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Effects of Season and Nutrition on Growth Hormone and Insulin-Like Growth Factor-I in Male Red Deer

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ABSTRACT

GH and insulin-like growth factor (IGF)-I are important components of the growth axis. We undertook to determine how plasma levels of these hormones altered with different seasonal and nutritional states in young male red deer to provide an insight into how the growth axis changes under these conditions. Growth rate alters dramatically with season in male red deer, providing an opportunity to sample the same animals at two different growth rates within a short period of time. GH was measured every 15 min for 24 h in the fed state and after a 48-h fast, during slow growth in winter (23 June to 16 July), and during rapid growth in spring (8 September to 2 October). At the end of each sampling period, the animals were treated with *N*-methyl-D, L-aspartic acid (NMDA) (5 mg/kg live weight) and sampled for a further 1 h, 45 min. Glucose and IGF-I were measured hourly during each sampling period. Live weight was measured at weekly intervals.

GH was secreted in a characteristic pattern in which pulses tended to occur in rapid succession, termed a volley, that was separated from the subsequent volley by a period of baseline GH levels, termed a latent period. There were more GH pulses/24 h in the fasted state than in the fed state in winter (12.4 *vs.* 7.8, standard error of the difference [SED] = 1.07, $P < 0.001$) and in spring (11.5 *vs.* 8.8, SED = 1.04, $P < 0.05$). The increased number of GH pulses in the fasted state could be attributed to a higher number of pulses per volley (winter = 3.7 *vs.* 2.5, SED = 0.16, $P < 0.001$; spring = 3.1 *vs.* 2.8, SED = 0.19). Consequently, the volleys were wider in the fasted state than the fed state (winter = 197 min *vs.* 122 min, SED = 25, $P < 0.05$; spring = 173 min *vs.* 154 min, SED = 24, $P > 0.05$), and the latent periods between volleys were shorter in the fasted state than the fed state (winter = 175 min *vs.* 280 min, SED = 14, $P < 0.001$; spring = 183 min *vs.* 262 min, SED = 11, $P < 0.001$). The main differences between seasons in the fed state were larger amplitude pulses (12.4 *vs.* 8.3 ng/ml, SED = 1.57, $P < 0.05$) and higher mean GH concentrations (4.1 *vs.* 2.3 ng/ml, SED = 0.44, $P < 0.01$) in spring than in winter. The number of volleys and the intravolley pulse interval did not change significantly with nutritional state or season. NMDA administration was followed by an increase in GH with higher GH levels found in the fed state than in the fasted state in both seasons. Fed animals also had a larger initial increase in GH (until 60 min post NMDA) than fasted animals in spring ($P < 0.01$). Plasma IGF-I was higher in the fed state than the fasted state in both winter (315 *vs.* 221 ng/ml, SED = 21.0, $P < 0.001$) and spring (651 *vs.* 494 ng/ml, SED = 37.5, $P < 0.001$) and in the fed state was higher in spring than in winter (SED = 29.1, $P < 0.001$). Blood glucose was higher in the fed state than fasted state in winter (6.1 *vs.* 5.5 mmol/l, SED = 0.07, $P < 0.001$) and there was a strong trend toward this same effect in spring although it did not reach statistical significance (6.0 *vs.* 5.7 mmol/l, SED = 0.26, $P > 0.05$). Growth rate in winter at 117 g/day was less than that in spring when 220 g/day was recorded (SED = 36.8, $P < 0.05$).

These results demonstrate that the secretory pattern of GH and plasma IGF-I levels alter in response to changes in season and nutrition. The alterations in response to a 48-h fast show that the control of GH and IGF-I secretion may be rapid and is probably a response to maintain energy balance, whereas alterations with season reflect long term control that underlies the seasonal growth pattern of the animal. (*Endocrinology* **137:** 698–704, 1996)

INTENSIVE blood sampling has shown that GH has a pulsatile secretory pattern in many species (1). The pattern of GH in plasma is an important determinant of growth rate in rats and humans (2–4). GH stimulates growth predominantly via stimulation of insulin-like growth factor-I (IGF-I), which acts in both an endocrine and paracrine manner (5), although direct effects of GH on growth also occur (6). Thus, a link between the GH secretory pattern, IGF-I, and growth should be apparent in a comparison between two markedly different growth states in the same animal. Species in which growth rate changes with season provide an opportunity for such a study. The young male red deer is ideal as it changes from a condition of slow growth to one of rapid growth within a period of 10 weeks from winter to spring (7). This change in growth rate coincides with a dramatic increase in voluntary feed intake (7). Seasonal changes in GH secretory patterns have been described in red deer (8). Mean GH concentrations were positively correlated with live weight gain with a delay of 1 month, but a low (30 min) sampling frequency prevented conclusions on the role of GH pulse frequency or amplitude.

