# Obesity increases free thyroxine proportionally to nonesterified fatty acid concentrations in adult neutered female cats

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## Abstract

The obese cat is a model for the study of the progression toward type 2 diabetes. In this study, the impact of obesity on the hypothalamic–pituitary–thyroid axis was examined in 21 domestic shorthair cats before and after the development of obesity, which significantly increased body mass index (BMI), % body fat (BF), and girth ($P<0{\cdot}0001$ for all). Serum total thyroxine ($TT_4$), tri-iodothyronine, free $T_4$ ($FT_4$) by direct dialysis, nonesterified fatty acids (NEFA), and leptin were measured, and $FT_4$ fraction ($FFT_4$) was calculated. Serum thyrotropin (TSH) concentrations were measured in nine animals by validating a heterologous canine TSH assay with recombinant feline TSH as a standard. $FT_4$, $FFT_4$, NEFAs, and leptin were significantly higher in obese cats. $FT_4$ had the strongest positive correlation with obesity indices BF, BMI, girth, NEFA, and leptin. Fatty acids oleate and palmitate were shown to inhibit $T_4$ binding to pooled cat serum *in vitro*, suggesting the possibility that this mechanism was also relevant *in vivo*. Serum $TT_4$ and TSH did not rise significantly. The implications for thyroid hormone (TH) action are not yet clear, but fatty acids have been proposed to inhibit the cellular uptake of TH and/or pituitary TH receptor binding, leading to TH resistance. Increased leptin may also alter sensitivity to negative feedback of TH. In conclusion, feline obesity is associated with a significant increase in $FT_4$ within the normal range; future investigation into the cellular thyroid status will be necessary to establish cause and effect in this obesity model.

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## Introduction

Cats are one of the few model species of human type 2 diabetes in which the progression towards the diabetic state can be studied longitudinally. They are the only non-primate species to develop β-cell deposits of the hallmark β-cell protein of human type 2 diabetes mellitus, amyloid (Hoenig *et al.* 2000). Thyroid hormones (THs) are involved in the regulation of metabolism, and regulate resting metabolic rate, thermogenesis, and lipolysis (Oppenheimer *et al.* 1991, Silva 1995). However, studies of thyroid function in obese people have produced inconsistent results. Obesity has resulted in either no changes in thyroid-stimulating hormone (TSH) or TH concentrations in the hands of some investigators (Glass & Kushner 1996, Roti *et al.* 2000), a moderate rise in total and free tri-iodothyronine ($T_3$) and TSH serum concentrations (Bray *et al.* 1976, Matzen *et al.* 1989, Stichel *et al.* 2000, Reinehr & Andler 2002), or an increase in total thyroxine ($TT_4$) and TSH (Iacobellis *et al.* 2005). A study of over 6000 human patients established a positive correlation between body mass index (BMI) and serum TSH concentrations (Nyrnes *et al.* 2006). Some studies are confounded by an underlying incidence of overt or subclinical hypothyroidism, as reflected by the observation of a low free $T_4$ ($FT_4$) and high TSH concentration (Knudsen *et al.* 2005), or low total $T_3$ ($TT_3$) and $TT_4$ with increased TSH and thyroid volume, suggesting a primary disruption of TH synthesis (Sari *et al.* 2003). A progressive increase in serum $T_3$ concentration and a concomitant fall in reverse $T_3$ concentration have also been observed (Davidson & Chopra 1979).

Clinical studies of spontaneous obesity in dogs have resulted in similar discrepancies: in a case study of 31 obese canine patients, 58% had results consistent with overt or equivocal primary hypothyroidism (Martin *et al.* 2006). In another study, serum $TT_4$ and $T_3$ concentrations were higher but only $T_3$ decreased with food restriction (Daminet *et al.* 2003).

It has been suggested that the cause of the increased serum TH concentrations together with an increase in TSH might be caused by hypothalamic–pituitary–TH resistance (Edupuganti *et al.* 1997). Supporting this hypothesis is the observation that $T_3$ receptors are decreased in obesity and the negative feedback of circulating THs on TSH is decreased (Burman *et al.* 1980). Several studies have tried to establish a link between TH and leptin on energy expenditure; however, others have shown that the action of leptin was not dependent on the presence of TH (Vettor 2005).

