Large body size in the dog is associated with transient GH excess at a young age

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Abstract

The main determinants of body size are GH and IGFs. The aim of this study was to investigate whether differences in adult body size of medium-sized and giant dog breeds can be explained by differences in GH release and/or in plasma IGF-I and IGF-II concentrations at a young age. The basal plasma concentrations of GH, IGF-I and IGF-II were determined once weekly in six Great Danes and six beagles from the age of 6 weeks until the age of 24 weeks. In addition, the 6 h secretory profile of GH was determined every 2 weeks.

Basal plasma GH concentrations as well as the total area under the curve (AUC) and the AUC above the baseline for GH were significantly higher in Great Danes than in beagles of the same age. In contrast, plasma IGF-I and IGF-II concentrations did not differ significantly between the two breeds. Compared with values in adults, the basal plasma GH concentrations were high until the age of 7 weeks in the beagles, whereas in the Great Danes the basal

plasma GH levels remained high during the entire observation period, albeit with a gradual decline. The mean frequency and the mean amplitude of GH pulses tended to be higher in Great Danes than in beagles, although a significant difference was only reached at the age of 19 and 23 weeks for the frequency and at the ages of 9, 11 and 13 weeks for the amplitude. An age-dependent decrease in pulse frequency occurred in the Great Danes.

The results of this study demonstrate that differences in adult body size of medium-sized and giant dog breeds are preceded by differences in GH release and not by differences in circulating IGF-I or IGF-II concentrations. Both young Great Danes and young beagles experience a period of high GH release, but this period persists much longer in Great Danes. It is discussed that this difference may be due to delayed maturation of the inhibitory influences of somatostatin on pituitary GH release in the latter dogs.

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Introduction

In no other species of mammals is there such wide variation in body size and shape as that among the breeds of domestic dogs. Adult dogs at the opposite ends of the scale differ nearly 100-fold in weight. Equally striking differences are seen in head shapes, body proportions, hair coats and behaviour. Stockard & Vicari (1941) put forward the idea that there is an endocrine basis for these differences in form and behaviour. This concept was based upon the similarities between some endocrine diseases known in man and some characteristics of dog breeds. For example, posture, voice, large size and skin overgrowth in breeds such as the St Bernard dog and the bloodhound made Stockard & Vicari think of acromegaly. In their first attempt to substantiate the hypothesis morphologically, they found no correlation between gross pituitary proportion and the type of dog, but on histological examination the anterior pituitary lobe of large dogs revealed an abundance of acidophilic cells (Stockard & Vicari 1941).

The first biochemical data to support the endocrine basis for differences in body size among dog breeds were provided by Eigenmann *et al.* (1984b). In a study in adult dogs of different breeds, circulating concentrations of insulin-like growth factor I (IGF-I) were found to be correlated with body size. Among genetic subgroups within one breed, i.e. standard, miniature and toy poodles, Eigenmann *et al.* (1984a) found plasma IGF-I levels in adults to parallel body size, while basal and clonidine-stimulated plasma concentrations of growth hormone (GH) levels were similar among dogs of different sizes.

More recently, Nap *et al.* (1993) reported on changes in plasma levels of GH and IGF-I during prepubertal growth of Great Dane pups and miniature poodle pups. The basal plasma GH concentrations in Great Dane pups were initially high and declined to the low levels of adulthood at about one-half year of age (Nap *et al.* 1993). In the miniature poodle pups the mean basal plasma GH levels were low and did not change significantly with time (Nap *et al.* 1992). In agreement with the observations of

Eigenmann *et al.* (1984a, b) the plasma IGF-I concentrations in the Great Danes were higher than those in the miniature poodles.

Thus there is evidence that the GH-IGF-I axis is an important determinant in body size in dogs of different breeds. The observations of Nap *et al.* (1992, 1993) suggest that the large body size and the heavy overgrown features of some large dog breeds are the result of transient juvenile GH excess, i.e. gigantism. Or in the words of Stockard & Vicari (1941): '...that a temporary glandular modification acting for only a short time during development may have impressed certain organs then at a critically susceptible stage'.

In order to explore this further we have studied GH secretory patterns and plasma IGF-I and IGF-II concentrations in young, growing Great Danes and in young, growing dogs of a medium-sized breed, the beagle.

Materials and Methods

Animals

Six beagles (three males and three females) and six Great Danes (three males and three females) were studied. The Great Danes originated from three different litters and the beagles from two different litters. All dogs were fed a commercial dog food, twice daily. Water was freely available. On the days of blood sampling the dogs were fed at 0900 h. The dogs were studied from the age of 6 weeks until the age of 24 weeks.