In this experiment, we aimed to determine in more detail how GH secretion and IGF-I levels change with season. We therefore examined the GH secretory pattern obtained by 15-min sampling and IGF-I levels at hourly intervals, over a 24-h period at two phases of the seasonal growth cycle: in winter when growth is at its slowest and then in spring when growth rate is faster.

GH secretion is sensitive to acute nutritional status and responds positively to fasting in humans (9, 10) and sheep (11). Recent studies in humans have used fasting to remove nutritional feedback, allowing the expression of underlying GH pulsatility and thus revealing aspects of the neuroendocrine mechanisms regulating GH secretion (9, 10). Therefore, in this study, we decided to perturb the nutritional axis with a 48-h fast and examine how the GH secretory pattern and IGF-I levels are altered, to give an insight into the mechanisms controlling GH and IGF-I secretion in response to acute changes in energy balance.

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N-methyl-D, L-aspartic acid (NMDA) is an excitatory amino acid (EAA) agonist that has been shown to stimulate the release of GH in a variety of species (12). Seasonal changes in the ability of NMDA to stimulate LH release were inversely related to endogenous LH secretion in rams suggesting a possible role for EAAs in regulating the activity of the reproductive axis (13). EAAs may play a similar role in activity of the growth axis in red deer; therefore, in this experiment we sought to test the ability of NMDA to increase GH levels in male red deer and determine if this action altered with growth rate or nutritional state.

Materials and Methods

General

This study was performed on 12 male red deer that were blood sampled on two occasions. The first sampling was in winter (23 June to 16 July) at 8 months of age, and the second sampling was in spring (8 September to 2 October) at 11 months of age. The animals were maintained indoors in two groups at Invermay Agricultural Centre and fed a pelleted diet supplemented with lucerne hay. Live weight was measured at weekly intervals. All procedures were approved by the Invermay Animal Ethics Committee.

Experimental design

To determine the effect of season and fasting on the pattern of GH secretion, each animal was blood sampled for 24 h in both fed and fasted states in winter and spring. The 12 animals were randomly allocated into 6 pairs and this pairing was maintained throughout the experiment, with the exception of 1 pair in which one animal died after the first sampling and was replaced with another for the remaining three samplings. The animals were sampled in these pairs, one animal fed and the other fasted, and then 14 days later sampling was repeated with the nutritional states of the two animals reversed. Fasted animals were denied access to food from 48 h before the start of sampling. At the end of each 24 h sampling, the effect of NMDA on GH secretion was determined during a further 1-h, 45-min sampling period.

Sampling

Evaluation of GH patterns in red deer may be hindered by the handling procedures, as stress has been shown to alter GH secretion in humans, rats, and cattle (1). We therefore developed a system for sampling blood continuously from unrestrained and undisturbed red deer. During the period of blood sampling, animals were placed in small stalls. Blood was removed continuously (15–20 ml/h) from a jugular catheter to a fraction collector in an adjacent room using a peristaltic pump. The fraction collector was set to collect 15 min samples of blood into glass tubes maintained at 4 C. Each animal was treated with 25,000 IU heparin (Multiparin, Fisons Pharmaceuticals, New South Wales, Australia) just before collection, followed by 10,000 IU/h during the collection period, diluted in physiological saline to be delivered at the same rate as blood removal. After 24 h of collection, each animal was treated with NMDA (5 mg/kg live weight, Sigma, St. Louis, MO) and sampling continued for a further 1 h, 45 min. Blood was kept chilled on ice until it was centrifuged (~every 8 h), and then plasma was removed and frozen until RIA for GH. IGF-I was measured in hourly plasma samples for the 24-h period before NMDA administration.

