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Investigation of the association between serum protein concentrations and concurrent chronic kidney disease in hyperthyroid cats

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Abstract

Our objective was to identify if changes in serum protein concentrations occur in hyperthyroidism and to assess their association with the development of azotaemia following treatment.

Initially non-azotaemic hyperthyroid cats and healthy older cats were included. Serum concentrations of protein fractions were determined by agarose gel electrophoresis and compared between; hyperthyroid and control cats, initially non-azotaemic hyperthyroid cats which developed azotaemia in a 4 month follow up period (masked-azotaemic) and those which remained non-azotaemic, and hyperthyroid cats before and at the time of restoration of euthyroidism. Data are presented as median [25^{th}, 75^{th} percentile].

Hyperthyroid cats (n = 56) had higher serum α_2 globulin concentrations (12.5 [10.9, 13.1] g/L vs. 9.8 [3.0, 11.4] g/L; $P < 0.001$) and lower serum γ globulin concentrations (11.4 [9.1, 13.3] g/L vs. 14.0 [12.4, 16.8] g/L; $P = 0.001$) than control cats (n = 26). Following treatment, serum total globulin concentration increased (from 38.6 [35.4, 42.8] g/L to 42.3 [39.0, 45.7] g/L; $P < 0.001$), serum α_2 globulin concentration decreased (from 12.5 [10.9, 13.9] g/L to 11.5 [10.1, 12.6] g/L; $P < 0.001$) and serum γ globulin concentration increased (from 11.4 [9.0, 13.3] g/L to 14.0 [12.4, 16.8] g/L; $P < 0.001$). Serum concentrations of total globulin or globulin fractions were not significantly different between masked-azotaemic and non azotaemic groups.

In conclusion, hyperthyroidism is associated with altered serum concentrations of the α_2 and γ globulin fractions, however these changes were not associated with the development of azotaemic chronic kidney disease following treatment.

Keywords: Azotaemia; Electrophoresis; Globulin; Protein; Serum

Introduction

Chronic kidney disease (CKD) is a common co-morbidity in hyperthyroidism, however hyperthyroidism will complicate the diagnosis of CKD due to the consequent increase in glomerular filtration rate (GFR) (Adams et al. 1997) and decrease in body muscle mass (Shiel and Mooney 2007). Hence, hyperthyroidism decreases serum creatinine concentrations, which 'masks' concurrent azotaemic CKD in these patients. Some hyperthyroid cats with concurrent CKD only develop azotaemia after treatment, once GFR and body muscle mass have returned to normal (for the cat). Identification of hyperthyroid cats with concurrent, but masked, CKD prior to treatment of hyperthyroidism would be beneficial because it would allow the institution of appropriate treatment strategies for CKD at an earlier time point.

Several studies have attempted to identify biomarkers of CKD in hyperthyroidism (Lapointe et al. 2008; Riensche et al. 2008; van Hoek et al. 2009a; van Hoek et al. 2009b; Williams et al. 2010; Williams et al. 2016), however, no single reliable test has been reported. In one previous study of 300 hyperthyroid cats, only (higher) plasma creatinine concentrations and (lower) plasma globulin concentrations were independent predictors of the development of azotaemia within 240 days of diagnosis of hyperthyroidism (Williams et al. 2010). These data suggest that plasma total globulin concentration, or the plasma concentration of a component of the globulin fraction, could be a marker of concurrent, but masked, azotaemic CKD in hyperthyroidism.

Agarose gel electrophoresis (AGE) is a technique which separates serum proteins into 4-6 major groups of one or more bands, based on the ability of the proteins to migrate through the agarose gel when an electrical field is applied. The distance of migration is

dependent on the electrical charge, mass and shape of the protein. By utilising this technique, the serum concentrations of individual globulin fractions can be elucidated, which could help to determine which serum protein is associated with the presence of concurrent, but masked, azotaemic CKD in hyperthyroid cats.

The first aim of this study was to establish if the results of our previous study were repeatable in an independent group of cats, and if so, to identify if any individual globulin fraction associated with the presence of concurrent, but masked, azotaemic CKD in hyperthyroid cats, since this could provide a novel biomarker for CKD in these cases. The second aim was to investigate if changes in serum protein concentrations occur in hyperthyroid cats (by comparison of serum concentrations of individual globulin fractions between hyperthyroid cats and healthy older cats, and before and after treatment of hyperthyroidism), since changes in serum concentrations of some proteins have been reported in animal models of hyperthyroidism previously (Farthing et al. 1960, Griffin and Miller 1973).

