Insulin-like growth factor I in the dog: a study in different dog breeds and in dogs with growth hormone elevation

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Abstract. A radioimmunoassay (RIA) devised for the measurement of human insulin-like growth factor I (IGF I) was employed for the measurement of canine IGF I. Canine IGF I was extracted from plasma specimens by gel chromatography. Columns were eluted with 1 M acetic acid and the fractions representing the 55 to 85% curve were pooled, lyophilized and reconstituted with assay buffer. Serial dilutions of canine IGF I from both normal and acromegalic dogs when added to the RIA system gave a similar displacement pattern of human [125I]IGF I as the one obtained by the addition of unlabelled human IGF I. The dose-response curve obtained by canine IGF I paralleled the one obtained by human IGF I. Logit-log transformation and least squares fitting resulted in straight line fitting of the standard curve between 0.039 and 5 ng IGF I added per tube. The within-assay coefficient of variation (CV) was 16.7% and the between-assay CV was 21.8%. Plasma IGF I concentrations in normal dogs appeared to be a function of body size. The concentrations were 36 ± 27 ng/ml in Cocker Spaniels, 87 ± 33 ng/ml in Beagles, 117 ± 34 ng/ml in Keeshonds, and 280 ± 29 ng/ml in German Shepherds (mean ± SEM). The mean IGF I level in a group of dogs with growth hormone (GH) elevation was 700 ± 90 ng/ml. Though this group of dogs comprised both small and large dogs, the mean IGF I level significantly differed from the one found in German Shepherds, the largest breed studied (P < 0.01). IGF I levels in dogs with GH elevation were similarly elevated in both dogs exhibiting acromegaly and dogs exhibiting GH-diabetes, but no signs of acromegaly. In dogs with GH elevation, drop in GH levels was associated with a significant drop in IGF I levels.

Insulin-like growth factors (IGF I and IGF II), earlier called non-suppressible insulin-like activity, are circulating small polypeptides tightly associated with plasma carrier proteins (Zapf et al. 1980). Both IGF I and IGF II appear to be controlled by growth hormone (GH) levels and to mediate the growth-promoting effects of GH. In adult human beings, the GH status appears to affect IGF I rather than IGF II concentrations (Zapf et al. 1981; Merimee et al. 1981). IGF I concentration is high in human acromegalics and low in patients affected by hypopituitarism. IGF II concentrations are normal in acromegalics but become subnormal in GH deficiency (Zapf et al. 1981). Recently, it has been shown that human IGF I given to hypophysectomized rats, provokes body growth similar to that obtained by GH administration (Schoenle et al. 1982). IGF I exhibits appreciable structural similarity with insulin and proinsulin, suggesting that these hormones have evolved from a common ancestral molecule (Blundell et al. 1978). These findings suggest that IGF I plays an important general growth-promoting role among mammalian species.

Recently, we have documented spontaneous, iatrogenically- and experimentally-induced GH elevation in the dog. Some dogs, when under progesterone exposure, during either a natural progesterone phase or administration of medroxyprogesterone acetate (MPA), develop increased levels of
circulating GH. These dogs, as a result of GH elevation, develop glucose tolerance and/or acromegaly (Eigenmann & Rijnberk 1981; Eigenmann & Eigenmann 1981b; Eigenmann 1981; Eigenmann & Venker van-Haagen 1981; Eigenmann et al. 1983). Growth hormone in the dog is known to be a powerful diabetogenic agent (Young 1953; Altszuler 1974).

The aims of this study were 1) to evaluate the applicability of a radioimmunoassay devised for the measurement of human IGF I for the measurement of IGF I extracted from canine plasma, 2) to investigate the circulating IGF I concentrations in normal dogs belonging to breeds that differ in size and 3) to investigate the GH-IGF I relationship in the plasma of dogs with GH elevation.

Materials and Methods

1. Hormones and chemicals

Highly purified canine GH (Lot D 1080A, Dr. A. E. Wilhelmi) was used in a radioimmunoassay (RIA) as described earlier (Eigenmann & Eigenmann 1981a). Pure human IGF I was used as tracer and standard in RIA procedures for the measurement of IGF I. Bovine serum albumin (BSA, Sigma) was of RIA grade. Anti-rabbit IgG antiserum and rabbit IgGs were purchased from Antibodies, Inc., Davis, CA.

