Prolonged fasting or clonidined can restore the defective growth hormone secretion in old dogs

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Abstract. Age-related changes in GH secretion were studied in the dog. In preliminary experiments, administration of GH-releasing hormone (GHRH-40, 2 μg/kg, iv) or the α2-adrenoceptor agonist clonidine (4 μg/kg, iv) elicited significantly higher plasma GH rises in 3 to 4 years old than in 10 to 14 years old beagle dogs. The pulsatile patterns of GH secretion in both young and old dogs under baseline conditions and after prolonged fasting or clonidine administration were studied. Samples were taken every 10 min from 09.00 to 15.00 h from five young and five old dogs of both sexes. Under baseline conditions, GH peak frequency, total peak area, and integrated GH secretion were significantly lower in old than in young dogs. In old dogs, 5-day complete fasting or 14-day clonidine administration (75 μg/dog, po, twice daily) increased the frequency and amplitude of spontaneous GH bursts, the total peak area, and the integrated GH secretion. After either stimulus, the GH secretory pattern was quantitatively and qualitatively indistinguishable from that of young dogs under baseline conditions. Similarly, the foregoing indices were significantly increased in young dogs by either stimulus, except for the inability of clonidine to affect peak frequency. These data demonstrate that the defective GH secretion in old dogs is not irreversible, since it is normalized when old dogs are exposed to central nervous system-directed stimuli.

Among the several causes proposed for the geriatric changes in structure and function, one is cessation of endogenous growth hormone secretion, which occurs in about half of the elderly population (1). There are some similarities between the alterations in ageing and those that occur in GH-deficient patients. In both, there are shrinkage of lean body mass, expansion of adipose mass, diminution of renal function, and decreased rates of cell division (1). In addition, both kinds of subjects have reduced bone mass and density, dental deficits, and a decrease in calcium absorption. Consistent with this proposition, one third of a large group of old subjects (55–80 years) had very low levels of somatomedin-C (2), the GH-related peptide that mediates most of the biological effects of GH (3).

If defective GH secretion is one of the pacemakers of ageing, it is of interest to study the mechanisms underlying reduced GH secretion in aged humans and animals (2, 4–6), with the ultimate goal being to develop suitable pharmacological means for counteracting the age-related phenomena. The present studies were performed in the dog, a species with many aspects of GH regulation resembling those in humans (7, 8).

After an initial assessment of the ability of old and young dogs to respond to acute challenges with GH-releasing hormone (GHRH) or clonidine, an α-adrenergic agonist (9), dogs were fasted for a long period or given clonidine, two known stimuli for GH secretion in this species (10–12), and the ability of these stimuli to alter the secretory pattern of GH was evaluated over a 6-h sampling period. Plasma somatomedin-C levels were also evaluated in young and old dogs before and at the end of fasting.
Material and Methods

Animals
Eight young (3 to 4 years old, 4 male and 4 female) and five old (10 to 14 years old, 3 male and 2 female) normal beagles were used for the acute GHRH and clonidine testing and five young (3 male and 2 female) and five old (3 male and 2 female) beagles for assessing the GH secretory pattern. All dogs, weighing between 9–15 kg, were trained over four weeks to lie on a comfortable pad in the laboratory for up to 7 h at a time. Experiments on each dog were scheduled at least 2 weeks apart, with continued training in between. The animals were exercised routinely and were fed normal dry dog food (Bobby Meat, Nossan, Corezzana, Italy) once a day, at 16.00 h with water ad libitum. They were on a 12-h light: 12-h dark regimen, with light on at 07.00 h. At the beginning of the study, body weights of all the dogs were stable and they had no observable diseases. The animals were brought to the laboratory at 08.00 h and experiments begun at 09.00 h. During the experiments, exercise was not permitted.

Experimental design
Acute GHRH and clonidine testing. The beagles of both sexes were randomly injected with GHRH (hpGRF-40, Bachem, Bubendorf, Switzerland, 2 μg/kg, iv), clonidine (ClO, Catapresan, Boehringer Ingelheim, Florence, Italy, 4 μg/kg, iv), or isovolumetric amounts of normal saline administered at time 0. After two baseline samples had been taken (−30 and −15 min), samples were obtained at 15, 30, 45 and 60 min.

Growth hormone secretory pattern. Blood samples (1 ml) were drawn between 09.00 and 15.00 h through an indwelling, non-thrombogenic intravenous catheter (Venisystem Butterfly-10, Abbott Ireland Ltd, Sligo, Republic of Ireland). The needle was positioned in the cephalic vein and fixed with an adhesive bandage 1 h before starting the experiment; the cannula was kept open by slow infusion of normal saline. Less than 40 ml blood was removed.