For the determination of the secretory profiles of GH, blood samples of 0.7 ml were collected at 15 min intervals for 6 h every 2 weeks. Blood sampling for the determination of the secretory profiles of GH always started at 0800 h. The samples for measurement of basal plasma concentrations of GH, IGF-I and IGF-II were collected once weekly at 0900, 0930, 1000, 1030 and 1100 h. Blood samples were collected by jugular venepuncture, immediately placed in chilled EDTA-coated tubes, and centrifuged at 4 °C at 2000 g. Plasma was stored at -20 °C until assayed. On the days of blood sampling body weight (BW) was recorded.

Hormone determinations

Plasma GH concentrations were measured in a homologous RIA with intra- and interassay coefficient of variation (CV) values of 3.8 and 7.2% respectively (Eigenmann & Eigenmann 1981). In adult dogs, a mean $\pm$ S.E.M. GH level of 1.92 ± 0.14 μ g/l has been reported (Eigenmann & Eigenmann 1981).

Total plasma IGF-I and IGF-II were measured after acid-ethanol extraction to remove interfering IGF-binding proteins (IGFBPs). Plasma IGFs were extracted using a mixture of 87.5% (v/v) ethanol and 12.5% 2 M

formic acid. Tubes containing 100 μ l plasma and 400 μ l of the ethanol-formic acid mixture were mixed thoroughly and incubated for 30 min at room temperature. After centrifugation for 30 min at 5500 g at 4 °C, a 50 μ l aliquot of the supernatant was diluted 1:50 with assay buffer containing 63 mM Na₂HPO₄ (pH 7.4), 13 mM Na₂EDTA, and 0.25% (w/v) BSA. The presence of interfering IGFBPs in the plasma extracts was investigated according to the protocol of Sota *et al.* (1996) by measuring the IGF-binding capacity before and after extraction using dextran-coated charcoal to separate free and bound radio-labelled IGF-II. There was clear IGF-binding capacity before extraction, whereas no remaining IGF-binding capacity could be established after extraction. Also, on Western ligand blots no IGFBP bands could be visualised in the extracts (not shown).

IGF-I concentrations were measured in a heterologous RIA validated for the dog as described previously (Nap *et al.* 1993). The intra- and interassay CV values were 4.7 and 15.6% respectively. IGF-I antiserum (UBK 487) was a generous gift of Drs Underwood and Van Wyk, University of North Carolina, USA, via the hormone distribution programme of the US National Institute of Diabetes, Digestive and Kidney Diseases.

IGF-II concentrations were measured with a heterologous RIA using monoclonal antibodies against rat IGF-II (Amano Enzyme Co., Troy, VA, USA). The antibody had a cross-reactivity of 100% with human IGF-II and of 10% with human IGF-I. The assay was performed by addition of 100 μ l standard or plasma extract to 100 μ l assay buffer with monoclonal antibody (0.2 ng IgG/100 μ l) and 100 μ l assay buffer containing 12 500 d.p.m. ¹²⁵I-IGF-II. After incubation for 1 day at 4 °C, antibody-bound IGF-II was precipitated by the addition of 100 μ l anti-mouse second-antibody-coated cellulose (Sac-Cel; IDS Ltd, Boldon, Tyne and Wear, UK). The interassay variation was 12.6% at an IGF-II concentration of 326 μ g/l.

Data analysis

The pattern of pulses in the 6 h profiles of GH was analysed using the Pulsar program developed by Merriam & Wachter (1982). The program identifies secretory peaks by height and duration from a smoothed baseline, using the assay S.D. as a scale factor. The cut-off parameters G1-G5 of the Pulsar program were set at 3.98, 2.40, 1.68, 1.24 and 0.93 times the interassay S.D. as criteria for accepting peaks 1, 2, 3, 4 and 5 points wide respectively. The smoothing time, a window used to calculate a running mean value, was set at 6 h. The splitting cut-off parameter was set at 2.7 and the weight assigned to peaks was 0.05. The A, B and C values of the Pulsar program, used to calculate the variance of the assay, were set at A=0.206, B=7.77 and C=18.4.

The values extracted from the Pulsar analyses included the number and amplitude of peaks and the area under the

Figure 1 BW (means $\pm$ S.E.M., $n=6$) of beagles ($\circ$) and Great Danes ($\bullet$) from the age of 6 weeks until the age of 24 weeks.

curve (AUC) above the zero level as well as the AUC above the baseline.

Statistical analysis

The median of the five once-weekly values was used as the basal value. These median values were used to calculate the mean values per group at each time point. Differences between groups were examined by a general linear model for repeated measures. Student's *t*-test was used to test the differences between both breeds at a particular age for significance. Correlation was calculated with the Spearman rank test. Calculations were performed by use of the statistical software program SPSS. Results are expressed as means $\pm$ S.E.M. $P < 0.05$ was considered significant.

