Serum Insulin-Like Growth Factor (IGF)-I Concentrations Are Reduced by Short-Term Dietary Restriction and Restored by Refeeding in Domestic Cats (*Felis catus*)1,2

Amanda Maxwell,3 Richard Butterwick,* Roger M. Batt† and Cecilia Camacho-Hübner

Departments of Endocrinology and Chemical Endocrinology, St. Bartholomew’s Hospital, London EC1A 7BE, UK; ‡WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, LE14 4RT, UK; and 1Department of Small Animal Medicine and Surgery, Royal Veterinary College, University of London, North Mymms AL9 7TA, UK

ABSTRACT Nutritional modulation of insulin-like growth factors (IGF) and their binding proteins (IGFBP) is well established. The effect of nutritional restriction on the serum IGF/IGFBP system of adult cats was investigated to evaluate serum IGF-I as a biochemical marker of nutritional status. Assays for measuring feline serum IGF and IGFBP were validated and normal ranges established in a study population of 46 healthy nonobese adult cats. Serum concentrations of IGF-I and IGF-II correlated significantly with body weight (*r* = 0.75, *P* < 0.0001 and *r* = 0.34, *P* < 0.03, respectively). Serum IGFBP profiles were similar to other species, including humans, dogs and guinea pigs. IGFBP-3 was the predominant binding protein reflecting IGF-I concentrations and body size. Serum IGFBP-2 concentrations were high relative to the normal human serum pool (NHS) control. Food withdrawal for 18 h followed by refeeding did not alter circulating IGF or IGFBP concentrations, including IGFBP-1, in nine cats. Short-term dietary restriction of nine adult cats to supply initially 56% (56%M) and then 42.5% (42.5%M) of calculated maintenance energy requirements for 14 d resulted in a significant weight loss (*P* < 0.01). However, serum IGF-I concentrations fell significantly (−51%, *P* < 0.01) only with 42.5%M restriction. Serum IGF-II, IGFBP, insulin and albumin concentrations were not altered during the study. We conclude that nutrition does modulate the adult feline IGF/IGFBP system, but to a lesser extent than in other species. Further evaluation is required before serum IGF-I can be used for the assessment of nutritional status in adult cats. J. Nutr. 129: 1879–1884, 1999.

KEY WORDS: • cats • insulin-like growth factor • nutritional restriction

The assessment of nutritional status and monitoring of the response to nutritional support in veterinary patients are complicated by a lack of validated methods that are appropriate for use in a small animal hospital setting. Anthropometric methods developed for human patients such as triceps skin-fold and mid-arm circumference measurement (Lukaski 1987) or zoometry e.g., body mass index (Nelson et al. 1990) and body condition scoring (Donaghue and Kronfeld 1994), have either not been validated for use in cats or may be insensitive as a result of wide interobserver variation. Assessment of whole-body metabolism, e.g., nitrogen balance, direct and indirect calorimetry (Long 1977) or body composition, e.g., dual emission X-ray absorptiometry scanning (Munday et al. 1994) and double-labeled water techniques (Balleve et al. 1994), although accurate, may not be practical in feline patients. Biochemical markers such as serum albumin, prealbumin (trans-thyretin), retinol binding protein and transferrin have shown good correlations with nutritional status in humans, but their relatively long half-lives and interference by factors other than nutrition have reduced their usefulness in monitoring response to nutritional support (Clemmons et al. 1985). Recently, serum creatinine kinase activities have been assessed for monitoring response to refeeding in hospitalized cats, and good correlations between cessation of anorexia and decreasing enzyme activity have been demonstrated (Fascetti et al. 1997). However, this marker may be influenced by muscle trauma such as surgery or venous catheter insertion. Insulin-like growth factor (IGF)-I, a single-chain polypeptide hormone with a structural similarity to proinsulin, is regulated predominantly by growth hormone (GH), but nutrition also plays a major role.


2 Supported by a grant from the WALTHAM Centre for Pet Nutrition, Leicestershire, UK.

3 To whom correspondence and reprint requests should be addressed at Department of Veterinary Basic Sciences, Royal Veterinary College, University of London, Royal College Street, London NW1 0TU, UK.

022-3166/99 $3.00 © 1999 American Society for Nutritional Sciences.