Despite the variety of prior studies, none have ascertained the mechanism of the increase in free and/or total TH


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concentrations in obesity. Most studies in man have been in spontaneously obese patients and interpretation has been complicated by the uncertainty of the underlying incidence of subclinical hypothyroidism. Cats are not prone to the development of spontaneous adult onset hypothyroidism to the extent of man or dog, having only a small incidence of congenital disease (Tobias & Labato 2001). We have previously shown that obese cats have decreased heat production and changes in fat metabolism (Hoenig *et al.* 2007*a*,*b*), raising the possibility that alterations in fat mass may impact hypothalamic–pituitary–thyroid axis function as well as calorigenesis. Therefore, in this study, we sought to study lean euthyroid cats before and again after the development of stable obesity to identify potential effects on the hypothalamic–pituitary–thyroid axis.

## Materials and Methods

### *Animals*

Twenty-one adult (aged 1–2 years) neutered purpose-bred female cats (Sinclair, Columbia, MO, USA and Harlan Sprague–Dawley, Madison, WI, USA) were used. All cats were maintained at the University of Georgia College of Veterinary Medicine Animal Care Facility, using standard colony conditions. Cats were housed separately in cages and were provided unlimited access to water. Animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with the guidelines established by the Animal Welfare Act and the National Institutes of Health. It was determined that the cats were healthy on the basis of results of physical examination and clinical laboratory tests. All cats were used to being handled daily. All cats were fed a commercially available diet (Iams Ocean Fish and Rice, Dayton, OH, USA) once daily and food intake was recorded at each feeding. Their weight was monitored weekly. BMI and % fat mass were measured as described (Hoenig *et al.* 2003) at the beginning of the study and after the cats had gained ~40% of fat mass. At both time points, blood was drawn, allowed to clot, and serum was collected after centrifugation at 500 ***g*** for 10 min. The serum was stored at −20 °C until assayed.

### *TH and NEFA assays*

Serum $TT_4$ and $TT_3$ were measured by previously described and validated procedures using in-house RIAs with commercially prepared antibodies (Endocrine Sciences, Tarzana, CA, USA) as described previously (Peterson *et al.* 1983, Ferguson & Peterson 1992). Free $T_4$ ($FT_4$) concentrations were measured by direct dialysis using the Nichols Institute (San Juan Capistrano, CA, USA) kit. Leptin was measured using the Linco multi-species leptin ELISA as validated for cats (Hoenig *et al.* 2003). Nonesterified fatty acids (NEFAs) were measured using an enzymatic test kit (Wako Diagnostic, Richmond, PA, USA). In the cats, the $FT_4$ fraction expressed as a percentage was calculated as: % $FT_4$ = $FT_4$ concentration (pmol/l)/$TT_4$ concentration (nmol/l) ×10.

Although each animal served as its own control, for purposes of comparison, the normal ranges for hormonal analytes as determined by values 2S.D. above and below the mean in 30 lean cats in our research colony were: $TT_4$ (12–29 nmol/l), $TT_3$ (0·06–1·1 nmol/l), $FT_4$ (15–40 pmol/l), and $FT_4$ fraction ($FFT_4$) (0·05–0·23%). The values demonstrated a parametric distribution.

