The response of the hepatic insulin-like growth factor system to growth hormone and dexamethasone in calves

H M Hammon, Y Zbinden, H Sauerwein¹, B H Breier², J W Blum and S S Donkin³

Division of Animal Nutrition and Physiology, Institute of Animal Genetics, Nutrition and Housing, Faculty of Veterinary Medicine, University of Berne, Bremgartenstr. 109a, CH-3012 Berne, Switzerland

¹Institute of Physiology, Biochemistry and Animal Hygiene, University of Bonn, Germany

²Liggins Institute for Medical Research, Faculty of Medical and Health Science, University of Auckland, Private Bag 92019, Auckland, New Zealand

³Department of Animal Science, Purdue University, West Lafayette, Indiana 47906, USA

(Requests for offprints should be addressed to H M Hammon; Email: harald.hammon@itz.unibe.ch)

Abstract

Glucocorticoids inhibit postnatal growth and yet can stimulate the somatotrophic axis around birth. The aim of the present study was to investigate the effects of dexamethasone on the somatotrophic axis and on the responses of the insulin-like growth factor (IGF) system to growth hormone treatment in calves. Calves ($n=24$) were randomly divided into four groups. Group DX was injected with dexamethasone (30 µg/kg body weight per day), group GH was injected with 500 mg slow-release bovine growth hormone at 14-day intervals, group GHDX was injected with dexamethasone and bovine growth hormone, and group CNTRL (serving as control) was injected with saline from day 3 to day 42 of life. Blood samples were taken on day 3 and blood and liver samples were obtained on days 7, 14, 28 and 42. Body weight increased in the CNTRL and GH groups up to the end of the study and in the DX and GHDX groups up to the fourth week. Dexamethasone treatment decreased ($P<0.05$) plasma IGF binding protein (IGFBP)-1 on days 7 and 14, but increased ($P<0.05$) plasma IGFBP-1, decreased ($P<0.05$) plasma IGF-I and IGFBP-3, and

decreased hepatic mRNA for growth hormone receptor (GHR) and IGF-I on day 42. Growth hormone treatment increased ($P<0.05$) plasma growth hormone concentrations on days 7 and 14, tended to increase ($P<0.1$) plasma IGF-I concentrations on day 42, and increased ($P<0.05$) hepatic mRNA levels of GHR on day 14 and IGF-I mRNA levels on days 7 and 14. The combined dexamethasone and growth hormone treatment increased plasma growth hormone concentrations on day 7 and resulted in the highest plasma concentrations of IGF-I and IGFBP-3 (day 7 to day 28) as well as the greatest abundance of hepatic GHR (day 14) and IGF-I (days 7 and 14) mRNA. Plasma IGFBP-1 concentrations in the GHDX group behaved in a similar manner as in the DX group. In conclusion, the response of the somatotrophic axis to growth hormone treatment could be greatly enhanced by dexamethasone treatment during the neonatal and early postnatal period, but body weight gain was not improved. Dexamethasone alone inhibited the somatotrophic axis and postnatal growth after the first month of life.

Journal of Endocrinology (2003) **179**, 427–435

Introduction

Although several hormones regulate postnatal growth, the somatotrophic axis becomes the dominant endocrine system (Spencer 1985, Etherton & Bauman 1998, Breier *et al.* 2000). Glucocorticoids inhibit prenatal (Newnham & Moss 2001) and postnatal growth, in part by direct interaction with the somatotrophic axis (Spencer 1985, Unterman & Phillips 1985, Bloomfield *et al.* 2001, Butler & LeRoith 2001, Renaville *et al.* 2002). However, in the perinatal period glucocorticoids are supposed to enhance the maturation of the somatotrophic axis. The prepartum cortisol surge plays an important role in initiating the

perinatal switch of the somatotrophic axis from the fetal to the postnatal status and function (Gluckman *et al.* 1999, Breier *et al.* 2000). Thus, cortisol stimulates hepatic growth hormone receptor (GHR) and insulin-like growth factor-I (IGF-I) mRNA levels in the sheep fetus (Li *et al.* 1996) and studies with hepatocytes indicated an increase in GHR and IGF-I expression and enhanced IGF-I response to growth hormone if combined with dexamethasone administration (Brameld *et al.* 1995). Recent studies in neonatal pigs report even enhanced postnatal growth and stimulation of the growth hormone (GH)–IGF system after dexamethasone treatment, lasting in part for the whole growth period (Carroll 2001, Gaines *et al.* 2002).

In addition, our own investigations in neonatal calves indicated a similar effect of dexamethasone on the somatotrophic axis (Sauter *et al.* 2003). In contrast, dexamethasone reduced IGF-I levels in adult cattle (Elsasser *et al.* 1997, Maciel *et al.* 2001). Therefore, the effects of glucocorticoids on the somatotrophic axis are inconsistent and may depend on the developmental stage.

