Paper presented at The First Arctic Ungulate Conference,
Nuuk, Greenland, 3-8 September, 1991.

Insulin-like growth factor 1 and growth seasonality in reindeer *(Rangifer tarandus)* - comparisons with temperate and tropical cervids

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Abstract: Growth in temperate and arctic deer is seasonal, with higher growth rates in spring and summer while growth rates are low or negative in autumn and winter. We have measured IGF1 concentrations in the plasma of reindeer calves exposed to a manipulated photoperiod, indoors, of either 16 hours light followed by 8 hours dark each day (16L:8D) (n=3) or 8L:16D (n=3) from about the autumnal to the vernal equinox, to determine whether the seasonal growth spurt normally seen in spring is associated with changes in the circulating level of IGF1. A high quality concentrate diet was available *ad libitum.* The animals were weighed, and bled every 2 weeks and plasma samples assayed for IGF1 by radioimmunoassay. 6-8 weeks after the start of the study those calves exposed to 16L:8D showed a significant increase in plasma IGF1 concentration which was maintained until the close of the experiment, 24 weeks after the start. In contrast IGF1 plasma concentrations in those calves exposed to a daylength of 8L:16D did not significantly alter during the study. The elevated IFG1 in the 16L:8D group was associated with rapid weight gain compared with the 8L:16D group. We have shown that the seasonal growth spurt is preceded by an elevation in plasma IFG1 concentration. Further, this elevation in IGF1 is daylength dependent. For comparison IGF1 and growth rate seasonal profiles from temperate and tropical deer are included. This comparison reveals that seasonal increases in IGF1 take place only in animals with a seasonal growth spurt. Thus IGF1 plasma level elevations seem most closely associated with the resumption of rapid growth in spring following the winter.

Key words: reindeer, photoperiod, IGF1, growth, seasonality

Rangifer, 13 (2): 91-97

Introduction

Arctic and temperate species of deer have a seasonal pattern of growth typified by a period of weight gain in spring and summer, weight loss in autumn and weight stasis in winter (French *et al.* 1956; McEwan and Whitehead, 1968; Ryg and Jacobsen, 1982). This growth pattern is not solely a consequence of available nutrition because it is expressed under the conditions of *ad libitum* feeding of high quality diets; deer vo-

luntarily reduce their food intake in winter (Suttie *et al.* 1983). In red deer (*Cervus elaphus*) daylength is known to be the primary cue for synchrony of the seasonal growth pattern with environmental changes, the long hours of daylight in spring-summer stimulating growth and *vice versa* (Simpson *et al.* 1984). However, the precise role of daylength in controlling seasonal events in reindeer has not been elucidated. In addition, in red deer the abrupt increase in growth rate in early spring has been shown to coincide with the increase in plasma levels of insulin like growth factor 1 (IGF1), a hormone known to stimulate bone growth and protein synthesis (Suttie *et al.* 1989). However, it was not clear whether photoperiod exerted a specific effect on IGF1 or whether IGF1 rose simply because growth rate increased. The present study offered the opportunity to investigate the role of daylength in controlling growth pattern in reindeer (*Rangifer tarandus*) and also determine whether daylength had any effect on plasma levels of IGF1. Additionally, to further emphasise the possible role of IGF1, growth rate data from arctic and temperate deer, showing a daylength eontrolled spring growth increase, have been compared with tropical deer which do not show clear seasonal changes.

Materials and methods

Animals and sampling

Reindeer - six male reindeer calves which were born in May 1987 at the Large Animal Research Station, Institute of Arctic Biology (IAB, Fairbanks, Alaska), 67°N, were weaned on September 15 and transported 2 days later to indoor pens at the Arctic Health Building, IAB. They were randomly allocated to either of two pens, (n=3 per group), held on a photoperiod of 12 hours of light followed by 12 hours of darkness (12L:12D) and fed a concentrate diet *ad libitum* (Quality Texture, Purina Mills Inc. St Louis, MO). On October 22, the daylength was abruptly changed to 16L:8D for one pen and 8 L:16D for the other. The animals were kept under these conditions for 24 weeks, until April 7. All calves were weighed and a blood sample removed into a preheparinised tube every 2 weeks.

Red deer - full details are given in Suttie *et al.* (1989). Briefly, 6 red deer stag calves were kept on natural photoperiod in indoor pens and fed *ad libitum* a high quality concentrate diet from 5–17 months of age. They were weighed and a jugular blood sample taken at monthly intervals.

