Evaluation of pulsatile plasma concentrations of growth hormone in healthy dogs and dogs with dilated cardiomyopathy

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Objective—To evaluate plasma concentrations of growth hormone (GH) and insulin-like growth factor I (IGF-I) in healthy dogs and large-breed dogs with dilated cardiomyopathy (DCM).

Animals—8 dogs with DCM and 8 healthy control dogs of comparable age and body weight.

Procedures—Blood samples for determination of the pulsatile plasma GH profile were collected from all dogs at 10-minute intervals between 8:00 AM and 8:00 PM. Plasma IGF-I concentration was determined in the blood sample collected at 8:00 AM.

Results—No significant differences in plasma IGF-I concentrations, basal plasma GH concentration, GH pulse frequency, area under the curve above the zero line and above the baseline for GH, and GH pulse amplitude were found between dogs with DCM and control dogs.

Conclusions and Clinical Relevance—Results did not provide evidence for an association between DCM in dogs and a reduction in plasma concentrations of GH or IGF-I. Therefore, reported positive effects of GH administration are most likely attributable to local effects in the heart. (Am J Vet Res 2011;72:59–63)

Dilated cardiomyopathy, characterized by impaired myocardial systolic function and ventricular chamber dilatation (ie, volume overload hypertrophy), is one of the most common heart diseases in large- and giant-breed dogs.1 The etiology of DCM in dogs is often unknown; however, there is a diverse spectrum of suspected and known causes of myocardial hypokinesis that include genetic factors. In humans, familial DCM may account for 20% to 50% of cases and is caused by mutations in several genes.2,3 Mutations responsible for DCM in dogs have not been identified yet.4 In addition to genetic factors, nutritional deficiencies, metabolic disorders, immunologic abnormalities, infectious diseases, drugs, toxins, sustained tachycardia, and biochemical alterations have been recognized as causes of DCM in dogs.5 Little attention is paid to whether an abnormality in the release of GH or IGF-I may play a role in the pathogenesis of DCM.

The GH–IGF-I axis deserves attention for several reasons. Growth hormone and IGF-I are known to be of importance in the maintenance of normal myocardial function, which is evident from observations in human patients with either acromegaly or GH deficiency. The GH excess of acromegaly leads to biventricular concentric hypertrophy and subsequent diastolic dysfunction from myocardial hypertrophy with interstitial fibrosis.6,7 At a late stage, this has been reported to progress to systolic dysfunction, decompensated eccentric hypertrophy, and heart failure.8 Growth hormone deficiency, conversely, is also associated with impaired cardiac performance.7,9 Moreover, cardiac function has been reported to improve markedly after GH replacement therapy in human patients with GH deficiency.10–12 Furthermore, in healthy humans, GH administered for a short time increases ventricular contractility.13 Also, in healthy dogs, GH has effects on myocardial structure.14 The apparent benefits of GH on cardiovascular function of patients with GH deficiency gave rise to extensive clinical and experimental studies on the cardiovascular...
effects of the GH–IGF-I axis in different pathological settings, including DCM. The activity of this axis has been reported to be decreased in human patients with DCM, especially when the severity of cardiac dysfunction is taken into account.15-20 Moreover, prolonged GH deficiency may contribute to the development of human DCM.12 There is, however, controversy about the beneficial effects of GH treatment in these patients.21-23

Large-breed dogs at a young age go through a period of GH excess.24 When longitudinal bone growth stops in these dogs, the plasma GH concentration decreases to adult values, which are similar for dogs of all sizes, whereas there is a strongly positive correlation between body size and plasma IGF-I concentrations.25 It can be hypothesized that this initially high setting of the GH–IGF-I axis (ie, juvenile hypsomatotropism) leads to a relative GH deficiency later in life, which could be an endocrine basis for the high prevalence of DCM in large-breed dogs. Alternatively, it may be hypothesized that myocardial dysfunction develops in adult life as a consequence of GH-induced cardiac remodeling in early life.14

The purpose of the study reported here was to evaluate plasma concentrations of GH and IGF-I in healthy dogs and large-breed dogs with DCM to test the hypothesis that an abnormality in GH or IGF-I release plays a role in the pathogenesis of DCM in large-breed dogs.