Results

In the beagles the mean BW increased from 3.6 ± 0.2 kg in week 6 to 10.8 ± 1.1 kg in week 24. In the Great Danes mean BW was 8.6 ± 0.7 kg in week 6 and increased to 30.1 ± 1.3 kg in week 24 (Fig. 1).

In the Great Danes basal plasma GH concentrations were 9.0 ± 2.1 $\mu\text{g/l}$ at 6 weeks of age and 4.6 ± 1.1 $\mu\text{g/l}$ at 24 weeks age. In the beagle dogs basal plasma GH concentrations were 4.4 ± 0.9 $\mu\text{g/l}$ at 6 weeks of age and 1.7 ± 0.1 $\mu\text{g/l}$ at 24 weeks of age. The mean basal plasma GH concentrations were significantly higher in the Great Danes than in the beagles (Fig. 2). The basal plasma GH concentrations in the Great Danes decreased slowly with time ($r = -0.82$, $P = 0.001$). In the beagles there was also

Figure 2 Basal plasma GH concentrations (means $\pm$ S.E.M., $n=6$) of beagles ($\circ$) and Great Danes ($\bullet$) from the age of 6 weeks until the age of 24 weeks.

a significant correlation between mean basal plasma GH concentrations and age ($r = -0.50$, $P = 0.029$). Compared with adult values, the basal plasma GH concentrations in the beagles were high until the age of 7 weeks, whereas in the Great Danes the basal plasma GH levels remained at higher levels during the entire observation period (Fig. 2).

In the Great Danes plasma IGF-I and IGF-II concentrations were 104 ± 32 and 77 ± 6.2 $\mu\text{g/l}$ respectively at 6 weeks of age, and 307 ± 31 and 171 ± 13 $\mu\text{g/l}$ respectively at 24 weeks of age. In the beagle dogs plasma IGF-I and IGF-II concentrations were 311 ± 35 and 134 ± 15 $\mu\text{g/l}$ respectively at 6 weeks of age, and 237 ± 52 and 152 ± 13 $\mu\text{g/l}$ respectively at 24 weeks of age. The plasma IGF-I or IGF-II concentrations did not differ significantly between breeds (Fig. 3).

The mean of the total AUCs for GH were significantly higher in the Great Danes than in the beagles, except for the total AUCs at 21 weeks of age (Fig. 4). Also the mean AUCs above the baseline for GH were significantly higher in the Great Danes than in the beagles except for the AUCs at 7, 17 and 21 weeks of age. In the Great Danes the mean total AUC for GH ($r = -0.75$, $P = 0.02$) and the mean AUC above the baseline for GH ($r = -0.80$, $P = 0.01$) decreased significantly with time (Fig. 4).

The mean number of GH peaks per 6 h tended to be higher in the Great Danes than in the beagles, but a significant difference was only reached at the ages of 19 and 23 weeks. The mean number of GH peaks decreased slowly with time in the Great Danes ($r = -0.89$, $P = 0.001$) but not in the beagles ($r = -0.65$, $P = 0.058$) (Fig. 5). The mean amplitude of the GH peaks was significantly higher in the Great Danes than in the beagles at the ages of 9, 11 and 13 weeks, and tended to be lower at the age of 23 weeks (Fig. 5).

Figure 3 Mean $\pm$ S.E.M. plasma concentrations of IGF-I (left panel) and IGF-II (right panel) in six beagles (○) and six Great Danes (●) from the age of 6 weeks until the age of 24 weeks.

Discussion

The results of this study demonstrate that the secretory profile of GH in Great Danes at young age is characterised by high basal plasma level, high total AUC, high AUC above the baseline, high pulse frequency, and high pulse amplitude compared with beagles of the same age. It is likely that these differences in pituitary GH release are responsible for the differences in body size between medium-sized and giant breeds of dogs.

During the entire observation period, basal plasma GH levels in the young Great Danes were higher than those reported for adult dogs (Eigenmann & Eigenmann 1981, French *et al.* 1987). With the same assay used in this study, Eigenmann & Eigenmann (1981) found in 63 healthy adult dogs a mean $\pm$ S.E.M. GH level of 1.92 ± 0.14 µg/l. In contrast, in the present young beagles high GH levels

were only present until 7 weeks of age. The neonatal period in humans is also characterised by relatively high GH concentrations (De Zegher *et al.* 1993). Similar to our observations in Great Danes and beagles, neonatal hyper-somatotropism in human beings is characterised by pulsatile GH secretion with a high pulse amplitude and a high pulse frequency. In premature or small for gestational age newborns even higher basal plasma GH concentrations are found together with significantly higher peak GH levels, which may contribute to the early catch-up growth of these children (Deiber *et al.* 1989, Wright *et al.* 1992).