2. Assay procedures

Growth hormone was assessed in an RIA as described earlier (Eigenmann & Eigenmann 1981a). Plasma IGF I concentrations were measured in an RIA using pure human IGF I (Zapf et al. 1981). In the IGF I RIA, bovine serum albumin rather than human serum albumin was used. Insulin was measured in an RIA as described by Hales & Randle (1963). Glucose was measured in a glucose analyzer.

3. Isolation of IGF I from canine plasma specimens

Insulin-like growth factor I was isolated from canine plasma by gel chromatography under acidic condition (Eigenmann et al. 1977a; Zapf et al. 1981). Specimens (0.5 ml) were passed over Sephadex G-50 columns. For all samples columns 1.5 × 100 cm with a total bed volume of 170–180 ml were used. Chromatography was developed in the cold room at 4°C with the columns being equilibrated with 1 M acetic acid. The flow rate was kept constant at a rate of 40 ml/h. Prior to use, each column was calibrated with a trace amount of [125I]IGF I in 0.5 ml pooled canine plasma. The fractions representing the 55 to 85% bed volume were pooled, an aliquot (20%) was lyophilized and reconstituted with 2 ml assay buffer (Zapf et al. 1981).

4. Calculations and statistics

Standard curve fitting and calculation of hormone concentrations in samples were performed using a programmable desk-top unit (T1 59 and a Pc-100c Printer, Texas Instruments). The logit-log transformation, as described by Rodbard & Lewald (1970) ordinate (y): logit B/Bo = log 1 - B/Bo; abscissa (x): log hormone concentration was used for least squares fitting of the standard curve and calculation of hormone concentrations in samples (B = binding at a given hormone concentration, Bo = binding in the absence of unlabelled hormone; B and Bo corrected for unspecific binding). For statistical analysis, Student's t-test was employed. The within- and between-assay coefficient of variation was calculated according to Abraham et al. (1971).

5. Sources of plasma specimens

Blood samples were obtained from mature experimental dogs, from patients and from normal adult dogs maintained in a breeding colony or kept as pets. Blood was drawn by jugular venipuncture after an overnight fast. Blood was transferred into ethylene diamine tetraacetic acid (EDTA)-containing tubes, centrifuged immediately and plasma was stored at -20 to -30°C.

Normal dogs. Twenty-six normal members of a breeding colony, including Beagles, Keeshonds and Cocker Spaniels were studied. The mean age was 4.8 ± 0.8 years for the Cocker Spaniels (± SD). Normal German Shepherds studied were kept as pet dogs. These dogs exhibited a mean age of 2.6 ± 1.7 years (± SD). Both male and female dogs were studied.

Dogs with GH elevation. Dogs with experimentally-induced GH elevation were all ovariohysterectomized females, which after oestradiol-priming, were given medroxyprogesterone acetate (Eigenmann & Eigenmann 1981a). This group included dogs aged from 8–13 years.

Dogs with GH elevation were all privately owned females. Their mean age was 8 ± 2.3 years (± SD). Among these dogs different breeds were prevalent, the smallest in size being the Dachshund and the largest the Dalmatian and the German Shepherd. All these dogs were presented with clinical signs of acromegaly and/or diabetes occurring either during MPA medication or spontaneously during a luteal (progesterone) phase. Among the 29 dogs studied, 15 had developed GH elevation spontaneously during the progesterone phase and 14 had developed GH elevation during MPA medication. Among the dogs with spontaneous GH elevation, 7 exhibited acromegaly and 8 exhibited diabetes alone (plasma glucose ≥ 10 mm). Two of the 7 acromegalic dogs exhibited a plasma glucose of ≥ 10 mm. Among the dogs with iatrogenically-
induced GH elevation. 12 exhibited acromegaly and 2 exhibited diabetes alone (plasma glucose ≥ 10 mM). Four of the acromegalic dogs exhibited a plasma glucose of ≥ 10 mM.