### *Serum TSH immunoassay*

The commercial canine TSH immunoassay (Immulite Canine TSH, Diagnostic Products Corporation Inc., (DPC) Los Angeles, CA, USA) was standardized for feline TSH using a standard curve of purified recombinant feline TSH prepared as described by Rayalam *et al.* (2006*a*,*b*). In brief, the protein concentration of the purified recombinant feline TSH (rfsTSH) standard was determined by bicinchoninic acid protein assay using bovine TSH as a protein standard. Purity of the recombinant hormone was established by densitometric analysis of a silver-stained PAGE. For rfTSH preparations in which this gravimetric analysis was performed in parallel with measurement in this commercial canine TSH assay, rfTSH was detected with 34·0±6·2% (mean±S.D.) efficiency. We noted that recombinant canine TSH prepared, expressed, and purified with the same techniques was detected with 73·6% efficiency. The rfTSH standards diluted in the serum 'blank' provided in the commercial assay showed linearity with the provided canine TSH standards ($R$=0·999) with an intercept indistinguishable from zero. Using this standardized assay, serum TSH concentrations were determined in nine animals during the lean and obese state. To facilitate comparison with other studies using this canine TSH assay to measure feline TSH, the values directly derived from the canine standard curve (i.e. uncorrected for efficiency of detection of rfTSH) were reported. For comparison, one laboratory has reported immunoreactive TSH in normal cats as measured in the DPC canine TSH assay to be 0·03–0·11 ng/ml with a detection limit of 0·03 ng/ml and a median of 0·05 ng/ml (Moore *et al.* 2004).

### *$FT_4$ fraction by tracer equilibrium dialysis: effect of added NEFAs*

To directly evaluate the effect of specific NEFAs on serum binding of $T_4$, a tracer dialysis procedure was used as described for dog serum (Ferguson & Peterson 1992). In brief, customized Plexiglas chambers with dialysand and dialysate chamber volumes of 1 ml were used. High specific activity $^{125}$I-$T_4$ was purchased from Perkin–Elmer (Wellesley, MA, USA) and added to a 5% solution of BSA and predialyzed against 0·15 M $NaH_2PO_4$, pH 7·4, overnight to remove iodide. Pooled normal cat serum was used for these *in vitro* studies and was assayed to have 0·159 mEq/l nonesterified fatty acids. Stock solutions of sodium oleate and sodium palmitate (Sigma Chemical Co.) were prepared in 1% BSA solution at 300 mEq/l and, by dilution, to 100, 60, 30,

and 10 mEq/l. Sera with added NEFAs were prepared by a 1:100 dilution of the stock, resulting in final concentrations of 0·159, 0·259, 0·459, 0·759, 1·159, and 3·159 mEq/l, and allowed to pre-incubate 30 min at room temperature. Ten microliters of the pre-dialyzed tracer (0·1–0·2 μCi) were then added to the serum and incubated for 30 min at room temperature. One milliliter of serum and of the dialysis buffer was added to each side of the chamber, sealed, and allowed to dialyze for 16–24 h. The dialysate buffer was then subject to magnesium precipitation to remove residual iodide in the dialysate and $FT_4$ fraction calculated as described previously (Ferguson & Peterson 1992).

### *Statistical analysis*

All data were analyzed using computer software (Prism software, GraphPad Software Inc, San Diego, CA, USA). The data are expressed as means ± S.D. unless otherwise stated. The significance of differences of means was evaluated by paired *t*-test. Values of $P<0·05$ were considered significant.

## Results

Body weight, fat mass, girth, and BMI were significantly higher between the lean and obese state (Table 1). Leptin increased from 327 ± 17 to 482 ± 48 (pmol/l; $P<0·004$), as did NEFAs. They were 0·33 ± 0·04 mEq/l in the lean state and 0·49 ± 0·05 mEq/l in the obese state ($P<0·002$).

$TT_4$, $TT_3$, and $FT_4$ concentrations, and % $FFT_4$ are shown in Fig. 1A–D. Although $TT_4$ trended to be higher in obese than lean cats ($P<0·08$), this was not significant. $TT_3$ also did not change significantly with an increase in fat mass; however, $FT_4$ was significantly higher in obese than lean cats ($P<0·0001$), as was $FFT_4$ ($P<0·004$). $FT_4$ correlated positively and significantly with all indices of obesity (body weight, $P<0·002$; % body fat (BF), $P<0·0001$, Fig. 2A; girth, $P<0·0001$; and BMI, $P<0·02$). $FT_4$ also correlated positively with NEFA ($P<0·025$, Fig. 2B) and leptin ($P<0·04$). $TT_4$ correlated positively and significantly with all indices of obesity (body weight, $P<0·001$; % fat, $P<0·02$; girth, $P<0·003$; and BMI, $P<0·03$), as well as with leptin ($P<0·002$) but not with NEFA. $TT_3$ correlated positively with weight ($P<0·004$), girth ($P<0·013$), and BMI ($P<0·04$). $FFT_4$ correlated significantly and linearly with NEFA concentration (Fig. 2C; $P<0·002$).

