Effects of neutering on hormonal concentrations and energy requirements in male and female cats

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Objective—To determine whether changes in concentrations of hormones involved in glucose and fatty acid homeostasis are responsible for the increased probability that neutered cats will develop obesity and diabetes mellitus.

Animals—10 male and 10 female weight-maintained adult cats.

Procedure—Results of glucose tolerance tests and concentrations of hormones and nonesterified fatty acids (NEFA) were examined before and 4, 8, and 16 weeks after neutering.

Results—Caloric requirements for weight maintenance were significantly decreased 8 and 16 weeks after neutering in females. Glucose concentrations during a glucose tolerance test did not change in neutered females or males. The area under the curve (AUC) for insulin was significantly higher in males, compared with females, before neutering. However, the AUC for insulin increased and was significantly higher 4 and 8 weeks after neutering in females. The AUC for insulin did not change in neutered male cats. Leptin concentrations did not change in females but increased significantly in males 8 and 16 weeks after neutering. Thyroxine concentrations did not change after neutering; however, free thyroxine concentration was significantly higher in females than males before neutering. Baseline concentrations of NEFA were significantly higher in female than male cats before but not after neutering. Suppression of NEFA concentrations after glucose administration decreased successively in male cats after neutering, suggesting decreased insulin sensitivity.

Conclusions and Clinical Relevance—Changes in NEFA suppression, caloric intake, and leptin concentrations may be indicators of, and possible risk factors for, the development of obesity in cats after neutering. (*Am J Vet Res* 2002;63:634–639)

In several species, body weight and adiposity correlate well with concentrations of gonadal hormones. This has been extensively studied in rats. In that species, ovariectomy leads to overeating and weight gain, whereas castration leads to lower body weight or no change in body weight.[1-3] In Zucker rats, ovariectomy led to overeating and weight gain in lean and genetically obese types, whereas estrogen treatment reversed obesity in the lean type but not the genetically obese rats. The obese rats continued to overeat and gain weight.[4] Mechanisms for the various responses are not clear. In 1 study,[5] it was found that loss of estrogen was initially associated with a decrease in leptin concentrations and weight gain in rats. However, in those same rats, leptin concentrations subsequently increased to higher concentrations than before ovariectomy, and the rats continued to gain weight. Leptin is believed to act as a lipostat regulating adipose tissue mass by suppressing appetite and causing a negative energy balance. Therefore, it seems the initial decrease in leptin concentrations may have caused the increase in food intake, as would be expected. However, it is unclear why the rats continued to gain weight despite the increase in leptin concentrations. Others have not seen a change in leptin concentrations after ovariectomy in rats.[6]

Neutered cats are at greater risk to become obese, compared to sexually intact cats, and are at almost twice the risk for developing diabetes mellitus.[7] It is possible that hormones involved in glucose and fat metabolism are altered after neutering to cause this effect. Hormonal changes after neutering have been examined in cats.[8] In that study, the cats had free access to food and gained weight because food intake increased after neutering. This is an important finding; however, those results do not allow clinicians to differentiate changes caused by neutering from changes caused by weight gain attributable to increased food consumption.

In contrast, the weight of cats in the study reported here was maintained within a narrow range. Cats were examined before and 4, 8, and 16 weeks after neutering to determine whether changes in concentrations of hormones involved in glucose and lipid metabolism play a role in the increased risk of becoming obese and developing diabetes mellitus independent of effects on food intake.

Materials and Methods

Ten male and 10 female adult purpose-bred cats were obtained from a commercial supplier[a] for use in the study. Cats were maintained at our 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 guidelines established by the Animal Welfare Act and the National Institutes of Health.[9]

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. Cats were fed a commercially available dry ration[b] twice daily. Food intake was recorded at each feeding for 4 weeks before neutering to obtain an accurate estimate of the energy requirements of the

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sexually intact cats and for use in the study after cats were neutered. Cats were weighed twice weekly, and body weight was maintained within 5%. Ovariohysterectomy and castration were performed, using standard methods.[10]

To enable collection of blood samples, catheters were inserted in a jugular or cephalic vein 15 to 17 hours before any procedures were performed. Patency of catheters was maintained by flushing with 0.5 ml of 0.38% citrate solution[c] every 6 hours. Blood samples were collected via the jugular catheter and were allowed to clot for harvest of serum. After centrifugation, serum was harvested and frozen at –20 C until assays were performed. Blood samples also were collected via the jugular catheter for routine CBC counts and biochemical analysis. For each sample collection, 15 ml of blood was obtained to provide serum and whole blood for analyses.

