Nutritional modulation of canine insulin-like growth factors and their binding proteins

A Maxwell, R Butterwick¹, M Yateman, R M Batt², A Cotterill and C Camacho-Hübner

Departments of Endocrinology, St Bartholomew's Hospital, London EC1A 7BE, UK, WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, UK and Department of Small Animal Medicine and Surgery, Royal Veterinary College, University of London, London, UK

(Requests for offprints should be addressed to C Camacho-Hübner, Department of Chemical Endocrinology, St Bartholomew's Hospital, 51–53 Bartholomew Close, London EC1A 7BE, UK)

Abstract

The response of canine insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) to moderate nutritional restriction followed by refeeding has not previously been studied in detail. The purpose of these studies was to examine the effects of nutritional restriction on the IGF system of adult dogs. Normal serum IGF values were established after validation of heterologous RIAs for measuring canine IGFs-I and -II. Canine serum IGFBP profiles were examined by Western ligand blotting (WLB), using radiolabelled recombinant human (rh) IGF-I as the ligand, and were found to be similar to those of other species. IGF-I and IGFBP-3 concentrations correlated with body weight, thus reflecting breed size as previously shown, whereas IGF-II concentrations did not. IGFBP-2 serum concentrations and band intensity on WLB were increased compared with normal human serum IGFBP-2. Overnight fasting had no effect on IGF or IGFBP concentrations, including IGFBP-1, nor did refeeding. Prolonged restriction to 56% and then 42·5% of maintenance energy requirements for 2 weeks decreased IGF-I concentrations by 20·4% and 32·7% respectively. Feeding of the same diet ad libitum for 2 weeks normalised IGF-I concentrations. There were no changes in IGF-II or insulin levels. Serum IGFBP-2 concentrations increased with 56% restriction of maintenance energy (P=0·03). We conclude that serum IGF-I is potentially a useful marker of short-term change in nutritional status in the adult dog.


Introduction

Studies of the role of nutrition in modulation of the growth hormone (GH)–insulin-like growth factor (IGF) axis have been carried out mainly in omnivores and herbivores. Certain aspects of glucose and carbohydrate metabolism in carnivores differ from those in animals taking omnivorous or herbivorous diets, particularly in the fasted state. Hepatic gluconeogenic activity is higher in carnivores and does not increase with fasting (Kettlehut et al. 1980). Insulin sensitivity and glucose tolerance are decreased in animals eating high protein diets compared with those eating carbohydrate-rich diets (Belo et al. 1976, Kettlehut et al. 1980).

Circulating somatomedin-like activity was reported by Van den Brande et al. (1974), who used canine plasma in human and rat cartilage bioassay models. This study suggested that the growth-promoting actions of this canine growth factor were similar to those in other species. Furthermore, Eigenmann et al. (1985) demonstrated that the dog showed changes in serum IGF-I in response to change in nutritional status that were similar to those in other species. Serum IGF-I concentrations decreased significantly after a 19 day fast, then increased rapidly to normal values with 9 days of refeeding.

The influence of nutrition on serum IGFs and IGF binding proteins (IGFBPs) has been demonstrated in many species. Fasting progressively reduces serum IGF-I concentrations in man (Clemmons et al. 1981) and in rats (Emler & Schalch 1987, Goldstein et al. 1991), which reflects changes in hepatic IGF-I mRNA. Fasting, either overnight or longer, results in a significant increase in IGFBP-1 concentrations in man (Busby et al. 1988, Cotterill et al. 1993) and rat (Rivero et al. 1995), which decline with feeding. In neonatal rats, this coincided with changes in hepatic mRNA expression. Other IGFBPs require a longer or more severe nutritional challenge before they are affected. A fast of 48 h was necessary to increase rat IGFBP-2 concentrations (Orlowski et al. 1990), whereas IGFBP-2 concentrations in obese human volunteers increased significantly only after 9 days of fasting (Clemmons et al. 1991). Restriction of dietary components, energy and protein, also affects the IGF/IGFBP system, resulting in decreased serum IGF-I and IGFBP-2 concentrations and band intensity on WLB were increased compared with normal human serum IGFBP-2. Overnight fasting had no effect on IGF or IGFBP concentrations, including IGFBP-1, nor did refeeding. Prolonged restriction to 56% and then 42·5% of maintenance energy requirements for 2 weeks decreased IGF-I concentrations by 20·4% and 32·7% respectively. Feeding of the same diet ad libitum for 2 weeks normalised IGF-I concentrations. There were no changes in IGF-II or insulin levels. Serum IGFBP-2 concentrations increased with 56% restriction of maintenance energy (P=0·03). We conclude that serum IGF-I is potentially a useful marker of short-term change in nutritional status in the adult dog.


