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Effects of dietary selenium and moisture on the physical activity and thyroid axis of cats

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Summary

Consumption of canned cat food is considered a risk factor for the development of feline hyperthyroidism. Because selenium and water are substantially higher in canned diets compared to dry diets, objectives of this study were to determine whether increased dietary selenium or water alters the function of the hypothalamic–pituitary–thyroid axis and leads to an increase in activity level. Employing a 28-day latin square design with a 14-day washout, six lean, neutered male domestic shorthair cats were fed (i) commercially available adult dry feline diet containing 0.8 ppm selenium (control), (ii) control diet with added sodium selenite to achieve a dietary selenium concentration of 1.125 ppm (selenium treatment) and (iii) the control diet with additional water to achieve a moisture content of 75% wt/wt (water treatment). Water consumption was determined using deuterium oxide washout. Actical activity monitors were placed on each cat's collar to allow quantification of the activity of each cat. Circulating serum T3 and T4 was measured on days 0, 14, and 28. On day 28, a thyrotropin-releasing hormone (TRH) stimulation test was conducted to determine treatment effects on serum concentrations of thyroid hormones. There was a significant increase in daily water consumption with dietary water treatment (192 ml ± 7.85 SEM) compared to the control (120 ml ± 20.4) and selenium (116 ml ± 14.6) treatments. Both water and selenium treatments were associated with greater ($p < .05$) activity over that of the control treatment by 20.5% and 11% respectively. Serum TT3 AUC concentrations (0–4 hr) of TRH stimulation tests were greater ($p < .05$) by 16% with water compared to control treatments. The results of this study indicate that dietary water content may alter the function of the thyroid axis and that this effect is associated with an increase in physical activity.

KEYWORDS

feline, hyperthyroidism, nutritional aetiology, thyroxine (T4), triiodothyronine (T3)

1 | INTRODUCTION

Hyperthyroidism is a condition observed in domestic cats defined by the abnormal elevation of circulating concentrations of one or more thyroid hormones. The elevation of circulating thyroxine (T4), the thyroid hormone most commonly elevated, occurs from hyperfunctioning single or multiple hyperplastic nodules in the feline thyroid gland that autonomously proliferate (Gerber, Peter, Ferguson, & Peterson, 1994; Peterson, Graves, & Cavanagh, 1987). Since the first case report of spontaneously occurring hyperthyroidism in cats in 1979 (Peterson, Johnson, & Andrews, 1979), the prevalence of feline hyperthyroidism has skyrocketed (Edinboro, Scott-Moncrieff, Janovitz, Thacker, & Glickman, 2004; Scarlett, Moise, & Rayl, 1988). Hyperthyroidism is now considered the most common feline endocrine disorder in the USA (Mooney & Peterson, 2012) with an estimated 10% of cats over 10 years old are diagnosed with the disease (Peterson, 2012). Beyond

the USA, feline hyperthyroidism is present worldwide with varying prevalence rates, ranging from 0.1% to 20.14% (Gójska-Zygner, Lechowski, & Zygner, 2014; Kohler, Ballhausen, Stockhaus, Hartmann, & Wehner, 2016), depending on the inclusion criteria for the study.

Many epidemiological studies have been undertaken in attempts to determine the etiopathogenesis and risk factors associated with feline hyperthyroidism. These studies have not identified a cause or a single risk factor. The veterinary community currently believes that the etiopathogenesis is likely multifactorial, with the risk factors being classified under two main categories: (i) nutritional and (ii) thyroid-disrupting compounds in the environment (van Hoek, Hesta, & Biourge, 2015; Peterson, 2012).

Epidemiological studies conducted in the USA have consistently identified consumption of canned commercial cat food to be a significant risk factor for development of feline hyperthyroidism (Edinboro et al., 2004; Kass et al., 1999; Martin, Rossing, Ryland, DiGiacomo, & Freitag, 2000; Scarlett et al., 1988). Some studies have found increasing risk if the canned food was in a pop-top can (Edinboro et al., 2004), and increasing if the flavour was fish or liver and giblets (Martin et al., 2000). The consumption of canned cat food has also been identified worldwide as a risk factor for feline hyperthyroidism (Gójska-Zygner et al., 2014; Kohler et al., 2016; Olczak et al., 2005; Wakeling, Everard, Brodbelt, Elliott, & Syme, 2009). However, a single study conducted in Hong Kong did not report canned food as a significant risk factor. The sample size of this study was limited to 12 hyperthyroid cats (De Wet, Mooney, Thompson, & Schoeman, 2009). With a lack of prospective studies, there have only been suggested hypotheses as to why the consumption of canned cat food is linked to an increase risk of hyperthyroidism.