An **IV glucose tolerance test (IVGTT)** was performed on each cat before and 4, 8, and 16 weeks after neutering. For each IVGTT, a solution of 50% dextrose (wt:vol) was injected into a cephalic vein, using a dosage of 1 g/kg. Blood samples for glucose determinations were collected 5, 10, 15, 30, 45, 60, 90, and 120 minutes after dextrose injection. Glucose measurements were performed, using a colorimetric glucose oxidase method.[d]

All samples for determination of hormone concentrations were processed in a single assay for each hormone. Serum insulin concentrations were measured as described elsewhere,[11] using a charcoal method; the intra-assay coefficient of variation was 3.6%. Measurement of leptin concentrations was performed by use of a radioimmunoassay.[c] All samples were tested in duplicate in a single assay; the intra-assay coefficient of variation was 4.9%. The standard curve for serial dilutions of serum from clinically normal cats was observed to be parallel to the standard curve for human leptin standards. Addition of 3 concentrations of human standard to feline serum resulted in mean ± SD recovery of 105.4 ± 8.2%. The assay had a working range of 62.5 to 3,125 pmol/L. Storage of serum samples for 4 months at –20 C did not alter leptin concentrations (data not shown).

Total serum thyroxine and free thyroxine concentrations were measured by use of equilibrium dialysis, as described elsewhere.[11,12] The intra-assay coefficient of variation was 11.1 and 10.5%, respectively.

Serum concentration of **nonesterified fatty acids (NEFA)** was measured by use of an enzymatic test kit.[f] The intra-assay coefficient of variation was 1.7%.

Measurement of **body mass index (BMI)** was calculated, using the following formula:

$$\text{BMI} = \text{Body weight (in kilograms)} / (\text{body length [in meters]} \times \text{height [in meters]})$$

where length was the distance from the point of the shoulder to the tuber ischium, and height was the distance from the point of the shoulder through the point of the elbow joint to the proximal boundary of the central metacarpal pad.[13]

Data were analyzed, using commercially available software.[g] Data were expressed as mean ± SD unless stated otherwise. Significant differences among means within a group were assessed by the use of the Student *t*-test for paired samples, whereas a 2-sample *t*-test was used to assess differences between groups. Values of $P < 0.05$ were considered significant.

Results

Body weight, BMI, and caloric intake of female and male cats were determined **(Table 1)**. Values were determined before and 4, 8, and 16 weeks after neutering.

Glucose concentrations before and 8 and 16 weeks after neutering in male and female cats were determined **(Fig 1)**. The **area under the curve (AUC)** for glucose during the IVGTT did not change after neutering in female and male cats. Values were 1,468 ± 304 and 1,541 ± 251 mmol/L/120 min in females and 1,220 ± 174 and 1,126 ± 146 mmol/L/120 min in males before and 16 weeks after neutering, respectively. We did not detect a change in the value for percentage glucose disappearance per minute after IV injection of glucose during the IVGTT; the value was 1.6 ± 0.3% before and 1.7 ± 0.3% 16 weeks after neutering in females and 1.9 ± 0.2% before and 2.1 ± 0.1% after neutering in males, respectively. Time required for the glucose concentration to decrease by half did not change after neutering (data not shown).

Changes in the **AUC** for insulin before and after neutering in male and female cats were calculated **(Fig 2)**. The AUC for insulin was 25.0 ± 6.5 nmol/L/120 min before neutering. It was significantly ($P < 0.001$) higher 4 (52.5 ± 16.8 nmol/L/120 min) and 8 (46.2 ± 16.3 nmol/L/120 min) weeks but not 16 weeks (33.0 ± 11.8 nmol/L/120 min) after neutering in female cats. The AUC for insulin was not significantly different in male cats before (34.2 ± 5.8 nmol/L/120 min and 16 weeks after (33.5 ± 12.9 nmoles/L/120 min) neutering. Male cats had significantly ($P = 0.004$) higher values for AUC for insulin than female cats before neutering, but values were not significantly different after neutering because of the increase in AUC for insulin 4 and 8