Little is known of how glucocorticoids interfere with the somatotrophic axis in the early growth period of calves. Furthermore, the effects of glucocorticoid treatment on growth in fetal animals and preterm infants are inconsistent (Newnham & Moss 2001). Plasma cortisol concentrations in calves were high around birth and then rapidly decreased with postnatal growth, whereas IGF-I concentrations decreased during the first week of life, but then increased (Kerr *et al.* 1991, Hammon & Blum 1997, 1998, Nussbaum *et al.* 2002). The present study was carried out to clarify the influence of dexamethasone on the somatotrophic axis during the first 6 weeks of life. We have tested the hypothesis that dexamethasone treatment modifies the somatotrophic axis and the response of the hepatic IGF system to growth hormone treatment in calves. Therefore, we have treated calves for 40 days with either dexamethasone or growth hormone alone or in combination.

Materials and Methods

Animals, husbandry, feeding and experimental procedures

All animal handling procedures were approved by the Purdue Animal Care and Use Committee. Twenty-four male Holstein calves, aged 1–2 days, were purchased from a single large commercial dairy farm and transported to the Purdue University Dairy Research and Education Center. Calves were born spontaneously, separated from their dams at birth, fed colostrum and housed individually. Calves were weighed upon receipt, blocked by initial body weight and randomly assigned within each block to one of four treatment groups ($n=6$ per group). The following treatments were initiated on day 3 of life: dexamethasone was administered subcutaneously (Azium, Schering-Plough, Terre Haute, IN, USA) at 30 µg/kg body weight per day (group DX); growth hormone, as 500 mg slow-release recombinant bovine somatotropin (Posilac, Monsanto, St Louis, MO, USA), was administered subcutaneously at 14-day intervals (on days 3, 17 and 31 of age; group GH); dexamethasone and Posilac (group GHDX) were administered in combination at the same concentrations as for the DX and GH groups and were injected subcutaneously; saline treatment acted as a control (group CNTRL). Treatments continued until 42 days of age. The dose of dexamethasone was chosen according to previous studies with neonatal calves (Sauter *et al.* 2003). The dose of bovine somatotropin is based on Holzer *et al.* (2000) and previous studies using neonatal calves

(Hammon & Blum 1997). A constant rate of growth hormone release during the 14-day period was assumed to provide 0.5 to 0.9 mg/kg body weight per day, which is comparable to the effective daily injection of growth hormone used in previous studies (Hammon & Blum 1997).

Calves were fed a milk replacer diet (Milk Specialties Company, Dundee, IL, USA), which contained 29% crude protein, 15% crude fat and less than 0.15% crude fiber at a rate of 2% of body weight on a dry milk replacer basis. Milk replacer was diluted with warm water to 15% solids and was fed in buckets twice daily at 0700 and at 1600 h. Calves were weighed weekly, and the amount fed was adjusted according to body weight. Fresh water was available at all times. Calves that failed voluntarily to consume milk replacer within 45 min were fed their scheduled allotment of milk replacer via stomach tube.

To protect against bacterial infections all calves were injected intramuscularly with 1.5 mg/kg body weight ampicillin twice daily (Polyflex; Wyeth, Fort Dodge Animal Health, Overland Park, KA, USA) and 1500 U/kg body weight penicillin G procaine daily (Penicillin G Procaine, G.C. Hanford, Syracuse, NY, USA) upon receipt and for the following 2 days. The health status was evaluated weekly based on the following clinical traits: rectal temperature, heart rate, respiratory rate, behavior, nasal discharge, respiratory sounds, appetite, fecal consistency and navel adspersion. Calves with symptoms of respiratory or gastrointestinal infections were treated as per instructions of the Veterinary School at Purdue University.

Blood sampling and analyses

Blood was taken from the jugular vein with evacuated tubes on days 3, 7, 14, 28 and 42 of life. Tubes were put on crushed ice until centrifuged at 550 g for 15 min. Supernatants were aliquoted and stored at -20°C . Tubes containing dipotassium-EDTA (1.8 g/l blood) were used for the determination of plasma concentrations of GH, IGF-I, IGF binding protein (IGFBP)-1 and IGFBP-3 four to five hours after food intake. Plasma IGF-I, GH and IGFBP-1 concentrations were measured by radioimmunoassays as described previously (Hammon & Blum 1997, Kaufhold *et al.* 2000). Plasma concentrations of IGFBP-3 were measured by enzyme immunoassay (EIA) as described by Hennies and Sauerwein (2003).

Analyses in liver

Liver biopsy samples (about 500 mg) were obtained on days 7, 14, 28 and 42 of life, 2 to 3 h after food intake for analysis of IGF-I and GHR mRNA. The biopsy technique was adapted from Greenfield *et al.* (2000). Calves were restrained using a small animal squeeze chute and tilt table combination and were placed on their left side. The area above the 11th and 12th intercostal space on a line

from the hip joint to the elbow was clipped, scrubbed with Betadine (Purdue Pharma L.P., Stamford, CT, USA) and swabbed with 70% ethanol. Lidocaine (10 ml; 2% lidocaine hydrochloride; Phoenix Pharmaceutical, St Joseph, MO, USA) was injected subcutaneously. Liver biopsy samples were obtained percutaneously. Samples were rinsed in saline and transferred to a fresh tube containing a guanidinium thiocyanate solution (4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.4), 0.5% sarkosyl and 0.1 M β -mercaptoethanol) and frozen in liquid nitrogen. All samples were stored at -80°C until analysis.