Introduction

Studies of the role of nutrition in modulation of the growth hormone (GH)–insulin-like growth factor (IGF) axis have been carried out mainly in omnivores and herbivores. Certain aspects of glucose and carbohydrate metabolism in carnivores differ from those in animals taking omnivorous or herbivorous diets, particularly in the fasted state. Hepatic gluconeogenic activity is higher in carnivores and does not increase with fasting (Kettlehut et al. 1980). Insulin sensitivity and glucose tolerance are decreased in animals eating high protein diets compared with those eating carbohydrate-rich diets (Belo et al. 1976, Kettlehut et al. 1980).

Circulating somatomedin-like activity was reported by Van den Brande et al. (1974), who used canine plasma in human and rat cartilage bioassay models. This study suggested that the growth-promoting actions of this canine growth factor were similar to those in other species. Furthermore, Eigenmann et al. (1985) demonstrated that the dog showed changes in serum IGF-I in response to change in nutritional status that were similar to those in other species. Serum IGF-I concentrations decreased significantly after a 19 day fast, then increased rapidly to normal values with 9 days of refeeding.

The influence of nutrition on serum IGFs and IGF binding proteins (IGFBPs) has been demonstrated in many species. Fasting progressively reduces serum IGF-I concentrations in man (Clemmons et al. 1981) and in rats (Emler & Schalch 1987, Goldstein et al. 1991), which reflects changes in hepatic IGF-I mRNA. Fasting, either overnight or longer, results in a significant increase in IGFBP-1 concentrations in man (Busby et al. 1988, Cotterill et al. 1993) and rat (Rivero et al. 1995), which decline with feeding. In neonatal rats, this coincided with changes in hepatic mRNA expression. Other IGFBPs require a longer or more severe nutritional challenge before they are affected. A fast of 48 h was necessary to increase rat IGFBP-2 concentrations (Orlowski et al. 1990), whereas IGFBP-2 concentrations in obese human volunteers increased significantly only after 9 days of fasting (Clemmons et al. 1991). Restriction of dietary components, energy and protein, also affects the IGF/IGFBP system, resulting in decreased serum IGF-I and IGFBP-2 concentrations and band intensity on WLB were increased compared with normal human serum IGFBP-2. Overnight fasting had no effect on IGF or IGFBP concentrations, including IGFBP-1, nor did refeeding. Prolonged restriction to 56% and then 42·5% of maintenance energy requirements for 2 weeks decreased IGF-I concentrations by 20·4% and 32·7% respectively. Feeding of the same diet ad libitum for 2 weeks normalised IGF-I concentrations. There were no changes in IGF-II or insulin levels. Serum IGFBP-2 concentrations increased with 56% restriction of maintenance energy (P=0·03). We conclude that serum IGF-I is potentially a useful marker of short-term change in nutritional status in the adult dog.


Introduction

Studies of the role of nutrition in modulation of the growth hormone (GH)–insulin-like growth factor (IGF) axis have been carried out mainly in omnivores and herbivores. Certain aspects of glucose and carbohydrate metabolism in carnivores differ from those in animals taking omnivorous or herbivorous diets, particularly in the fasted state. Hepatic gluconeogenic activity is higher in carnivores and does not increase with fasting (Kettlehut et al. 1980). Insulin sensitivity and glucose tolerance are decreased in animals eating high protein diets compared with those eating carbohydrate-rich diets (Belo et al. 1976, Kettlehut et al. 1980).

Circulating somatomedin-like activity was reported by Van den Brande et al. (1974), who used canine plasma in human and rat cartilage bioassay models. This study suggested that the growth-promoting actions of this canine growth factor were similar to those in other species. Furthermore, Eigenmann et al. (1985) demonstrated that the dog showed changes in serum IGF-I in response to change in nutritional status that were similar to those in other species. Serum IGF-I concentrations decreased significantly after a 19 day fast, then increased rapidly to normal values with 9 days of refeeding.