Table 1—Mean ± SD values for body weight, body mass index, and caloric intake in 10 male and 10 female cats before and 4, 8, and 16 weeks after neutering

Cats	Time	Body weight (kg)	Body mass index (kg/m²)	Caloric intake (kcal/kg)
Males				
	Before neutering	4.0 ± 0.6[a]	42.0 ± 2.8[e]	68.3 ± 4.6
	4 weeks after	4.0 ± 0.6[b]	ND	68.2 ± 4.6
	8 weeks after	4.1 ± 0.7[c]	41.1 ± 3.1	69.5 ± 9.5[f]
	16 weeks after	4.0 ± 0.6[d]	40.4 ± 5.4	58.5 ± 15.6
Females				
	Before neutering	3.4 ± 0.3[a]	37.6 ± 2.9[e]	65.6 ± 4.1
	4 weeks after	3.4 ± 0.4[b]	ND	67.9 ± 2.5[g,h]
	8 weeks after	3.5 ± 0.4[c]	38.8 ± 3.3	60.1 ± 6.1[f,g]
	16 weeks after	3.4 ± 0.4[d]	37.0 ± 3.9	57.0 ± 7.7[h]

[a-h]Within a column, values with the same superscript letter differ significantly ([a-e]$P < 0.05$; [f]$P = 0.021$; [g]$P = 0.037$; [h]$P = 0.035$).
ND = Not determined.

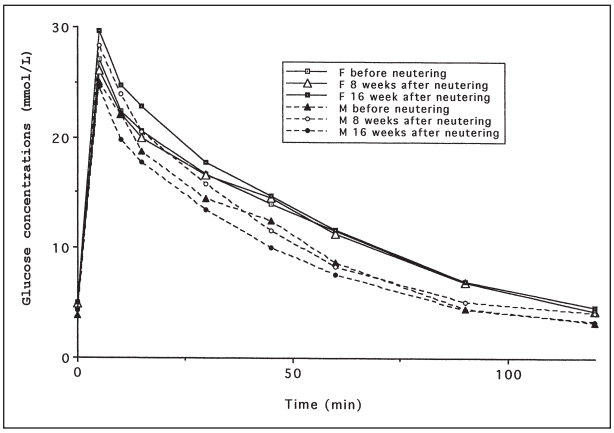

Figure 1—Mean glucose concentration after IV injection of 1 g of dextrose/kg in 10 male (M) and 10 female (F) cats before and 8 and 16 weeks after neutering.

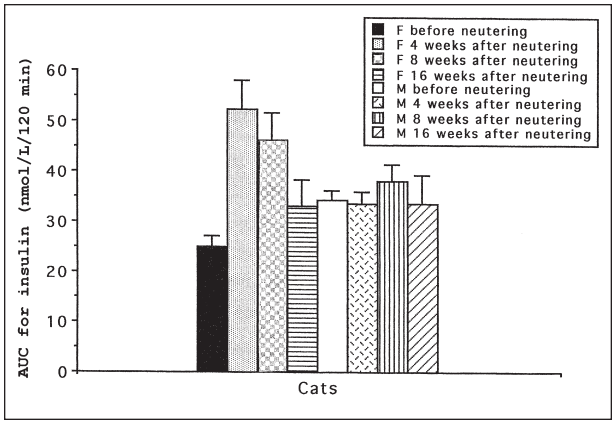

Figure 2—Mean ± SD value for area under the curve (AUC) for insulin in response to an IV glucose tolerance test (1 g of dextrose/kg) in 10 male (M) and 10 female (F) cats before and after neutering. In female cats, the AUC for insulin was significantly higher 4 ($P < 0.001$) and 8 ($P = 0.001$) weeks after neutering, compared with values before neutering. Male cats had a significantly ($P = 0.004$) higher AUC for insulin, compared with female cats, before neutering.