Isolation of RNA and Northern blotting

Total RNA was extracted from biopsy samples and 20 μg were separated by electrophoresis through a 1% agarose gel and transferred to a Genescreen membrane (NEN Life Science Products, Boston, MA, USA) and prehybridized as described (Donkin *et al.* 1996). The cDNA probes for GHR (total GHR) and IGF-I were kindly provided by Dr Matt Lucy (University of Missouri, MO, USA) and have been described recently (Kobayashi *et al.* 1999). The ^{32}P -labeled cDNA probes were prepared using ^{32}P [d-CTP] and the Ready-to-Go DNA labeling beads dCTP random oligonucleotide priming kit (Pharmacia, Piscataway, NY, USA) to a specific activity of approximately 109 c.p.m./ μg DNA. Membranes were probed sequentially for IGF-I, GHR and 18S rRNA. Membranes were exposed to Kodak X-Omat film for 1 to 3 days at -80°C . Labeled probes were stripped by boiling for 60 min in a buffer containing 1% sodium dodecyl sulfate, 15 mM sodium chloride and 1.5 mM sodium citrate. The removal of labeled probes from membranes was verified using a Geiger counter. Abundance of mRNA for each transcript was quantified from digital scans of autoradiographic images using Kodak Digital Science 1D Image Analysis software (Eastman Kodak Co, Rochester, NY, USA). Differences in loading and transfer of RNA among samples were adjusted by normalizing the GHR and IGF-I data for each calf to the corresponding intensity of the 18S signal for each sample. A pooled RNA sample was used for correction between blots. The size of the transcript was determined by the migration distance relative to RNA standards. Northern blots of GHR and IGF-I resulted in one single band at 4.2 kb for GHR and 7.5 kb for IGF-I and were supported by findings by others (Vandelaar *et al.* 1995).

Statistical procedures

Values of body weight, feed intake, blood traits and mRNA were expressed as means $\pm$ S.E.M. and the data were evaluated using the RANDOM and REPEATED methods of the MIXED procedure (SAS 1995). The procedure analyses of repeated measurements considers

Figure 1 Body weight during the experimental period in control calves (CNTRL), calves treated with dexamethasone (30 $\mu\text{g}/\text{kg}$ body weight/day; DX), calves treated with growth hormone (500 mg/14 days; GH), and calves treated with dexamethasone and growth hormone (GHDX) in the same amounts as the DX and GH groups. Means with different uppercase letters (A, B) are significantly different ($P < 0.05$) at the specific time points.

random effects in the statistical model and permits modeling of the covariance structure of the data. Dexamethasone, growth hormone treatment and time were fixed effects and the individual calves were considered random effects. For the evaluation of differences in time-dependent changes within dexamethasone or growth hormone treatments, interactions (dexamethasone $\times$ time; growth hormone $\times$ time) were included in the model. Treatment and time effects were localized by Bonferroni *t*-test ($P < 0.05$). Due to heteroscedasticity of IGFBP-1, the data were transformed to log values prior to analysis, and the reported values reflect the untransformed means.

Results

Food intake, body weight and health status

Body weight (Fig. 1) increased ($P < 0.05$) in the CNTRL and GH groups up to week 6 and in the DX and GHDX groups up to week 5. Different treatments affected ($P < 0.05$) body weight with time. Calves of the DX and GHDX groups ceased growing after week 4 of life, and in week 6 body weight was higher ($P < 0.05$) in the CNTRL and GH groups than in the DX and GHDX groups. Food intake increased in accordance with body weight in all groups from 5.3 ± 0.3 to 8.6 ± 0.4 kg milk per day from week 1 to week 6. Food intake was influenced differently ($P < 0.05$) by different treatments during the experimental period and was reduced ($P < 0.05$) in week 6 by dexamethasone treatment (8 ± 0.5 and 9.2 ± 0.3 kg milk per day for dexamethasone-treated and non-dexamethasone-treated calves respectively). Two calves from the DX and GHDX groups respectively were excluded from the study

Figure 2 Plasma growth hormone (GH) concentrations on days 3, 7, 14, 28, 42 of life in control calves (CNTRL), calves treated with dexamethasone (30 µg/kg body weight/day; DX), calves treated with growth hormone (500 mg/14 days; GH), and calves treated with dexamethasone and growth hormone (GHDX) in the same amounts as the DX and GH groups. Arrows indicate the times of growth hormone treatment. Means with different uppercase letters (A, B, C) are significantly different ($P < 0.05$) at the specific time points.

after day 28 because of health problems. All other calves were healthy up to the end of the study.