Assays

GH was measured by homologous double-antibody RIA. Antiserum (IVY9303) was generated in rabbits using cervine (c) GH (AFP10316B) conjugated (10% wt/wt) to bovine thyroglobulin and Span Tween Markol adjuvant. Resulting antiserum was diluted in protein buffer (0.01 M PBS + 0.05 M EDTA + 0.5% BSA + 0.1% azide) containing 1:220 normal rabbit serum and used at a final tube dilution of 1:24,000. Cross-reactivities for the antisera were ovine (o) GH 78.4%, oLH 0.06%, rat (r) GH 0.05%, oFSH < 0.01%. Serial dilutions of plasma cGH were parallel to the standard curve. Recovery of cold cGH from deer plasma averaged 95–105%. The standard curve ranged from 0.31 ng/ml to 100 ng/ml cGH (AFP10316B) in protein buffer. Radioiodination was carried out using the method of Greenwood (14) and involved incubating 5 μg cGH (AFP10316B), 20 μl 0.5 M phosphate buffer, pH 7.4, 0.5 mCi I^{125} and 5 μg chloramine-T for 40 sec. The incubation was terminated with 5 μg sodium metabisulphite. The labeled cGH was separated using a Sephadex G50 (1.5 cm × 15 cm) column and used at a dilution of 20,000 cpm/100 μl added to each tube. Sample (100 μl), protein buffer (100 μl), and antiserum (100 μl) were added to tubes in duplicate and incubated for 24 h at 4 C. Tracer was added and after a further 24 h at room temperature, 100 μl second antibody (sheep antirabbit, SAR4, 1:20 dilution) was added followed by 1 ml 4% polyethylene glycol. Tubes were left for at least 2 h at 4 C, then centrifuged at 3000 rpm for 30 min, the supernatant discarded, and the bound pellet counted for 60 sec. Mean intra/interassay coefficients of variation over eight assays were 9.5/9.8, 12.0/13.4, 7.4/7.5, and 8.2/9.1% for plasma pools of 2.5, 3.9, 5.8, and 18.3 ng/ml. Mean assay sensitivity, calculated as two standard deviations from assay blanks, was 0.5 ng/ml.

IGF-I was extracted from plasma using the method of Moore and Mylek (15). Briefly, 100 μl of plasma were diluted in 900 μl of 20 mM HCl containing 0.02% Triton X-100. After 10 min, 800 μl of the diluted plasma were added to a small column containing 1.7 ml Sephacryl HR100 (Pharmacia LKB, Uppsala, Sweden). The liquid was run onto the column, and then the binding proteins were eluted with 800 μl 20 mM HCl, 800 μl, pH 3.5, 18 mM phosphate buffer and 1000 μl of phosphosaline BSA buffer, pH 7.5. The IGF-I was then eluted with 1600 μl of phosphosaline BSA buffer. Serially, diluted extracts were parallel to the standard curve. The extracted IGF-I was measured using a double antibody RIA. Antibody to IGF-I was raised in sheep against a conjugate of chicken egg albumin and N-Met-IGF-I. This antiserum (Y32) had negligible cross-reactivity with either c- or oIGF-II. Extracts (100 μl) were assayed in duplicate using a competition assay. Bound and free fractions were separated with a preprecipitated donkey antisheep second antibody (Pel Freez Biologicals, Rogers, Arkansas) and human recombinant IGF-I CGP 35 126 (Ciba Geigy, Basle, Switzerland) was used for both standards and iodinations. Intraassay variation of red deer plasma control pools was 12.7, 11.2 and 7.7%, whereas interassay variation was 19.1, 18.3, and 13.0% at 236, 647, and 1181 ng/ml. Assay sensitivity averaged 20 ng/ml. Sample extracts stored for up to 2 weeks at 4 C showed no change in measured concentration. The IGF-I International Reference Preparation 87/518 was used to calibrate the assay results.

Blood glucose was measured hourly by Reflolux S Reflectance photometer (Boehringer Mannheim, Germany).

Data analysis

The peak-detection program Cluster (J. D. Veldhuis and M. L. Johnson, Charlottesville, VA) (16) was used to locate GH pulses and calculate summary GH secretory profile characteristics. Peak and nadir clusters had minimum sizes set to 1 and 2 points, respectively. T statistics for upstrokes and downstrokes were set at 2.0. The Cluster output was then analyzed in two different ways. First, the summary statistics for each profile, GH mean, interpulse interval, pulse width, number of peaks and mean peak amplitude, were analyzed by ANOVA. After a preliminary analysis showed no evidence of a cross-over effect, the ANOVA was conducted for each month with animal pair as the block structure and nutritional state and week as the treatment structure. Comparisons between month were made for the fed state only.