Materials and methods

Hyperthyroid cats seen between March 2010 and June 2013 at two first opinion practices in London were included in the study. All were non-azotaemic (plasma creatinine concentration < 177 μmol/L) at the time of presentation. Cats with other known significant systemic disorders except hypertension (based on clinical examination, serum biochemistry and urinalysis), including significant systemic inflammatory disease (for example inflammatory bowel disease) were excluded. Included cats were not, however, screened for FeLV or FIV infection. Following informed consent by the owner, blood and urine samples were taken from the cats as part of a geriatric screening programme (Royal Veterinary College Ethics and Welfare Committee approval number and date; URN 20131258, 2nd December

2013). Blood was obtained by jugular venepuncture, placed in heparinised or non-anticoagulated tubes, and stored at 4 ^{o}C until sample processing (within 6 h). Urine samples were taken by cystocentesis. Biochemical analysis was performed by IDEXX Laboratories (Wetherby, UK) using heparinised plasma. Residual serum was stored at -80 ^{o}C and subsequently submitted to Central Diagnostic Services (Cambridge, UK) for batch measurement of serum TT4 by enzyme immunoassay (Williams and Archer 2016), serum total protein by biuret reaction, and AGE analysis. Urine specific gravity (USG) determination (by refractometry), urine dipstick, and urine sediment analysis was also performed in-house. The presence of bacteriuria or pyuria (>5 white blood cells per 1000x field) was an exclusion criterion for the study.

All hyperthyroid cats were treated with the aim of attaining a plasma TT4 < 40 nmol/L. Cats were initially treated with anti-thyroid medication (usually methimazole), with some animals also undergoing thyroidectomy following initial stabilisation. Cats were re-examined and plasma TT4 repeated every 4 weeks until restoration of euthyroidism was documented (defined as a plasma TT4 < 40 nmol/L). Once euthyroidism had been achieved, the cats were monitored for a 4 month period, with further blood and urine samples taken at the end of this monitoring period. Cats were defined as masked-azotaemic if they had a plasma creatinine concentration > 177 µmol/L with a concurrent USG < 1.035 at the end of this four month monitoring period. Cats with persistent azotaemia without obvious evidence of dehydration (pre-renal azotaemia) were also classified as masked-azotaemic. All other hyperthyroid cats were classified as non-azotaemic. Samples taken at the time of diagnosis of hyperthyroidism and at the time of first documentation of euthyroidism were used for AGE analysis.

Healthy older cats that presented to three first opinion practices in the South-East of England between March 2013 and April 2015 were also included. These cats all had no clinical history of disease (except the presence of tartar with or without associated gingivitis, or degenerative joint disease), no significant haematological or biochemical abnormalities, were on no long term medications (except anti-parasitic medications) and were at least 8 years old. Included cats were not, however, screened for FeLV or FIV. Blood and urine samples were obtained by practitioners following informed consent as part of a geriatric screening programme (Ethics and Welfare Committee of the Department of Veterinary Medicine at the University of Cambridge approval number and date; CR56, 4th August 2012) and submitted to Central Diagnostic Services within 3 days of sampling. Full haematology, serum biochemistry (including TT4 by enzyme immunoassay) and urinalysis (including urine protein:creatinine ratio [UPC]) was performed, and any animals with azotaemia (defined as a serum creatinine concentration > 153 μmol/L), proteinuria (defined as UPC >0.4), borderline or overt hyperthyroidism (defined as a serum TT4 > 40 nmol/L), bacteriuria, pyuria (defined as > 5 white blood cells per 1000x field) or other significant haematological or biochemical abnormalities were excluded from the cohort. Residual serum was stored at -80C until batch AGE analysis.

Agarose gel electrophoresis

AGE was performed with agarose gels (HydraGel 7 β1β2, Sebia), according to the manufacturer's instructions. A normal cat control sample was run on each gel and paired samples from each individual hyperthyroid cat (hyperthyroid and euthyroid time points) were run on the same gel. The electrophoretograms were read using a densitometer and Phoresis software (Sebia). Densitometric readings from each lane of the gel were displayed as a curve, and the various bands (represented by peaks on the curve) were resolved manually by one

investigator (TW) with the percentage of albumin and the individual globulin fractions calculated based on the area under each part of the curve divided by the total area under the curve. Absolute concentrations of the proteins were determined by multiplication of the percentage of that protein by the serum total protein concentration (determined by biuret reaction).

Statistical analysis

Statistical analysis was performed with SPSS v21.0 (IBM). The Mann Whitney *U* test was used to compare serum concentrations of total protein, albumin, total globulin and the individual globulin fractions between; hyperthyroid and healthy older cats, and masked-azotaemic and non-azotaemic hyperthyroid cats. The Wilcoxon signed rank test was used to compare serum concentrations of proteins in hyperthyroid cats before treatment and at the time of establishment of euthyroidism. Correlations between baseline serum concentrations of total protein, albumin, globulin and the individual globulin fractions and age, serum concentrations of creatinine and TT4, were assessed by Spearman's correlation co-efficient. Correlations were classified as weak if $r_s < 0.5$, moderate if r_s was 0.5-0.7, and strong if $r_s > 0.7$. The proportion of cats in the hyperthyroid and healthy older cat groups which had serum concentrations of the various protein fractions outside of the reference intervals reported in a previous study (Taylor et al. 2010) were compared using the Fisher's Exact test. Data are presented as median [25^{th}, 75^{th} percentile] and statistical significance was defined as $P < 0.05$.