Baseline. On the day of the experiments, blood samples were taken from all dogs, fasted since 16.00 h of the preceding day, every 10 min for 6 h. No food was given. Each dog was studied twice, at least two weeks apart, and the results of the two experiments, being similar, were pooled.

Fasting. In these experiments, dogs were fasted completely for 5 days, with water available ad libitum. Blood samples were taken as previously described, from 09.00 to 15.00 h on the sixth day, while still fasting.

Clonidine. In these experiments, dogs with free access to food and water were given 75 μg ClO, po, at 09.00 and 19.00 h for 14 days, and at 09.00 of the fifteenth day, 14 h after the last ClO administration, blood sampling was begun.

GH radioimmunoassay. Blood was collected into tubes containing EDTA and immediately chilled. Plasma was frozen until assayed for GH by double-antibody radioimmunoassay (8). Highly purified canine GH (batch 1080A), obtained through the courtesy of Dr A. E. Wilhelmi, Emory University, Atlanta, GA, was used for iodination and as standard. Intra- and inter-assay variabilities were 4 and 18%, respectively. The sensitivity of the assay was 0.4 μg/l.

Plasma somatomedin levels. Sm-C was measured by radioimmunoassay of acid-extracted samples (13), taken at 09.00 and 09.10 h, using the immunochemicals and tracer provided by Nichols Institute (San Juan Capistrano, CA) for human use; the standard was a pure (Thr-59)-Sm-C preparation obtained by DNA recombinant technology (Amgen, Thousand Hoaks, CA). The sensitivity of this assay is 4.5 pg/tube. The inter-assay coefficient of variation is 0.15 at a concentration of 78 pg/tube. Sm-C recovery by this acid extraction procedure, determined after overnight incubation of pooled samples with labelled Sm-C, was 89.9 ± 2.5% (mean ± sd). Test samples were diluted to give concentrations of 5 to 500 pg/tube. The heterologous assay was validated by showing that after administration of human GH (2.5 mg/day) for 5 days to a young dog, basal Sm-C levels rose from 35.7 to 89.2 μg/l.

Plasma cortisol levels. Plasma cortisol levels under baseline and fasting (6th day) conditions were determined in the two initial samples (i.e. 09.00 and 09.10 h) with a commercial kit (Radim, Pomezia, Italy).

Data analysis
The patterns of GH secretion in the different experiments were analysed by 'cluster analysis' to search for significant increases and significant decreases within the data series (14). A significant increase was judged in relation to a specified nadir width, by using a moving nadir that began at onset of the experimental series. The individual values making up the nadir cluster were compared against the values making up a possible peak, defined as a second set of consecutive samples of specified number immediately after the test nadir (cluster size: two nadir values against two peak values). Nadir and peak clusters were compared by a pooled t-test, using the actual experimental replicates present in the test nadir and peak (3 replicates/point). A peak was considered to have occurred only when there was significant increase followed by a significant decrease.

All the other measures reported were calculated by conventional methods. Statistic evaluation of basal GH peak frequency, mean peak amplitude, integrated GH secretion (IC-GH), and total peak area in young vs old dogs was performed by the Mann-Whitney-U two-tailed test. Since age-related differences in these indexes were found, the non-parametric Friedman two-way analysis by rank was applied to establish whether the experimental conditions investigated (fasting and clonidine) had induced significant global differences in young and old
dogs. Finally, differences between basal and experimental conditions in both young and old dogs were evaluated by Wilcoxon-signed rank test. Data obtained in the acute studies were analysed by Dunnett t-test. The Student's t-test for paired data was used to compare cortisol and Sm-C data before and after fasting. Regression analysis was used to look for linear relationship between basal values of Sm-C and the different indices of the GH secretory pattern.

Results

Acute GHRH and clonidine testing

Administration of normal saline did not modify the basal secretion of GH in young nor in old dogs (data not shown). Administration of either GHRH or CLO evoked significantly greater responses in young than in old dogs (peak values: 20.2 ± 5.5 vs 10.1 ± 1.4 μg/l, p<0.05 and 16.2 ± 1.9 vs 4.6 ± 0.6 μg/l, p < 0.01; Fig. 1).

Growth hormone secretory patterns

Baseline. At least one burst of GH secretion was observed in all dogs but one during the experiments, the exception being an old female dog with non-pulsatile secretion. In old dogs, GH peak frequency, total peak area and integrated GH secretion were smaller than in young dogs (Table 1).

Fasting and clonidine. In old dogs, either fasting or CLO administration significantly increased all the secretory indices (Table 1, Fig. 2) and rendered them indistinguishable from those of young dogs, under basal conditions (Table 1). The same indices were significantly increased by both experimental conditions in young dogs, the only exception being the inability of CLO to affect GH peak frequency (Table 1).