Blood parameters

Plasma growth hormone concentrations (Fig. 2) increased ($P < 0.05$) in the GH and GHDX groups from day 3 to day 7 and then decreased ($P < 0.05$) up to day 28. Different treatments had different effects ($P < 0.001$) on growth hormone concentrations with time; concentrations on day 7 were highest ($P < 0.01$) in the GH group and were higher ($P < 0.01$) in the GHDX group than in the CNTRL and DX groups. On day 14, concentrations were highest ($P < 0.01$) in the GH group.

Plasma IGF-I concentrations (Fig. 3) increased ($P < 0.05$) in the CNTRL group from day 14 to day 42, increased ($P < 0.05$) in the GH group from day 3 to day 42, and increased in the GHDX group ($P < 0.05$) from day 3 to day 28 but thereafter decreased ($P < 0.05$) to day 42. Different treatments changed ($P < 0.001$) IGF-I concentrations during the experimental period. Plasma IGF-I concentrations on days 7 and 14 were higher ($P < 0.05$) in the GHDX group than in the CNTRL and DX groups, and on day 14 tended to be higher ($P < 0.1$) in the GHDX group than in the GH group. On day 28, IGF-I concentrations were higher ($P < 0.05$) in the GHDX group than in all other groups and were lower ($P < 0.05$) in the DX than in the GH group. On day 42, concentrations were lowest ($P < 0.05$) in the DX group and tended to be higher ($P < 0.1$) in the GH than in the CNTRL groups.

Plasma IGFBP-1 concentrations (Fig. 4) increased ($P < 0.01$) in the CNTRL and GH groups from day 3 to

Figure 3 Plasma IGF-I concentrations on days 3, 7, 14, 28, 42 of life in control calves (CNTRL), calves treated with dexamethasone (30 µg/kg body weight/day; DX), calves treated with growth hormone (500 mg/14 days; GH), and calves treated with dexamethasone and growth hormone (GHDX) in the same amounts as the DX and GH groups. Arrows indicate the times of growth hormone treatment. Means with different uppercase letters (A, B, C) are significantly different ($P < 0.05$) at the specific time points.

Figure 4 Plasma IGFBP-1 concentrations on days 3, 7, 14, 28, 42 of life in control calves (CNTRL), calves treated with dexamethasone (30 µg/kg body weight/day; DX), calves treated with growth hormone (500 mg/14 days; GH), and calves treated with dexamethasone and growth hormone (GHDX) in the same amounts as the DX and GH groups. Arrows indicate the times of growth hormone treatment. Means with different uppercase letters (A, B, C) are significantly different ($P < 0.05$) at the specific time points.

day 7 and then decreased ($P < 0.01$) up to day 42. Concentrations increased ($P < 0.05$) in the DX and GHDX groups from day 28 to day 42. Different treatments changed ($P < 0.001$) IGFBP-1 concentrations with time. Dexamethasone decreased IGFBP-1 concentrations on days 7 and 14 and increased IGFBP-1 concentrations on day 42, whereas growth hormone had no effect on IGFBP-1 plasma concentrations.

Plasma IGF-3 (Fig. 5) concentrations increased ($P < 0.05$) in the CNTRL and GH groups up to day 42

Figure 5 Plasma IGFBP-3 concentrations on days 3, 7, 14, 28, 42 of life in control calves (CNTRL), calves treated with dexamethasone (30 µg/kg body weight/day; DX), calves treated with growth hormone (500 mg/14 days; GH), and calves treated with dexamethasone and growth hormone (GHDX) in the same amounts as the DX and GH groups. Arrows indicate the times of growth hormone treatment. Means with different uppercase letters (A, B, C) are significantly different ($P < 0.05$) at the specific time points.

and in the GHDX group up to day 28, but decreased ($P < 0.05$) from day 28 to day 42. Different treatments changed ($P < 0.001$) IGFBP-3 concentrations with time. Plasma concentrations on day 7 were higher ($P < 0.05$) in the GHDX group than in the CNTRL group and on days 14 and 28 were higher ($P < 0.05$) than in all other groups. On day 28 concentrations were higher ($P < 0.05$) in the GH group than in the DX group and on day 42 concentrations were lowest ($P < 0.05$) in the DX group.

Abundance of IGF-I and GHR mRNA in the liver (Table 1)

Abundance of GHR mRNA in liver tended to increase ($P < 0.1$) in the CNTRL and GH groups from day 7 to day 42. Different treatments changed ($P < 0.05$) GHR mRNA levels with time. On day 14 growth hormone and the combined dexamethasone and growth hormone treatment increased ($P < 0.05$) GHR mRNA levels. On day 42, dexamethasone and the combined dexamethasone and growth hormone treatment decreased ($P < 0.05$) GHR mRNA levels.

Abundance of IGF-I mRNA levels in liver increased ($P < 0.05$) in the CNTRL and GH groups from day 14 to day 42 and in the DX group from day 14 to day 28. Different treatments tended to change ($P < 0.1$) IGF-I mRNA levels with time. On days 7 and 14 growth hormone increased ($P < 0.01$) IGF-I mRNA levels and mRNA levels on day 14 were higher ($P < 0.05$) in the GHDX group than in the CNTRL and DX groups.

Dexamethasone decreased ($P < 0.05$) IGF-I mRNA levels on day 42.

