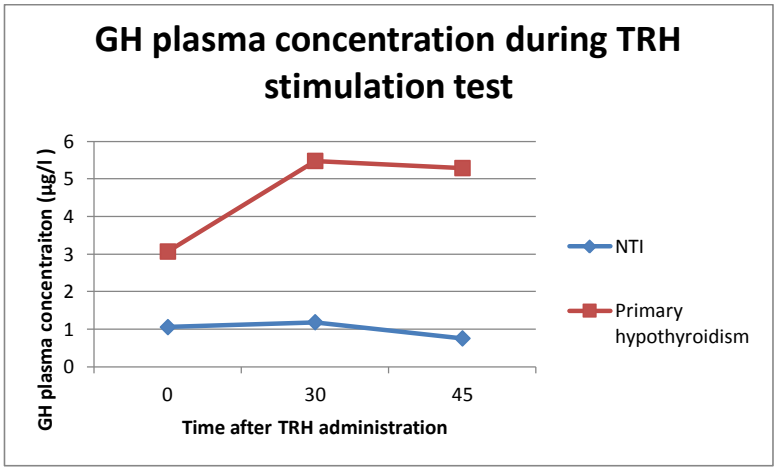

Figure 4. Mean GH plasma concentration (µg/l) during TRH stimulation test.

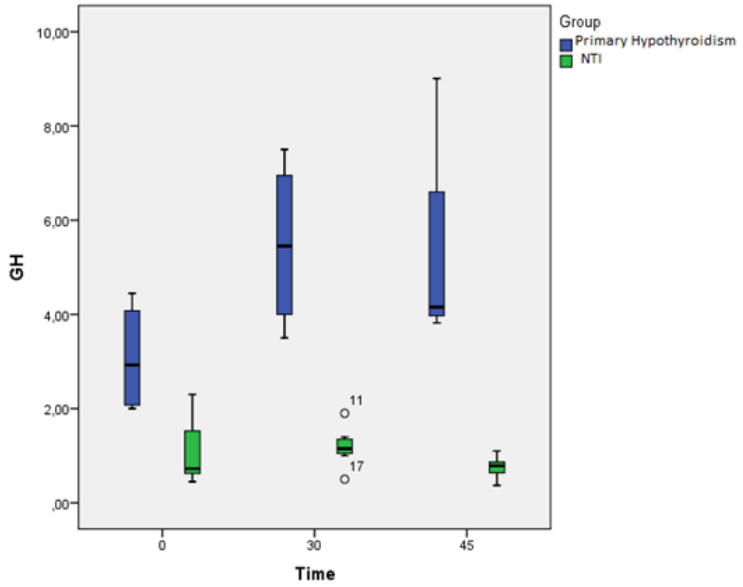

Figure 5. Boxplot with GH plasma concentrations in µg/l

At every time point in this experiment the plasma concentrations of TSH do overlap between the two groups (Figure 3). Therefore the differences in increment in plasma concentrations needed to be tested. 30 minutes after TRH administration the mean increase in TSH plasma concentration in dogs with NTI was greatest, i.e. 0.52 µg/l versus 0.39 µg/l at 45 minutes after TRH administration. The difference in increase in TSH plasma concentration between dogs with NTI and dogs with primary hypothyroidism was statistically significant ($p = 0.006$ between basal level and 30 minutes after TRH administration and $p = 0.007$ between basal

level and 45 minutes after TRH administration). The minimum increase in TSH plasma concentration in dogs with NTI was 0.11µg/l, where the maximum increase in TSH plasma concentration in dogs with primary hypothyroidism was 0.03µg/l between basal plasma concentration and 30 minutes after TRH administration. Figure 6 shows the differences in increment in TSH plasma concentration during the TRH stimulation test.