The second analysis of Cluster output looked at within profile details. It was noted that GH secretion for each profile exhibited a distinctive cyclical pattern (Fig. 1) in which several pulses occurred in rapid succession (referred to as a volley) followed by a period of baseline GH levels (referred to as a latent period), and that the combination of a volley and its latent period (which we termed a cycle) was of very regular length. This characteristic suggested a dual approach, in the frequency domain using the periodogram, and in the time domain using the stratification of pulses (or intervals) within volleys, within cycles, and within profiles.

The periodogram for each profile was calculated and averaged for fed and fasted nutritional states in winter and spring. Averaging perio-

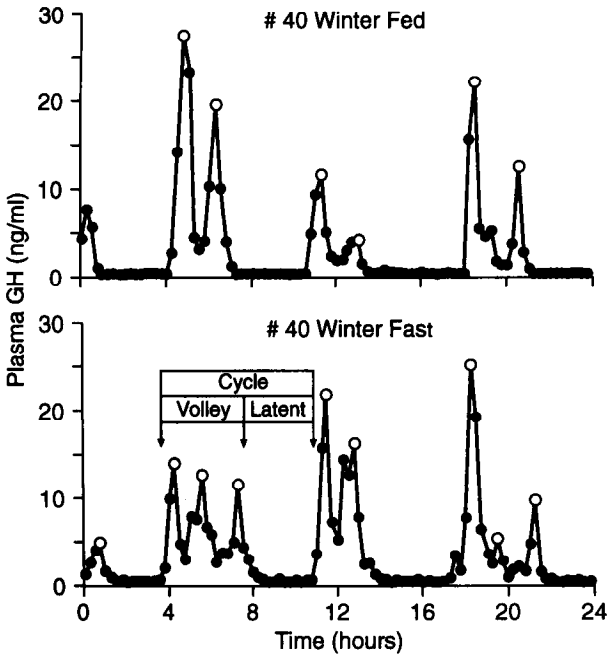

FIG. 1. GH profiles of a representative stag during winter illustrating how the profile was divided into cycles that consisted of a volley followed by a latent period of baseline GH levels and how this pattern can change with nutritional state. Pulses identified by the Cluster algorithm are represented by *open circles*. In the *top panel*, (fed profile) there were three volleys, each with two pulses. In the *lower panel*, (fasted profile) there were three volleys with three, two, and three pulses respectively, from left to right.

dograms over treatments in this way enables a more precise estimation of the spectrum than that obtained from individual periodograms (17). Fisher's (18) exact test of the ratio of the maximum to mean periodogram ordinates and the Kolmogorov-Smirnov test for the cumulative periodogram were used to assess each periodogram in relation to white noise. The ordinate ratio was used to assess differences between treatments.

Each GH profile was then divided into secretory cycles, using the frequency of the periodogram for that profile to interpret ambiguities. Incomplete cycles at the start and end of profiles were not considered. For each cycle, the order and size of each GH peak within the volley, the interval between each successive GH pulse within a volley (intravolley pulse interval), the time from the first GH peak to the last GH peak (volley duration) and the time from the last GH peak until the first GH peak of the subsequent cycle (latent period) were obtained. Where five or more pulses occurred in a volley, the data were combined. These variables were then analyzed by residual maximum likelihood [REML, (19)] separately for each season, with cycle within profile within animal as the random effects, and nutritional treatment, and (if applicable) pulse order and their interaction, as fixed effects.

GH values after NMDA were log-transformed for analysis, which was done for each sample, and the mean was also calculated over an early phase of the first 60 min and a late phase of the next 45 min.

IGF-I and glucose values were analyzed for each season by ANOVA, with animal pair as the block structure and nutritional state and week as the treatment structure.

Growth rates over a 2-week period just before each sampling period were analyzed by ANOVA with animal as the block structure and season as the treatment structure.

Results

The pattern of GH secretion differed dramatically between nutritional states in both winter and spring (Table 1 and Fig 2). The number of GH pulses/24 h was higher in the fasted than in the fed state in winter and in spring. GH profiles in the fasted state were also characterized by a higher mean GH concentration (winter only) and narrower pulses than in the fed state, although there was no difference in pulse amplitude with nutritional state.

A seasonal change in GH secretion was apparent in fed animals with higher mean GH concentrations and higher amplitude pulses in spring than in winter.