All dogs were studied during their active disease. Seventeen out of the 29 were available for a second study when GH levels had lowered 2–4 months after MPA withdrawal/ovariohysterectomy. At that time all dogs with signs of acromegaly had appreciably improved and in dogs with diabetes, glucose tolerance also had dramatically improved.

Results

Evaluation of the IGF I assay

Iodination of IGF I resulted in specific activities ranging from approximately 40 to 100 μCi/μg IGF I.

The dilution of the second antibody giving maximal precipitation was chosen for assay procedures. Addition of the second antibody was shown to result in maximal precipitation after 1 h. Maximal precipitation persisted up to 2 h but slightly declined at 3 or more hours after addition of the second antibody. Thus, immunoprecipitates were harvested 1 h after adding anti-rabbit IgG antiserum. As shown in Fig. 1, linearization of the standard curve was obtained for concentrations between 0.039 and 5 ng added IGF I per tube. Serial dilutions of IGF I material extracted from canine plasma by acidic gel chromatography resulted in a displacement pattern of human [125I]IGF I parallel to the one obtained by unlabelled human IGF I (Fig. 1). Identical dose-response curves were obtained for IGF I extracted from plasma of normal and acromegalic dogs. For 23 samples the within-assay coefficient of variation (CV) with values ranging from 0.05–4.9 ng/tube was 16.7%. The between-CV for 21 samples with values ranging from 0.05–3.7 ng/tube was 21.8%. In 23 samples chosen at random, IGF I was determined in the undiluted and in the diluted (1:2) solution. The mean IGF I concentration in the undiluted samples was 1.37 ± 0.91 ng/ml and 1.53 ± 0.91 ng/ml in the diluted samples (mean ± sd; \( P = 0.1 \)).

![Fig. 1.](image)

Standard curve obtained by logit-log transformation. Ordinate: logit B/Bo. Abscissa: log hormone concentration. • = displacement obtained by the addition of human IGF I, △ = displacement obtained by canine IGF I extracted from canine plasma by gel chromatography.
**Fig. 2.**
Plasma IGF I concentrations as observed in different dog breeds of different sizes and in dogs with GH elevation

+ = mean ± SEM. Bars = mean body weight ± SEM.

**Fig. 3.**
GH and IGF I plasma levels in dogs with acromegaly, dogs with GH-diabetes and in dogs with experimental GH-elevation. Open bars represent IGF and filled bars represent GH.
Normal dogs

The mean IGF I concentration in plasma samples from normal dogs was lowest in Cocker Spaniels. Beagles had higher IGF I concentration than Cocker Spaniels but lower IGF I concentration than Keehonds. The highest IGF I concentrations were found in German Shepherds. The mean IGF I concentration was 36 ± 27 ng/ml in Cocker Spaniels, 87 ± 33 ng/ml in Beagles, 117 ± 34 ng/ml in Keeshonds, and 280 ± 23 ng/ml in German Shepherds (± SD).

Dogs with GH elevation

Dogs with GH-elevation had a significantly higher mean IGF I level than German Shepherds. The mean IGF I level in dogs with GH elevation was 700 ± 90 ng/ml while German Shepherds exhibited a mean IGF I level of 280 ± 23 ng/ml (mean ± SEM; \( P < 0.01 \)) (Fig. 2). IGF I and GH concentrations were similar in dogs with acromegaly and in dogs with GH-diabetes. Dogs with experimentally-induced GH elevation had both lower mean IGF I and GH concentrations but the differences were not significant. Acromegalic dogs had a mean IGF I level of 679 ± 116 ng/ml and a mean GH level of 112.8 ± 47.2 ng/ml. Diabetic dogs had a mean IGF I level of 751 ± 154 ng/ml and a mean GH concentration of 70.5 ± 25.2 ng/ml (mean ± SEM) (Fig. 3). In acromegalic dogs the mean insulin concentration was 147 ± 26 \( \mu \text{U/ml} \) and the mean glucose concentration was 7.3 ± 0.4 mM (mean ± SEM). In diabetic dogs the mean insulin concentration was 173 ± 46 \( \mu \text{U/ml} \) and the mean glucose concentration was 18.5 ± 3.3 mM (mean ± SEM). In dogs with experimentally-induced GH elevation the mean IGF I concentration was 419 ± 60 ng/ml and the mean GH concentration was 17.7 ng/ml (mean ± SEM) (Fig. 3). There was no correlation between GH and IGF I levels \((P = 0.5)\). Seventeen of the 29 dogs studied during