Isoflavones, selenium and iodine are known to be incorporated into feline canned diets at highly variable concentrations (Court & Freeman, 2002; Johnson, Ford, Tarttelin, & Feek, 1992; Simcock, Rutherford, Wester, & Hendriks, 2005). These three dietary constituents are routinely hypothesized to contribute to the pathogenesis of feline hyperthyroidism (van Hoek et al., 2015). Simcock et al. (2005) reported canned cat food contains significantly higher concentrations of selenium than dry cat diets, with "seafood" and "chicken and seafood" flavoured diets consistently containing the highest selenium concentrations. This association is of particular interest since consumption of fish, liver and giblets-flavoured canned cat food further increases risk of hyperthyroidism (Martin et al., 2000). Dietary selenium content has been shown to influence the conversion of T4 to triiodothyronine (T3) through the selenoenzymes, iodothyronine deiodinase types I, II and III (Wedekind et al., 2003). It is unknown whether selenium directly alters the function of the hypothalamic–pituitary–thyroid axis.

Most domestic pet cats are maintained on dry-expanded diets, canned diets or a combination of both diets. It is unknown if the high dietary moisture content of canned diets alters the production, secretion or metabolism of thyroid hormones. Water, as a nutrient, is often overlooked in research studies (Sawka, Cheuvront, & Carter, 2005); however, water intake can have profound impacts on a variety of physiological processes including metabolism (Boschmann et al., 2003). Deng, Iwazaki, Suchy, Pallotto, and Swanson (2014) demonstrated that a diet with 70% moisture content increased the activity of cats when compared to a diet with a moisture content of 8% w/w. The mechanism of this increased activity has not been determined, but perhaps the increased dietary water consumption altered the production of thyroid hormones resulting in the observed increase in activity level. Increased activity, or hyperactivity, is clinically observed in cats with hyperthyroidism (Thoday & Mooney, 1992) which is also observed in rats rendered hyperthyroid through administration of T3. However, the mechanism as to how thyroid hormones increase spontaneous physical activity is unknown (Levine, Nygren, Short, & Nair, 2003).

This study sought to determine whether the dietary nutrients, selenium and water alter the function of the hypothalamic–pituitary–thyroid axis. This objective was encompassed by three hypotheses. The first hypothesis was that a diet high in selenium would result in an increase in T3 production by the thyroid gland, and that this production would be revealed by a thyrotropin release hormone (TRH) stimulation test. The second hypothesis was that the moisture content of a typical feline canned diet (~75% w/w) would alter the function of the hypothalamic–pituitary–thyroid axis and cause an increase in T4 production. The third hypothesis was that the increase secretion of thyroid hormones from the dietary treatments would result in a significant increase in physical activity.

2 | MATERIALS AND METHODS

All procedures performed were approved by the University of Missouri Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011), Public Health Service policy (Public Health Service, 2002) and the Animal Welfare Act and Regulations (United States Department of Agriculture, 2008).

2.1 | Animals

Six, adult (3–5 yr), lean (16%–25% body fat [w/w] based upon deuterium oxide body composition and a BCS of 4-5/9), purpose bred, neutered male, domestic short-haired cats, were deemed healthy on physical examination, complete blood cell count and serum clinical biochemistry (University of Missouri Veterinary Diagnostic Laboratory, Columbia, MO, USA) findings. All cats had ad lib access to water and were socially housed in a room (3.2 × 4.8 m) with free access to four adjacent cubicles (1.1 × 1.6 × 2.4 m). Two cats were individually housed in stainless steel cages (0.9 × 0.7 × 1.2 m), and four cats were individually housed in cubicles during food presentation. All cats were consistently housed in the same cubicle or cage during food presentation.

2.2 | Diets

A commercially available feline adult maintenance dry-expanded diet (Purina Pro Plan Urinary Tract Health Formula, Nestlé Purina PetCare

TABLE 1 Proximate analysis and select nutrients of the control diet

Nutrient	Amount
Crude protein g/100 g	31
Crude fat g/100 g	14
Crude fibre g/100 g	2
Moisture g/100 g	10
Ash g/100 g	6.2
Selenium ppm[a]	0.857
Iodine ppm[b]	2.27

[a]As determined by the South Dakota Agricultural Laboratories Agricultural Experiment Station Chemical Lab, on a dry matter basis.
[b]As determined by the University of Missouri-Columbia Research Reactor Center, on dry matter basis.
Ingredients: Corn gluten meal, chicken, wheat flour, brewers rice, ground yellow corn, animal fat preserved with mixed-tocopherols (form of vitamin E), egg product, sodium caseinate, phosphoric acid, calcium carbonate, potassium chloride, animal digest, salt, L-lysine monohydrochloride, dried whey, choline chloride, dicalcium phosphate, taurine, zinc sulphate, ferrous sulphate, manganese sulphate, vitamin E supplement, niacin, citric acid, vitamin A supplement, calcium pantothenate, thiamine mononitrate, copper sulphate, riboflavin supplement, vitamin B-12 supplement, pyridoxine hydrochloride, folic acid, vitamin D-3 supplement, calcium iodate, biotin, menadione sodium bisulphite complex (source of vitamin K activity) and sodium selenite.