The mean periodograms for both nutritional states in winter and spring (Fig. 3) all feature maxima at the fourth ordinate, corresponding to GH secretory volleys occurring with a frequency of approximately 6 h. These maxima are all higher ($P < 0.05$) than their mean with the exception of the winter-fasted periodogram. All cumulative periodograms deviate massively ($P < 0.001$) from white noise. The periodogram analysis found no evidence of a difference ($P > 0.05$) in the number of GH secretory cycles between fed and fasted periodograms in either season. However, the composition of each secretory cycle was altered (Table 1). The number of pulses per volley (Fig. 4) was higher in the fasted state than in the fed state in winter and to a lesser extent in spring. The volleys were wider in the fasted state than the fed state and the latent period between volleys was shorter in the fasted state than the fed state in both seasons. The amplitude of the pulses within a volley declined from the first to the last, and this decline was not affected by nutrition (Fig. 5). The intravolley pulse interval was also unaffected by nutrition in both winter and spring. No differences in volley width, latent period or intravolley pulse interval were found between seasons in the fed state.

NMDA administration was followed by an increase in GH with higher levels found in the fed state than in the fasted

TABLE 1. Mean GH secretory characteristics for animals in the fed and fasted states in winter and spring

	Winter			Spring			Winter *vs.* Spring
	Fed	Fast	SED	Fed	Fast	SED	SED
Mean GH (ng/ml)	2.3	2.8	0.26[a]	4.1	5.5	0.74 NS	0.44[b]
GH pulses/24 h	7.8	12.4	1.07[c]	8.8	11.5	1.04[a]	0.48 NS
Pulse width (min)	66	56	3.1[a]	75	63	3.7[b]	4.0 NS
Pulse height (ng/ml)	8.3	7.4	1.33 NS	12.4	13.8	1.95 NS	1.57[a]
Pulses per volley	2.5	3.7	0.16[c]	2.8	3.1	0.19[a]	0.18[a]
Volley width (min)	122	197	25[a]	154	173	24 NS	27 NS
Latent period (min)	280	175	14[c]	262	183	11[b]	22 NS
Intravolley pulse interval (min)	87	80	5.0 NS	89	87	6.7 NS	2.1 NS

Within each season, the SED between the mean of the fed and fasted states is given along with the significance level between the means ([a], $P < 0.05$; [b], $P < 0.01$; [c], $P < 0.001$; NS, $P > 0.05$). At the *right* of the table (winter *vs.* spring) are the SED and significance levels between seasons in the fed state only.

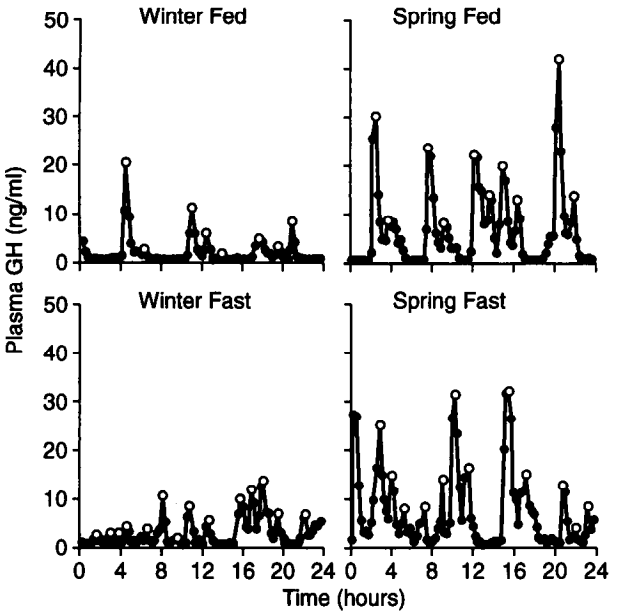

FIG. 2. GH profiles of a representative stag in the fed and fasted state during winter and spring. Pulses identified by the Cluster algorithm are represented by *open circles*. In the winter-fed profile there were 8 pulses with a mean amplitude of 7.0 ng/ml and a mean GH level of 1.88 ng/ml. The winter fast profile had 14 pulses with a mean amplitude of 6.6 ng/ml and a mean GH level of 3.1 ng/ml. The spring fed profile had 10 pulses with a mean amplitude of 19.4 ng/ml and a mean GH level of 6.7 ng/ml. The spring fast profile had 12 pulses with a mean amplitude of 15.2 ng/ml and a mean GH level of 7.5 ng/ml.