Results

Fifty six hyperthyroid cats and 26 healthy older cats were included in the study. In the hyperthyroid group, 21 cats developed azotaemia during the follow up period (masked-

172 azotaemic group) and 35 cats were classified as non-azotaemic. Selected clinicopathological
173 data for the hyperthyroid and healthy older cat groups are shown in Table 1.
174
175 The comparison of serum concentrations of total protein, albumin, total globulins and
176 globulin fractions between the masked-azotaemic and non-azotaemic hyperthyroid groups are
177 shown in Table 2. Serum total protein concentration was higher in masked-azotaemic
178 hyperthyroid cats compared to those which remained non-azotaemic ($P = 0.049$), however no
179 significant differences in the serum concentrations of α_1, α_2, β, γ or total globulin were evident
180 between the masked-azotaemic and non azotaemic groups.
181
182 Serum concentrations of total protein, albumin, total globulins and globulin fractions in
183 the hyperthyroid and healthy older cat groups are summarised in Table 1. Hyperthyroid cats
184 had significantly higher serum α_2 globulin concentrations ($P < 0.001$, Figure 1) and lower serum
185 γ globulin concentrations ($P = 0.001$, Figure 2) than healthy older cats. The proportion of cats
186 with a serum α_2 globulin concentration above the reference interval reported in a previous study
187 (Taylor et al. 2010) was significantly greater in the hyperthyroid group compared to the healthy
188 older cat group (50/56 vs. 11/26; $P<0.001$). All cats had a serum γ globulin concentration within
189 or above the previously reported reference interval, however there was no significant difference
190 in the proportion of cats with a serum γ globulin concentration above the reference interval
191 between the hyperthyroid and healthy older cat groups (4/56 vs. 3/26 respectively; $P=0.673$).
192 Hyperthyroid cats also had significantly lower serum albumin concentrations than healthy older
193 cats ($P = 0.008$), however the proportion of cats with a serum albumin concentration below the
194 reference interval reported in a previous study (Taylor et al. 2010) was not significantly
195 different between the hyperthyroid and healthy older cat groups (6/56 vs. 4/26 respectively;
196 $P=0.718$). In hyperthyroid cats (at baseline), serum γ globulin concentrations were weakly

positively correlated with plasma creatinine concentration (r_s = 0.356, n = 54; P = 0.008) and weakly negatively correlated with TT4 (r_s = -0.415, n = 56; P < 0.001). No parameter was correlated with age in the hyperthyroid group alone, however when healthy older cats were included in the analysis, serum albumin concentration was weakly negatively associated with age (r_s = -0.236, n = 80; P = 0.035). When only healthy older cats were considered, a moderate positive correlation between age and serum γ globulin concentrations was evident (r_s = 0.522, n = 25; P = 0.008).

Changes in serum concentrations of total protein, albumin, total globulins and globulin fractions in the hyperthyroid group after treatment (n = 55) are shown in Table 3. Following treatment of hyperthyroidism, serum total globulin concentration increased (P < 0.001), serum α_1 globulin concentration increased (P = 0.031), serum α_2 globulin concentration decreased (P < 0.001, Figure 3) and serum γ globulin concentration increased (P < 0.001, Figure 4). Serum concentrations of albumin did not change significantly following treatment (P = 0.378).

Discussion

The primary aim of the current study was to identify potential biomarkers of CKD in hyperthyroidism. In a previous study, decreased plasma globulin concentrations were independent predictors of the development of azotaemia within 240 days of diagnosis of hyperthyroidism (Williams et al. 2010), however despite the evidence of altered serum protein concentrations in hyperthyroid cats in this study, no association between the serum concentrations of total globulin or the globulin fractions and the development of azotaemic CKD following treatment of hyperthyroidism was identified. Total protein concentrations were significantly higher in cats with concurrent, but masked, azotaemic CKD than cats which remained non-azotaemic, which is in contrast to the previously reported study

(Williams et al. 2010). This discrepancy could reflect a type I statistical error in this or the previous study, or could reflect differences in the sample types used. The present study investigated *serum* globulin concentrations, whereas the previous study reported the *plasma* globulin concentrations. The only difference between these samples would be the absence or presence of fibrinogen respectively, however it is possible that changes in circulating fibrinogen concentrations could account for the observed differences between this and the previous study (Williams et al. 2010). Elevated serum fibrinogen concentrations occur in human hyperthyroid patients (Dörr et al. 2006; Popławska-Kita et al. 2013), therefore evaluation of serum fibrinogen concentrations and their association with the development of azotaemic CKD following treatment of hyperthyroidism could be warranted. Whilst it could be speculated that the higher total protein concentrations observed in cats in the masked-azotaemic group in this study reflects lower extracellular fluid volume of those animals, perhaps secondary to concurrent polyuria associated with CKD, this is considered unlikely given the lack of a concurrent increase in serum albumin concentration in these cats. Furthermore, a recent study suggested that extracellular fluid volume is not different between non-hyperthyroid cats with and without azotaemic CKD (Finch et al. 2015).