Company, Saint Louise, MO, USA) that was nutritionally complete and balanced as substantiated from passage of feeding trials as described by American Association of Feed Control Officials (AAFCO) served as the base and control diet (Table 1). Reverse osmosis water which did not contain substantive amounts of selenium or iodine was continuously provided. The base/control diet contained selenium at 0.8 ppm and 10% moisture. There were two dietary nutrient variables, selenium and water. The increased selenium content diet of 1.25 ppm selenium was made by top-dressing the base diet with sodium selenite (Dyets Inc, Bethlehem, PA, USA) dispersed in dyetrose (Dyets Inc, Bethlehem, PA, USA) to ensure even distribution and coating of the kibble during batch mixing. The increased moisture content diet of 75% (w/w) water was achieved by adding water purified by reverse osmosis to the base diet the evening before presentation. The food remained covered and refrigerated (4°C) overnight to allow the kibble to absorb the water. Selenium contents of the diets were determined by the South Dakota Agricultural Laboratories (Brookings, SD, USA) and proximate analysis of the diets was completed at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Iodine contents of the diets were determined by neutron activation analysis (Spate et al., 1995) by the University of Missouri-Columbia Research Reactor Center (Columbia, MO, USA).

2.3 | Study design

Using a latin square design, each cat was randomly assigned to one of three dietary treatments, (i) control (0.8 ppm selenium, 10% moisture), (ii) high selenium (1.25 ppm, 10% moisture) or (iii) high moisture (0.8 p.m., 75% moisture). Diet-presentation blocks were 28 days in duration. Following each block were 14-day washout periods during which cats were presented with the base diet. Cats were presented daily an allotted amount of diet to maintain a stable weight and an ideal body condition score of 5 of 9 (Laflamme, 1997). The dry matter amount of diet offered to each cat was not changed during the study, and dietary intake was recorded daily. Cats were weighed weekly. Jugular venous blood was collected on days 0, 14 and 28 of each dietary block. Blood was allowed to clot at room temperature for 1 hr prior to centrifugation at 1,200 × *g* for 10 min. Extracted serum was stored in 1 ml aliquots at −20°C until analyses.

2.4 | Thyroid-releasing hormone (TRH) stimulation tests

On day 28 of each diet block, a thyrotropin-releasing hormone (TRH) stimulation test was conducted using previously described methods (Peterson, Broussard, & Gamble, 1994; Sparkes, Jones, Gruffydd-Jones, & Walker, 1991). Briefly, jugular venous blood was collected prior to each cat intravenously receiving 0.1 mg/kg protirelin (TRH) (Wedgewood Pharmacy, Swedesboro, NJ, USA) (time 0). Jugular venous blood was thereafter collected at 0.5, 1, 2 and 4 hr post-TRH injection. Blood was kept on ice for up to 2.5 hr before being centrifuged at 1,200 × *g* for 10 min to extract serum, which was stored at −20°C until analyses of thyroid hormones.

2.5 | Activity monitoring

Three weeks prior to the start of the study, all cats were habituated to wearing collars to which was attached an accelerometer (Actical Activity Monitoring Devices, Mini Mitter, Bend, OR, USA). The accelerometer devices were previously validated for use in measuring the physical activity of cats (Lascelles et al., 2008). Motion was registered as an activity count, a unit-less measure. Activity counts were continuously logged at 15 s intervals throughout each diet block on days 1 through 27. Each cat wore the same Actical unit for the duration of the study. Sums of activity counts per day were determined for each cat for each block with and without periods of human interaction. A period of human interaction was defined as when any husbandry staff or laboratory staff entered the housing room which was tracked by key card access and a room door sign-in sheet.

2.6 | Thyroid hormone and selenium analysis

Serum samples were sent overnight on dry ice to Michigan State University's Diagnostic Center for Population and Animal Health (DCPAH) for measurements of total T4 (TT4), and total T3 (TT3) on days 0 and 14; and TT4, TT3, TSH, free T4 (fT4), and free T3 (fT3) on day 28. Days 0 and 28 serum samples were sent on dry ice to South Dakota Agricultural Laboratories (Brookings, SD, USA) for selenium analysis.

TABLE 2 Feed intake, metabolizable energy, weight and percentage weight change over the duration of each dietary treatment block

Dietary treatment	Dietary moisture (% w/w)	Dietary Se (ppm)[a]	Food intake (g DM/day)	Metabolizable energy (kcal/day)	Weight day 0 (kg)	Weight day 28 (kg)	Weight change (%)
Control	10	0.8	52 ± 3.1	87 ± 5.1	4.7 ± 0.23	4.7 ± 0.28	−0.02 ± 0.03
Selenium	10	1.25	52 ± 3.0	87 ± 5.0	4.7 ± 0.35	4.8 ± 0.24	0.01 ± 0.04
Moisture	75	0.8	52 ± 3.2	86 ± 5.3	4.8 ± 0.20	4.7 ± 0.27	−0.02 ± 0.02

[a]Provided as sodium selenite on a per dry matter basis.