It has a half-life in humans of 14–18 h in circulation (Guler et al. 1989) and is not stored before release from the liver. Serum IGF-I concentrations have been used in humans to monitor response to nutritional support in which they reflected improving nitrogen balance (Hawk et al. 1987). Serum IGF-I concentrations were significantly more sensitive to improving nutritional status compared with serum prealbumin, retinol binding protein, transferrin and albumin (Clemons et al. 1985). Nutritional modulation of IGF and their binding proteins (IGFBP) has been established in many species (Thissen et al. 1994) but not in domestic cats. This study was undertaken to investigate nutritional modulation of the feline IGF/IGFBP system in order to assess the potential of serum IGF-1 concentrations as a marker of nutritional status in adult cats.

MATERIALS AND METHODS

Animals. Animals used in these studies were healthy nonobese adult cats (domestic short-haired) that were bred on-site. Females were excluded if pregnant, lactating or in estrus. Cats were group-housed unless required otherwise for recording voluntary food intake. Body weight was recorded daily. Health of the cats was ensured throughout the study by regular veterinary examination and monitoring of blood biochemistry and hematology. Blood for analysis of IGF, IGFBP, insulin and albumin was taken from the cephalic vein after overnight food withdrawal, unless otherwise indicated. Serum samples were stored in aliquots at −20°C until assay. Care of animals and experimental use conformed to UK Home Office guidelines under the Animals (Scientific Procedures) Act 1986.

Diet. All diets were fed at maintenance requirements unless indicated. Water was available at all times and was not restricted.

Study protocols. Samples for validation studies were taken from 46 cats [11 male neuter (mn), median age, 2.24 y, range 2.16–8.23 y; 35 female (f), median age, 5 y, range 1.8–9.1 y]. All cats were receiving adequate maintenance diets based on previously established nutritional guidelines (Earle and Smith 1991) in which daily maintenance energy requirements are 293 kJ/kg body weight. A modification for inactivity due to kenneling further reduced the daily energy supplied in the rations by ~14%.

Nine cats (5 f, 4 mn, median age, 7.1 y, range 2.8–9.3 y; median weight, 4.6 kg, range 2.8–6.9 kg) were studied to investigate the effects of overnight food withdrawal followed by refeeding. Samples were taken after food had been withdrawn for 18 h and again 3 h after feeding. Direct observation confirmed that food had been eaten by each cat.

The effects of nutritional restriction for 14 d were studied in nine nonobese healthy adult cats [6 f, 2 mn, male neuter (fn) and 1 mn, median weight, 4.8 kg, range 3.7–5.9 kg; median age, 5.4 y, range 3.1–6.9 y]. Short-term dietary restriction was followed by free access to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet. Cats were fed maintenance rations (M) for 5 d before restriction to first 56% (56%M) and then 42.5% (42.5%M) of caloric intake to the diet.

RESULTS

Assay validation. Serial dilutions of feline serum were parallel to standard curves in the IGF and IGFBP-2 RIA as shown in Figure 1. Examination of extracted serum by WLB revealed residual IGFBP, predominantly IGFBP-3 and -2 (data not shown). The potential interference of these residual binding proteins in the RIA for IGF was investigated by assessing recovery of unlabeled IGF from serum samples pre- and post-FAE, and also by comparison with acid gel chromatography. Recovery of unlabeled IGF for NHS was 103 ± 15% of pre-FAE (mean ± sd) and 14 ± 7% of post-FAE for IGF-I corresponding values in IGF-II were 87 ± 14 and 94 ± 9%, respectively. For feline serum, similar recovery rates of 91 ± 14 and 108 ± 8% for IGF-I and 94 ± 14 and 90 ± 11% for IGF-II were obtained. Acid-gel chromatography of NHS and feline serum resulted in similar elution profiles (data not shown). Comparison of the two methods of IGFBP extraction showed...
that between 107 and 126% of IGF-I and 88 and 102% of IGF-II measured in acid-gel chromatographed NHS samples were measured after FAE. The corresponding results for feline serum were 118 and 121% and 70 and 95%, respectively. Furthermore, serial dilution curves of serum from two cats with high concentrations of IGF-I and from one cat with a low IGF-I concentration were parallel to recombinant human standard curves (data not shown). Further analysis of RIA data generated from all validation curves showed a high correlation between observed and predicted values ($r = 0.98$, $P < 0.001$).