**Fig. 4.**

Plasma GH and IGF levels in acromegalic dogs, dogs with GH-diabetes and dogs with experimentally-induced GH-elevation during and after a stage of GH elevation. Open bars = IGF. Filled bars = GH. \( *P < 0.05; **P < 0.01; ***P < 0.001 \).
GH elevation (acromegalics and dogs with GH-diabetes) became available for a second study after ovariolhysterectomy/progestagen withdrawal. At this time, GH levels had significantly decrease in both acromegalic dogs and in dogs with GH diabetes. The mean IGF I level in acromegalic dogs was 558 ± 80 ng/ml during and 154 ± 18 ng/ml after GH elevation (n = 9; mean ± SEM; P < 0.001). In dogs with GH diabetes the mean IGF I concentration during the stage of GH elevation was 746 ± 175 ng/ml and 188 ± 66 ng/ml after GH levels had lowered (n = 8; mean ± SEM; P < 0.01). In dogs with experimentally-induced GH elevation a drop in GH levels was also associated with a drop in IGF I concentrations. Mean IGF I concentration in this group of dogs was 419 ± 60 ng/ml during and 164 ± 54 ng/ml after GH elevation (n = 6; mean ± SEM; P < 0.01) (Fig. 4).

Discussion

Under the assay conditions described in this report, linearization of the standard curve was obtained for IGF I amounts of 0.039–5 ng/ml. In most of the samples obtained and diluted as described under Materials and Methods, IGF I values fell within the linear range of the standard curve. Only a few samples from acromegalic dogs had to be diluted and reasayed. Thus, the methodological steps chosen appeared appropriate for most of the samples obtained from either normal dogs or dogs with GH elevation. Dilutions of canine IGF I preparations resulted in a parallel dose-response to the one obtained with human IGF I thus demonstrating similar immunoreactivities of human and canine IGF I. Thus, canine IGF I cross-reacts with human IGF I. Other investigators have found that canine serum cross-reacted in their human somatedin assay system (Bala & Bhaumnick 1979). The latter findings suggest that the structure of the IGF I molecule is likely to be, at least partly, conserved among certain mammalian species. Moreover, earlier we have shown that IGF I (earlier called non-suppressible insulin-like activity) extracted from canine specimens exhibits cross-reactivity in a protein-binding assay (Zapf et al. 1977) and that canine IGF thus extracted exhibits biologic activity (fat pad assay) similar to the one obtained by human IGF. Furthermore, there was a good correlation between the IGF activity measured in the two assay systems (Eigenmann et al. 1977a).

In our preliminary study on IGF I levels in dogs belonging to breeds that differ in size, circulating IGF I levels appeared to increase with body size. Thus, our findings suggest that the different IGF I levels found in dogs of different sizes may be, at least partly, responsible for the differences in size. This is in keeping with the fact that GH does not exert in vitro growth-promoting activity on cartilage (Daughaday et al. 1972) and that IGF I exerts both in vitro and in vivo growth-promoting activity (Froesch et al. 1976; Schoenle et al. 1982). However, from our study it cannot be excluded that the primary difference is in GH secretory rate, which in turn may account for the different IGF I concentrations found in different dog breeds. The mean IGF I level found in normal German Shepherds closely resembled the one found in normal human beings (Zapf et al. 1981). Thus, German Shepherds appear to have the full IGF I potential while other, smaller breeds appear to have a selected, weaker IGF I potential.