2.7 | Water consumption

Water consumption was estimated using the deuterium oxide (D2O) washout method (Lifson & McClintock, 1966). Body composition was determined as previously described (Backus, Cave, Ganjam, Turner, & Biourge, 2010). Briefly, on day 0 of each block, jugular venous blood was collected and subsequently cats received 0.7 g/kg of sterile filtered, salinated (9 g/L sodium chloride), D2O (99.8% Sigma-Aldrich, St. Louis, USA) subcutaneously. Jugular venous blood was collected 4–6 hr after injection of the D2O for determination of D2O enrichment in serum after equilibration. Jugular venous blood was collected again on days 14 and 28. Aliquots of serum were stored in 1.5-ml microcentrifuge screw top tubes (USA Scientific, Ocala, FL, USA) at −20°C until analysis. In duplicate, deuterium oxide enrichment was determined in water distilled from serum using Fourier transform infrared spectrophotometry (Jennings, Bluck, Wright, & Elia, 1999). A first-order washout curve was assumed to correctly model rate of water turnover of the cats. Water consumption during each block in millilitres per day was determined from the fractional change in D2O enrichment during blocks and from body water mass estimated from D2O dilution on day one of blocks. Body lean mass of cats was assumed to equal body water mass divided by 0.732, the fractional moisture mass of lean tissue reported applicable to many species. Body fat mass was assumed to equal body weight minus body lean mass.

2.8 | Statistical analyses

We used SAS 9.3 software package (SAS Institute Inc., Cary, NC, USA) to perform all statistical analysis, and significance was calculated at alpha = 0.05. All outcome variables were determined to be normally distributed, except for the activity data which was transformed through log10. The Proc GLM procedure and differences of the least square means were used to compare the variables of interest: TT4, TT3, TSH, fT4, fT3, activity counts, water consumption, diet consumption, body weight, and cat and the interactions of cat*dietary treatment, dietary treatment*block, dietary treatment*block*time.

3 | RESULTS

The dietary treatments did not significantly ($p > .05$) alter the amount of diet consumed or weights throughout the study (Table 2).

When the cats were given the high moisture diet, their total water consumption was significantly increased ($p < .001$) by a mean of 72.6 ml per day compared to the control diet. One cat consumed more water than the other cats ($p < .01$) when they were on the high selenium and control diets (Figure 1). When data from this cat were excluded from analyses, the mean water consumption among the other cats was greater ($p < .001$) by a mean of 86.2 ml per day when they were when given the high moisture diet compared to when they were given the control diet. Urinalysis and serum chemistry analysis were repeated for the cat with extraordinary water consumption. Serum creatinine and urea nitrogen concentrations were within the reference interval of the clinical laboratory. Results of the urinalysis revealed a low urine osmolality with no bacteria or crystals observed in sediment. It is possible the cat with extraordinary water consumption was in a very early stage of chronic renal failure. Nevertheless, observations from this cat were included in

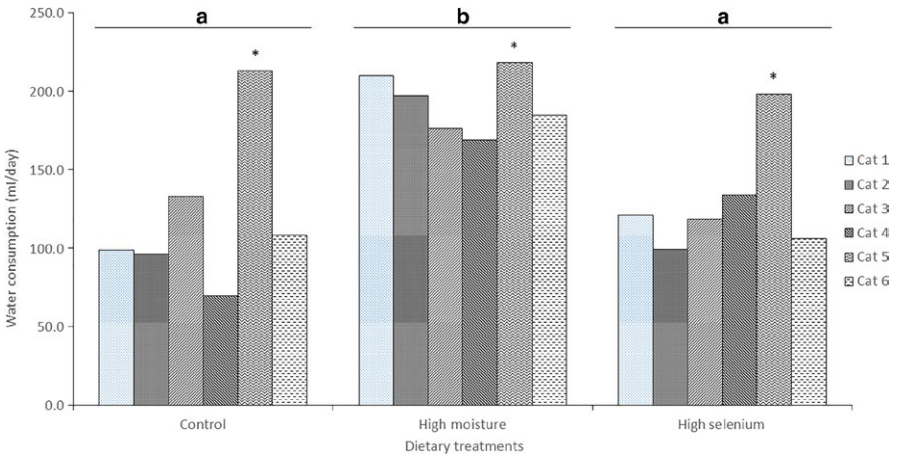

FIGURE 1 Daily water consumption of each cat as measured by deuterium oxide dilution method. The cats consumed significantly more water on the high moisture diet compared to the control ($p < .01$) and high selenium ($p < .01$) diets. Cat five consumed more water than all other cats (*$p < .01$) [Colour figure can be viewed at wileyonlinelibrary.com]

all statistical analyses as removing his data did not change the outcomes of the study.

Throughout each dietary block, serum selenium concentrations were highly variable within each cat. The mean serum selenium concentration did not significantly increase ($p = .98$) when the cats were on the selenium-added dietary treatment (Table 3).

The TT4 and TT3 serum concentrations of the cats were not significantly different ($p > .05$) between any of the dietary treatments on any of the sampling days during the treatment blocks (days 0, 14 and 28) (Table 3).

On day 28 of dietary blocks, prior to TRH injection of TRH stimulation tests, baseline serum concentrations of TT4, TT3, fT4, fT3 and TSH were not significantly different ($p > .05$) between the control, high selenium and high moisture dietary treatments. Immediately after each cat received their dose of TRH for TRH stimulation test, the cats immediately began to exhibit ptyalism with one to two of the cats vomiting frothy fluid each block. During the first 2 hr, all the cats exhibited lethargy, and during the second 2 hr, only two cats remained lethargic.