Results for NHS and feline serum were similar and the assays were considered appropriate.

Serum IGFBP concentrations were analyzed as described. IGFBP-3, a doublet at 39- and 43-kDa molecular weight, was the predominant binding protein (Fig. 2) and was further identified by immunoblotting (data not shown). Serum IGFBP-3 concentrations analyzed by WLB reflected body weight and IGF-I concentrations (Fig. 2). Compared with NHS, feline serum IGFBP-2 band intensity was increased by WLB analysis; this was confirmed by immunoblotting (Fig. 3) and RIA using sera from cats in the initial validation population ($n = 7$ cats, range 13–31 nmol/L; NHS pool 9–11 nmol/L). This was a consistent finding throughout the study.

With validation, normal ranges for IGF-I and IGF-II were established for the study population of 46 cats. Both serum IGF-I ($r = 0.75$, $P < 0.0001$) and IGF-II concentrations ($r = 0.34$, $P < 0.03$) correlated significantly with body weight as shown in Figure 4, although the normal ranges for this population were wide. Male cats were heavier ($m = 5.1$ kg...
4.1–6.8; f = 3 kg, 2.2–4.9, P < 0.0001) and this was reflected by higher serum IGF-I (m = 50 nmol/L, 18–83; f = 17 nmol/L, 11–74; P < 0.0001) and IGF-II concentrations (m = 56 nmol/L, 22–89; f = 37 nmol/L, 6–95; P < 0.02). There were no significant correlations between age and IGF concentration. Increased IGFBP-3 band intensity [m = 33.6 ± 5.1 (sd) densitometry units, f = 21.9 ± 8.6 densitometry units] was evident on WLB as shown in a representative autoradiograph (Fig. 2).

**Overnight food withdrawal.** There were no changes in serum IG or IGFBP concentrations, including IGFBP-1, with overnight food withdrawal or 3 h after refeeding (data not shown).

**Short-term dietary restriction.** Feeding records indicated incomplete ration uptake during the study (Table 1). Therefore, expressed as a percentage, energy restriction was shown to be first 67% and then 45% of actual intakes during the maintenance periods.

Serum samples were taken and weight recorded on d 1 and 5 during maintenance feeding at the start of the study to establish a baseline. Serum IGF concentrations and body weights did not differ significantly at the start of the study during the 5-d maintenance feeding period.

After 14 d of 56%M restriction, weight was reduced (−7.1%, P < 0.01) from 4.7 kg (3.5–5.5) to 4.3 kg (3.2–5.4) and after 14 d of 42.5%M restriction (−8.5%, P < 0.01) from 4.5 kg (3.4–5.5) to 4.1 kg (3.1–5.2). Body weights at the start of each restriction period did not differ significantly as a result of weight gain during the intervening 14-d refeeding period. Serum IGF-I concentrations fell by 37%, from 78 nmol/L (29–87) to 50 nmol/L (32–61) with 56%M restriction (Fig. 5). However, concentrations normalized with refeeding; thus there was no difference at the start of each restriction period. Serum IGF-I concentrations fell significantly only after 14 d of 42.5%M restriction (−51%, P < 0.01) from 71 nmol/L (29–106) to 34 nmol/L (15–60).

Serum IGFBP-II concentrations were not altered during each period of dietary restriction or refeeding, but concentrations had risen significantly by the end of the study (−44%, P < 0.02) from 50 nmol/L (19–76) to 72 nmol/L (50–98).

**DISCUSSION**

We have shown that the adult feline serum IGF/IGFBP system is similar to that of other species (Jones and Clemmons 1995); however, it appears to be less sensitive to modulation by nutrition (Thissen et al. 1994).

The absence of raised IGFBP-1 concentrations after overnight food withdrawal in adult cats is also seen in adult dogs (Maxwell et al. 1998), but these findings differ from similar studies in humans (Busby et al. 1988, Cotterill et al. 1993) and rats (Rivero et al. 1995). Similarly, feline serum IGFBP-1 concentrations did not change during prolonged dietary restriction or ad libitum consumption of the diet, but we have not assessed the influence of prolonged food withdrawal. Although we have not positively identified the 29-kDa band on WLB autoradiographs as IGFBP-1, specific immunoblotting has shown that it is not IGFBP-2. It is also unlikely to be a fragment of IGFBP-3 because under the denaturing WLB conditions used, the lower-molecular-weight fragments of IGFBP-3 do not bind radiolabeled IGF-I (Suikkari and Baxter 1995); however, it appears to be less sensitive to modulation by nutrition (Thissen et al. 1994).