Rusa deer (tropical) (Cervus timorensis) - 14 male rusa deer calves were kept on high quality pasture, supplemented with sorghum grain and lucerne hay from 5–17 months of age. They were weighed and a jugular blood sample taken at 4 weekly intervals.

Assay

IGF1 in all plasma samples was analysed by radioimmunoassay, but assay details vary. For the reindeer and red deer, reagents and methodology followed Suttie *et al.* (1989). IGF was separated from binding proteins by acid ethanol extraction followed by cryoprecipitation. The antiserum used (878/4) was raised in rabbits against recombinant methuman (h) IGF1. This has an association constant of 151/nM and was used at a final tube dilution of 1/150,000. The minimal detectable dose was 0.03 ng/tube, and the inter and intra assay coefficients of variation were 12 % and 7 %, respectively. The cross-reaction with IGFII was less than 0.5 %.

Rusa deer plasma samples were diluted with an equal volume of 0.9 % saline and acidified with formic acid before extraction on a reverse phase μBondapak HPLC column which gives a thorough separation from IGF binding proteins. Plasma to which radiolabelled IGF1 was added was used to calibrate the column. The fraction collected for analysis was dried in a vacuum centrifuge and reconstituted in phosphosaline BSA buffer before being analysed in a second antibody radioimmunoassay. CIBA GEIGY recombinant, human, authentic sequence IFG1 was used for standards and to prepare iodinated ligand. This material was also used to raise antisera to IGF in sheep (Y32, provided by S. R. Hodgkinson). The second antibody was donkey anti-sheep. Cervine plasma samples analysed at 3 different volumes exhibited parallelism and control samples with means of 210 and 430 ng/ml had CVs of 12.4 and 9.2 % respectively (n=12). Extracts were stable when stored at 4°C for up to one week. Assay sensitivity was typically 5 ng/ml.

Biometrics

Plasma IGF1 and live weight gain data were analysed using ANOVA and the LSD (least significant difference, 5 %) calculated.