The influence of nutrition on serum IGFs and IGF binding proteins (IGFBPs) has been demonstrated in many species. Fasting progressively reduces serum IGF-I concentrations in man (Clemmons et al. 1981) and in rats (Emler & Schalch 1987, Goldstein et al. 1991), which reflects changes in hepatic IGF-I mRNA. Fasting, either overnight or longer, results in a significant increase in IGFBP-1 concentrations in man (Busby et al. 1988, Cotterill et al. 1993) and rat (Rivero et al. 1995), which decline with feeding. In neonatal rats, this coincided with changes in hepatic mRNA expression. Other IGFBPs require a longer or more severe nutritional challenge before they are affected. A fast of 48 h was necessary to increase rat IGFBP-2 concentrations (Orlowski et al. 1990), whereas IGFBP-2 concentrations in obese human volunteers increased significantly only after 9 days of fasting (Clemmons et al. 1991). Restriction of dietary components, energy and protein, also affects the IGF/IGFBP system, resulting in decreased serum IGF-I and

0022–0795/98/0158–0077 $08.00/0

Downloaded from Bioscientifica.com at 11/11/2018 09:38:37AM via free access
altered binding-protein profiles. In contrast, IGF-II appears not to be regulated by nutrition, except with extreme restriction (Davenport et al. 1988).

Apart from studies on canine IGF-I and fasting, there are no reports on the effects of more moderate energy restriction on canine serum IGF and IGFBP values. The IGFBPs have important roles in modulating IGF bioavailability to tissue receptors, and are known either to potentiate or to inhibit the actions of IGFs in vitro (Jones & Clenmmons 1995). Regulation of IGFBP concentrations is believed to be important in modulation of IGF bioactivity. The aim of the present study was to examine the effect of moderate energy restriction and refeeding on the canine IGF/IGFBP system and to evaluate the potential of serum IGF-I as a marker of nutritional status in adult dogs.

**Materials and Methods**

**Animals**

Adult dogs used in the following procedures were healthy, non-obese and kennel-adapted. Females were excluded from the study if they were pregnant, lactating, in oestrus or in dioestrus. Blood was taken from the cephalic vein after an 18 h overnight fast, unless otherwise indicated, for analysis of IGFs, IGFBPs, insulin and albumin. Serum samples were stored in aliquots at −20 °C until required for assay. Health of the animals was ensured throughout the studies by regular veterinary physical examination and monitoring of blood biochemistry and haematology. Animals that failed to regain adequate body weight when refeeding *ad libitum* after caloric restriction were withdrawn from the remainder of the study.

**Study protocols**

Fasting samples for validation studies were taken from the 33 dogs (male, female and neuter) of various breeds described in Table 1. All dogs were receiving adequate maintenance feeding (maintenance energy requirements in kcal given by the formula 110 × BW^0.75^ where BW is body weight in kg) according to previously established nutritional guidelines (Burger 1994, 1995).

Nine female Labrador Retrievers, age 3·4 ± 0·5 years, weight 27·9 ± 0·8 kg (mean ± s.e.m.) were studied to investigate the effects of short-term fasting followed by refeeding. Samples were taken after an 18 h overnight fast and 3 h after feeding.

The effects of more prolonged nutritional restriction were studied in the 10 dogs of various breeds described in Table 2. Details of the study protocol are given in Fig. 1, which shows caloric restriction for 14 days and subsequent refeeding for 14 days by allowing access to the diet *ad libitum*. During the periods of nutritional restriction, only the energy was reduced below maintenance requirements. This was accomplished by a straightforward reduction in the amount of the diet fed. Although this affected availability, all other nutrients were provided in amounts equal to or greater than established maintenance requirements (Table 3). The values chosen were based on those in similar studies in other species and current recommendations for weight reduction in the dog (Markwell & Butterwick 1994). Throughout the study, all other nutrient requirements, including those for protein, were met.

**Assays**

Canine serum IGF-I and -II concentrations were measured in heterologous RIA, with procedures modified from those described by Blum et al. (1988), Holly et al. (1988), and Morrell et al. (1989), using monoclonal antibodies to human IGF-I (Blood Products Ltd, Elstree, Herts, UK) and rat IGF-II (Upstate Biotechnology Incorporated, Lake Placid, NY, USA). Serum samples underwent formic acid–acetone extraction (Bowsher et al. 1991) before assay to separate IGFs from their binding proteins. After overnight equilibration with antibody and [125I]rhIGF, the bound and free fractions were separated using Sac–Cel (Immunodiagnostic Systems, Boldon, Tyne and Wear, UK) and compared with a standard curve produced by rhIGF standards (a gift from Pharmacia and Upjohn, Stockholm, Sweden). For the IGF-I RIA the