When the cats were on the high dietary moisture treatment, serum TT3 concentrations were greater ($p < .001$) post-TRH infusion (Figure 2) compared to when they were on the other diets ($p < .01$). Consumption of the high moisture diet resulted in a sixteen per cent increase in serum TT3 area under the curve when compared to the control dietary treatment. There were no significant dietary treatment effects on the serum concentrations of TT4 ($p = .94$), fT4 ($p = .88$) and fT3 ($p = .68$) following TRH injection (Figure 3).

There was no significant dietary treatment effects seen with serum TSH concentrations ($p = .80$) following TRH injection. Each cat's TSH concentrations peaked at 30 min post-TRH infusion; however, there was great between-individual variability in serum TSH concentrations among the cats with two cats consistently exhibiting the highest of serum TSH concentrations during each dietary block (Figure 4, Fig. S1–S2).

The cats accepted the Actical activity monitors on their collars. One monitor malfunctioned on days 14–28 of the high moisture dietary treatment; therefore, only data from five cats were included for the activity analysis of this dietary treatment. Additionally, there was one cat whose collar was found to be on the floor during an approximately 4-hr time span. The data for all cats during this specific 4-hr time span during each dietary block were eliminated prior to analysis. Activity counts were highly variable between cats and within cats, and activity counts greatly varied each day ($p < .01$). A significant increase in physical activity without human interaction was observed in the high moisture dietary treatment and the high dietary selenium treatment compared to the control dietary treatment ($p < .05$, Figure 5). Activity counts were not found to significantly vary with dietary treatment unless activity counts recorded during periods of human interaction were omitted from analyses. The authors would like to highlight that with our data, there was large variability between days and between cats which lead us to incorporate the entire block into our analysis rather than only the last week. We also chose to use the sum of the daily activity counts instead of the mean activity counts.

TABLE 3 Serum Se, TT4 and TT3 over the duration of each dietary treatment block

Dietary Treatment	Serum Se day 0 (μmol/L)	Serum Se day 28 (μmol/L)	Serum TT4 day 0 (nmol/L)	Serum TT4 day 14 (nmol/L)	Serum TT4 day 28 (nmol/L)	Serum TT3 day 0 (nmol/L)	Serum TT3 day 14 (nmol/L)	Serum TT3 day 28 (nmol/L)
Control	6.6 ± 1.3	6.3 ± 0.93	18 ± 4.9	16 ± 4.5	20 ± 5.0	0.83 ± 0.39	0.73 ± 0.12	0.70 ± 0.09
Selenium	6.6 ± 0.73	6.8 ± 0.75	18 ± 3.8	17 ± 1.9	17 ± 2.1	0.72 ± 0.14	0.75 ± 0.18	0.63 ± 0.05
Moisture	6.6 ± 0.71	7.1 ± 0.93	17 ± 4.4	16 ± 3.9	18 ± 3.0	0.67 ± 0.12	0.73 ± 0.10	0.73 ± 0.14

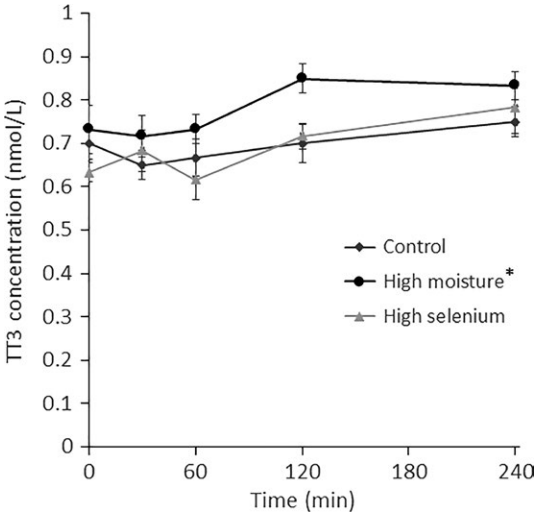

FIGURE 2 Mean total T3 serum concentration prior to intravenous administrations of TRH and post-injection. There was a significant difference found between the high dietary moisture diet compared to the control diet (*$p < .05$)

4 | DISCUSSION

With nearly all epidemiological studies universally identifying commercial canned cat food as a risk factor for feline hyperthyroidism (Gójska-Zygner et al., 2014; Kohler et al., 2016), there has been much speculation on whether the increased risk is due to the nutrient composition of canned food or contaminants in the diet (van Hoek et al., 2015). It has been largely assumed that canned cat food is a "causation" factor whereas few have reflected on the possibility that it may be simply "guilty by association" rather than an actual cause of hyperthyroidism. Most studies that investigate possible causes of feline hyperthyroidism have focused on measuring blood biomarkers (nutrients or contaminants) in hyperthyroid and euthyroid cats. One such example is Foster et al. (2001) who measured the plasma concentrations of selenium and glutathione peroxidase (GPX) activity. These studies are of limited value because they report on effects pre- and post-diagnosis rather than physiological changes that are occurring during the early pathogenesis of feline hyperthyroidism.