The second aim of this study was to identify if alterations in serum globulin concentrations were present in hyperthyroid cats. This is the first study to report a number of changes in the serum concentrations of globulin fractions in hyperthyroid cats when compared with healthy older cats, which mostly resolved following treatment, thus suggesting that hyperthyroidism causes changes in the concentrations of some of the globulin fractions as determined by AGE. However, the observed changes in serum concentrations of the globulin fractions in hyperthyroid cats were mild compared with the more marked

changes that would be expected in inflammatory diseases (which were not fully excluded in cats included in this study).

In the present study, serum concentrations of α_2 globulins were higher in hyperthyroid cats than healthy older cats, and they also decreased following successful treatment of hyperthyroidism, suggesting that hyperthyroidism increases serum α_2 globulin concentrations. Proteins that will migrate in the α_2 globulin band on AGE include α_2 macroglobulin and haptoglobin (Baker and Valli 1988). It is possible that the increased serum α_2 globulin concentrations observed in feline hyperthyroidism are secondary to increased hepatic synthesis of haptoglobin, as has been demonstrated in rats with experimentally induced hyperthyroidism (Griffin and Miller 1973), however further studies utilising methods that can specifically measure serum haptoglobin, for example ELISA (Kajikawa et al. 1999), would be necessary to determine this.

Hyperthyroid cats in the present study also had significantly lower serum γ globulin concentrations, which increased following successful treatment of hyperthyroidism. These results also suggest that hyperthyroidism causes a decrease in serum γ globulin concentrations in cats. Proteins that migrate in the γ globulin band include IgG and IgM (Gerou-Ferriani et al. 2011). In one previous experimental study, the catabolism of ^{131}I-labelled homologous γ globulins (IgG) was increased in hyperthyroid rats (Farthing et al. 1960), therefore it is possible that the same process occurs in hyperthyroid cats, however further evaluation of serum IgG concentrations, by IgG specific techniques, would be necessary to confirm if the changes in serum γ globulin concentrations are secondary to changes in IgG. Furthermore, in this study, increased urinary or gastrointestinal losses of immunoglobulin were not excluded.

The clinical significance of the observed changes in serum concentrations of specific globulin fractions in hyperthyroid cats is unclear, but given the relatively mild changes observed in the electrophoretic fraction then they are unlikely to be clinically relevant.

Serum albumin concentrations were lower in hyperthyroid cats than in healthy older cats, but did not change following treatment. Although hyperthyroidism accelerates albumin turnover in human patients and rats (Kekki 1964; Blomstedt and Likjedahl 1967), altered serum albumin concentrations in hyperthyroid human patients have not been demonstrated (Blomstedt and Likjedahl 1967). In the current study, hyperthyroid cats were significantly older than the healthy older cat group, therefore the lower serum albumin concentration in hyperthyroid cats might reflect reduced albumin synthesis associated with chronic inflammatory diseases (such as dental disease), which are likely to be more common in older cats. The significant positive correlation between serum γ globulin concentrations, which are also associated with inflammatory disease, and age that was identified in the healthy older cats would support this hypothesis. Alternatively, the lower serum albumin concentrations in hyperthyroid cats could reflect increased urinary protein loss, since hyperthyroidism is associated with proteinuria (van Hoek et al. 2009a; Williams et al. 2010).

This study had a number of limitations; firstly the healthy older cat and hyperthyroid groups were not age matched, which might have confounded some of the comparisons in the cross sectional analysis, however a strength of the study was that the hyperthyroid cats were evaluated after successful treatment which enabled the determination of which serum globulin fractions might be influenced by hyperthyroidism *per se*. Secondly, haematological evaluation was not performed in hyperthyroid cats, due to financial restrictions at the time of