In our study in dogs with GH elevation the breeds comprised both very small (Dachshund) and very large (German Shepherds) breeds. Since normal animals were not studied for all of the different breeds affected by GH elevation, an estimate of the true IGF I elevations, i.e. per cent of normal, could not be given for each affected dog. However, the fact that the mean IGF I level in the group with GH elevation was significantly higher than the mean found in German Shepherds, a breed with large body size, and the fact that IGF I concentrations appear to be size-dependent points to the fact that acromegalic dogs indeed have elevated IGF I levels. In these dogs the mean IGF I concentrations was similar to that observed by Zapf et al. (1981) in human acromegalics.

The fact that in dogs with GH elevation there was no correlation between GH and IGF I concentrations is not surprising. A similar lack of correlation exists in human patients suffering from GH elevation (Zapf et al. 1980).

It is interesting to find that in our study all dogs with GH elevation had elevated IGF I levels but only a portion had acromegaly; some dogs had elevated GH and IGF I levels and diabetes, but no obvious signs of acromegaly. Actually, the dogs with more severe hyperglycaemia tended to
have less commonly acromegaly (Eigenmann et al. 1983). Thus, hyperglycaemia per se does not appear to abolish IGF I overproduction. In an earlier study we have shown that total IGF concentration in the insulin-deficient, pancreatetectomized dog drops to very low levels and that these low levels are restored toward normal by insulin substitution (Eigenmann et al. 1977b). The fact that the diabetic dogs investigated in this study had high rather than low IGF I levels is not at variance with our earlier findings. Rather, it appears that in the diabetic dog, insulin deficiency or any other metabolic change caused by lack of insulin but not hyperglycaemia per se causes IGF I levels to drop. This is in keeping with the belief that in the dog insulin is a prerequisite for normal IGF I production. It is possible that in the hyperinsulinaemic dog with GH diabetes, the catabolic situation invariably associated with pronounced/prolonged diabetes eventually outstrips the anabolic effects normally exerted by IGF I. In the dog with experimentally-induced GH diabetes, body weight initially increases. Later, when the signs of diabetes become more severe, despite continued GH administration, the body weight decreases (Young 1953).

In our dogs with GH elevation, IGF I levels in response to a decrease in GH concentrations invariably dropped. This is at slight variance with findings in human acromegals where acromegaly and elevated IGF I levels can be found in patients with normal GH levels (Zapf et al. 1980), or where there has been a poor correlation between somatomedin, GH and clinical response (Stonesifer et al. 1981). However, it should be emphasized that GH activity measured radioimmunologically does not always parallel the GH activity measured biologically (Ellis et al. 1978). Moreover, the treatment of GH elevation in man differs from the one in the dog. In acromegalic man, irradiation or surgery of the pituitary which both may lead to inadequate GH suppression, are commonly employed. In the female dog with GH elevation, GH overproduction appears to result from progestagen exposure (MPA administration/natural progesterone phase) and withdrawal of progestagens and/or ovariohysterectomy almost invariably leads to a reduction of GH levels (Eigenmann & Rijnberk 1981; Eigenmann & Eigenmann 1981b; Eigenmann 1981; Eigenmann & Venker van-Haagen 1981; Eigenmann et al. 1983). Thus, in the dog with GH elevation caused by progestagen exposure the measurement of IGF I may be more helpful than in human beings suffering from signs of GH-elevation. The clear-cut drop in IGF I levels after the drop in GH levels in our dogs supports the somatomedin hypothesis: GH exerts its anabolic action via somatomedin (IGF I) and GH controls the level of somatomedin (IGF I).

The following conclusions can be drawn from this study:

1) canine IGF I extracted by acidic gel chromatography cross-reacts with human anti-IGF I antisemur in a specific and sensitive way, 2) in normal dogs that differ in size, circulating IGF I concentrations increase with body size, and 3) IGF I concentrations in the dog as in man are subject to the control of circulating GH levels; high GH levels induce high IGF I levels and lowering of high GH levels leads to a reduction of IGF I levels. Additional investigations in the dog should disclose some aspects of the relationship between GH, IGF I and body size, and underline the importance of IGF I as an in vivo growth-promoting hormone.

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References


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