Our study was unique as it examines potential nutritional aetiologies of hyperthyroidism in a prospective investigation. The aims were to determine whether high dietary selenium or high dietary moisture, equivalent to those typically found in canned cat foods (Simcock et al., 2005), alter the function of the hypothalamic–pituitary–thyroid axis, leading to an increase in thyroid hormone production, and this increase in thyroid hormone would be reflected in the measurable outcome of increased physical activity, as increased activity is a commonly described clinical sign of feline hyperthyroidism.

Our hypothesis that the increase secretion of thyroid hormones from the dietary treatments would result in a significant increase in physical activity was partially supported by our results as we observed a significant increase ($p < .05$) in activity when the cats were on the high selenium and high dietary moisture treatments compared to the control treatment, but only when they were socially housed without human interaction (Figure 5). Including human interaction time along with when the cats were housed socially together, yielded results

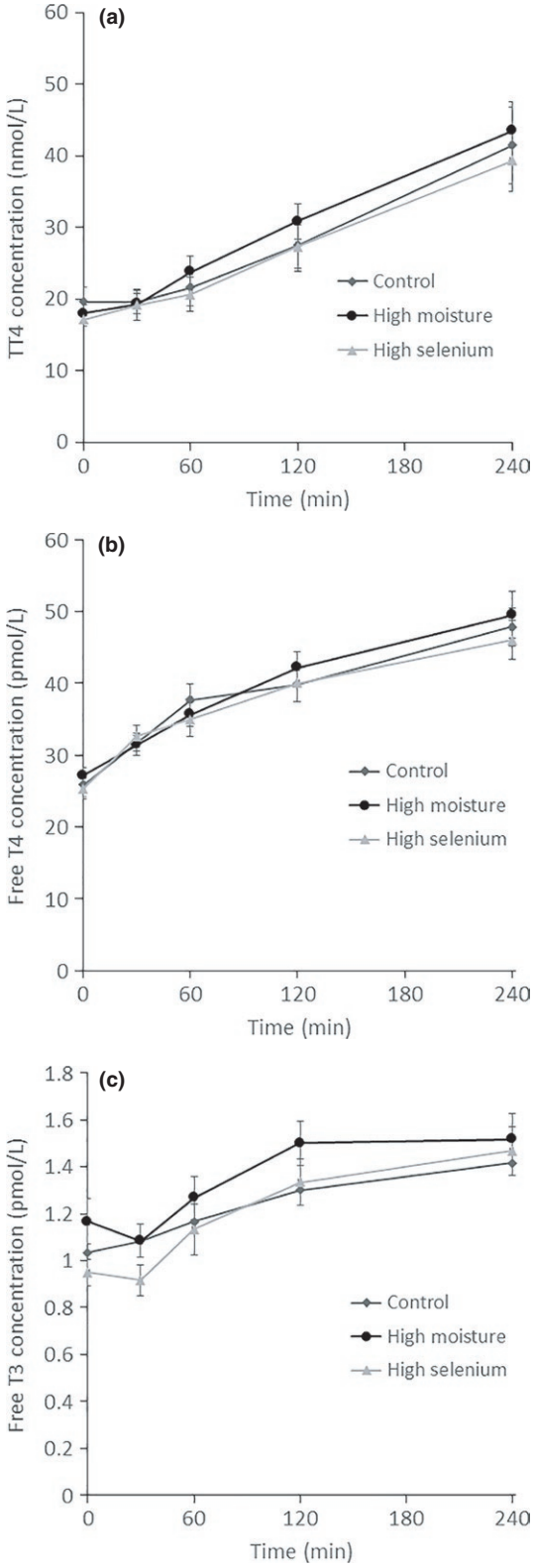

FIGURE 3 Mean total T4 (A), free T4 (B) and free T3 (C) serum concentration prior to intravenous administration of TRH and post-injection

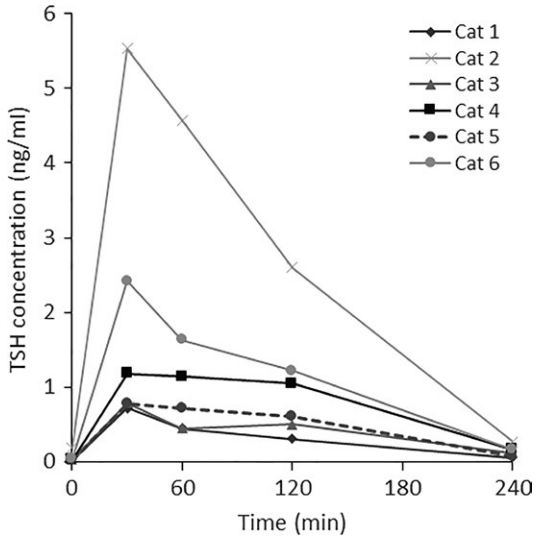

FIGURE 4 Individual serum TSH concentrations prior to intravenous administration of TRH and post-injection when cats were receiving the control diet