296 sampling, however cats with suspected co-morbidities (except for 'masked' CKD and
297 hypertension) were excluded from the study. We also did not exclude all possible
298 inflammatory causes of altered serum protein concentrations, most notably FeLV and FIV
299 infection, in the cats included in this study, which could have confounded our results given
300 the effect of these conditions on the electrophoretogram (Hofmann-Lehmann et al. 1997).
301 However, the prevalence of FeLV and FIV in our population is likely to be low (Hosie et al.
302 1989; Murray et al. 2009; Juvet et al. 2011), therefore we do not feel that this would have
303 significantly altered our results. Furthermore, the electrophoretic fractions will be mostly
304 influenced by the presence of concurrent (usually inflammatory) diseases, and therefore we
305 cannot fully exclude the possibility that the differences in the globulin fractions observed
306 between the hyperthyroid and healthy groups in the present study could actually reflect
307 differences in the prevalence of concurrent diseases (that influence the electrophoretogram)
308 between the two groups. Serum urea and creatinine concentrations for the healthy and
309 hyperthyroid groups were also determined in different laboratories, although azotaemia for
310 each group was defined based on a value above the laboratory reference interval specific to
311 the testing laboratory, as is recommended. In addition, some of the healthy older cats and
312 non-azotaemic hyperthyroid cats could have had subclinical, non-azotaemic CKD, which
313 could not be diagnosed without direct assessment of glomerular filtration rate. Serum protein
314 electrophoresis (SPE) is also a relatively insensitive way of determining the changes in serum
315 concentrations of the various components of the globulin fraction, and it is possible that
316 changes in the serum concentrations of one or more individual components of the globulin
317 fraction were associated with the development of azotaemic CKD following treatment of
318 hyperthyroidism, but the abundance of these proteins was too low to be detected by SPE.
319 Further evaluation of specific acute phase proteins and immunoglobulin concentrations in

hyperthyroid cats and their association with the presence of concurrent, but masked, CKD would be necessary to further evaluate this possibility.

Conclusions

Hyperthyroid cats demonstrate mild changes in the serum concentrations of some globulin fractions, most notably in the serum α_2 and γ globulin fractions, when compared with old non-hyperthyroid cats, however the cause of these changes was not identified in the current study. The mild changes in the globulin fractions are also unlikely to be of clinical relevance in most cases. Serum concentrations of total protein, total globulin or globulin fractions were not associated with the presence of concurrent, but masked, azotaemic CKD in hyperthyroid cats, suggesting that serum globulin concentrations are not a reliable marker of concurrent CKD in hyperthyroidism.

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440 **Table 1**

441 Selected baseline clinicopathological data and serum concentrations of total protein, albumin,
442 total globulin and globulin fractions in hyperthyroid cats (n=56) and healthy older cats
443 (n=26).

Variable	Hyperthyroid[a]	Healthy older cats[a]	Significance[b]
Age (years)	14.8 [12.8, 16.1]	12.0 [10.4, 16.1]	**<0.001**
Serum total thyroxine concentration (nmol/L)	104 [68, 152]	28 [18, 27]	**<0.001**
Serum/plasma urea concentration (mmol/L)	5.0 [4.1, 6.2]	4.6 [4.1, 5.6]	0.576
Serum/plasma creatinine concentration (mmol/L)	106.1 [88.4, 123.8]	132.6 [114.9, 141.4]	**<0.001**
Serum total protein concentration (g/L)	69.7 [65.9, 73.4]	71.5 [69.0, 76.0]	0.106
Serum albumin concentration (g/L)	29.9 [28.6, 32.0]	33.0 [29.0, 35.0]	**0.006**
Serum total globulin concentration (g/L)	38.5 [35.5, 42.8]	38.0 [35.8, 43.0]	0.858
Serum α_1 globulin concentration (g/L)	5.9 [5.1, 6.4]	5.9 [4.6, 6.7]	0.854
Serum α_2 globulin concentration (g/L)	12.5 [10.9, 13.9]	9.8 [9.0, 11.4]	**<0.001**
Serum β globulin concentration (g/L)	5.8 [4.9, 6.8]	5.6 [5.0, 6.8]	0.936
Serum γ globulin concentration	11.4 [9.1, 13.3]	14.0 [12.4, 16.8]	**0.001**

444 [a] Data are presented as median [25th, 75th percentiles].

445 [b] Mann Whitney U test was used to compare values in hyperthyroid and healthy older cat
446 groups.

447

448

Table 2

Comparison of serum concentrations of total protein, albumin, total globulin and globulin fractions between initially non-azotaemic hyperthyroid cats which develop azotaemic chronic kidney disease within four months of establishment of euthyroidism (masked-azotaemic, n=21) and hyperthyroid cats that remain non-azotaemic following treatment (n=35).

Variable	Masked-azotaemic[a]	Non-azotaemic[a]	Significance[b]
Serum total protein concentration (g/L)[c]	70.8 [67.2, 75.9]	68.1 [65.5, 72.2]	**0.049**
Serum albumin concentration (g/L)[c]	30.7 [28.0, 32.1]	29.6 [29.0, 32.0]	0.806
Serum total globulin concentration (g/L)[c]	39.8 [37.3, 45.8]	37.9 [33.8, 42.4]	0.104
Serum α_1 globulin concentration (g/L)[c]	5.8 [5.0, 6.3]	6.0 [5.2, 6.8]	0.326
Serum α_2 globulin concentration (g/L)[c]	13.0 [11.7, 14.4]	12.2 [10.9, 13.7]	0.426
Serum β globulin concentration (g/L)[c]	5.7 [5.3, 6.4]	5.9 [4.8, 7.0]	0.912
Serum γ globulin concentration[c]	12.3 [9.5, 17.4]	10.8 [8.7, 12.9]	0.092

[a] Data are presented as median [25th, 75th percentiles].