Materials and Methods

Animals—Eight healthy dogs (3 anestrous females, 1 neutered female, 3 sexually intact males, and 1 castrated male) with ages ranging from 3 to 12 years (mean ± SD, 6.3 ± 3.1 years) and body weights ranging from 31 to 60 kg (mean ± SD, 47 ± 8 kg) entered the study as control dogs. The control group was composed of 4 crossbreed dogs, 2 Great Danes, 1 Scottish Deerhound, and 1 Irish Wolfhound. The dogs were considered healthy on the basis of their medical history and results of physical examination, ECG, and echocardiography. The control dogs were pet dogs owned by hospital personnel and students, who gave informed consent for use of the dogs in the study.

A diagnosis of DCM was made in 8 privately owned dogs (7 sexually intact males and 1 neutered female) with ages ranging from 3 to 10 years (mean ± SD, 6.5 ± 2.0 years) and body weights ranging from 26 to 45 kg (mean ± SD, 38 ± 6 kg). The diagnosis was based on clinical and radiographic evidence of congestive heart failure and echocardiographic evidence of LV dilation, a normal to decreased LV wall thickness, and an FS < 25% in the absence of other congestive heart failure–related lesions determined by use of 2-D echocardiography. This group was composed of 4 Bouvier de Flandres, 1 Doberman Pinscher, 1 Newfoundland, 1 German Shepherd Dog, and 1 German Shorthaired Pointer. After owners’ informed consent was obtained, blood samples for GH and IGF-I measurements were collected. All dogs were being treated with drugs, including furosemide, enalapril, or digoxin. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University.

Experimental protocol—in the healthy dogs, for the determination of the secretory GH profile, blood samples were collected via jugular venipuncture at 10-minute intervals between 8:00 AM and 8:00 PM. In the dogs with DCM, blood samples for the determination of the secretory profiles of GH were collected according to the same schedule from a jugular vein by means of an indwelling catheter® aseptically inserted just before the start of sampling. Immediately after each sample collection, the jugular catheter was filled with saline (0.9% NaCl) solution containing 5 U of heparin/mL.

Immediately after collection, the blood was placed in chilled EDTA-coated tubes and centrifuged at 4°C for 10 minutes at 1,500 × g. Plasma was stored at −20°C until assayed. The 8:00 AM sample was also used for measurement of the concentration of IGF-I.

Hormone concentration determinations—Plasma GH concentration was measured by use of a specific homologous radioimmunoassay.26 The intra- and interassay coefficients of variation were 3.8% and 7.2%, respectively, and the lower limit of quantitation was 0.3 µg/L.

Total plasma IGF-I concentration was measured after acid-ethanol extraction to remove interfering binding proteins. Plasma IGF-I was extracted by use of a mixture of 87.5% (vol/vol) ethanol and 12.5% 2M formic acid. One hundred microliters of plasma and 400 µL of the ethanol–formic acid mixture were placed in tubes, mixed thoroughly, and incubated for 30 minutes at 21°C. After centrifugation for 30 minutes at 5,500 × g at 4°C, a 50-µL aliquot of the supernatant was diluted 1:50 with assay buffer containing 63mM Na2HPO4 (pH, 7.4), 13mM Na2EDTA, and 0.25% (wt/vol) BSA. The extraction efficiency was 92.5 ± 5.7%.24 The plasma IGF-I concentration was measured by use of a heterologous radioimmunoassay validated for use in dogs.27 The intra- and interassay coefficients of variation were 4.7% and 15.6%, respectively. The IGF-I antiserum AFP4892898 and human IGF-I for iodination were obtained from the National Hormone and Peptide Program.9

Echocardiography—Echocardiography was performed in conscious dogs, with each dog in right lateral recumbency, by use of a high-definition ultrasonography system equipped with a 3- to 5-MHz broadband phased-array transducer and simultaneous ECG recording. Under the guidance of 2-D images, standard M-mode and ECG recordings were made to measure the thickness of the LV free wall and the interventricular septum and the LV diameter in both systole and diastole on the short-axis view. The FS was calculated by the computer software of the ultrasonography machine. The Ao was measured at the end of diastole and the LA at its maximal upward excursion near the end of systole, and these values were used to determine the LA diameter:Ao ratio.28

Data analysis—The 12-hour pulsatile profiles of plasma GH concentrations were analyzed by use of a software program.29 The program identifies secretory peaks by height and duration from a smoothed baseline by use of the assay SD as a scale factor. The cutoff
Results

Mean body weight and mean age of the dogs with DCM were not significantly different from those of the control dogs. Differences in mean LV dimensions in diastole and systole, mean FS, and mean LV wall and mean interventricular septum thickness in diastole and systole were significantly different between groups (Table 1). Differences in mean LA diameter:Ao ratios between the 2 groups were not significant.