<table>
<thead>
<tr>
<th>Breed</th>
<th>Weight (kg)</th>
<th>Age (years)</th>
<th>IGF-I (ng/ml)</th>
<th>IGF-II (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irish Wolfhound (n=2)</td>
<td>61·7 ± 7·3</td>
<td>4·2 ± 0·8</td>
<td>461 ± 87·7</td>
<td>498 ± 110·3</td>
</tr>
<tr>
<td>Large Munsterlander (n=2)</td>
<td>30·2 ± 5·2</td>
<td>2·8 ± 0·1</td>
<td>167·9 ± 17·0</td>
<td>600 ± 17·0</td>
</tr>
<tr>
<td>Labrador Retriever (n=15)</td>
<td>26·8 ± 0·7</td>
<td>4·6 ± 0·6</td>
<td>306·6 ± 27·6</td>
<td>494± 20·4</td>
</tr>
<tr>
<td>Golden Retriever (n=2)</td>
<td>25·9 ± 2·0</td>
<td>3·3 ± 0·5</td>
<td>187·6 ± 4·9</td>
<td>597 ± 2·1</td>
</tr>
<tr>
<td>English Springer Spaniel (n=2)</td>
<td>16·4 ± 2·7</td>
<td>3·4 ± 1·3</td>
<td>118·7 ± 33·2</td>
<td>573 ± 80·6</td>
</tr>
<tr>
<td>Beagle (n=4)</td>
<td>13·8 ± 1·3</td>
<td>6·9 ± 0·2</td>
<td>107 ± 12·1</td>
<td>513 ± 4·5</td>
</tr>
<tr>
<td>Miniature Schnauzer (n=6)</td>
<td>7·3 ± 0·7</td>
<td>2·9 ± 0·2</td>
<td>165·9 ± 16·2</td>
<td>432 ± 47·5</td>
</tr>
</tbody>
</table>

*Journal of Endocrinology (1998) 158, 77–85*
intra- and interassay coefficients of variation (CVs) were 6.8% and 12% respectively, for a pooled serum source of 1.92 ng/ml. For the IGF-II RIA, the CVs were 8.9% and 6.8% respectively, for 477 ng/ml.

IGFBP concentrations were assessed by Western ligand blotting (WLB) techniques as described by Hossenlopp et al. (1986), using 125I-rhIGF-I as the ligand, followed by densitometric analysis of autoradiographs (Bio Rad Laboratories Ltd, Hemel Hempstead, Herts, UK). Individual IGFBPs were identified by immunoblotting using Enhanced Chemiluminescence (Amersham International plc, Amersham, Bucks, UK). Bovine IGFBP-2 antiserum (kindly provided by Dr D Clemmons, University of North Carolina, Chapel Hill, USA) at 1:4000 and human IGFBP-3 antiserum (Upstate Biotechnology Incorporated) at 1:2000 were used as described previously (Camacho-Hübner et al. 1992). Serum IGFBP-2 concentrations were measured using a commercial radioimmunoassay kit (DSL-7100, Diagnostic Systems Laboratories Inc., Webster, TX, USA), which was validated for canine serum.

Serum albumin and insulin were assayed at external laboratories using established methods that had been validated for canine serum.

**Table 2** Energy restriction study population

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Age (years)</th>
<th>Energy allowance (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LM</td>
<td>mn</td>
<td>33.8</td>
<td>2.7</td>
<td>1825 1022 775</td>
</tr>
<tr>
<td>2</td>
<td>LM</td>
<td>mn</td>
<td>26.5</td>
<td>2.9</td>
<td>1825 1022 775</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>m</td>
<td>16</td>
<td>6.5</td>
<td>1155 647 490</td>
</tr>
<tr>
<td>4</td>
<td>ESS</td>
<td>mn</td>
<td>14.5</td>
<td>2.4</td>
<td>1155 647 490</td>
</tr>
<tr>
<td>5</td>
<td>ESS</td>
<td>mn</td>
<td>18.3</td>
<td>4.3</td>
<td>990 554 420</td>
</tr>
<tr>
<td>6</td>
<td>GR</td>
<td>fn</td>
<td>24.5</td>
<td>2.9</td>
<td>1180 661 502</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>m</td>
<td>10.8</td>
<td>7</td>
<td>990 554 420</td>
</tr>
<tr>
<td>8</td>
<td>L</td>
<td>fn</td>
<td>24.8</td>
<td>9</td>
<td>1155 647 —</td>
</tr>
<tr>
<td>9</td>
<td>B</td>
<td>f</td>
<td>12.6</td>
<td>7-2</td>
<td>660 370 —</td>
</tr>
<tr>
<td>10</td>
<td>GR</td>
<td>mn</td>
<td>27.3</td>
<td>3.6</td>
<td>1815 1016 —</td>
</tr>
</tbody>
</table>

Dogs 1–7 underwent both periods of caloric restriction. M* = maintenance energy requirements calculated according to Burger (1994, 1995). LM, Large Munsterlander; B, Beagle; ESS, English Springer Spaniel; GR, Golden Retriever; L, Labrador Retriever; m, male; f, female, n, neuter.