Baseline plasma Sm-C levels did not differ significantly in young and old dogs; after fasting, there was a significant decrease in Sm-C levels in old dogs, but the decrease in young dogs was not significant (Table 2). A linear correlation between GH peak areas and plasma levels of Sm-C was found in old dogs either under baseline conditions (r = 0.98, p < 0.008) or after fasting (r = 0.83, p < 0.05), but not in young dogs.

During fasting, body weights of young and old dogs decreased by about 1—1.4 kg (8.3—11.2%), but there were no changes in body weight following CLO administration. Repeated administration of CLO induced no overt adverse reactions.

Baseline plasma cortisol levels did not differ significantly between young and old dogs and were not significantly increased in either group on the sixth day of fasting (Table 2).

Discussion

Both man and laboratory animals show profound age-related alterations in the pattern of spontaneous GH secretion, consisting mainly in decreases
Table 1.
Growth hormone secretion during a 6-h period in young (3 to 4 years old) and old (10 to 14 years old) dogs under baseline conditions and after prolonged fasting or clonidine treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>GH peak frequency (No./6h)</th>
<th>Mean peak amplitude (μg/l)</th>
<th>IC-GH (μg·l⁻¹·(6h)⁻¹)</th>
<th>Total peak area (μg·l⁻¹·(6h)⁻¹)²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.8 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td>629.5 ± 72.6</td>
<td>196.3 ± 48.1</td>
</tr>
<tr>
<td>Fasting</td>
<td>5.0 ± 0.4</td>
<td>4.6 ± 0.8</td>
<td>931.5 ± 191.0</td>
<td>407.5 ± 55.1</td>
</tr>
<tr>
<td>Clonidine</td>
<td>3.5 ± 0.5</td>
<td>5.3 ± 2.0</td>
<td>731.8 ± 118.6</td>
<td>270.5 ± 50.8</td>
</tr>
<tr>
<td><strong>Old</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.5 ± 0.6</td>
<td>1.6 ± 0.1</td>
<td>343.3 ± 67.3</td>
<td>31.2 ± 17.1</td>
</tr>
<tr>
<td>Fasting</td>
<td>3.8 ± 0.7</td>
<td>2.5 ± 0.3</td>
<td>513.2 ± 69.4</td>
<td>175.6 ± 35.8</td>
</tr>
<tr>
<td>Clonidine</td>
<td>3.3 ± 0.2</td>
<td>3.0 ± 0.7</td>
<td>410.2 ± 100.6</td>
<td>160.0 ± 67.8</td>
</tr>
</tbody>
</table>

* Dogs were fasted for 5 days or received clonidine (75 μg po twice daily for 14 days); each point is the mean ± SEM of 5 determinations, except for basal values which refer to 10 determinations.

² p < 0.05 vs young. ³ p < 0.05 vs baseline. ⁴ Sum of peak areas recorded during 6 h.

Fig. 2.
Effect of a 5-day-fast (middle) or 14-day-clonidine (75 μg/dog, po, twice daily) administration (bottom) on baseline pulsatile GH secretion (top) in two young (No. 1 and 2) and two old (No. 3 and 4) dogs. Blood samples were taken every 10 min for 6 h. Arrows indicate the GH peaks detected by the 'cluster analysis' method. Note that in young dogs the effect of fasting and clonidine was evident in dog No. 2, but not in dog No. 1. See text for further details.
in pulse amplitude and frequency and marked blunting of the GH response to pituitary or central nervous system (CNS)-directed stimulation (15–18). In vitro studies have shown that pituitary cells from aged rats secrete smaller amounts of GH and respond poorly to the stimulant effects of GHRH, both GH release and stimulation of synthesis of cAMP, the main intracellular transducer for GHRH signalling in the pituitary (17,19). However, Sonntag et al. (20) were unable to demonstrate in vitro and Wehrenberg & Ling (21) in vivo any change in pituitary responsiveness of the aged rat to hypothalamic GHRH. Interestingly, in vivo pretreatment of old rats with GHRH does not blunt, as it does in young rats, the GH-responsiveness to GHRH and the ability of the latter to increase cAMP formation, but instead stimulates it (19). In all, these findings might indicate that old rodents have a (primary?) deficiency of GHRH synthesis and/or release leading to their reduced GH pool and the defective GH responses to secretory stimuli. Supporting this view is the observation that GHRH immunoreactivity (22) and GHRH mRNA (23) are considerably reduced in the median eminence of old rats.

Regarding the involvement of somatostatin, a wealth of evidence has been presented for the existence of an increased hypothalamic somatostatinergic tone in ageing rodents (16, 20, 24–26). However, a major role of somatostatin in the decreased pituitary GH synthesis of aged rats (see above) seems unlikely, as this neuropeptide, at least under acute experimental conditions, inhibits GH release but not synthesis (27,28).