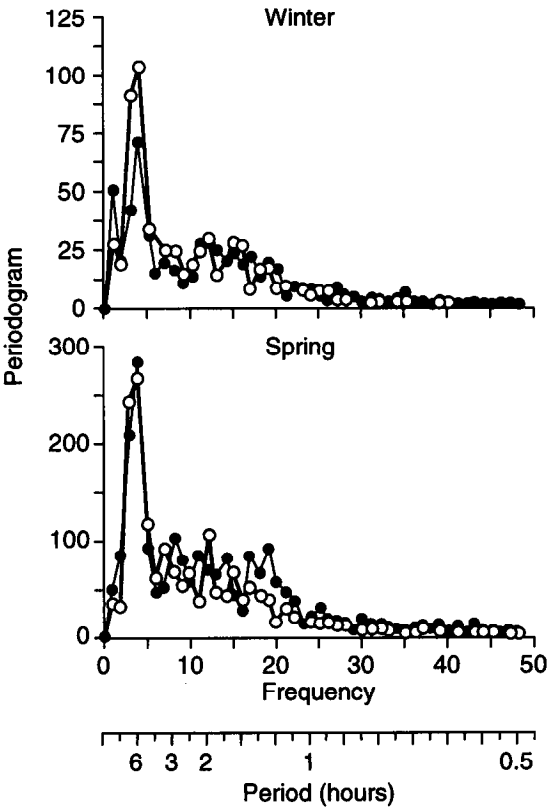

FIG. 3. Mean periodogram for fed (○) and fasted GH (●) profiles in winter and spring. High values indicate a significant frequency within the profiles with a period shown in hours on the lower x-axis.

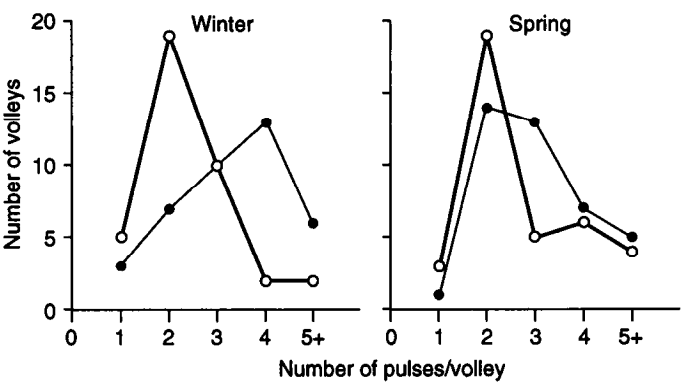

FIG. 4. Distribution of the number of GH volleys that contained one, two, three, four, and five or more or more pulses in fed (○) and fasted (●) states in winter and spring.

state at certain sampling times (Fig. 6). The GH response appeared to consist of two phases, with an initial increase until 60 min post NMDA and then a second sustained increase to the end of the sampling period. Fed animals had a larger initial phase than fasted animals in spring ($P < 0.01$).

Plasma IGF-I was higher in the fed state than the fasted state in both winter (315 *vs.* 221 ng/ml, standard error of the difference [SED] = 21.0, $P < 0.001$) and spring (651 *vs.* 494 ng/ml, SED = 37.5, $P < 0.001$) and was higher in the fed state in spring than in winter (SED = 29.1, $P < 0.001$).

Blood glucose was higher in the fed state than fasted state in winter (6.1 *vs.* 5.5 mmol/l, SED = 0.07, $P < 0.001$) and there was a strong trend toward this same effect in spring although it did not reach statistical significance (6.0 *vs.* 5.7 mmol/liter, SED = 0.26, $P > 0.05$).

Growth rate just before the winter sampling period was less than that recorded before the spring sampling (117 g/day *vs.* 220 g/day, SED = 36.8, $P < 0.05$).

Discussion

This study has confirmed the pulsatile nature of GH secretion in red deer and demonstrated that the pattern of GH secretion varies with both season and nutritional state. Higher GH pulse amplitudes and IGF-I levels in spring than in winter were associated with a faster growth rate and a 48- to 72-h fast was associated with a faster GH pulse frequency and lower IGF-I levels in both seasons. These changes indicate that the control of the GH secretory pattern and circulating IGF-I levels in male red deer is sensitive to both long term (seasonal) and short term (fasting) feedback.