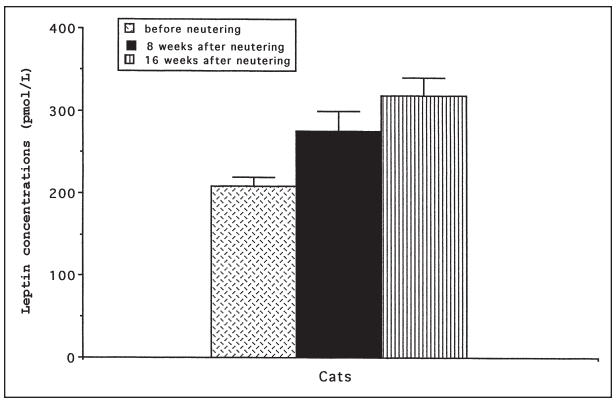

Figure 3—Mean ± SD AUC for leptin in 10 male and 10 female cats before and 8 and 16 weeks after neutering. The AUC was significantly greater 8 ($P = 0.026$) and 16 ($P = 0.008$) weeks after neutering in male cats, compared with values for male cats before neutering. Values for female cats did not differ significantly before and after neutering.

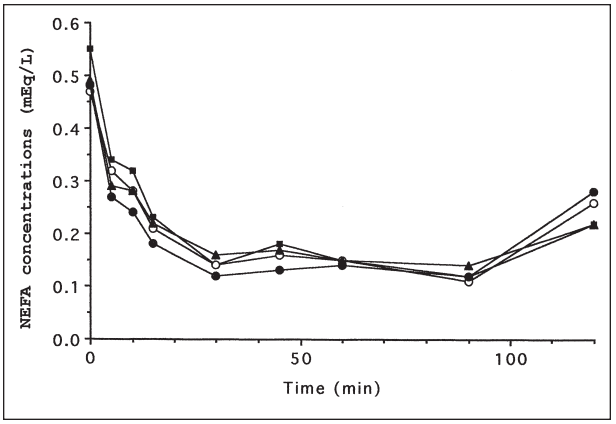

Figure 4—Suppression of nonesterified fatty acid (NEFA) concentrations in response to an IV glucose tolerance test (1 g of dextrose/kg) in 10 female cats before (solid square) and 4 (open circle), 8 (solid triangle), and 16 (solid circle) weeks after neutering. Time 0 = Time of glucose administration.

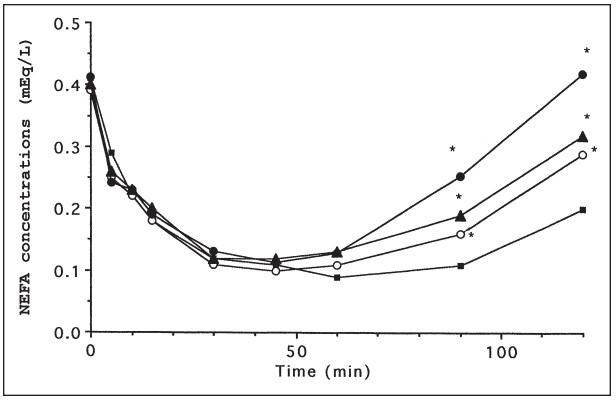

Figure 5—Suppression of NEFA concentrations in response to an IV glucose tolerance test (1 g of dextrose/kg) in 10 male cats before (solid square) and 4 (open circle), 8 (solid triangle), and 16 (solid circle) weeks after neutering. *Values at 90 and 120 minutes after glucose administration were significantly ($P = 0.02$) less after neutering, compared with concentrations at those time periods before neutering. Time 0 = Time of glucose administration.

weeks after neutering in female cats. Female cats had significantly ($P = 0.008$) higher insulin concentrations than male cats 4 weeks after neutering. Male and female cats had similar values for AUC for insulin 16 weeks after neutering.

Leptin concentrations increased significantly ($P = 0.026$) in male cats from 208.0 ± 36.3 pmol/L before neutering to 275.3 ± 76.7 pmol/L 8 weeks after neutering. They were significantly ($P = 0.008$) higher 16 weeks after castration (318.5 ± 47.4 pmol/L; **Fig 3**). Leptin concentrations in female cats were 243.0 ± 66.8 pmol/L before neutering and did not change significantly 8 (225.9 ± 48.3 8 pmol/L) and 16 (234.2 ± 66.1 pmol/L) weeks after neutering.

Thyroxine concentrations did not differ significantly before and after neutering in male and female cats. They ranged from 21.4 to 25.1 nmol/L in male cats and from 20.8 to 22.0 nmol/L (average) in female cats. Free thyroxine concentrations were significantly ($P = 0.022$) higher in female cats than male cats before neutering (2.5 ± 0.7 vs 1.8 ± 0.7 pmol/L) but not after neutering, because the concentrations in males increased after neutering, although not significantly.