**Table 3** Nutritional analysis: pedigree canine calorie control, dry

<table>
<thead>
<tr>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Energy</td>
</tr>
</tbody>
</table>

**Figure 1** Protocol for the energy restriction study. Dogs were fed their maintenance energy requirements for 6 days (M) before energy restriction. The same diet was then fed at a reduced level for 2 weeks to supply first 56% of maintenance energy requirements (56%M) and then 42.5% of maintenance energy requirements (42.5%M). After each restriction period dogs were allowed to eat the same diet ad libitum for 2 weeks (ad lib.) before proceeding to the next part of the study. Blood samples (†) were taken during the study periods as indicated.

**Statistical analysis**

Normally distributed data were analysed by Student’s paired t-test. Other data were examined using non-parametric tests, Wilcoxon’s signed rank test for two related samples, as given in SPSS 6.0 for Windows.

**Results**

**Validation**

Serial dilution curves of canine serum were parallel to the standard curves of rhIGFs (Fig. 2) and supplied IGFBP-2 standards (data not shown) in each RIA. Recovery of unlabelled IGF in the IGF assays added to canine serum before and after extraction was compared with reference normal human serum pool (NHS). Pre- and postextraction recovery from canine serum was approximately 90–102%, compared with 88–114% for NHS. These assays were used to establish normal ranges for the study population (Table 1). Serum IGF-I concentrations correlated well with body weight (r=0.7, P<0.01) in normal adult, nutritionally replete dogs (Fig. 3).
WLB analysis of canine serum revealed four bands similar to the NHS lane (Fig. 4). The IGFBP-3 doublet was the most abundant binding protein present in serum; this was confirmed by immunoblotting (data not shown). There were no differences in IGFBP profile between the sexes, including neutered dogs (data not shown); however, IGFBP-3 concentrations appeared to increase with breed size (Fig. 4). The second most abundant IGFBP in canine serum was IGFBP-2, which was confirmed by immunoblotting (Fig. 5b). Concentrations of this IGFBP were greater than the reference NHS lane as shown by WLB and immunoblotting (Fig. 5); this was a consistent finding throughout our studies. Bands present at 29 and 24 kDa probably represent IGFBPs-1 and -4 respectively.

**Overnight fasting**

There was no significant change in serum IGF-I concentrations with overnight fasting and refeeding in a group of Labrador Retrievers. Serum concentrations were (mean ± s.d.) 361·1 ± 90·7 ng/ml after an 18 h overnight fast and 345·4 ± 86·9 ng/ml 3 h after feeding. Serum IGF-II likewise did not alter with refeeding (after fasting, 592 ± 150·8 ng/ml; after refeeding, 606·7 ± 119·6 ng/ml). Serum IGFBP concentrations as shown by WLB were not affected by fasting or refeeding (data not shown). Insulin concentrations (n=8) increased from 8·15 µIU/ml (range 4·0–13·4 µIU/ml) to 20·4 µIU/ml (range 5·7–36·1 µIU/ml) 3 h after feeding (P<0·02).

**Energy restriction**

During restriction of maintenance energy requirement (M) to 56% (56%M), body weight decreased by 7·1% (P<0·01); it decreased by 9% (P<0·01) with 42·5%M restriction (Fig. 6a). There was no difference between starting weights for each restriction period in the seven dogs that continued the study. There were no changes in serum insulin concentrations (Table 4). Median IGF-I concentrations decreased during each period of nutritional restriction. With 56%M restriction (n=10 dogs), IGF-I decreased from a baseline of 144·2 ng/ml (range 92·4–204·4 ng/ml) to 114·8 ng/ml (range 75·6–161 ng/ml; −20·4%, P<0·01). With 42·5%M restriction (n=7 dogs), it decreased from 154 ng/ml (range 84–186·2 ng/ml) to 103·6 ng/ml (range 70–158·2 ng/ml; −32·7%, P<0·01). With feeding ad libitum, IGF-I concentrations normalised so that there was no difference between values at the start of each restriction period. In order to minimise the influence of breed size on circulating IGF-I concentrations, the percentage changes relative to baseline for each dog were examined (Fig. 6a). After 56%M restriction (n=10 dogs), the median percentage decrease was 21·3% (range 7·3–37·9%, P=0·005); after 42·5%M restriction (n=7 dogs), it was 29·2% (range 7–47·3%, P<0·03).