[b] Mann Whitney U test was used to compare values in masked-azotaemic and non-azotaemic hyperthyroid cats.

[c] Total protein was determined by biuret reaction and all other protein concentrations were determined by agarose gel electrophoresis.

Table 3

Changes in serum concentrations of total protein, albumin, total globulin and globulin fractions in hyperthyroid cats before treatment and at time of establishment of euthyroidism (n=55). Total protein was determined by biuret reaction and all other protein concentrations were determined by agarose gel electrophoresis.

Variable	Before treatment[a]	Establishment of euthyroidism[a,b]	Significance[c]
Serum total protein concentration (g/L)	69.9 [65.9, 73.5]	72.6 [69.6, 76.6]	**<0.001**
Serum albumin concentration (g/L)	29.6 [28.6, 32.0]	30.3 [27.9, 32.5]	0.378
Serum total globulin concentration (g/L)	38.6 [35.4, 42.8]	42.3 [39.0, 45.7]	**<0.001**
Serum α_1 globulin concentration (g/L)	5.9 [5.0, 6.4]	6.0 [5.3, 7.0]	**0.031**
Serum α_2 globulin concentration (g/L)	12.5 [10.9, 13.9]	11.5 [10.1, 12.6]	**<0.001**
Serum β globulin concentration (g/L)	5.7 [4.9, 6.8]	5.6 [4.7, 6.5]	0.626
Serum γ globulin concentration	11.4 [9.0, 13.3]	14.5 [12.0, 18.4]	**<0.001**

[a] Data are presented as median [25th, 75th percentiles].

[b] Time between before treatment and establishment of euthyroidism time points was 42 [35, 68] days.

[c] Wilcoxon signed rank test was used to compare values in hyperthyroid cats before treatment and at time of establishment of euthyroidism.

Figure legends

Figure 1. Box and whisker plots of serum α_2 globulin concentrations in a group of untreated hyperthyroid cats (n=56) and a group of healthy older cats (n=26). Whiskers represent the 5th and 95th percentiles and circles represent outliers. Serum α_2 globulin concentrations were higher in hyperthyroid cats compared to healthy older cats (P<0.001).