The percentage increment in TSH plasma concentration has also been investigated with a T-test. The percentage increment in dogs with NTI 30 minutes after TRH administration was 565 % ± 539 %, compared to a 3.0 % ± 10 % increase of TSH plasma concentration in dogs with primary hypothyroidism. The difference shows statistical significance (p = 0.021). The minimum percentage of increase in TSH plasma concentration for dogs with NTI was 94 %, 30 minutes after TRH administration and the maximum percentage of increase was 1457 %. The minimum percentage of increase was -11.1% and the maximum increment in TSH plasma concentration for dogs with primary hypothyroidism was 12.5%. Figure 7 shows a boxplot with the comparison of the two groups in percentage of increment in TSH plasma concentration during TRH stimulation test.

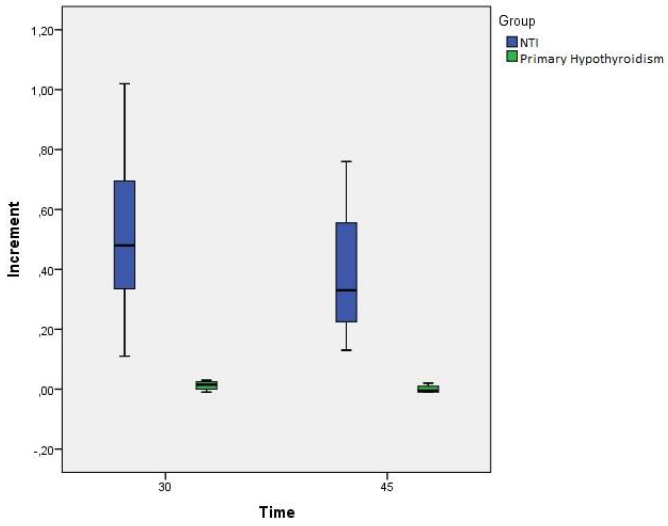

Figure 6. Boxplot with increment in TSH plasma concentrations in µg/L between basal plasma concentration and the plasma concentration 30 respectively 45 minutes after TRH administration.

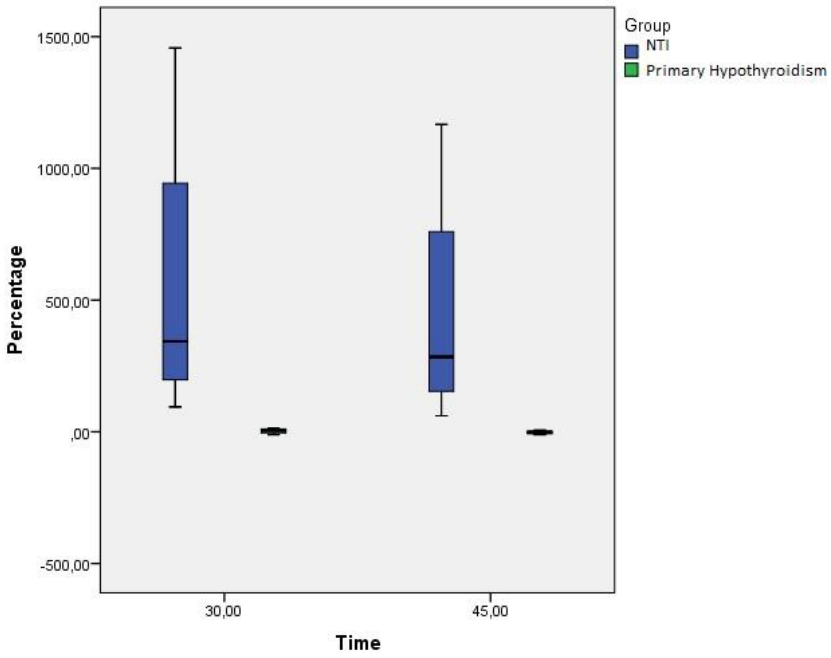

Figure 7. Boxplot with percentage of increment in TSH plasma concentrations in µg/L between basal plasma concentration and the plasma concentration 30 respectively 45 minutes after TRH administration.