Correlations between tissue and blood parameters

During the experimental period body weight correlated with hepatic mRNA abundance of IGF-I ($r = 0.56$; $P < 0.001$) and GHR ($r = 0.44$; $P < 0.001$) and with plasma concentrations of IGF-I ($r = 0.52$; $P < 0.001$), growth hormone ($r = -0.29$; $P < 0.01$), IGFBP-1 ($r = -0.28$; $P < 0.01$) and IGFBP-3 ($r = 0.56$; $P < 0.001$). Levels of IGF-I mRNA in liver correlated with GHR mRNA levels ($r = 0.73$; $P < 0.001$) and with plasma concentrations of IGF-I ($r = 0.62$; $P < 0.001$), IGFBP-1 ($r = -0.28$; $P < 0.01$) and IGFBP-3 ($r = 0.49$; $P < 0.01$). Levels of GHR mRNA in liver correlated with plasma concentrations of IGF-I ($r = 0.41$; $P < 0.001$), IGFBP-1 ($r = -0.29$; $P < 0.01$) and IGFBP-3 ($r = 0.29$; $P < 0.01$). Plasma concentrations of IGF-I correlated with plasma concentrations of IGFBP-1 ($r = -0.31$; $P < 0.01$) and IGFBP-3 ($r = 0.85$; $P < 0.01$). Plasma concentrations of IGFBP-1 correlated with plasma concentrations of IGFBP-3 ($r = -0.29$; $P < 0.01$). Almost the same pattern of correlations was seen in the CNTRL and GH group calves, whereas the number of correlations in the DX and GHDX groups were smaller (data not shown).

Discussion

The present study was performed to clarify the influence of dexamethasone on the somatotrophic axis and the response of the hepatic IGF system to growth hormone treatment during the neonatal and early postnatal period in calves. We observed that the developmental increase in body weight in CNTRL calves was associated with an increase in plasma IGF-I and IGFBP-3 concentrations and with hepatic GHR and IGF-I mRNA levels, whereas plasma IGFBP-1 concentrations decreased. These findings support the general concept that the somatotrophic axis is basically associated with growth performance during the early postnatal period (Kerr *et al.* 1991, Etherton & Bauman 1998, Breier *et al.* 2000, Renaville *et al.* 2000). However, plasma IGF-I and IGFBP-3 concentrations as well as hepatic mRNA levels of GHR and IGF-I in CNTRL calves did not start to increase before day 14, indicating a delayed response of the somatotrophic axis in postnatal growth regulation. Our findings in calves support the view that the somatotrophic axis in neonatal calves is not fully competent at birth and that the postnatal rise in IGF-I levels is delayed (Hammon & Blum 1997, Nussbaum *et al.* 2002). The reasons for the low plasma concentrations of IGFBP-1 in all groups on day 3 are not known. The interval between feeding and blood sampling was the same for all groups and across sampling days. In addition,

Table 1 Hepatic mRNA levels (arbitrary units) of GHR and IGF-I in calves treated with 0.9% NaCl (CNTRL), 30 µg/kg body weight dexamethasone (DX), 500 mg/14 days growth hormone (GH), and 30 µg/kg body weight dexamethasone and 500 mg/14 days growth hormone (GHDX). Values are means ± S.E.M. (pooled standard error). Main effects of dexamethasone (δ), growth hormone (ϕ) and dexamethasone × growth hormone ($\delta \times \phi$) are shown

Parameter	Day	Group				Main effects (<i>P</i> -values)		
		CNTRL	DX	GH	GHDX	δ	ϕ	$\delta \times \phi$
GHR	7	0.98 ± 0.2	1.1 ± 0.22	1.07 ± 0.16	1.0 ± 0.18	NS	NS	NS
	14	0.73 ± 0.09	0.82 ± 0.18	1.06 ± 0.19	1.37 ± 0.29	NS	<0.05	<0.05
	28	1.7 ± 0.21	1.65 ± 0.18	1.62 ± 0.23	1.63 ± 0.15	NS	NS	NS
	42	2.0 ± 0.31	1.05 ± 0.42	1.71 ± 0.14	1.06 ± 0.29	<0.001	NS	<0.01
IGF-I	7	0.39 ± 0.11	0.47 ± 0.15	1.03 ± 0.32	1.31 ± 0.59	NS	<0.05	<0.1
	14	0.19 ± 0.04	0.2 ± 0.12	0.6 ± 0.26	1.64 ± 0.44	NS	<0.01	<0.05
	28	1.91 ± 0.42	1.98 ± 0.31	1.7 ± 0.52	1.87 ± 0.37	NS	NS	NS
	42	2.27 ± 0.42	1.91 ± 0.79	2.95 ± 0.13	1.88 ± 0.59	<0.05	NS	NS

NS, not significant.

the order of sampling was uniformly balanced across treatments, therefore differences in IGFBP-1 are not attributable to short term nutritional status. Despite the lower IGFBP-1 in all groups on day 3, the plasma concentrations in the range of 5 to 50 mg/l, measured in the present study, correspond to postprandial plasma concentrations in neonatal calves (Sauter *et al.* 2003).