Mean (± S.D.) TSH concentration (ng/ml) measured in nine cats during the lean state was 0·038 ± 0·016 ng/ml, and in the obese state was 0·048 ± 0·024 ng/ml, an insignificant rise. All samples were above the lower limit of detection of 0·01 ng/ml against the canine TSH standard.

When specific NEFAs were examined for effects of displacing tracer $T_4$ from pooled cat serum, oleate and palmitate increased $FFT_4$ at a concentration above 1 mEq/l (Fig. 3). This *in vitro* effect appears to confirm a direct inhibitory effect of common circulating NEFAs on $T_4$ binding to cat serum proteins, an effect seen at NEFA concentrations observed in obese cats, and one which would account in significant part for the elevations in $FT_4$ concentrations by direct dialysis seen in obese patients.

## Discussion

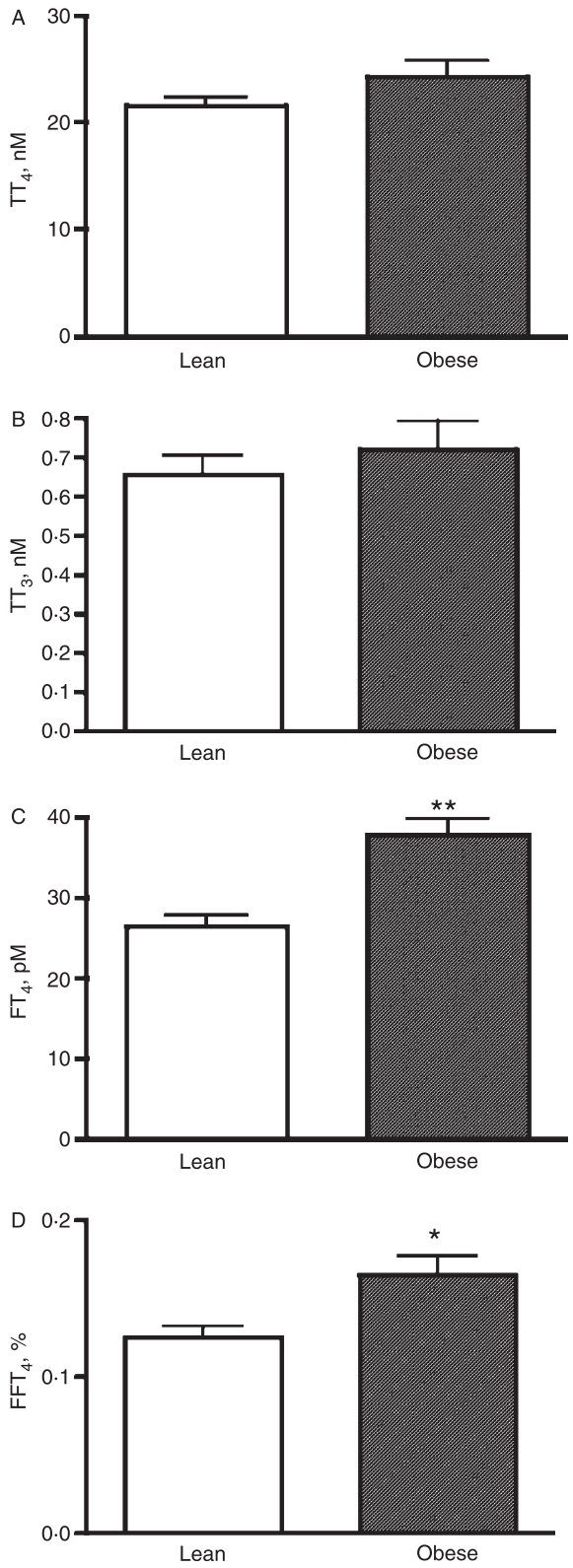
The observation of an increase in $FT_4$ in cats developing obesity is similar to those reported in a recent study of obese women. Serum concentrations of TSH, $T_4$, $T_3$, and $FT_4$ were observed to be elevated (Kozlowska & Rosolowska-Huszcz 2004). This study did not see the $FFT_4$ fall during weight loss and attributed it to potentially increased non-esterified fatty acids, although they were not measured, nor was $FT_4$ measured by a dialysis procedure. However, while multiple studies have demonstrated a positive correlation between TSH and BMI, they generally have noted a negative association between BMI and $FT_4$ concentrations, albeit with changes within normal range. It should be noted that non-dialysis techniques were used to estimate $FT_4$ concentration (Knudsen *et al.* 2005).