The increment and percentage of increment of GH have also been calculated. The increment in plasma GH concentration in dogs with primary hypothyroidism was 1.13 µg/l ± 1.68 µg/l compared to 0.78 µg/l ± 2.34 µg/l in dogs with NTI 30 minutes after TRH administration. The difference between the two groups was not statistically significant ($p = 0.797$). The increment of GH plasma concentration after 45 minutes was 0.48 µg/l ± 0.88 µg/l in dogs with primary hypothyroidism and 0.56 µg/l ± 2.92 µg/l . Again no statistical significant difference ($p = 0.955$) could be detected. The increment between 30 and 45 minutes after TRH administration was -0.65 µg/l ± 1.16 µg/l in dogs with primary hypothyroidism and -0.19 µg/l ± 0.81 µg/l in dogs with NTI. There was no statically significant difference between the two groups ($p = 0.412$). Figure 8 shows a boxplot with the increment in GH plasma concentration in both groups between basal plasma concentration and 30 respectively 45 minutes after TRH administration.

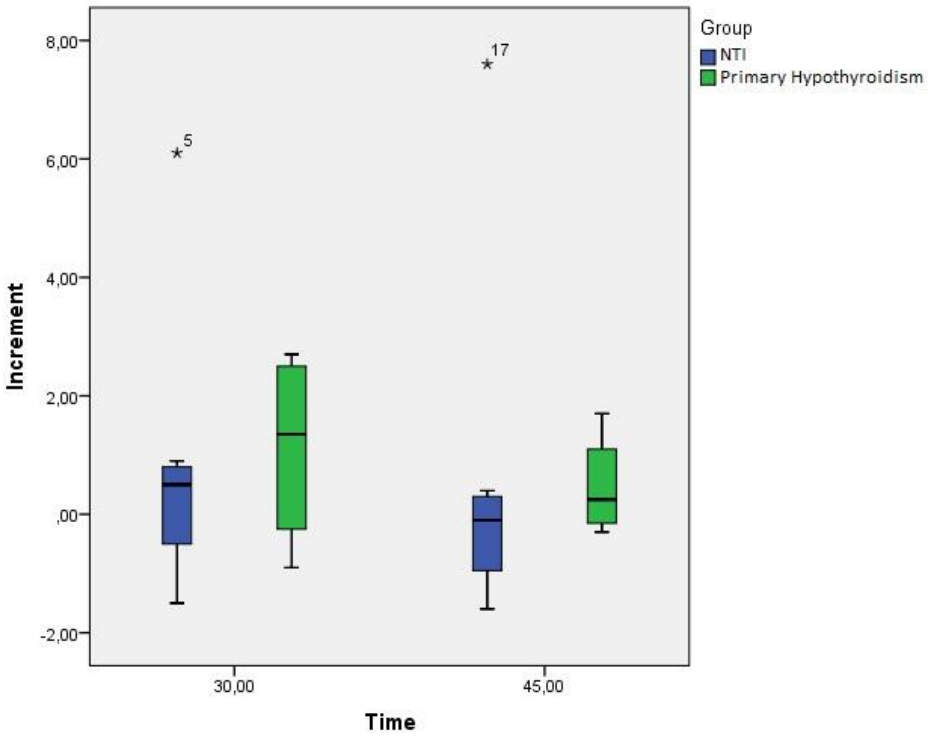

Figure 8. Boxplot with increment in GH concentration in µg/l 30 respectively 45 minutes after TRH administration.

The percentage of increase in GH plasma concentration has also been investigated using an independent T-test. The percentage of increment in dogs with primary hypothyroidism 30 minutes after TRH administration was 53 % ± 55 %, 45 minutes after TRH administration was 22 % ± 38 % and between 30 and 45 minutes after TRH administration it was -16 % ± 23.4%. In dogs with NTI the percentage of increment 30 minutes after TRH administration was 96 % ± 160 % , 45 minutes after TRH administration it was 52 % ± 195 % and between 30 and 45 minutes after TRH administration it was -20 % ± 43 %. None of the three differences were statistically significant ($p = 0.624$, $p = 0.774$ and $p = 0.846$ respectively for the difference in percentage of increment 30 respectively 45 respectively between 30 and 45 minutes after TRH administration). Figure 9 shows a boxplot with the percentage of increase in GH concentration 30 respectively 45 minutes after TRH administration for both groups.