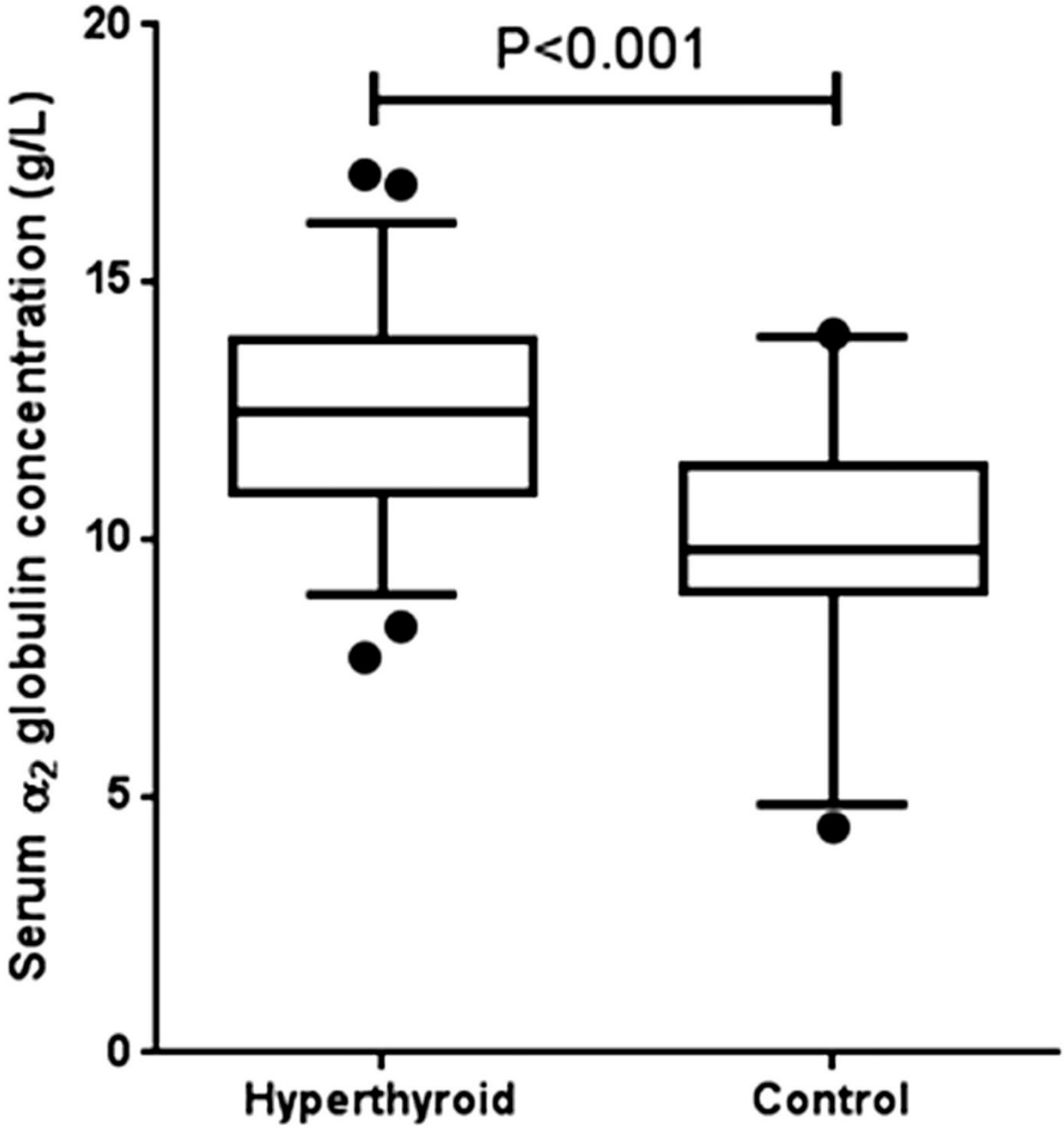

Figure 2. Box and whisker plots of serum γ globulin concentrations in a group of untreated hyperthyroid cats (n=56) and a group of healthy older cats (n=26). Whiskers represent the 5th and 95th percentiles and circles represent outliers. Serum γ globulin concentrations were higher in hyperthyroid cats compared to healthy older cats (*P*=0.001).