Our studies of developing obesity in cats suggest that a primary alteration in thyroid function is an alteration of $FFT_4$ induced by the increase in NEFAs, and that this effect can be mimicked in pooled cat serum by the addition of the exogenous NEFAs oleic or palmitic acid. It is possible that a larger sample size might have demonstrated a significant increase in $TT_4$ and $TT_3$, and it is notable that both correlated significantly with the obesity indices of weight, girth, and BMI, and $TT_4$ also correlated with BF and leptin. The lack of significant changes in $TT_3$ or $TT_4$ is inconsistent with studies in man, which have generally shown an increase in $T_3$ production and $rT_3$ degradation rates, with no change in net $T_4$ production or degradation, suggesting an increase in the Type I 5′-deiodinase (D1) enzyme activity (Roti *et al.* 2000). Studies in the dog

**Table 1** Body weight (kg), body fat (%), girth (m), and body mass index (kg/m$^2$) in 21 neutered female adult cats before (lean) and after becoming obese (obese). Variances are S.D.

| | **Body weight** (kg) | **Body fat** (%) | **Girth** (m) | **Body mass index** (kg/m$^2$) |
|---|---|---|---|---|
| Lean | 4·2 ± 0·5 | 23·6 ± 6·2 | 0·37 ± 0·04 | 44·3 ± 6·2 |
| Obese | 5·0 ± 0·7$^{\dagger}$ | 36·8 ± 5·9$^{\dagger}$ | 0·43 ± 0·01$^{*}$ | 54·7 ± 7·1$^{\dagger}$ |

Significance when compared in paired analysis with same parameter in the lean state $^{*}P<0·004$, $^{\dagger}P<0·0001$.




showed an increase in $T_3$, but no change in reverse $T_3$ concentrations (Daminet *et al.* 2003). Conversely, the Type II 5′-deiodinase (D2) was shown to be decreased in white adipose tissue of obese human patients (Nauman *et al.* 1990).

Establishing cause and effect for the observed changes is difficult with this study alone. However, it should be noted that the tightest correlations to $FT_4$ were with % BF and between $FFT_4$ and NEFAs (Fig. 2). Furthermore, the changes in the calculated $FFT_4$ *in vivo* are made more plausible by the *in vitro* evidence that NEFAs can increase $FFT_4$ at concentration ranges achieved during obesity.

TSH is a very sensitive marker of altered thyroid status in man with exponential increases or decreases with linear changes in $FT_4$ concentrations. If the change in plasma NEFA concentration was the initial and sole effect, one would predict that $FT_4$ would be transiently increased, TSH would suppress transiently, and then $TT_4$ would fall, until $FT_4$ normalized. However, to explain the results, it is necessary to postulate that there is a concomitant change in the sensitivity of the hypothalamus and/or pituitary to negative feedback. In cats progressing to the obese state, $FT_4$ remained elevated, $TT_4$ was unchanged (with a trend toward elevation, not depression), and TSH did not change, and in no case in which it was measured, was it undetectable as is observed in spontaneous hyperthyroidism. Measurement of serum TSH in additional cats might have uncovered a significant change in TSH, but TSH measurements were not initially planned because recombinant feline TSH was not yet available to standardize the canine assay.

We propose that obesity induces a relative state of TH resistance, either caused by the effect of leptin or by the effect of increased NEFA concentrations, or both. The diagnostic criterion for TH resistance is an elevated $FT_4$ with a normal or elevated TSH concentration, and we believe that these observations are consistent with this criterion (Brucker-Davis *et al.* 1995, Larsen & Davies 2003). Most often when TSH and TH concentrations are elevated in obese human subjects, they are still within the normal range (Reinehr & Andler 2002, Michalaki *et al.* 2006, Nyrnes *et al.* 2006).