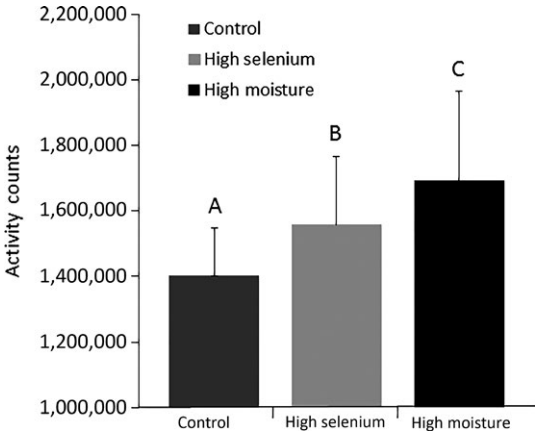

FIGURE 5 Mean sum of the activity counts during the entire dietary treatment block. Control and high selenium dietary treatments $n = 6$ whereas high dietary moisture $n = 5$ due to the malfunction of one of the units

similar to Deng et al. (2014), who reported a trending increase in the mean activity of cats fed a 70% (w/w) high moisture diet compared to the control dry diet when fed twice daily. As our cats' personalities are highly variable, with only some of the cats seeking attention and playing with the care staff and laboratory staff, it was important to eliminate this uncontrollable variable by controlling access to the vivarium room and eliminating all data that were collected while there was a human in the room. This allowed us to find a difference between dietary treatments. A limitation of our study was the lack of video recording to pair with the activity data in order to determine the type of physical activity the cats engaged in; however, with a 20% increase in total non-human interaction activity when the cats were on the high moisture diet, we do not believe it was solely due to increased litter box usage as Deng et al. (2014) suggests.

Our results warrant further investigation into the mechanisms underlying the increase in activity that was observed, particularly when cats consume a diet high in selenium as there was not a significant increase in T4 or T3 during the dietary treatment blocks nor did we detect an increase in serum selenium (Table 3). While only TSH has been found to be impacted by food consumption in humans (Nair, Mahadevan, Muralidharan, & Madhavan, 2014), in cats it has been shown that TT4 increases 4 hr post-prandial, and TT3 increases post-prandial 4, 8, 12 and 16 hr (Hooper, Mori, & Backus, 2014). All of the thyroid hormones measured within this study were performed on fasted samples. Perhaps if we had measured post-prandial samples, an increase in TT3 may have been recorded that could be directly linked to the increased activity level.

Additionally, we did not observe any changes in the peripheral serum TT3:TT4 ratio when the cats were on the high dietary selenium treatment. This could reflect directly on the deiodinase activity not increasing as to the uptake of selenium, as detected by the selenium content of the blood, did not change significantly between day 0 and day 28 (Table 3). As we utilized sodium selenite which is absorbed through passive diffusion, the blood selenium content may have increased if an organically bound selenium was incorporated into the diet as this form is actively transported in the cat (Todd, Thomas, & Hendriks, 2012). Despite not observing an increase in serum content, the tissue selenium concentration could have been increased, resulting in increased TT3 cellular concentration; however, the tissue levels were not measured. Alternatively, the lack of changes in TT3 could be attributed to the deiodinase enzymes already being fully activated on the control diet as our dietary selenium concentration of 0.8 mg/kg was greater than the breakpoint of 0.05 mg/kg for the TT4/TT3 ratio that is reported for kittens (Wedekind et al., 2003). The iodothyronine deiodinases are present in both the tissue and the thyroid gland (Salvatore, Tu, Harney, & Larsen, 1996). Iodothyronine deiodinase I (IDI) is attributed to the main production source of plasma TT3 in human hyperthyroid patients and has been documented in the thyroid gland of some species (Bianco & Kim, 2006). IDI has not been found in the feline thyroid gland (Foster et al., 2001) and could help explain why we did not detect an increase in circulating T3. Iodothyronine deiodinase II (IDII) mRNA has been detected in the thyroid gland of humans (Salvatore et al., 1996), but there have been no reports of the IDII enzyme detected in the feline thyroid gland and typically tissue only expresses one type of deiodinase at a given time (Arthur & Beckett, 1999). Although IDII is a major source of plasma T3 in euthyroid patients (Bianco & Kim, 2006), it would be of value to determine whether the feline thyroid contains IDII. Iodothyronine deiodinase III (IDIII) is not found in the thyroid gland but solely in the tissue, and is responsible for converting T4 to rT3 and T3 to T2 (Bianco & Kim, 2006).

To determine whether selenium upregulated the deiodinases within the thyroid or altered the function of the hypothalamic–pituitary–thyroid axis, we conducted a TRH stimulation test at the end of each dietary block. This allowed differentiation between increased T3 production by the thyroid versus increased peripheral conversion of T4 to T3. Contrary to our hypothesis, the high dietary selenium treatment did not significantly affect the serum TT3 concentration during the TRH stimulation test (Figure 2). The selenium content of the thyroid is one

of the highest out of all the organs in the body (Kohrle, 1999), which may be due to its role as an antioxidant. In humans, there is no correlation established between selenium content of thyroid tissues and the expression of functional IDI activities (Kohrle, 1999), which along with the lack of IDI enzymes within feline thyroids (Foster et al., 2001), could explain why there was no impact seen during the TRH stimulation test. Alternatively, the lack of effect could be due to the excessive selenium promoting iodine excretion as has been shown in the mouse (Xu et al., 2011). If iodine was reduced in the thyroid, it may reduce the amount of thyroid hormone produced. Future research on the interaction of iodine with other dietary nutrients such as selenium would be of value.