With increasing age in the Great Danes there was not only a decrease in the basal GH level and AUC for GH but also an age-dependent decrease in the frequency of GH pulses. It is very likely that the pattern of excessive juvenile secretion of GH is related to the final adult body size. However, the fundamental questions are how this

Figure 4 Total AUC for GH in six beagles (open bars) and six Great Danes (shaded bars) (left panel), and AUC for GH above the baseline in six beagles (open bars) and six Great Danes (shaded bars) (right panel). Plasma samples were collected at 15 min intervals for 6 h. Values are expressed as means $\pm$ S.E.M. *Indicates a significant difference between breeds at the same age.

Figure 5 Number of GH peaks in a 6 h period in six beagles (open bars) and six Great Danes (shaded bars) (left panel), and peak amplitude of GH pulses in six beagles (open bars) and six Great Danes (shaded bars) (right panel). Values are expressed as means $\pm$ S.E.M. *Indicates a significant difference between breeds at the same age.

excessive GH secretion is regulated and what mechanism causes the decreasing secretion pattern of GH with increasing age. It is generally acknowledged that the pulses in GH release are caused by the withdrawal of somatostatin from the pituitary portal circulation, whereas the pulse amplitude greatly depends on bursts of GH-releasing hormone (GHRH) (Cella *et al.* 1996, Rigamonti *et al.* 1998). GH in turn inhibits the release of GHRH directly at the hypothalamic level or indirectly through feedback at the hypothalamic and/or the pituitary level by inhibitory effects of GH-induced plasma IGF-I concentrations. As no differences were found in plasma IGF-I or IGF-II concentrations between the Great Danes and beagles, and as increased sensitivity to endogenous IGF negative feedback is not a cause of the decline in GH secretion with ageing (Chapman *et al.* 1997), the hypersomatotropism of the Great Danes must be attributed to regulatory pathways in the hypothalamic–pituitary system. The high basal GH concentrations in Great Danes point to a low sensitivity to inhibition by somatostatin at the pituitary level, or low hypothalamic release of somatostatin. The fact that the reduction in total GH exposure with increasing age in Great Danes is mainly caused by lowering of the basal GH levels may be attributed to the development of an enhanced somatostatin inhibitory pathway. Maturation of the GH control system usually begins after birth. In the foetal period there is also high GH release, which is partly caused by pituitary insensitivity to somatostatin (Torronteras *et al.* 1997) and is associated with enhanced GHRH receptor expression (Korytko *et al.* 1996).

Although IGF-I has been identified as a growth modulator (Glasscock *et al.* 1992), the results of this study indicate that it is not circulating IGF-I or IGF-II but rather GH that is the predominant mediator of somatic growth. The IGF-I values in young, growing Great Danes are in accordance with the values reported previously in this breed (Nap *et al.* 1993). The IGF-I concentrations in plasma in young beagles were not different from those in Great Danes at the same age, but were higher than those

in miniature poodles of the same age (Nap *et al.* 1992). Thus the high correlation between plasma IGF-I concentration and BW observed in adult dogs (Eigenmann *et al.* 1984a) seems to be less pronounced in young dogs.

IGFs circulate in the plasma compartment mainly bound to IGFBPs. Chapman *et al.* (1998) showed in healthy fasting adults a dominant role for free IGF-I, and not IGFBP-bound IGF-I in suppressing GH release. Binding of IGF-I to IGFBP-3, the major binding protein in plasma, in a 150 kDa complex together with the acid-labile subunit, serves to trap IGF-I in the circulation and inhibits the effects of IGF-I exerted outside the vascular space. Preliminary data on the distribution pattern and concentrations of IGFBPs in plasma samples of Great Danes and beagles by Western ligand blot analyses did not reveal differences in either the concentration or nature of the IGFBPs present (J A Mol, unpublished observations). These data are in agreement with the absence of differences in total IGF-I or IGF-II concentrations in the plasma samples of these immature dogs.

There is a large body of experimental evidence indicating that GH effects on growth are mediated by circulating IGF-I. However, this is not an exclusive pathway, for in infusion experiments the actions of GH and IGF-I are quantitatively different (Hunziker *et al.* 1994). It is now known that peripheral tissues such as skeletal muscle and chondrocytes can synthesise IGF-I and that the response of the peripheral tissues to GH can be mediated in a paracrine and an autocrine manner through IGF-I produced *in situ*. Our observations in large and medium-sized dogs, like those in young rats (Palmer *et al.* 1993), do not support the concept that total plasma IGF-I plays a crucial role as an endocrine mediator of GH action.

In conclusion, the results of this study demonstrate that differences in final body size between medium-sized and giant dog breeds are associated with differences in GH release at young age and not with differences in circulating IGF-I or IGF-II levels. In both the Great Danes and the beagles initially there is a period of juvenile

hypersomatotropism. The relatively long persistence of this high GH release in the Great Danes may be caused by delayed maturation of the inhibitory influences of somatostatin on GH release.

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