Dexamethasone treatment depressed plasma concentrations of IGFBP-1 in the neonatal period, but did not depress mRNA levels of GHR and IGF-I and plasma concentrations of IGF-I and IGFBP-3 before day 42. In the present study, chronic dexamethasone treatment failed to stimulate the somatotrophic axis in the neonatal and early postnatal period, in contrast to recent findings in neonatal calves and pigs (Carroll 2001, Sauter *et al.* 2003). However, in these studies dexamethasone was injected immediately after birth and was administered only once (pigs; Carroll 2001) or for 4 days only (calves; Sauter *et al.* 2003). Obviously, the response of the somatotrophic axis to dexamethasone treatment depends on age, dose, and duration of the administration (Bloomfield *et al.* 2001, Butler & LeRoith 2001). In human hepatocytes short-term dexamethasone treatment stimulated, whereas long-term treatment inhibited GHR gene expression (Vottero *et al.* 2003). The depression of the hepatic IGF system by dexamethasone on day 42 corresponds with findings in preterm infants (Bloomfield *et al.* 2001), during postnatal growth and in adults (Unterman & Phillips 1985, Burrin *et al.* 1999), including cattle (Elsasser *et al.* 1997, Maciel *et al.* 2001). Dexamethasone treatment may also affect growth hormone clearance (plasma growth hormone concentrations were lower in the GHDX group than in the GH group on days 7 and 14) possibly by interfering with growth hormone binding proteins (Gabrielsson *et al.* 1995).

Plasma growth hormone concentrations increased in the GH group at the beginning of the study, but concen-

trations did not remain elevated after day 14 of life. Although the blood sampling protocol did not permit a detailed evaluation of growth hormone status, the lack of plasma growth hormone responses in the GH and GHDX groups from day 28 onwards was unexpected (Holzer *et al.* 2000). The clearance rate of growth hormone may increase with age, because plasma growth hormone concentrations were elevated on day 14 of life (11 days after the first injection of growth hormone), but not on days 28 and 42 (11 days after the second and third injections of growth hormone respectively). The postnatal maturation of the somatotrophic axis greatly depends on the competency of hepatic GHRs, which are low during fetal development and gradually increase after birth (Badinga *et al.* 1991, Min *et al.* 1999, Sauter *et al.* 2003). Growth hormone treatment up-regulates hepatic growth hormone binding sites in sheep, but not in lambs (Sauerwein *et al.* 1991, Min *et al.* 1999). Although growth hormone stimulated hepatic IGF-I mRNA levels up to day 14 in our study, plasma concentrations of IGF-I in the GH group did not increase before day 42 in our study, as is known for cattle (Elsasser *et al.* 1989, Holzer *et al.* 2000, Smith *et al.* 2002). Because hepatic IGF-I is the main source for circulating IGF-I in cattle (Pfaffl *et al.* 1998, Cordano *et al.* 2000), it may take some time before the mature IGF-I peptide is delivered to the circulation.

Most impressively, the combined growth hormone and dexamethasone treatment markedly stimulated the somatotrophic axis up to day 28 of life. The IGF-I response to growth hormone treatment depends on the degree of maturation of the somatotrophic axis and increases with age and nutrient intake (Breier *et al.* 2000, Holzer *et al.* 2000). Our findings suggest that dexamethasone greatly increases the hepatic IGF-I response to growth hormone treatment in the neonatal period. Studies with porcine hepatocytes, too, showed increased responsiveness of IGF-I mRNA to growth hormone after dexamethasone treatment

(Brameld *et al.* 1995), but in rats dexamethasone inhibited the IGF-I response to growth hormone (Luo & Murphy 1989, Beauloye *et al.* 1999). Because plasma insulin concentrations were elevated in the GHDX group (Hammon *et al.* 2003), insulin might, in part, have stimulated hepatic IGF-I production, as seen in rats and cows (Böni-Schnetzler *et al.* 1991, McGuire *et al.* 1995). Furthermore, elevated plasma IGF-I may result from reduced clearance rates due to increased IGFBP-3 and decreased IGFBP-1 plasma concentrations, which are closely associated with IGF-I and insulin concentrations. The IGFBP-3 retains IGF-I in the circulation by binding with the acid-labile subunit, whereas IGFBP-1 is able to leave the circulation (Clemmons 1997). The enhanced response of the somatotrophic axis to growth hormone following dexamethasone treatment was abolished at the end of the study on day 42. Therefore, the enhanced growth hormone effects on the hepatic IGF system due to dexamethasone treatment might be age-dependent or might be a consequence of the chronic dexamethasone treatment.