In contrast, no changes in IGF-II (Fig. 6a) or IGFBP concentrations were revealed by WLB (Fig. 5a), with
energy restriction or refeeding. Densitometric analysis showed a trend towards decreased IGFBP-3 and increased IGFBP-2 values (Fig. 5). In the seven dogs that completed both caloric restriction periods, serum IGFBP-2 concentrations measured by RIA increased to 752 ± 3 ng/ml (range 553·4–1047 ng/ml) from baseline values of 611 ng/ml (range 457–716 ng/ml, \( P = 0.028 \)) during 56%M restriction. During 42·5%M, concentrations increased from 593·1 ng/ml (range 431·5–810·9 ng/ml) to 728·8 ng/ml (range 492·7–936·4 ng/ml, \( P = 0.06 \)). The slight decrease in IGFBP-3 concentrations was not due to IGFBP-3 protease induction (data not shown).

Serum albumin concentrations in the seven dogs that took part in the entire study (Fig. 6d) increased from 29·4 g/l (range 27·6–32·1 g/l) to 31·9 g/l (range 29·5–34·6 g/l) with 56%M feeding (\( P < 0.02 \)) and then decreased to 24·5 g/l (range 20·8–32 g/l) with the 14 days of feeding \( \text{ad libitum} \) that followed (\( P < 0.05 \)). Overall, serum concentrations decreased from the start of the study to 22·9 g/l (range 19·2–29·6 g/l) at the end of the entire study (\( P = 0.06 \)).

Discussion

Results from the present study indicate that changes in nutritional status modulate the adult canine IGF/IGFBP system in a manner similar to that found in other species. Although nutritional modulation of adult canine IGF-I has been demonstrated previously (Eigenmann et al. 1985), this is the first report characterising IGFBPs and IGF-II during moderate energy restriction.

We validated heterologous assays and immunoblotting techniques using peptides and antibodies developed for other species. Our results suggest that there is a high degree of homology, consistent with reports of structural analysis of IGFs and IGFBPs in other species. Delafontaine et al. (1993) and, more recently, Hayakawa et al. (1996) have shown that canine IGF-I cDNA shares more than 82% homology with pig, human, mouse and rat IGF-I cDNAs. There are no published reports on canine IGFBP structure but analyses of IGF-binding proteins from other species show a high degree of homology. In the present study, immunoblotting results showed that this structural conservation may include canine IGFBPs.

Our results also complement earlier reports by Eigenmann et al. (1984a,b,c) that adult canine IGF-I was
Figure 6 Changes in weight (a), serum IGF-I (b), serum IGF-II (c) and serum albumin (d) during energy restriction, presented as percentages of baseline values. Data are medians with interquartiles (shaded areas). Solid bars on the x-axis indicate periods of energy restriction. Values significantly different from baseline: \( * P < 0.05 \), \( ** P < 0.01 \).
affected by body size in addition to GH secretion. The 41 dogs from four different breeds (Cocker Spaniel, Beagle, Keeshond and German Shepherd Dog) studied by Eigenmann ranged in size from 12 to 38 kg and had serum IGF-I concentrations between 36 and 280 ng/ml, which correlated well with body weight. This correlation in serum IGF-I and weight was further demonstrated using miniature, toy and standard Poodles as examples of different size within a breed. Furthermore, 29 dogs with increased GH concentrations (experimentally or iatrogenically induced as a result of administration of medroxyprogesterone acetate (MPA), or arising spontaneously during dioestrus) had serum IGF-I concentrations more than double those found in normal, healthy German Shepherd Dogs, despite being of mixed and smaller breed sizes. Those that received treatment, ovariohysterectomy or cessation of MPA to decrease circulating GH concentrations showed a corresponding decrease in serum IGF-I concentrations concurrent with resolution of clinical signs, for example improved glucose tolerance. In our study, we found that IGFBP-3 concentrations, like those of IGF-I, correlated with body size. This was not unexpected, as IGFBP-3 is the predominant IGF-binding protein in serum, and in other species carries the majority of circulating IGF-I in conjunction with the acid-labile subunit. Serum IGF-II concentrations showed no correlation with breed size. The reason for the increased concentrations of adult canine serum IGFBP-2 compared with our normal human serum pool is unclear, although it is possible that a greater proportion of IGF circulates bound to this IGFBP. Increased IGFBP-2 concentrations have previously been found in neonatal pigs (McCusker et al. 1991) and certain pathological conditions (Counts et al. 1992, Frystyk et al. 1995). Francis et al. (1990) reported that IGFBP-2 was the predominant serum IGFBP in chickens, although this was later disputed, and increased values were seen only after food restriction (Kirk et al. 1996). In adult canine serum, IGFBP-2 is consistently increased compared with that in a normal human serum pool and healthy adult subjects by WLB, immunoblotting and RIA (C Camacho-Hübner, unpublished data).