In this study, we first showed that administration of GHRH and CLO elicited in old dogs strikingly lower GH responses than in young counterparts, as is true for old rats (16, 17) and elderly humans (4,29,30). We then studied whether fasting or CLO, two GH releasers that act via the CNS (31, 32), could restore the presumably low somatotropic function of old dogs.

Dogs have spontaneous bursts of GH secretion, although they are somewhat smaller and rather less frequent than those in rats (33, 34). In our study, under baseline conditions, the GH peak frequency, total peak area and integrated GH secretion were considerably lower in old than in young dogs, whereas there was no significant difference in the mean peak amplitude.

Thus, the GH secretory profile of old dogs was similar to that of elderly humans, for whom a smaller total peak area has been reported, particularly during sleep, when the height of the pulses may be significantly decreased or they may be completely absent (35). In rats, instead, there is no significant difference between young and old animals in pulse frequency, the height is 3 times greater in the young rats (15,18).

Prolonged fasting and treatment with CLO effectively altered the GH secretory pattern of old dogs, augmenting the GH peak frequency, the mean peak amplitude, and the total secretion of GH. Thus, quantitative and qualitative changes in the GH secretory pattern were elicited, with a profound shift from a non-pulsatile to a pulsatile type of secretion. It is worth recalling that in rats pulsatile plasma GH promotes growth more effectively than constants GH levels (36).

Quantitative and qualitative changes in the GH secretory pattern were elicited by prolonged fasting and treatment with CLO also in young dogs, except for the inability of CLO to alter GH peak frequency.

How fasting increases pulsatile GH secretion in old dogs is obscure. A non-specific stress effect of

### Table 2.

Effect of fasting on plasma cortisol and Sm-C levels in young and old dogs.

<table>
<thead>
<tr>
<th></th>
<th>Cortisol (µg/l)</th>
<th>Sm-C (µg/l)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Fasting</td>
</tr>
<tr>
<td>Young (5)</td>
<td>22.1 ± 1.2</td>
<td>26.8 ± 3.9</td>
</tr>
<tr>
<td>Old (5)</td>
<td>17.8 ± 2.3</td>
<td>16.8 ± 1.4</td>
</tr>
</tbody>
</table>

\( P < 0.01 \) vs baseline.

Number of determinations in parentheses.
the food deprivation would be ruled out by the normal plasma cortisol levels. Similarly to our data in young dogs, Thorner et al. (31) have shown that after a 5-day fast, a healthy 37-year-old male volunteer had increased GH pulse frequency, maximal pulse amplitude, and integrated GH concentration. The conclusion of Thorner and associated was that a reduction in serum Sm-C was probably the reason for the ultradian rhythm of GH secretion in the adult human.

It is known that fasting causes a profound decrease in circulating Sm-C levels in man (37) and dogs (12) and that Sm-C in the systemic circulation feeds back to the pituitary and/or hypothalamus to regulate the release of GH (38). Thus, the enhanced pulsatile secretion of GH elicited by fasting might be due to an impairment of this GH autoregulatory mechanism.

In our study, baseline plasma levels of Sm-C were not significantly different in young and old dogs, which might be due to the wide spread of individual Sm-C levels in the old dogs. During the 5-day fast, Sm-C levels significantly decreased in old but not in young dogs, in spite of a significant increase in GH mean peak areas induced by food deprivation in both groups. The data obtained in young dogs are consistent with the small decrease in plasma Sm-C levels reported for young dogs fasted for 4 days (12).

All in all, it seems unlikely that Sm-C plays a role in the GH secretory pattern of fasted old dogs. The similarity between the effects of administration of CLO and fasting in modifying the pulsatile GH secretion in old dogs would indicate a common or similar mechanism of action. Norepinephrine turnover has been reported to be diminished in the basal hypothalamus of rats after the second day of starvation (39) and it is known that CLO, by stimulating \(\alpha_2\)-presynaptic receptors, has the same effect (9). Although there is proof that CLO releases GH in rats by activating \(\alpha_2\)-postsynaptic receptors (40), evidence obtained in dogs suggests an \(\alpha_2\)-presynaptic mechanism of action for CLO (10). Thus, it may be hypothesized that both stimuli act via inhibition of (inhibitory) catecholaminergic neurons impinging on GHRH-secreting structures. In addition, it has been shown that CLO acts via increased hypothalamic release of GHRH (32). In our study, increased release of GHRH after either of the two stimuli might account for the augmented amplitude of GH secretory bursts, whereas the increased GH pulse frequency might result from a concomitant change in the output of hypothalamic somatostatin (41–43).

In summary, whatever the mechanism(s) of action by which fasting or CLO triggers GH release, our present data demonstrate that in old dogs, as in old rats, the defective secretion of GH is not irreversible, since it can be restored by 'physiologic' or pharmacological maneuvers directed at the CNS. Studies are in progress to ascertain whether some of these findings may be extrapolated to aged man.

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**References**


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