One of the most interesting findings of this study was that the consumption of high dietary moisture resulted in a significant increase in TT3 production during the TRH stimulation test (Figure 2). The 16% difference in the TT3 AUC between the high moisture versus the control diet found during the TRH stimulation test may provide a potential mechanism for the observed increased activity level (Figure 5), as only in the past few years has it become recognized that the thyroid hormones have a large role in the central nervous system, and do not just act peripherally (Lopez, Alvarez, Nogueiras, & Dieguez, 2013; Mullur, Liu, & Brent, 2014). To the authors' knowledge, there are no known reports of increased water consumption altering the production or regulation of thyroid hormones. However, our results should be carefully interpreted. Our findings do not indicate that the high moisture diets induce feline hyperthyroidism, especially when considering cats consuming prey are consuming diets with a similar moisture content. Rather our results indicate future studies on the relationship between water consumption and feline physiology including the thyroid axis are needed.

In cats, increase in production of T3 has been only attributed to an upregulation of the selenoenzymes iodothyronine deiodinases, which convert T4 to T3 (Wedekind et al., 2003). Upregulation of these iodothyronine deiodinases has been solely attributed to increased dietary intake of selenium (Wedekind et al., 2003), as consumed selenium is metabolized by the body into hydrogen selenide, which in turn can be utilized for selenoprotein synthesis (Suzuki, 2005), hence the hypothesis that increased dietary selenium would alter the function of the thyroid axis.

As our study was conducted in a high selenium area of the USA (United States Geological Survey, 2016), the water provided to the cats ad lib as well as the water added to the base diet, during creation of the high moisture diet, underwent filtering followed by reverse osmosis. Reverse osmosis water purification is routinely used effectively in mining operations to remove selenium (Santos, Ungureanu, Boaventura, & Botelho, 2015) and ensured our cats did not consume any additional selenium. Therefore, our TRH stimulation test results showing an increase in TT3 production when the cats were fed a diet with high dietary moisture (Figure 2) were not likely due to increased selenium intake nor an upregulation of deiodinases secondary to selenium ingestion. Future studies should be conducted to determine what physiological alterations may be occurring both on the central as well as peripheral or cellular level with the increased water consumption.

While no dietary treatment effects were found on the other thyroid parameters measured during the TRH stimulation test, the TSH results are of particular interest. There were significant intercat differences found as four of the cats exhibited similar TSH levels throughout the test while two individuals had repeatedly higher TSH values (Figure 4, Fig. S1–S2). Although it is difficult to conduct long-term studies, it may be worthwhile to design a lifetime prospective study to determine whether cats with high TSH values are at an increased risk for the development of hyperthyroidism. It is suspected that cats have a long subclinical state of hyperthyroidism, before developing overt hyperthyroidism (Wakeling et al., 2007). During this subclinical state, TT4 and TT3 will be within the normal range while TSH will be low or undetectable (Peterson, 2014; Wakeling et al., 2007). However, due to the lack of available feline specific TSH assays (Peterson et al., 1994), laboratories use canine TSH assays as feline TSH has 96% homology to canine TSH (Rayalam, Eizenstat, Hoenig, & Ferguson, 2006). These assays unfortunately lack sensitivity, and cause both hyperthyroid and some euthyroid cats to both have TSH below the limit of quantification (Wakeling et al., 2007) which the authors have experienced. As an attempt to avoid this, we predicted that the TRH stimulation test would to allow us to measure TSH in our research colony cats. While this was successful, our results also suggest cats may have individual set points for thyroid function similar to humans as it has been documented that individuals have different set points which result in unique thyroid functions (Andersen, Pedersen, Bruun, & Laurberg, 2002). There has been recent interest in using cats as an animal model for human thyroid research, particularly for toxic multinodular goitre as the subclinical, clinical and histological progression of the human disease is nearly identical to feline hyperthyroidism (Peterson, 2014; Wakeling et al., 2007), and our results also provide further support that cats may be an excellent animal model for thyroid diseases.

As canned cat food diets are considered a risk factor for developing feline hyperthyroidism (Gójska-Zygner et al., 2014; Kohler et al., 2016), our study sought to provide insights into the role selenium and moisture may have into the etiopathogenesis of feline hyperthyroidism. This study highlighted that high dietary moisture is capable of altering the thyroid hormone production in cats and could be the underlying mechanism explaining why increased dietary moisture has been linked to an increase in physical activity (Deng et al., 2014). Additionally, to the author's knowledge, this is the first report of TSH being described during a TRH stimulation test in cats. Our results suggest cats may have individual set points for thyroid function, which should be investigated to determine whether different set points can be attributed to an increased risk of hyperthyroidism or if high TSH indicates early stages of feline hyperthyroidism. Further studies into the mechanisms of this increased TT3 would also be of great value, including the risks of long-term increased TT3 production as excessive T4 production, thyrotoxicosis, is attributed to multiple health conditions such as hypertension and osteoporosis (Bassett et al., 2007; Kobayashi, Peterson, Graves, Nichols, & Lesser, 1990).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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