Baseline NEFA concentrations were significantly ($P = 0.025$) higher in female than in male cats before neutering (0.55 ± 0.19 vs 0.35 ± 0.09 mEq/L). Concentrations of NEFA did not change in response to the IVGTT in female cats after neutering (**Fig 4**). However, NEFA concentrations were significantly ($P = 0.02$) less suppressed 90 and 120 minutes after glucose administration 4, 8, and 16 weeks after neutering in male cats (**Fig 5**).

Discussion

A decrease in concentrations of sex hormones as a result of surgical procedures or the aging process has often been linked to a change in body composition and obesity, although the exact reasons for this change are unclear. Ovariohysterectomy increases food intake and body weight in rats, whereas castration decreased food intake in some studies.[2,4] In 1 study,[3] castration increased food intake during the light cycle but decreased it during the dark cycle, and a net change in

food intake was not detected. In dogs, results have been controversial. In 1 study,[14] male dogs had a greater increase in food intake and weight gain after neutering than did female dogs, whereas in another study,[15] food intake did not change in dogs after neutering. It has been suggested that mechanisms other than an increase in food intake are responsible for weight gain after neutering, because ovariectomized rats can gain weight without overeating.[16,17] This differs from results described in another study.[18] In that study, energy gain in ovariectomized rats was entirely caused by an increase in food intake and not caused by a reduction in energy expenditure. Heidenberger and Unshelm[14] reported that after neutering, male dogs became less active than female dogs, contributing to the increase in weight. Fettman et al[8] reported that male and female cats gain weight after neutering because of increased food intake. The weight of the cats in our study was strictly controlled, because we wanted to study changes that were independent of weight gain. Although we did not specifically study the amount of activity for the cats, it did not appear to change after neutering. The fact that the caloric intake of the female cats in our study had to be decreased to maintain body weight after neutering suggests that neutering decreased their energy expenditure. Another less likely possibility would be that neutering increased nutrient digestibility. We are not aware of data that would support this possibility. Therefore, when cats are allowed to eat the same amount or even increase their food intake as a consequence of the neutering process, weight gain will follow.

Results of the study reported here are in agreement with earlier suggestions that mechanisms other than an increase in food intake play a role in weight gain after neutering. One possible mechanism relates to the change in concentrations of pituitary hormones. Infusion of a **gonadotropin-releasing hormone (GnRH)** agonist leads to an increase in energy deposition in neutered female rats.[1,19] Interestingly, administration of GnRH in conjunction with thyrotropin-releasing hormone can lead to a shift from catabolism to anabolism.[20] It is unclear as to the role these hypothalamic factors and their target hormones play in metabolism of cats. Additional studies are needed to examine their role in obesity.

Female cats in the study reported here had transient insulin resistance but not glucose intolerance. Insulin resistance is also prevalent in postmenopausal woman[21,22] and can be treated by administration of estrogen at low doses, whereas higher doses of estrogen or the addition of progestins decreases insulin sensitivity.[21,23] Insulin resistance may be caused by an increase in gluconeogenesis or an increase in function of islet α cells.[24] Hyperglucagonemia has been described in humans with gonadal dysgenesis.[25] It also has been reported[26] that estrogen has a suppressive effect on α cells in rats. The fact that the insulin resistance was only transient in the cats of our study suggested adaptive changes occurred after neutering that alleviated the demand for hyperinsulinemia. Changes in insulin secretion were not seen in the male cats in our study, which is consistent with findings in rats[27] and dogs.[28] However, the change in NEFA suppression after neutering seems to indicate that uptake of fatty acids becomes less sensitive to the effect of insulin. It remains to be examined whether this is an early marker of insulin resistance that precedes any change in glucose disposal.

Our finding that neutering leads to hyperleptinemia in male cats is similar to observations that hypo gonadal men have higher leptin concentrations. In a study[29] of elderly men, serum leptin concentrations were inversely correlated with serum testosterone concentrations. In a study[30] of healthy young men, the administration of testosterone suppressed leptin concentrations, which returned to pretreatment concentrations after cessation of the testosterone treatment. Testosterone substitution decreases the high concentrations of leptin in hypogonadal men.[31] Similarly, when sex steroids are suppressed in healthy men and boys, leptin concentrations increase almost 3-fold, whereas they decrease when androgenic steroids are given.[32,33] All of these changes are independent of changes in body fat mass. Up-regulation of androgen receptor binding in fat cells in response to testosterone has been documented in male rats,[34] and leptin receptors have been identified in testes of rats,[35] suggesting the existence of a feedback loop. It could be concluded, therefore, that testosterone is a regulator of leptin secretion in men and cats, making cats an excellent choice for use in studying this mechanism.