Accurate measurement of TSH is critical to the accurate interpretation of the physiological significance of the increased $FT_4$ concentrations in obesity. This is the first report of the use of a commercially available canine TSH assay to measure serum feline TSH with documentation of detection efficiency of a feline TSH standard of known gravimetric purity. It is apparent that detection of feline TSH by the Immulite canine TSH assay is less complete (46%) than that observed with recombinant canine TSH. We would note that the predicted normal range for this small sample of lean

**Figure 1** Serum concentrations of total $T_4$ (A), total $T_3$ (B), direct dialysis free $T_4$ ($FT_4$; C), and calculated free fraction of $T_4$ ($FFT_4$; D) in 21 cats before (lean) and after becoming obese (obese). Error bars are S.D. Values with the same superscript letter differ significantly. Significance when compared in paired analysis with same parameter in the lean state: $^{**}P<0{\cdot}0001$, $^{*}P<0{\cdot}004$.

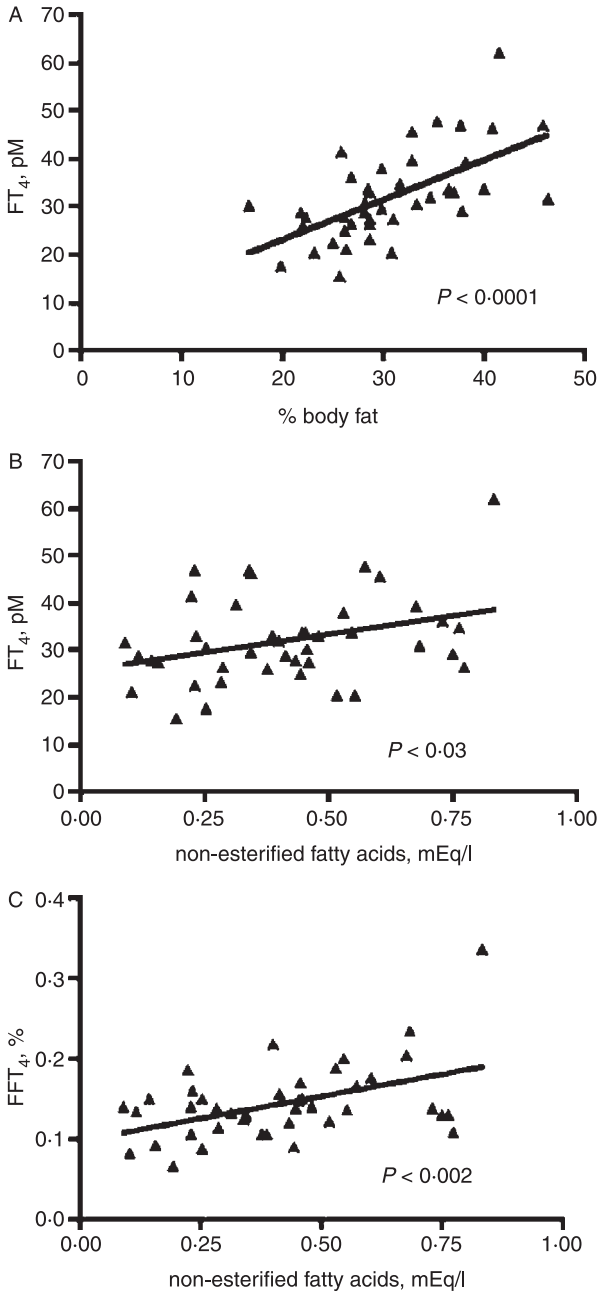



**Figure 2** (A) Correlation between $FT_4$ (pM) and body fat (%) in 21 neutered female adult cats before and after becoming obese ($r^2 = 0{\cdot}38$, $P<0{\cdot}0001$). (B) Correlation between $FT_4$ (pM) and nonesterified fatty acids (mEq/l) in 21 neutered female adult cats before and after becoming obese ($r^2 = 0{\cdot}11$, $P<0{\cdot}03$). (C) Correlation between $FFT_4$ (%) and nonesterified fatty acids (mEq/l) in 21 neutered female adult cats before and after becoming obese ($r^2 = 0{\cdot}22$, $P<0{\cdot}03$).