Pulsatile secretion of GH was observed in all control dogs and in 7 of the 8 dogs with DCM (Figure 1). Characteristics of the 12-hour plasma GH profiles and the mean plasma IGF-I concentration in the dogs with DCM and control dogs were determined (Table 2). Mean basal plasma GH concentrations and mean plasma IGF-I concentrations in the dogs with DCM were not significantly different from those in the control dogs, nor were mean AUC$_c$ and mean AUC$_b$. Mean GH pulse amplitude in dogs with DCM was lower than that in the control dogs, but this difference was not significant (P = 0.07). Mean GH pulse frequency was not significantly different between the dogs with DCM and the control dogs.

Discussion

Results of the present study indicated that there were no significant differences in the pulsatile plasma GH profile in dogs with DCM, compared with control dogs. Consequently, the data did not provide compelling evidence for alterations in GH release as an underlying cause of DCM. It may still be hypothesized that myocardial dysfunction develops in adult life as a consequence of GH-induced cardiac remodeling in early life. Therefore, the results provide a starting point in the design of future studies in this area.

Differences in GH pulse amplitudes between the dogs with DCM and the control dogs just failed to reach significance, with lower GH pulse amplitudes in the dogs with DCM. Growth hormone pulses are mainly the result of the pulsatile release of GHRH from the hypothalamus. Consequently, a lower GH pulse amplitude is most likely caused by reduced GH secretion in response to GHRH. In line with this supposition, humans with DCM have reduced somatotroph responsiveness to GHRH. Because of small sample sizes and variance that differed between groups, differences between groups were assessed by use of the nonparametric Mann-Whitney test. A value of P < 0.05 was considered significant. Results are reported as mean ± SD unless otherwise indicated.

Table 1—Echocardiographic characteristics (mean ± SD) in 8 dogs with DCM and 8 control dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DCM</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDd (mm)</td>
<td>74 ± 13</td>
<td>44 ± 7</td>
<td>0.006</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>64 ± 10</td>
<td>29 ± 8</td>
<td>0.006</td>
</tr>
<tr>
<td>FS (%)</td>
<td>12.2 ± 5.8</td>
<td>33.2 ± 7.4</td>
<td>0.006</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>8 ± 2</td>
<td>13 ± 3</td>
<td>0.010</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td>12 ± 2</td>
<td>18 ± 3</td>
<td>0.005</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>8 ± 2</td>
<td>13 ± 3</td>
<td>0.008</td>
</tr>
<tr>
<td>IVSS (mm)</td>
<td>10 ± 1</td>
<td>15 ± 4</td>
<td>0.008</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>45 ± 12</td>
<td>34 ± 7</td>
<td>0.09</td>
</tr>
<tr>
<td>LAD:Ao ratio</td>
<td>1.83 ± 0.75</td>
<td>1.20 ± 0.40</td>
<td>0.18</td>
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IVSd = Interventricular septum in diastole. IVSs = Interventricular septum in systole. LAD = LA diameter. LVDd = LV diameter in diastole. LVDs = LV diameter in systole. LVPWd = LV posterior wall in diastole. LVPWs = LV posterior wall in systole.

Table 2—Characteristics of 12-hour plasma profiles of GH and mean plasma IGF-I concentrations in the same dogs as in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DCM</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_c$ for GH (µg/L·12 h)</td>
<td>5.6 ± 4.8</td>
<td>8.6 ± 4.2</td>
<td>0.13</td>
</tr>
<tr>
<td>AUC$_b$ for GH (µg/L·12 h)</td>
<td>17.5 ± 14.3</td>
<td>20.4 ± 7.8</td>
<td>0.46</td>
</tr>
<tr>
<td>Basal plasma GH concentration (µg/L)</td>
<td>1.2 ± 0.9</td>
<td>1.0 ± 0.7</td>
<td>0.92</td>
</tr>
<tr>
<td>GH pulse frequency (peaks/12 h)</td>
<td>3 (0–8)</td>
<td>3 (2–7)</td>
<td>0.87</td>
</tr>
<tr>
<td>GH pulse amplitude (µg/L)</td>
<td>3.1 ± 2.5</td>
<td>6.3 ± 2.3</td>
<td>0.07</td>
</tr>
<tr>
<td>IGF-I concentration</td>
<td>148 ± 137</td>
<td>180 ± 71</td>
<td>0.29</td>
</tr>
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</table>