The effect of estrogen on leptin concentrations in women is not clear. In studies[36-38] of elderly women, leptin concentrations were not associated with serum estrogen concentrations or use of estrogen replacement therapy. In contrast, it was found in another study[39] that hormone replacement therapy (estrogen or estrogen and progesterone) increases leptin concentrations in postmenopausal women. More specifically, it was found that leptin concentrations are higher in premenopausal women than in postmenopausal women and that leptin concentrations are significantly higher during the luteal phase, compared with concentrations during the follicular phase.[40,41] In rats, serum leptin concentrations decrease after ovariohysterectomy, an effect that is reversed by estrogen treatment.[41] In the study reported here, we did not detect an effect of neutering in weight-maintained female cats, which suggests that estrogens do not control leptin expression in this species.

We detected significantly higher free thyroxine concentrations when there was not a change in total thyroxine concentration in sexually intact female cats, compared with concentrations in male cats. The ratio of concentrations for free thyroxine to total thyroxine or the free thyroxine fraction also was significantly increased. Because female cats also had a higher NEFA concentration before neutering, the difference in free thyroxine concentration in serum was largely caused by the increase in NEFA concentration, which can interfere with binding of thyroxine to serum proteins such as albumin in humans and dogs.[42,43,h,i] The equilibrium constant for oleic acid inhibition of thyroxine binding to bovine serum albumin is 98 μM, well within the range of NEFA concentrations observed in cats.[43] In serum obtained from dogs, concentrations of oleic

acid achieved in some metabolic conditions (3 m*M*) increase the free thyroxine fraction by > 300%.[h] However, an increase in free thyroxine fraction in the female cats of our report does not explain the fact that the average total thyroxine concentration is not decreased as would be expected by increased negative feedback on the pituitary gland attributable to an increase in free thyroxine concentrations. Fatty acids in moderate concentrations also can inhibit cellular uptake of thyroxine in cultured rat pituitary glands and renal tubules.[43,44] The primary effect seen at low NEFA concentrations (< 100 μ*M*) is inhibition of thyroxine uptake, whereas higher NEFA concentrations result in severe inhibition of thyroxine deiodination. When intrapituitary conversion of thyroxine to triiodothyronine is similarly inhibited by NEFA, the increased serum free thyroxine concentration would not result in increased pituitary gland uptake of thyroxine or 5'-deiodination of thyroxine to triiodothyronine, which mediates negative feedback at nuclear receptors.

The study reported here has documented the differences in the response of male and female cats to neutering. The previously reported observation that cats increase food intake after neutering together with our finding that there is a decrease in energy requirement for neutered female cats provides an explanation for the increased risk of obesity in neutered female cats. The role of hyperleptinemia in the increased risk of obesity in neutered male cats is yet to be determined. The finding that testosterone controls leptin secretion in male cats independent of fat mass may allow investigators to use cats when studying mechanisms involved in this feedback regulation of leptin. Additional studies are needed to examine mechanisms underlying the increase in food intake and decreases in caloric requirement of female cats after neutering.

[a]Sinclair Research Center, Columbia, Mo.
[b]Purina Pro Plan, Ralston Purina, St Louis, Mo.
[c]Citric acid, trisodium salt dihydrate, Sigma Chemical Co, St Louis, Mo.
[d]Glucose trinder kit, Sigma Chemical Co, St Louis, Mo.
[e]Linco Multi-species RIA kit, Linco, St Charles, Mo.
[f]NEFA C, Wako Diagnostic, Richmond, Pa.
[g]Data Desk software for Macintosh, Data Description Inc, Ithaca, NY.
[h]Ferguson DC. Influence of common drugs on the free thyroxine fraction in canine serum (abstr), in *Proceedings*. American College of Veterinary Internal Medicine, 1989;A32.
[i]Nowak G, Longino MR, Ferguson DC. Effects of nonesterified fatty acids on uptake and deiodination of thyroxine by rat renal proximal tubules (abstr). *Thyroid* 1991;1(suppl 1):S-74, A146.

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