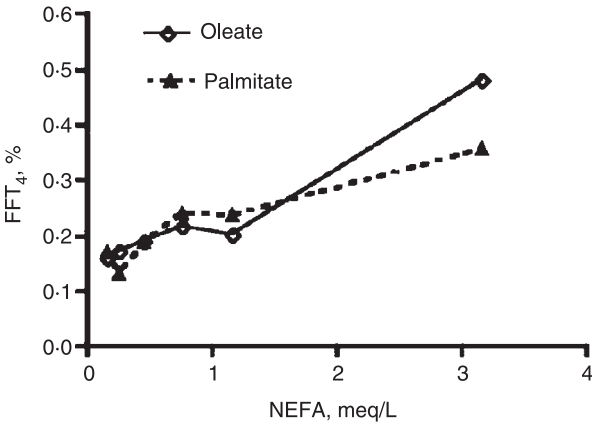



**Figure 3** Effect of added oleic and palmitic acids on the displacement of $^{125}$I-$T_4$ from pooled cat serum as reflected by the free $T_4$ fraction (%) determined by equilibrium dialysis *in vitro*. Points represent the average three replicate dialyses per concentration.

cats would predict a normal range with 95% confidence limits for TSH of 0·01–0·21 ng/ml, much closer to those observed in the normal dog (<0·5 ng/ml) or human (0·02–0·32 ng/ml, assuming 0·08 ng/microunit bioactivity (Nyrnes *et al.* 2006). Higher concentrations would be more consistent with the concentration range observed for the IC50s for bovine TSH displacement of $^{125}$I-bovine TSH from the feline TSH receptor (0·19 nmol/l or 5·5 ng/ml; Nguyen *et al.* 2002) and for recombinant feline TSH stimulation of adenylate cyclase in cells expressing the human TSH receptor (10·7 vs 4·9 ng/ml for bovine TSH; Rayalam *et al.* (2006*a*,*b*).

The current study is the first to identify a relationship between the rise in $FT_4$ associated with obesity being linked to the rise in NEFA. There may be several reasons that the elevation of $FT_4$ had not previously been identified. First with the exception of one study of dogs (Daminet *et al.* 2003), non-dialysis analog procedures for determining $FT_4$ have generally been employed. Analog $FT_4$ assays are not as likely to distinguish the effect of low-affinity inhibitors of serum TH binding such as NEFAs (Nelson *et al.* 2005). In studies of healthy euthyroid dogs, a good correlation between dialysis and analog $FT_4$ immunoassays has been observed (Schachter *et al.* 2004, Martin *et al.* 2006). However, this correlation tends to degrade when sick animals are evaluated suggesting that these assays may be incapable of discerning the effects of weak circulating weak inhibitors of serum TH binding (Schachter *et al.* 2004).

Secondly, the current study evaluated the same cat as it progressed from the lean to the obese state. It is also worth noting that, for most of the prior studies, $FT_4$ and, when it was measured, TSH generally remained within the normal range. Given intersubject variation, this pathophysiological mechanism may have been obscured when lean and obese groups were distinct populations.

Thirdly, cats are a species that have very low concentrations of specific serum thyroid-hormone-binding proteins such as thyroxine-binding globulin (TBG), which dominate serum binding of $T_4$ in primates (Refetoff *et al.* 1970). As such, changes in NEFA concentrations may have a greater tendency to impact TH binding in serum. Given the close similarity of the composition of serum thyroid-hormone-binding proteins in

dogs and cats, it is interesting to compare the results in cats with a study of separate groups of lean and obese laboratory beagles (Daminet *et al.* 2003). The obese dogs had significantly elevated serum $TT_4$ and $T_3$ concentrations, but neither TSH nor $FT_4$, measured by equilibrium dialysis, was increased. The investigators suggest that the $T_4$ increase in obesity could be the result of an increase in the serum concentration of thyroid-hormone-binding proteins, specifically TBG. However, they also show that the maximum serum $T_4$ response following TSH administration was not different. From this, one would conclude that the thyroid functional reserve is unchanged in obesity, and the results are more suggestive of a change in hypothalamic–pituitary set point than that of a change in binding protein capacity (Daminet *et al.* 2003).