Most parameters of the somatotrophic axis measured in this study were closely related to body weight, as expected. However, contrary to chronic dexamethasone treatment in preterm infants and during postnatal growth, dexamethasone treatment in our study did not affect body weight gain before day 28 after birth (Spencer 1985, Bloomfield *et al.* 2001, Newnham & Moss 2001, Renaville *et al.* 2002). The lack of effects on growth performance during the first month of life in the DX group was associated with unresponsiveness of the somatotrophic axis to dexamethasone. In neonatal pigs, a single dose of dexamethasone treatment after birth improved postnatal growth performance (Carroll 2001, Gaines *et al.* 2002), whereas chronic dexamethasone administration reduced growth rates (Burrin *et al.* 1999). Based on this information, the effects of glucocorticoids on growth performance in the neonatal period seem to depend on dose and duration of glucocorticoid administration, in accordance with studies in preterm infants (Bloomfield *et al.* 2001). Growth hormone treatment had no significant effect on body weight up to 6 weeks, but average daily gain was improved in the GH group when calves were studied during the first 2 months of life (Hammon & Donkin 2002). Previous studies in veal calves that were administered growth hormone daily up to the time of slaughter (age about 140 days) could not demonstrate any marked growth enhancement either (Ceppi & Blum 1994), indicating that it takes a long period of time before growing cattle become fully responsive to growth hormone. Administration of slow-release growth hormone to about 180-day-old calves resulted in a more distinct growth stimulation (Holzer *et al.* 2000). The enhanced hepatic IGF response to growth hormone treatment by dexamethasone failed to stimulate growth performance during the first month of life. Furthermore, the disappear-

ance of stimulatory effects on the somatotrophic axis after day 28 in the GHDX group was combined with a cessation of body weight gain.

In conclusion, dexamethasone administration improved the response of the IGF system to growth hormone treatment during the first month of life, but neither growth hormone nor dexamethasone alone or in combination stimulated postnatal growth during this time period. Dexamethasone and the growth hormone/dexamethasone combination depressed body weight gain and the somatotrophic axis after day 28 of life, indicating that the response of the somatotrophic axis to dexamethasone treatment in calves depends on age or at least on the duration of dexamethasone treatment.

Acknowledgements

Many thanks are due to Mike Grott and his team at the Purdue Dairy Research and Education Centre for daily calf care. Thanks are also extended to Juan Carlos Velez, Stacy Crowder and Jon Townsend for assistance with liver biopsy sampling and Tracy Wiegand for technical assistance with metabolite assays.

Funding

This work was supported in part by funds from the Indiana Agricultural Research as part of the North Central Regional project NC-1009. H M Hammon was supported by the University of Berne during his residence at Purdue University. There is no conflict of interest that would prejudice the impartiality of this work.

References

- Badinga L, Collier RJ, Thatcher WW, Wilcox CJ, Head HH & Bazer FW 1991 Ontogeny of hepatic bovine growth hormone receptors in cattle. *Journal of Animal Science* **69** 1925–1934.
- Beauloye V, Ketelslegers JM, Moreau B & Thissen JP 1999 Dexamethasone inhibits both growth hormone (GH)-induction of insulin-like growth factor-I (IGF-I) mRNA and GH receptor (GHR) mRNA levels in rat primary cultured hepatocytes. *Growth Hormone and IGF Research* **9** 205–211.
- Bloomfield FH, Knight DB, Breier BH & Harding JE 2001 Growth restriction in dexamethasone-treated preterm infants may be mediated by reduced IGF-I and IGFBP-3 plasma concentrations. *Clinical Endocrinology* **54** 235–242.
- Böni-Schnetzler M, Schmid C, Meier PJ & Froesch ER 1991 Insulin regulates insulin-like growth factor I mRNA in rat hepatocytes. *American Journal of Physiology* **260** E846–E851.
- Brameld JM, Weller PA, Saunders JC, Buttery PJ & Gilmour RS 1995 Hormonal control of insulin-like growth factor-I and growth hormone receptor mRNA expression by porcine hepatocytes in culture. *Journal of Endocrinology* **146** 239–245.
- Breier BH, Oliver MH & Gallaher BW 2000 Regulation of growth and metabolism during postnatal development. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp 187–204. Ed PB Cronjé. New York: CABI Publishing.