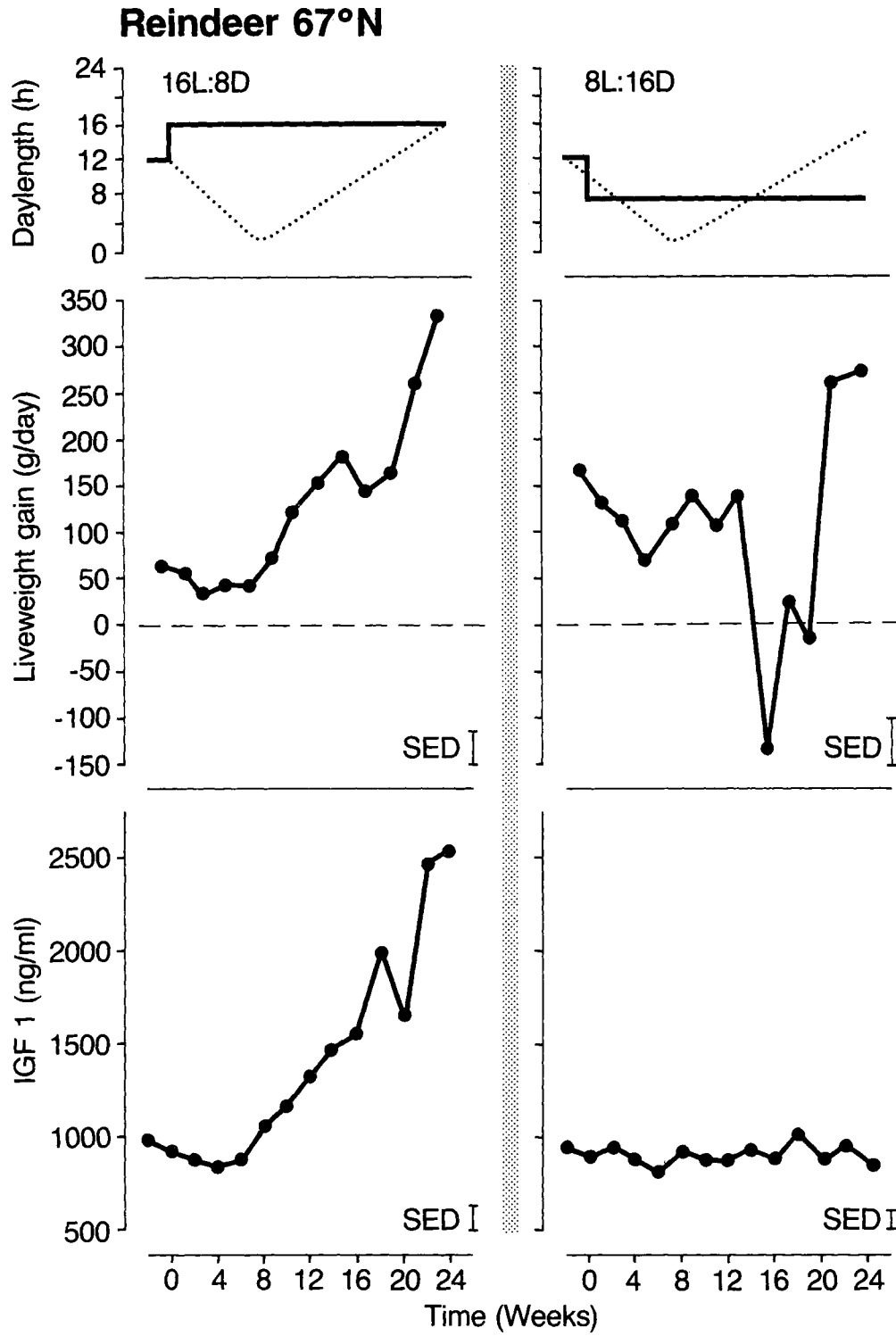

Fig. 1. Insulin-like growth factor 1 (IGF1) (ng/ml), liveweight gain (g/day), experimental indoor daylength (hours) (solid line) and outdoor daylength (broken line) for 2 groups of reindeer at 67°North. The experiment began in late September, treatments were imposed in October and the study closed in April. The broken line in the live weight gain panels denotes weight maintenance; sed indicates standard error of the difference.

Results

After 6 weeks exposure to the 16L:8D photoperiod the reindeer calves showed an increase in plasma IGF1 which slightly preceded an increase in live weight gain (Figure 1). Both IGF1 and live weight gain generally increased throughout the study. In contrast plasma IGF1 levels remained unchanged throughout the study in the group exposed to 8L:16D. In addition there was an absence of a clear growth spurt; rather growth rates remained higher than those of animals on 16L:8D for the first 12 weeks of the study and the 8L:16D group lost weight from 14 to 18 weeks of the study at about the time they might have been expected to show a growth spurt had they been on natural photoperiod. There was an increase in growth rate in the 8L:16D group towards the end of the study but neither the weight loss nor gain was accompanied by a change in IGF1 secretion.

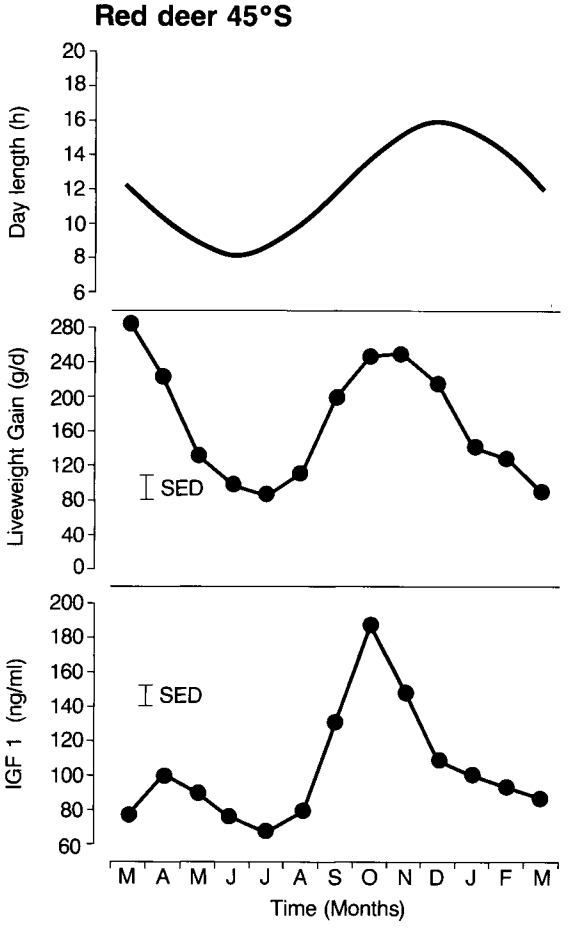

Fig. 2. IGF1 (ng/ml), live weight gain (g/day) and daylength (hours) with time in months for red deer on natural photoperiod indoors at 45°South; sed indicates standard error of the difference.