Aside from the hypothesis of TH resistance associated with increased NEFA concentrations, the increase in $FT_4$ observed in the cats during the obese phase of this study could be the result of other factors changing the hypothalamic–pituitary set point for negative feedback. Some studies have demonstrated an increase in free TH as well as TSH concentrations in humans (Duntas *et al.* 1991). TSH increases in another study were shown to be proportional to BMI (Nyrnes *et al.* 2006). In support of this theory is the fact that in obesity, nuclear $T_3$ receptors are decreased in number (Burman *et al.* 1980) and, presumably, the negative feedback between the TSH and the peripheral THs and TSH secretion, mediated by the TRβ nuclear thyroid receptor of the hypothalamus and pituitary, is decreased. As demonstrated in the rat, some compounds may inhibit binding of TH to TRβ without affecting TRα, resulting in tissue hyperthyroidism where the latter receptor subtype exists (Zoeller *et al.* 2005). Our study of cats showed no significant increase in TSH concentration with obesity. This does not preclude the presence of an altered set point for negative feedback as a normal TSH concentration is inappropriately high in the face of the elevation of $FT_4$ concentration. In the obese dog, the elevated baseline $T_4$ but identical maximal response of $T_4$ to TSH also suggests that there was a change in hypothalamic–pituitary set point, albeit with a normal baseline TSH concentration (Daminet *et al.* 2003). It has been suggested that measurement of the serum response of TSH to administered TRH would be useful to ascertain whether pituitary sensitivity is altered in obesity. However, at least in one study of obese patients, the response of TSH to i.v. TRH was not altered (Duntas *et al.* 1991). Future studies will be necessary to address this question in the cat.

Leading theories suggest that leptin and the adrenergic neurotransmitters are key regulators of the hypothalamic–pituitary–thyroid axis. When human subjects were sampled every 10 min for leptin and TSH concentrations, there was a linear relationship between the 24-h leptin and TSH, with a significantly higher 24-h rate in obese but a lower rate in fasted patients. Indeed, leptin has been shown to also correlate with thyroid volume (Ghizzoni *et al.* 2001). However, leptin and TSH concentrations both correlate with the degree of obesity, obscuring whether there is a cause–effect relationship. Reduced dopamine 2 (D2) receptors in the brain have been observed in obesity (Pinkney *et al.* 1998, Kok *et al.* 2005), and production of TSH is also regulated by transmitters and hormones, which regulate body weight and satiety, such as the neurotransmitters neuropeptide Y, α-melanocyte-stimulating hormone, and the agouti-related peptide, which interact with hypothalamic TRH neurons (Fekete *et al.* 2000, 2001, 2002, Guo *et al.* 2004).

In summary, the development of obesity in the cat is associated with a significant increase in $FT_4$ within the normal reference range, a change that correlated with the increase in plasma NEFA concentrations. We propose that the effect of obesity on $FT_4$ is primary as $FT_4$ and $FFT_4$ were most tightly correlated with BF and plasma NEFA concentrations. However, the set point for negative feedback on TSH secretion must also be altered as $TT_4$ and TSH concentrations remained unchanged, and $TT_4$ did not fall inversely with the rise in $FT_4$. Furthermore, we were able to demonstrate that the *in vitro* addition of NEFAs led to a significant increase of the $FT_4$ fraction. It is difficult to ascertain whether the cats developed a change in their tissue thyroid status upon the development of obesity.

Further investigation into the cellular thyroid status will be necessary to establish cause and effect in this animal model of obesity. It will require the measurement of markers of TH action in peripheral tissue such as muscle or adipose tissue to evaluate whether there might be tissue-specific changes in the TH's action resulting from the increased serum $FT_4$ concentration.

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