The 15-min sampling regime used in this study revealed a higher pulse frequency than that previously reported from sampling at 30-min intervals (8). This is due to the unmasking of frequent GH pulses that comprise the main volleys of secretion. A similar effect of sampling frequency on pulse frequency has been observed in humans (20). Our finding that the predominant effect of fasting is on GH pulse frequency is different from reports in other ruminants in which pulse frequency did not change but amplitude and mean GH increased in response to long term reduced feed intake (21,

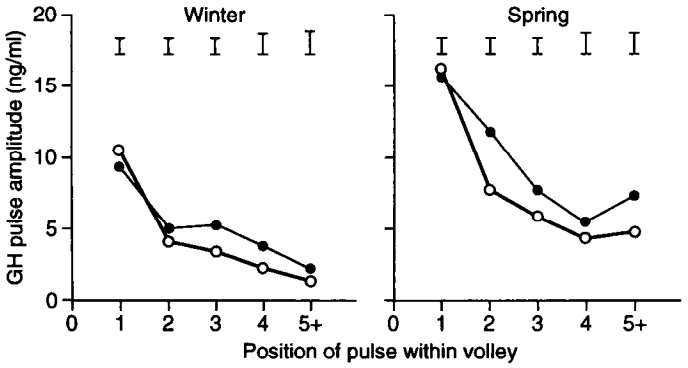

FIG. 5. Geometric mean pulse amplitude of GH pulses in each position from first to fifth or higher in the GH volley, in the fed (○) and fasted (●) states in winter and spring. *Vertical bars* indicate the SED.

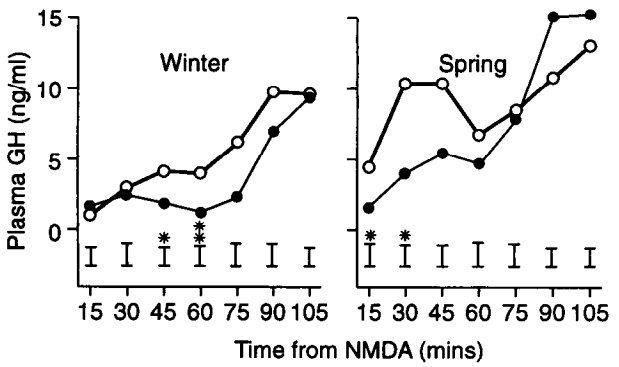

FIG. 6. GH response to NMDA (5 mg/kg live weight) given iv at time 0 in the fed (○) and fasted (●) states during winter and spring. GH values are the geometric means at each sampling point with the SED represented by the *vertical bars*. *Asterisks* indicate significant differences between treatments (*, $P < 0.05$; **, $P < 0.01$).

22). This may reflect a difference in response to acute fasting *vs.* chronic dietary reduction as a short duration (10 h) fast of sheep increased both pulse amplitude and frequency (11).

The pattern of GH secretion is thought to culminate from the interplay of two hypothalamic peptides, GRF and somatostatin (SRIF), which are released 180° out of phase. GH is secreted in response to pulses of GRF, which occur during periods of decreased SRIF secretion (23). The GH pulse profiles obtained in this experiment could be interpreted in a way that is consistent with this pattern of control. In this model a period of low SRIF secretion would allow multiple GRF pulses, giving rise to a volley of GH pulses in peripheral blood. Each volley is separated from the next by elevated SRIF. Attempts to test this model in sheep (24–26) have failed to demonstrate such a clear relationship between GRF and SRIF in the control of GH secretion. These studies do suggest that the majority of GH pulses are associated with GRF release into hypophyseal portal blood, however.

External and internal effects on the GH axis may be manifest at the level of the hypothalamus via changes in the secretion of GRF and/or SRIF. In the present study, we have demonstrated a change in the pattern of GH in response to both growth rate and nutrition. In the absence of portal blood samples, we must use the characteristics of the GH pulse pattern and the model for hypothalamic control of GH secretion described above to predict the causes of the change in GH secretion.

Fasting for 48 h caused an increase in the number of GH pulses/24 h. The number of GH secretory volleys was unaffected by fasting and thus the increased number of pulses in the fasted state was due to an increase in the number of GH pulses per volley. The GH pulse frequency within the volleys was also unaffected by fasting, and consequently volley width increased to accommodate more pulses. In terms of hypothalamic control, it may be predicted that fasting reduced the period of elevated SRIF, thereby allowing a longer period during which GRF pulses were secreted with the underlying frequency of GRF pulses within a volley remaining unchanged. Such a change in hypothalamic control of GH leads to the following questions: 1) what is the stimulus for the change in control of GH secretion? and 2) what are effects of the change in GH secretion?