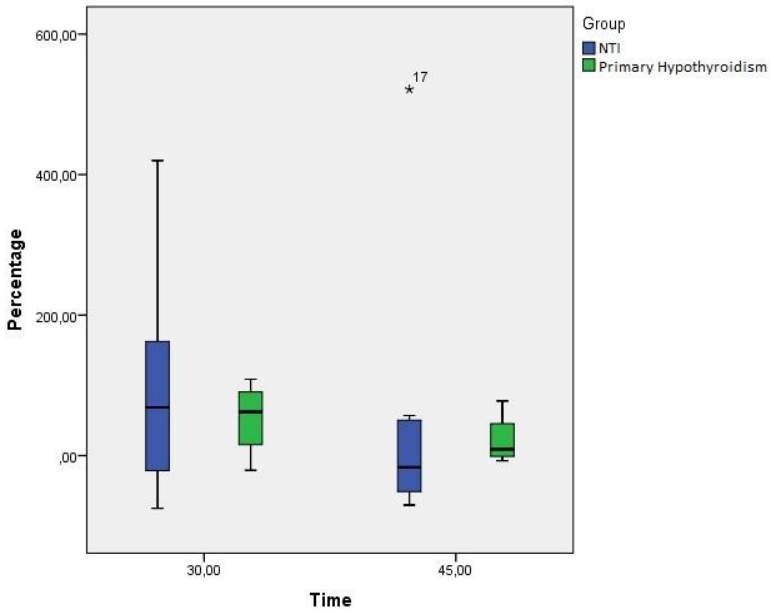

Figure 9. Boxplot with percentage of increment in GH plasma concentrations in µg/l between basal plasma concentration and the plasma concentration 30 respectively 45 minutes after TRH administration.

The GH/TSH ratio has been calculated for the basal plasma concentrations and the plasma concentrations 30 and 45 minutes after TRH administration for each dog. (Table 4).

	GH/TSH ratio		
Dog	**Basal**	**T=30**	**T=45**
1	23,33	9,07	3,69
2	6,04	27,93	37,54
3	7,86	1,10	0,95
4	15,33	1,52	1,70
5	32,00	5,97	3,76
6	1,10	1,17	0,34
7	6,67	0,54	1,08
8	23,33	2,48	2,89
9	1,47	1,15	0,96
10	14,23	22,79	16,76
11	6,52	13,21	10,91
12	49,44	44,00	51,50

Table 4. GH/TSH ratio of basal plasma concentrations and plasma concentrations 30 and 45 minutes after TRH administration

Differences in GH/TSH ratios between the two groups have been investigated using a independent sample t-test. The basal GH/TSH ratio in dogs with primary hypothyroidism was

19 ± 21 compared to 14 ± 11 in dogs with NTI. The difference in basal GH/TSH ratio was not statistically significant (p = 0.581). The GH/TSH ratio 30 minutes after TRH administration was 27 ± 13 in dogs with primary hypothyroidism and 2.9 ± 3.0 in dogs with NTI. The difference was statistically significant (p = 0.031). Additionally the GH/TSH ratio 45 minutes after TRH administration was 29 ± 19 in dogs with primary hypothyroidism and 1.9 ± 1.3 in dogs with NTI, however the difference was not significant (p = 0.062). Figure 10 shows the mean GH/TSH ratios for both groups during TRH stimulation test. This figure shows that the GH/TSH ratios differ the most between the two groups 45 minutes after administration of TRH. The minimum GH/TSH ratio in dogs with primary hypothyroidism at this point is 11, where the maximum GH/TSH ratio in dogs with NTI is 3.7.