Overnight or more prolonged fasting results in increased IGFBP-1 concentrations in man (Busby et al. 1988, Cotterill et al. 1988, 1993). In the rat (Rivero et al. 1995), increased serum concentrations are associated with altered hepatic IGFBP-1 mRNA transcription. The 29 kDa band on WLB of canine serum is likely to be IGFBP-1; although specific immunoblotting was not possible, this band did not correspond with any of the bands obtained after immunoblotting with antisera to IGFBP-3 or -2. The canine IGFBP-1 band did not change after overnight fasting or with more prolonged energy restriction, suggesting either different regulation of our putative IGFBP-1 or an altered metabolic environment compared with that in man or rat.

Energy restriction in other species has resulted in similar changes in IGFBPs-2 and -3. Energy restriction to 40% of maintenance decreased IGFBP-3 concentrations in young rats (Oster et al. 1995), whereas IGFBP-2 concentrations increased after only 4 days of similar dietary modification in meat-type chickens (Kitka et al. 1996). In adult human volunteers, IGFBP-3 was decreased and IGFBP-2 increased by short-term restriction of protein, and subsequently normalised with refeeding (Smith et al. 1995). Similar, but not significant, changes in canine serum IGFBPs-2 and -3 with caloric restriction were seen on WLB. IGFBP-2 concentrations measured by RIA increased significantly with 56% caloric restriction and decreased with refeeding.

In seven adult male and female Beagles, 19 days of starvation resulted in a significant reduction in serum concentrations of IGF-I, which normalised rapidly with 9 days of refeeding (Eigenmann et al. 1985); this was similar to findings in other species. Energy restriction in rats by fasting for one day is associated with decreased serum IGF-I concentrations and hepatic GH receptor downregulation (Maes et al. 1983). In the present study, restriction of energy intakes to 56% and 42.5% of maintenance requirements also resulted in decreased serum IGF-I concentrations but, as in man (Davenport et al. 1988), serum IGF-II concentrations were not affected.

### Table 4 Fasting insulin (Ins), IGF-I and IGF-II concentrations in six dogs that completed the entire energy restriction study

<table>
<thead>
<tr>
<th>Dog</th>
<th>M ( µL/m)</th>
<th>IGF-I (ng/ml)</th>
<th>IGF-II (ng/ml)</th>
<th>42.5% (µL/ml)</th>
<th>IGF-I (ng/ml)</th>
<th>IGF-II (ng/ml)</th>
<th>R (µL/ml)</th>
<th>IGF-I (ng/ml)</th>
<th>IGF-II (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5</td>
<td>162</td>
<td>696</td>
<td>8.6</td>
<td>158</td>
<td>696</td>
<td>6.0</td>
<td>161</td>
<td>600</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>179</td>
<td>732</td>
<td>3.7</td>
<td>119</td>
<td>720</td>
<td>1.6</td>
<td>165</td>
<td>588</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>126</td>
<td>576</td>
<td>4.6</td>
<td>81</td>
<td>564</td>
<td>5.7</td>
<td>162</td>
<td>648</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>102</td>
<td>480</td>
<td>3.1</td>
<td>70</td>
<td>492</td>
<td>2.9</td>
<td>76</td>
<td>528</td>
</tr>
<tr>
<td>5</td>
<td>4.2</td>
<td>154</td>
<td>876</td>
<td>4.3</td>
<td>119</td>
<td>732</td>
<td>2.1</td>
<td>164</td>
<td>636</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>186</td>
<td>336</td>
<td>3.8</td>
<td>104</td>
<td>336</td>
<td>6.5</td>
<td>235</td>
<td>336</td>
</tr>
</tbody>
</table>