**FIGURE 5** Serum insulin-like growth factor (IGF)-I and albumin concentrations in nine cats during short-term dietary restriction and refeeding. Data are shown as the median (line) with interquartile values shaded as the range. Restrictions to 56% (56%M) and 42.5% (42.5%M) of maintenance energy requirements are indicated by boxes on the x-axis. Closed brackets indicate significant change in serum IGF-I concentrations with 42.5%M restriction relative to baseline (P < 0.01).

**TABLE 1**

<table>
<thead>
<tr>
<th>energy (calculated vs. actual) and protein intakes by nine cats during short-term dietary restriction and refeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
</tr>
<tr>
<td>Calculated</td>
</tr>
<tr>
<td>Energy, kJ/kg</td>
</tr>
<tr>
<td>(31–59)</td>
</tr>
<tr>
<td>Restricted</td>
</tr>
<tr>
<td>(29–34)</td>
</tr>
<tr>
<td>Refed</td>
</tr>
<tr>
<td>(43–80)</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>Calculated</td>
</tr>
<tr>
<td>Energy, kJ/kg</td>
</tr>
<tr>
<td>(47–60)</td>
</tr>
<tr>
<td>Restricted</td>
</tr>
<tr>
<td>(25–26)</td>
</tr>
<tr>
<td>Refed</td>
</tr>
<tr>
<td>(49–94)</td>
</tr>
</tbody>
</table>

1 Data are shown as median actual intakes with the range in brackets during the 5-d maintenance period (M), energy restriction (Restricted) and free access (Refed).

2 Energy intakes were restricted to first 56% (56%M) and then 42.5% (42.5%M) of calculated daily energy requirements.
In humans (Busby et al. 1988) and rats (Rivero et al. 1995), IGFBP-1 is inversely regulated by serum insulin. During overnight or longer-term food withdrawal, concentrations of insulin fall and IGFBP-1 increases, whereas rising postprandial insulin concentrations down-regulate hepatic IGFBP-1 mRNA transcription. Feline serum IGFBP-1 concentrations may not rise with overnight food withdrawal because of an insufficient decrease in serum insulin concentrations, which could be compounded by increased sensitivity to the suppressive effects of insulin. It also may not be regulated by insulin.

A significant decrease in serum IGF-I concentrations was seen only after 14 d of 42.5%M restriction. In other species, there is a threshold energy intake below which serum IGF-I concentrations decrease, and this was expected with 56%M restriction for 14 d in the feline study population, as shown previously in dogs (Maxwell et al. 1998). The apparent discrepancy is partially resolved by analyzing the feeding records and expressing restriction concentrations as a percentage of intake during each preceding maintenance period (Table 1). Cats did not eat their full diet allowances during each maintenance feeding period. Therefore, if the subsequent restriction period intakes are expressed as a percentage of actual intake recorded during previous maintenance feeding, cats were restricted only to 67% and 45%. Comparison with studies in other species (Oster et al. 1995, Smith et al. 1995) shows that restriction below 67%M is required to decrease serum IGF-I. Hirsch et al. (1978) found that 10 cats with free access to food for 10 d consumed a median of 39.5 kcal/(kgd) (range 25.9–68.4), which was considerably lower than expected. It is possible that dietary energy requirements for our study population were overestimated, perhaps because of the effect of kenneling on activity. Alternatively, the significant decrease in serum IGF-I concentrations during the second period (42.5%M) of nutritional restriction was a result of priming by the first (56%M) restriction. Although this explanation is unlikely due to normalization of serum IGF-I concentrations with the intervening refeeding period, a crossover study would address this question.

During 42.5%M restriction, in addition to energy, protein intake was reduced to the recommended minimum dietary protein content for adult cats (Table 1). Dietary protein restriction also modulates the IGF/IGFBP system, resulting in lower serum IGF-I and higher IGFBP-1 and -2 concentrations in rats (Lemozy et al. 1994). It is possible therefore that feline serum IGFBP-1 concentrations may be more sensitive to dietary protein than caloric restriction. Dietary protein requirements in adult cats have been extrapolated from growth studies in kittens and may therefore overestimate maintenance levels.