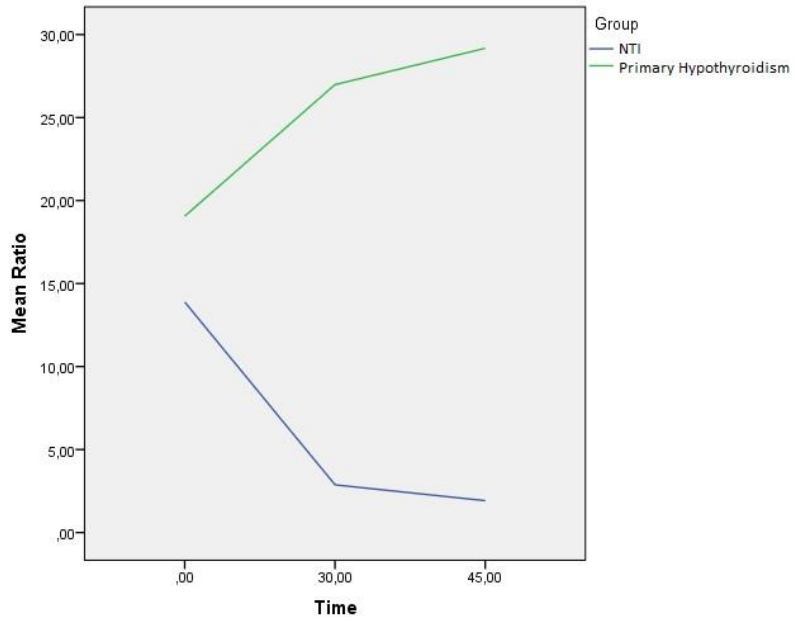

Figure 10. Mean GH/TSH ratio during TRH stimulation test.

DISCUSSION

In this study, the differences in plasma TSH and GH concentrations before and after administration of TRH have been investigated to see whether it is possible to differentiate between dogs with primary hypothyroidism and dogs with NTI. The hypothesis was that the plasma concentration of GH would increase and that of TSH will not increase in dogs with primary hypothyroidism after TRH has been injected. The other way around, it was hypothesized that in dogs with NTI, the plasma concentration of GH would not increase significantly and the plasma TSH concentration would increase after administration of TRH. All the expected has been found in this study, however the increase of plasma GH concentration did not show statistical significance. This could be due to small amount of dogs that has been used in this study. It is expected that the increase would become statistical

significant when more dogs can be used. For this reason more dogs with hypothyroidism have to be included.

The basal plasma concentration of GH showed a statistical significant difference between dogs with hypothyroidism and dogs with NTI. However, the basal plasma concentration of GH does show some overlap between the two groups. For this reason, the basal plasma concentration of GH cannot differentiate between the two groups, so a TRH stimulation test is necessary to differentiate. No significant increment could be found in plasma GH concentration by the TRH stimulation test. It was not expected that there would not be a significant difference between GH plasma concentration before and after TRH administration in dogs with primary hypothyroidism. The lack of significance could be due to the small amount of dogs with primary hypothyroidism in this study. Therefore more dogs with primary hypothyroidism should be recruited to proof the increase of GH plasma concentration after TRH administration.

Previous studies showed that the plasma GH concentration in dogs with primary hypothyroidism reaches the highest level 45 minutes after administration of TRH.[8] In this experiment all dogs with primary hypothyroidism have a plasma concentration of GH that is 3.82 µg/l or higher 45 minutes after TRH administration (Figure 5). All dogs with NTI have a plasma concentration of 1.10 µg/l or less at this point. Figure 5 shows that there is no overlap in GH plasma concentration between the two groups of dogs 30 and 45 minutes after TRH administration. Based on that a diagnostic test could be developed. Saying that when the plasma GH concentration rises above 3.0 µg/l 45 minutes after TRH administration the dog should be considered having primary hypothyroidism. The other way around, if the plasma concentration GH does not reach 3.0 µg/l the dog should be considered having NTI.