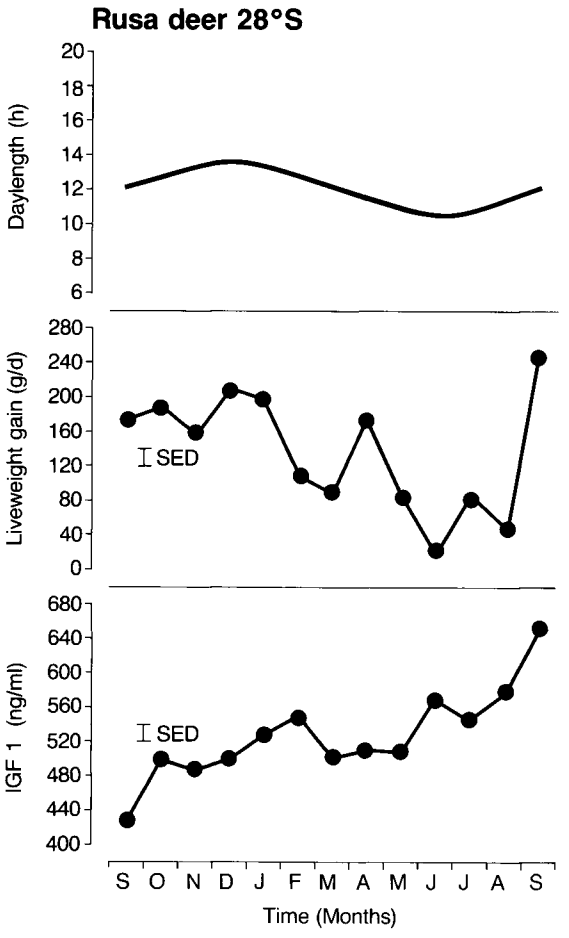

Fig. 3. IGF1 (ng/ml) live weight gain (g/day) and daylength (hours) with time in months for rusa deer grazing pasture at 28°South; sed indicates standard error of the difference.

The red deer (Figure 2) showed a robust increase in plasma IGF1 in spring (September to November, Southern Hemisphere) which was closely associated with the spring increase in live weight gain.

The rusa deer (Figure 3) showed neither a clear seasonal pattern of IGF1 nor live weight gain. Rather IGF1 broadly increased throughout the study and live weight gain did not markedly alter except for a reduction in the May to August period.

Discussion

A potential problem of interpretation exists in the present studies because different assays (with different standard preparations) were used to analyse plasma samples for IGF1. This has meant that absolute plasma levels appear to differ between the species studied, but this is likely to be an artefact of the assays used. However, all samples from one species were analysed in the same assay, so all within speeies comparisons are valid. Therefore between species comparisons of relative summer-winter levels and the presence or absence of rhythms are possible, and the different assay systems do not invalidate the studies as a whole. The comparisons are at least as valid as those where data from several quite different studies are considered. This is the case in many review papers.

After a lag of 6–8 weeks, long daylength (16L:8D) after 12L:12D caused an increase in the secretion of IGF1 in the reindeer, which was not observed in calves held on 8L:16D. This lag of about 50 days is close to 55 days reported by Suttie *et al.* (1984) for red deer responding to a change from 8L:16D to 16L:8D with regard to peak food intake. It seems that the amount of time to physiologically respond to a change from an inhibitory to a stimulatory photoperiod is remarkably consistent in deer. In the present study reindeer calves with photoperiodically induced rapid weight gain had higher mean levels of IGF1 than those which had lower weight gains. It is known that articular cartilage has receptors for IGF1 (McQuillan *et al.* 1986) and that local production of IGF1 by fibroblasts occurs (Adams *et al.* 1983), so it seems very likely that bone development is under IGF1 control. Muscle development may also come under IGF1 control because there are IGF1 receptors in skeletal muscle (Zapf *et al.* 1989). The carcass size of transgenic mice expressing IGF1 was greater than controls (Matthews *et al.* 1989), indicating that IGF1 probably does control body size. There are then valid reasons for postulating that photoperiodically induced growth in deer such as takes place in spring-summer is partly stimulated by the seasonal elevation in plasma IGF1. Several studies have shown that other hormones, e.g., prolactin (Ryg and Jacobsen 1982a) and growth hormone (Ryg and Jacobsen 1982b) are also likely to be involved with the spring growth spurt. The precise inter-relationships among these are the subject of further study.