Figure 1—Twelve-hour plasma profiles of GH in a 6-year-old male dog with DCM (upper panel) and a 4-year-old female control dog (lower panel). *Significant (P < 0.05) increase in GH concentration.
hexarelin, a synthetic GH secretagogue, is preserved in humans with DCM.10 Synthetic GH secretagogues elicit their effect on GH release by acting through receptors different from those for GHRH.33,35 This indicates that the reduced somatotroph responsiveness to GHRH is not caused by impairment of the releasable GH pool in pituitary somatotrophs, but rather by a reduced number or function of adenohypophyseal GH receptors or by post-GHRH receptor hypofunction. A possible explanation for the slightly lower GH pulse amplitudes in dogs with DCM, compared with the control dogs, is prolonged illness, which in humans is associated with a reduced pulsatile secretion of anterior pituitary hormones.35 Because DCM in the dogs was often associated with anorexia and weight loss for weeks to months, reduced GH pulse amplitudes could be attributable to chronic disease. Another explanation for lower GH pulse amplitude in dogs with DCM may be the medication that the dogs received. All dogs with DCM were treated with a combination of furosemide, digoxin, or an angiotensin-converting enzyme inhibitor. These drugs may have effects on somatotroph function. In humans, digoxin reduces the somatotrophic responsiveness to GHRH.34

Many of the somatotrophic effects of GH are mediated through the production of IGF-I. In contrast to what has been reported in humans with DCM,17,19,21 the plasma IGF-I concentration in the dogs with DCM was not significantly different from that in the control dogs. Reduced plasma IGF-I concentration in humans with DCM may be a consequence of the impaired nutritional status that is frequently seen in end-stage DCM.35,36 The reduced basal plasma IGF-I concentration in humans with DCM may also be caused by peripheral GH resistance,30,37 although the IGF-I response to low rhGH doses is still preserved in these patients.16

Because of the many similarities in DCM between humans and dogs, GH treatment seems to be an appealing treatment in dogs with DCM. However, before such endocrine treatment is started, it needs to be established whether the pulsatile secretory pattern of GH in dogs with DCM is changed. Because we did not find evidence for GH deficiency, it appears that treatment of canine DCM with GH is not a substitution treatment. It rather creates a state of supraphysiologic GH exposure that may have beneficial effects on the myocardium of dogs with DCM. The rationale for using GH therapy in humans with DCM is the fact that GH is a physiologic regulator of myocardial growth and performance and that GH deficiency or an inadequate response of the heart to circulating GH may play a role in the pathogenesis of DCM.38 Beneficial effects from rhGH treatment have indeed been reported in human patients with DCM,39–42 although the positive effects of rhGH administration have not been confirmed in other studies.35,44 Theoretically, an increase in local IGF-I concentration induced by GH treatment might inhibit apoptosis of cardiac myocytes caused by high myocardial wall stress in DCM.55–66 In addition, GH has a direct protective effect on cardiac myocytes against apoptosis.47 However, GH administration to patients with normal somatotroph function is not without risk. Administration of GH disturbs the physiologic pulsatile release of endogenous GH, which may affect GH receptor expression.30 In addition, prolonged GH treatment may cause arrhythmias.49 Results of a study30 with cardiomyopathic hamsters even suggest that chronic treatment with GH, starting at an early stage of lesion development, is associated with reduced cardiac performance in the terminal stage of the disease.

The study reported here had some limitations. An important limitation was the low number of affected dogs and low power of the study, which might have caused differences between groups to be missed. Nevertheless, the data provide important and relevant findings on GH secretion in dogs with DCM. The groups were not identical in terms of sex and neutering, but confounding effects seem unlikely because dogs lack sex-associated differences in the ultradian secretory patterns of GH.35 Only in healthy cyclic and pregnant bitches does the pulsatile secretion pattern of GH change, which occurs during progression of the luteal phase or pregnancy.52 Female dogs used in this study were in anestrus or neutered. Finally, we cannot rule out confounding effects of the drugs used in the dogs with DCM, which could affect GH secretion.34 Nevertheless, results of the present study did not provide evidence for an association between canine DCM and a reduction in circulating concentrations of either GH or IGF-I.

References