To make such a diagnostic test more reliable, the increase in plasma TSH concentration could also be a parameter in this test. In this study no difference was found between the two groups based on their basal plasma TSH concentration. However this was not expected as one of the selection criteria was a TSH plasma concentration within the reference range of <0.60 µg/l. When it comes to the plasma TSH concentration in the test with TRH administration, there is a difference between both groups 30 and 45 minutes after TRH administration. This difference was expected as in dogs with primary hypothyroidism TSH producing cells in the pituitary gland become desensitized for TRH.[1] Figure 3 shows that the two groups overlap in plasma concentration at every time point. Therefore it is impossible to set a certain plasma concentration that would differentiate between the two groups. Because of that, the increment in TSH plasma concentration is more important. In this study the largest increase in TSH plasma concentration in dogs with NTI was 30 minutes after administration of TSH. The absolute increase was 0.52 µg/l ± 0.29 µg/l with a minimum of 0.11 µg/l. The dogs with primary hypothyroidism showed an increase of 0.01 µg/l ± 0.02 µg/l with a maximum of 0.03 µg/l. Therefore the diagnostic test could differentiate between the two groups based on the increment in TSH plasma concentration after TRH administration. When the plasma TSH concentration increases more than 0.10 µg/l the dog is most likely a NTI patient. The other way around, whether the TSH plasma concentration does not reach an increase of 0.10 µg/l after TRH administration the dog most likely suffers from primary hypothyroidism.

The increment and percentage of increment in GH during the TRH stimulation test have also been investigated. Surprisingly no statistically significant difference was detected between the two groups. It was expected that the dogs with primary hypothyroidism would have a significant greater increment in plasma GH concentration after administration of TRH. However the results do not approve this hypothesis. Therefore more dogs with primary hypothyroidism should be included in this study to proof the TRH stimulated increment in GH plasma concentration is larger in dogs with primary hypothyroidism than in dogs with NTI.

GH/TSH ratios have also been calculated and differences between the two groups have been investigated with an independent sample T-test. Figure 10 shows that the mean greatest difference in GH/TSH ratio is reached 45 minutes after TRH administration. However, the difference at this time point did not show statistical significance. The difference in GH/TSH ratio between the two groups did show statistical significance 30 minutes after TRH administration. The GH/TSH ratio could probably be a way to differentiate between a dog with primary hypothyroidism and a dog with NTI. The minimum GH/TSH ratio in dogs with primary hypothyroidism 45 minutes after TRH administration, where the difference seems the greatest (figure 10), is 11, where the maximum GH/TSH ratio in dogs with NTI is 3.8. A diagnostic test could be formed saying that when the GH/TSH ratio is 10 or higher 45 minutes after TRH administration the dog most likely has primary hypothyroidism. The other way around, when the GH/TSH ratio stays below 10 the dog most likely has a non-thyroid illness.

CONCLUSION

To differentiate between dogs with primary hypothyroidism and dogs with NTI, a TRH stimulation test could be a diagnostic method in the future. A combination of a GH plasma concentration of 3.0 µg/l or more at 45 minutes after TRH administration and a TSH plasma concentration increment below 0.10 µg/l could be diagnostic for primary hypothyroidism. The other way around, when the increment of plasma TSH concentration is 0.10 µg/l or more and the plasma GH concentration stays below 3.0µg/l a dog most likely has a normal thyroid function and therefore a non-thyroidal illness. However, the increment of GH after TRH administration did not reach statistical significance in dogs with primary hypothyroidism so for that reason more dogs should be recruited to proof the increment in GH plasma concentration. The GH/TSH ratio could also be used for diagnostic testing. All dogs with primary hypothyroidism had a GH/TSH ratio of 11 or higher at 45 minutes after TRH administration. Additionally all dogs with NTI had a GH/TSH ratio of 3.7 or lower. Therefore when the GH/TSH ratio is 10 or higher in a dog at 45 minutes after TRH administration, the dog most likely has primary hypothyroidism. The other way around, when the GH/TSH ratio is below 10, 45 minutes after TRH administration, the dog most likely has NTI.

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