The animals kept on 8L:16D showed a reduction in growth rate from weeks 14–18 followed by an increase. This fluctuation was not accompanied by a change in IGF1. The causes of the weight fluctuation are unknown, however, the fact that IGF1 did not alter would seem to reinforce the view that it was suppressed, possibly by the photoperiod. Were that the case then this might explain why no growth spurt was observed in these reindeer; IGF1 only being related to the photoperiodically controlled spring growth increase.

In a previous study Ringberg *et al.* (1978) measured IGF1 (then called somatomedin) in free-ranging semi-domesticated reindeer using a chick pelvis cartilage bio-assay. They found higher levels of IGF1 in winter compared with summer which contrasts with the present findings. This discrepancy is most likely due to the assay systems. It is now known that IGF1 in the plasma is bound to a number of binding proteins which modulate its activity. IGF1 must be separated from the binding proteins before assay in order to accurately measure it; this was not done by Ringberg *et al.* (1978).

The seasonal pattern of weight gain typical of boreal deer most likely evolved in response to the profoundly seasonal pattern of food supply. In that plant growth is predictable from year to year, deer have adapted to seasons of plenty and seasons of famine by not attempting to achieve rapid growth at all times but rather, the strategy is for rapid growth in spring/summer and maintenance in winter. Physiologically this strategy has meant that deer proceed through different growth phases throughout the year in an endogenous manner. During growth phases, body protein is laid down and energy reserves, as fat, are deposited to meet energy demands during periods when dietary energy is inadequate. These are effective survival strategies for life in an environment with predictable seasons. However, just because the year may be divided into separate portions of a growth cycle does not logically indicate that the precise physiological control mechanisms for all of these stages must be the same. It is probably not meaningful to attempt to study changes in seasonal pattern of growth rate, voluntary food intake or metabolic rate in isolation or with a view to identifying a «master» controller. Rather it is

more meaningful to study seasonal patterns as related but separate components which must be synchronised and co-ordinated to achieve seasonal growth. Likewise no one hormone or hormonal system is likely to be the major controller of the live weight seasonal pattern we observe. It is clear from many studies that prolactin (Suttie and Kay, 1985; Ryg and Jacobsen, 1982b), insulin (Larsen *et al.* 1985), growth hormone (Ryg and Jacobsen, 1982a, Suttie *et al.* 1989) thyroxine (Ryg and Jacobsen 1982a,b; Nilssen *et al.* 1984) all play separate but co-ordinated roles. The present study has identified IGF1 as the likely cause of the transition between winter and spring growth states and the spring growth spurt. Although plasma levels of IGF1 preceded the growth spurt, whether the growth spurt would have occurred in the presence of an isolated increase in IGF1 but without synergistic elevations in other hormones (e.g., prolactin) is not known.

In the present study in reindeer a long photoperiod indicative of spring/summer daylength conditions increased growth rate and IGF1 but a short photoperiod indicative of winter daylength did not permit a growth spurt or increase in IGF1 although it did not suppress growth. Applying these experimental results to the natural environment it seems that the role of photoperiod may be two-fold. Firstly, an increase in daylength permits the deer to alter its metabolism in preparation to take advantage of the spring flush to herbage production with no time delay to maximise growth. Secondly, a short daylength may prevent the animal from altering its metabolism prematurely to attempt an inappropriate growth spurt when dietary energy was inadequate but not prevent opportunistic growth at a lower level if nutrition should be adequate. The role of photoperiod in timing the spring onset of growth is seen as critical for the control of the seasonal growth pattern. A parallel may be drawn between the requirement for short days in fall to time reproduction.

The comparisons among arctic, temperate and tropical deer reveal that in environments where little seasonal fluctuation in growth take place, no seasonal pattern of IGF1 is measured. Rusa deer in Queensland are capable of reproduction in all times for year (Woodford, 1991) so the lack of growth seasonality has a parallel in reproduction. The growth strategy of tropical ruminants may rely on opportunistic growth when nutrition is adequate thus not necessitating a growth spurt. Alternatively their environment may not be sufficiently predictable to have evolved a physiological strategy to exploit it.

The fact that two species of deer which had a spring growth spurt had elevated IGF1 at that time and one species which lacked a growth spurt also lacked an increase in IGF1 tends to emphasise the role of IGF1 as a part controller of the growth spurt. Had IGF1 been elevated in rusa deer in spring and no growth spurt been observed or *vice versa* this would have cast doubt on the hypothesis.

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Manuscript accepted 25 November, 1992.