The increased GH pulse frequency in the fasted state may be due to feedback from several sources. Fasting in humans stimulates lipolysis and is associated with a rise in plasma FFA and a decline in glucose, insulin (27), and IGF-I (9). Hypoglycemia stimulates GH secretion in humans (28), and a falling glucose level was considered a stimulus for GH secretion in sheep (29). The lower blood glucose level in fasted animals in this experiment may therefore have contributed to the increased GH pulse frequency. FFA are raised during fasting in sheep (30), but increased FFA produced by lipid infusion reduced mean GH and GH pulse frequency in that species (31). Feedback from changing glucose or FFA on GH appear to be mediated centrally (32), in part by inducing changes in SRIF and/or GRF secretion (33, 34). Changes in plasma IGF-I may also be a signal to alter GH secretion. In the present experiment, IGF-I levels were lower in the fasted state as has been found previously in sheep (35). This effect also occurs in humans, and it is considered that nutritional state is a more important regulator of IGF-I levels than GH secretion (36). Central administration of IGF-I to sheep failed to show an effect on GH secretion (37), although IGF-II administration suppressed GH in sheep (38). Effects of plasma IGFs on GH may not be mediated by absolute plasma levels but by IGF availability, which is determined by binding proteins, or by receptor numbers. For example, IGFBP-I was increased by fasting in humans (39), and IGF-I receptors in the median eminence of rats were increased in response to feed restriction and a decrease in plasma IGF-I (40).

The main effect of GH is thought to be on nutrient partitioning, and in the growing animal this leads to reduced lipogenesis; but in a restricted intake situation, the dominant effect of GH is to mobilize lipid stores (41). Thus, with regard to the second question of what the effects of the increased GH pulse frequency in the fasted state are, it is likely that GH response to fasting is linked to the hormone's role in lipolysis.

In the present experiment, GH pulse amplitude and mean GH levels were higher in spring than they were 11 weeks earlier in winter. The higher GH pulse amplitude in spring was associated with faster body growth than in winter, and evidence indicates that the elevated pulse amplitude was responsible for this increased growth rate. A GH secretory pattern consisting of high amplitude pulses separated by low baselines stimulated growth in rats more effectively than a

low amplitude high baseline pattern (6). GH pulse amplitude, and more specifically the rate of change of GH during the upswing of a pulse (4), were found to be major determinants of growth rate around puberty in children.

It is likely that at least some of the effects of GH on growth are mediated by circulating IGF-I (42). The enhanced GH secretion during spring in the present experiment may have contributed to the higher IGF-I at that time, as GH administration increased plasma IGF-I in sheep (35). IGF-I levels in red deer were positively correlated with growth rate during spring (43), and IGF-I administration increased body growth in rats (44). The stimulation of growth by IGF-I administration to castrate sheep was only small, however (45).

The pronounced seasonal growth and food intake pattern in male red deer is well documented (7, 46) and is entrained by photoperiod (47) via melatonin secretion (48). It is feasible that the melatonin secretory pattern in spring may stimulate GH pulse amplitude, which in turn increases IGF-I levels and results in a faster growth rate. In support of this, GH secretion in rams (49) and IGF-I secretion, food intake, and growth rate in reindeer (50) were altered by photoperiodic manipulation, and melatonin treatment of male red deer altered the seasonal cycle in IGF-I levels (51).

The stimulation of GH secretion by NMDA was affected by nutritional state and was greater in the fed state than fasted. It is likely that NMDA exerts its effect on GH secretion via stimulation of GRF neurons (12). The NMDA response was lower when endogenous GH secretion (judged by GH pulse frequency) was higher. This is a similar effect to that seen with NMDA on LH secretion in rams (13). The reduced GH response in the fasted state may therefore be due to the higher endogenous GH (and presumably GRF) at this time, resulting in less GRF available for release, or an increase in endogenous EAA activity causing a reduced sensitivity of GRF neurons to NMDA.

In conclusion, this study has extended our knowledge of the control of GH and IGF-I secretion in male red deer. It has shown that GH is secreted in pulses that are grouped into volleys and are separated from other volleys by baseline levels. This pattern of GH secretion alters with changes in season and nutritional state. The increase in GH pulse amplitude and time above baseline level are a likely cause of the elevated IGF-I levels in spring compared with winter, and all of these changes are associated with the increased growth rate at that time. The increased GH pulse frequency and fall in IGF-I during fasting are more likely to be a direct nutritional effect and form part of a response to regulate metabolism. Further work is needed to determine how the GH pattern is controlled at the level of the hypothalamus and how this pattern determines the growth state of the animal.

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