Feline serum IGFBP-II concentrations were unaffected by short-term dietary restriction, as also demonstrated in humans (Davenport et al. 1988), but rose overall throughout the study. The reason for this is unclear but may be related to decreased serum IGF-I concentrations.

Serum IGFBP concentrations by WLB analysis and RIA were also unchanged with short-term dietary restriction, which differs from other species (Oster et al. 1995, Smith et al. 1995). In common with adult dogs, adult cats have greater concentrations of serum IGFBP-2 when nutritionally replete compared with humans, rats and guinea pigs. The role of IGFBP-2 has not been established but the protein may have a regulatory role. Increased IGFBP-2 concentrations have been noted in porcine neonates (McCusker et al. 1991), anorexia (Couzens et al. 1992), endotoxemia (Rodríguez-Aniazo et al. 1996), hyperthyroidism (Frystyk et al. 1995) and nutritional restriction (Clemmons et al. 1991), which in rats is due to up-regulation of hepatic IGFBP-2 mRNA (Straus and Take-moto 1990). Increased hepatic gluconeogenesis or increased catabolism may be important in these physiologic and pathologic states.

Cats are obligate carnivores, and it is possible that differences in nutritional modulation of their IGF system may be due to their carnivorous metabolism. The majority of energy is obtained by carnivores from dietary protein and there is no requirement for carbohydrate. Overall, an obligate carnivore’s metabolism is directed to using amino acids as energy with the elimination of nitrogen as a by-product. The rate of hepatic gluconeogenesis varies according to the dietary protein content and is up-regulated during nutritional restriction. Compared with rats, this already proceeds at a greater rate, even with optimal nutrition (Belo et al. 1976, Kettlehut et al. 1980), and down-regulation due to nutritional restriction in cats is less (Kettlehut et al. 1980). In vitro studies have shown a lack of adaptation to nutrient deprivation by feline hepatic cells compared with other species (Silva and Mercer 1986 and 1991). During long-term voluntary fasting caused by provision of a highly unpalatable diet, obese cats improved nitrogen conservation, but not as efficiently as obese humans and rats (Biourge et al. 1994). Therefore, relative to other species, nitrogen conservation is either not practiced or is inefficient, resulting in the demand for high protein diets. Additionally, certain feline metabolic pathways, e.g., urea cycle and bile salt conjugation, are entirely dependent on a dietary supply of a single amino acid, e.g., arginine, taurine (Knopf et al. 1978, Morris 1985). The low level of nutritional modulation of the feline IGF/IGFBP system may be a protective response that conserves body resources more efficiently than do omnivores or herbivores or as a specific adaptation to an obligate carnivore lifestyle in which metabolic pathways are less responsive to nutrient change or depletion. However, our previous studies on short-term energy restriction of adult dogs have demonstrated a greater sensitivity of canine serum IGF-I to nutritional restriction (Maxwell et al. 1998). Dogs are classed as carnivores but, unlike cats, do not have a dietary requirement for animal protein and may more correctly be described as omnivores. Differences between the two species in nutritional modulation of serum IGF-I may be explained by this observation or indeed may simply represent overestimation of feline daily maintenance energy requirements.

In conclusion, serum IGF-I concentrations are modulated by nutrition in adult cats and reflect short-term changes in nutrition more sensitively than serum albumin. However, the wide range of normal values in our study population precludes its use for the diagnosis of malnutrition in an individual. Furthermore, its apparent insensitivity to declining nutrition raises doubts over its suitability as a biochemical marker of nutritional status in this species, although evaluation of the maintenance energy requirements of adult cats is necessary. Investigation into the effect of dietary components, especially protein, on serum IGF and their binding proteins is also indicated. However, the rapid normalization of serum IGF-I during refeeding after nutritional restriction suggests that serial measurements may be of use in monitoring the response of an individual to nutritional therapy.

ACKNOWLEDGMENTS

The authors thank Andrew Cotterill and Martin Yateman for useful discussions during the preparation of this manuscript, and the kennel staff at the WALTHAM Center for Pet Nutrition for sample collection and animal care.
LITERATURE CITED