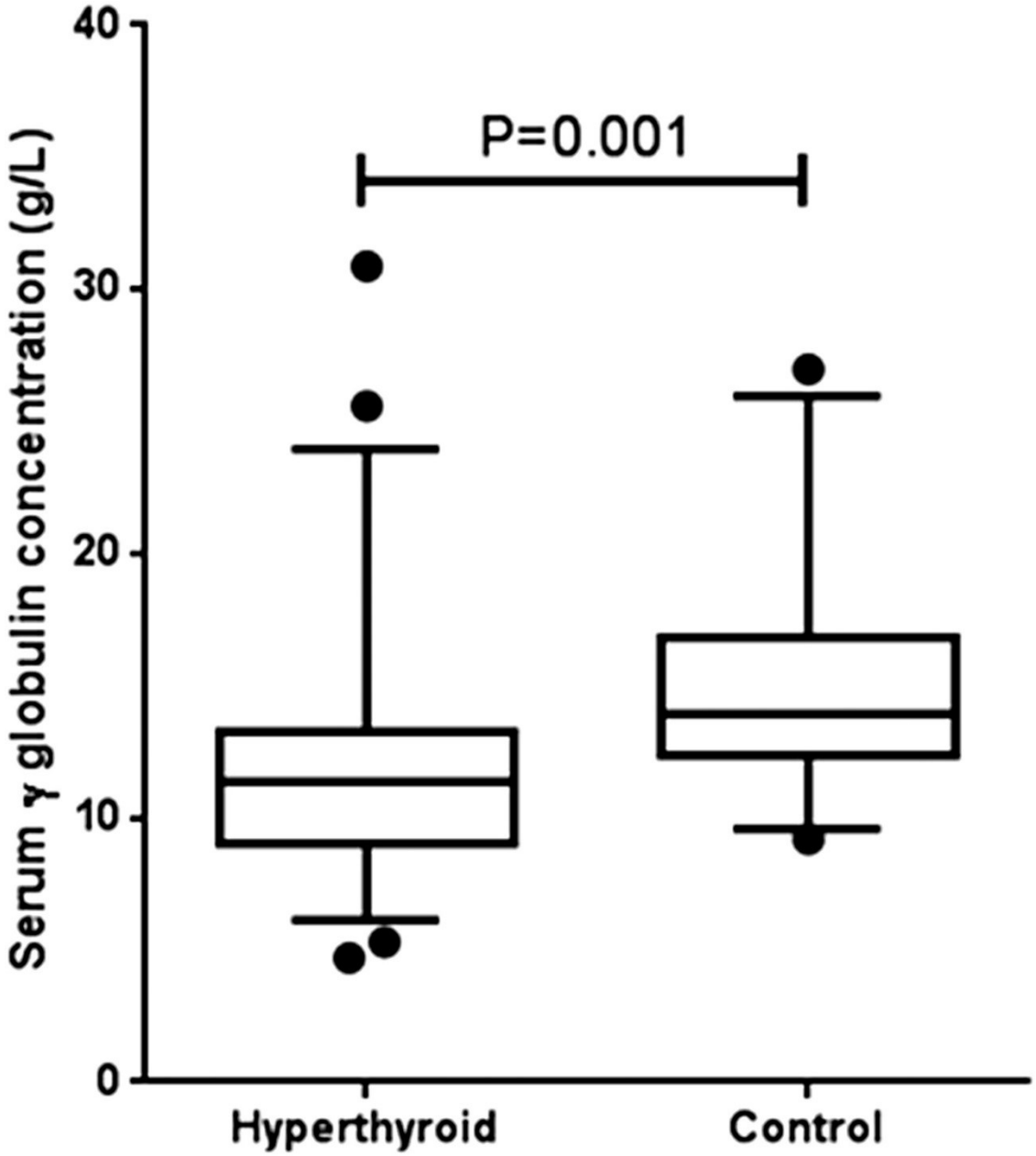

Figure 3. Line chart showing serum α_2 globulin concentrations in hyperthyroid cats before treatment (pre-treatment) and at time of establishment of euthyroidism (post-treatment). Serum α_2 globulin concentrations decreased significantly following treatment ($P<0.001$).

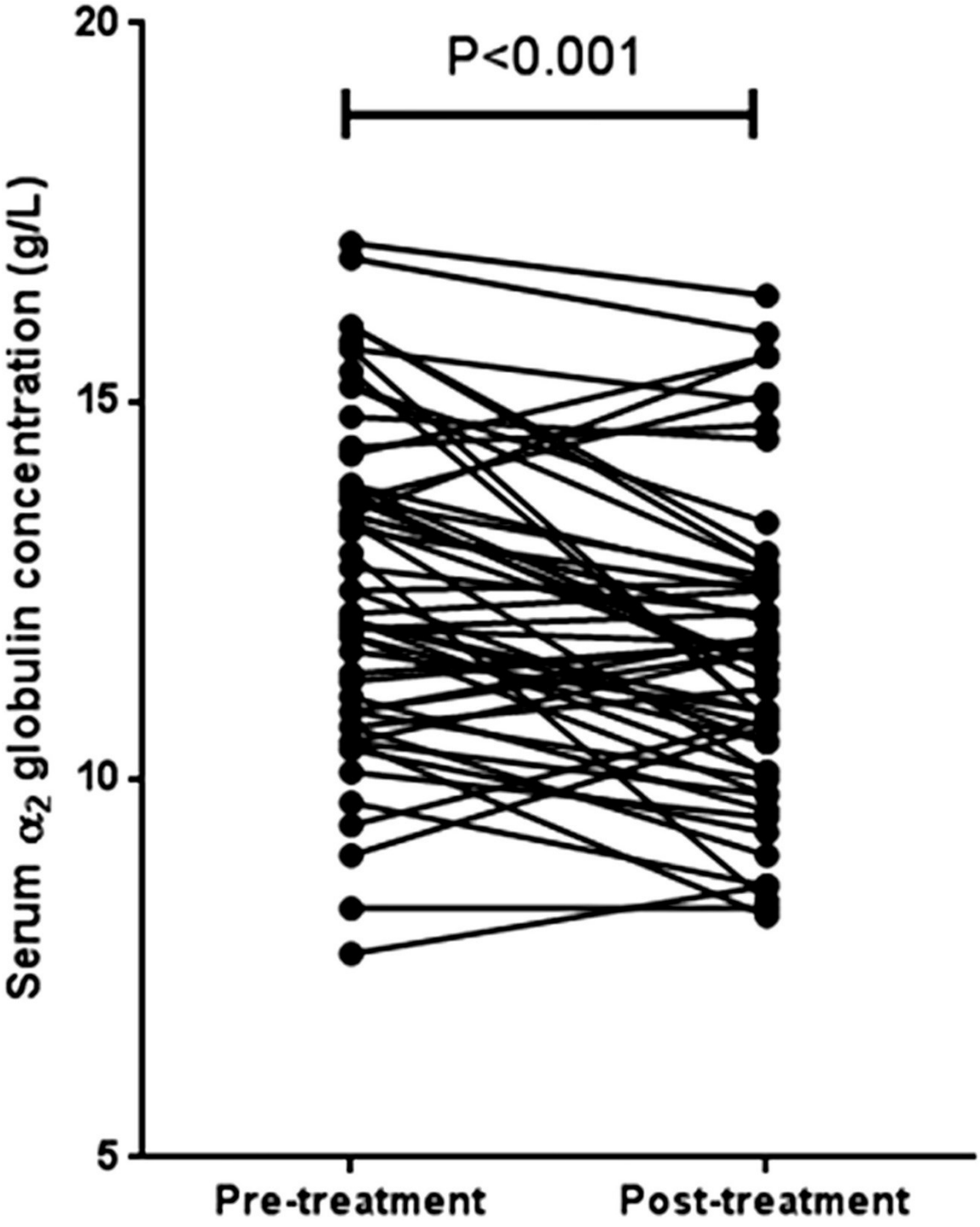

Figure 4. Line chart showing serum γ globulin concentrations in hyperthyroid cats before treatment (pre-treatment) and at time of establishment of euthyroidism (post-treatment). Serum γ globulin concentrations increased significantly following treatment (P<0.001).