M, sample taken on day 5 of maintenance feeding; 42.5%, sample taken on day 14 of 42.5%M restriction; R, sample taken on day 14 of refeeding ad libitum.
Comparison of results from the present studies, involving overnight fasting and short-term caloric restriction, with those from other nutritional studies suggests that the adult dog exhibits a more moderate response to energy restriction with regard to serum IGF and IGFBP concentrations than do other species. This may reflect their evolution originally as pack animals for which irregular food availability may have resulted in periods of involuntary fasting. The IGF/IGFBP system in this carnivore may have adapted to cope with infrequent feeding. Fasting does not result in as much hypoinsulinaemia or hypoglycaemia in the dog as it does in man (de Bruijne 1979), although GH responses are comparable (Eigenmann et al. 1985, Marks et al. 1965). Indeed, in our study, a proportion of the dogs showed an increase in circulating insulin concentrations in response to moderate energy restriction. Insulin may regulate canine IGFBP-1; however, a greater decrease in circulating concentrations may be required before changes in IGFBP-1 are seen. Alternatively, canine IGFBP-1 may be more sensitive to the suppressive effects of insulin, as was suggested by Smith et al. (1995) to be the case in energy-restricted children, or regulated by factors other than insulin or glucose. Canine liver glycogen reserves are not depleted as rapidly during fasting as are those in man and rat (Kettlehut et al. 1980), and prolonged starvation is associated with less ketogenesis and more efficient use of ketone bodies as energy substrates (de Bruijne et al. 1981). Efficient adaptive responses to dietary restriction may mean that body resources are utilised more efficiently, and the requirement to change bioavailability of free serum IGF-I through alteration of circulating IGFBP concentrations is not as great in adult dogs as in other species.

Protein restriction also affects the IGF/IGFBP system. In rats it is associated with lower serum IGF-I concentrations, partly because of increased IGF-I hepatic mRNA fragility (Straus & Takemoto 1990) and altered serum IGFBP profiles (increased IGFBPs-1 and -2, decreased IGFBPs-3 and -4) reflecting hepatic mRNA transcription (Lemozy et al. 1994, Takenaka et al. 1996). In carnivores, protein may be more important than energy in modulation of IGF and IGFBP concentrations, as it is in GH secretion. Hepatic gluconeogenesis proceeds at a greater rate in carnivores than in omnivores or herbivores (Belo et al. 1976, Kettlehut et al. 1980), and is less responsive to variation in dietary levels of protein. However, in this study, dietary protein requirements were consistently exceeded, despite decreased ration size. Dogs therefore were unlikely to have been in negative nitrogen balance, although this was not determined.

Comparison with serum albumin, a conventional nutritional marker, shows that serum IGF-I may be a more sensitive marker for monitoring short-term change in nutritional status of the adult dog. Serum albumin concentrations increased during the first restriction period (56%M) and decreased during the subsequent refeeding period. The reason for this is unclear, but we believe it may reflect the 7–10 day half-life of canine albumin. These studies were conducted in healthy, non-obese adult dogs and showed that components of the IGF system in this species are modulated by nutrition. Serum IGF-I concentration decreased significantly with energy restriction and normalised with feeding ad libitum, independent of breed size. Nutritional support of veterinary critical care cases is of increasing importance; however, the efficacy of such therapy is difficult to assess because of a lack of effective techniques for monitoring short-term changes in nutritional status. Although serum IGF-I concentrations vary widely with breed size, results from our study indicate the potential of serum IGF-I concentration as a biochemical marker of short-term changes in nutritional status in healthy adult dogs. This merits further evaluation in pathological states.

Acknowledgements

The authors thank Dr Robert McCusker (Department of Animal Sciences, University of Illinois) for his critical review of the manuscript. This work was supported by a grant from the WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, UK.

References

Belo PS, Romros DR & Leveille GA 1976 Influence of diet on glucose tolerance, on the rate of glucose utilisation and on gluconeogenic enzyme activities in the dog. Journal of Nutrition 106 1465–1467.


de Bruijne JJ, Altszuler N, Hampshire J, Visser IJ & Hackeng WHL 1981 Fat mobilisation and plasma hormone levels in fasted dogs. Metabolism 30 43–47.


Burger I 1995 Updated feeding recommendations for the canine diet. Waltham Focus 5 32.


Canine IGFs and IGFBPs during nutritional restriction

A MAXWELL and others


Received 5 March 1997
Revised manuscript received 14 October 1997
Accepted 16 February 1998