- Burrin DG, Wester TJ, Davis TA, Fiorotto ML & Chang X 1999 Dexamethasone inhibits small intestinal growth via increased protein catabolism in neonatal pigs. *American Journal of Physiology* **276** E269–E277.
- Butler AA & Le Roith D 2001 Control of growth by the somatotrophic axis: growth hormone and the insulin-like growth factors have related and independent roles. *Annual Review of Physiology* **63** 141–164.
- Carroll JA 2001 Dexamethasone treatment at birth enhances neonatal growth in swine. *Domestic Animal Endocrinology* **21** 97–109.
- Ceppi A & Blum JW 1994 Effects of growth hormone on growth performance, haematology, metabolites and hormones in iron deficient veal calves. *Journal of Veterinary Medicine. Series A, Physiology, Pathology, Clinical Medicine* **41** 443–458.
- Clemmons DR 1997 Insulin-like growth factor binding proteins and their role in controlling IGF actions. *Cytokine and Growth Factor Reviews* **8** 45–62.
- Cordano P, Hammon HM, Morel C, Zurbriggen A & Blum JW 2000 mRNA of insulin-like growth factor (IGF) quantification and presence of IGF binding proteins, and receptors for growth hormone, IGF-I and insulin, determined by reverse transcribed polymerase chain reaction, in the liver of growing and mature male cattle. *Domestic Animal Endocrinology* **19** 191–208.
- Donkin SS, McNall AD, Swencki BS, Peters JL & Etherton TD 1996 The growth hormone dependent decrease in hepatic fatty acid synthase mRNA is the result of a decrease in gene transcription. *Journal of Molecular Endocrinology* **16** 151–158.
- Elsasser TH, Rumsay TS & Hammond AC 1989 Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. *Journal of Animal Science* **67** 128–141.
- Elsasser TH, Kahl S, Steele NC & Rumsey TS 1997 Nutritional modulation of somatotrophic axis-cytokine relationships in cattle: a brief review. *Comparative Biochemistry and Physiology A. Molecular and Integrative Physiology* **116** 209–221.
- Etherton TD & Bauman DE 1998 Biology of somatotropin in growth and lactation of domestic animals. *Physiological Reviews* **78** 745–761.
- Gabrielsson BG, Carmignac DF, Flavell DM & Robinson IC 1995 Steroid regulation of growth hormone (GH) receptor and GH-binding protein messenger ribonucleic acid in the rat. *Endocrinology* **136** 209–217.
- Gaines AM, Carroll JA, Allee GL & Yi GF 2002 Pre- and postweaning performance of pigs injected with dexamethasone at birth. *Journal of Animal Science* **80** 2255–2262.
- Gluckman PD, Sizonenko SV & Bassett NS 1999 The transition from fetus to neonate – an endocrine perspective. *Acta Paediatrica Scandinavica Supplementum* **428** 7–11.
- Greenfield RB, Cecava MJ & Donkin SS 2000 Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during transition to lactation. *Journal of Dairy Science* **83** 1228–1236.
- Hammon H & Blum JW 1997 The somatotrophic axis in neonatal calves can be modulated by nutrition, growth hormone, and long-R3-IGF-I. *American Journal of Physiology* **273** E130–E138.
- Hammon H & Blum JW 1998 Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. *Journal of Nutrition* **128** 624–632.
- Hammon HM & Donkin SS 2002 Growth hormone influences growth performance, but does not affect gluconeogenesis from lactate or propionate in 60-day-old veal calves. *Journal of Dairy Science* **85** (Suppl 1) 337.
- Hammon HM, Blum JW & Donkin SS 2003 Effects of dexamethasone (DEXA) and growth hormone (ST) on glucose production in calves. *Journal of Animal Science* **81** (Suppl 1) 310.
- Hennies M & Sauerwein H 2003 Purification of bovine IGFBP-3 and the development of an enzyme immunoassay for the protein. *Journal of Immunological Methods* **281** 9–15.
- Holzer Z, Aharoni Y, Brosh A, Orlov A & Buonomo F 2000 The influence of recombinant bovine somatotropin on dietary energy level-related growth of Holstein-Friesian bull calves. *Journal of Animal Science* **78** 621–628.
- Kaufhold JN, Hammon HM, Bruckmaier RM, Breier BH & Blum JW 2000 Postprandial metabolism and endocrine status in veal calves fed at different feeding frequencies. *Journal of Dairy Science* **83** 2480–2490.
- Kerr DE, Laarveld B, Fehr MI & Manns JG 1991 Profiles of serum IGF-I concentrations in calves from birth to eighteen months of age and in cows throughout the lactation cycle. *Canadian Journal of Animal Science* **71** 695–705.
- Kobayashi Y, Boyd CK, Bracken CJ, Lamberson WR, Keisler DH & Lucy MC 1999 Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by specific down-regulation of GHR 1A that is associated with decreased insulin-like growth factor I. *Endocrinology* **140** 3947–3954.
- Li J, Owens JA, Owens PC, Saunders JC, Fowden AL & Gilmour RS 1996 The ontogeny of hepatic growth hormone receptor and insulin-like growth factor I gene expression in the sheep during late gestation: developmental regulation by cortisol. *Endocrinology* **137** 1650–1657.
- Luo J & Murphy LJ 1989 Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduced IGF-I mRNA abundance in the intact rat. *Endocrinology* **125** 165–171.
- McGuire MA, Dwyer DA, Harrell RJ & Bauman DE 1995 Insulin regulates circulating insulin-like growth factors and some of their binding proteins in lactating cows. *American Journal of Physiology* **269** E723–E730.
- Maciel SM, Chamberlain CS, Wettemann RP & Spicer LJ 2001 Dexamethasone influences endocrine and ovarian function in dairy cattle. *Journal of Dairy Science* **84** 1998–2009.
- Min SH, Mackenzie DDS, Breier BH, McCutcheon SN & Gluckman PD 1999 Ontogeny of IGF-I responsiveness to bGH in young lambs. *Domestic Animal Endocrinology* **17** 409–419.
- Newnham JP & Moss TJ 2001 Antenatal glucocorticoids and growth: single versus multiple doses in animal and human studies. *Seminars in Neonatology* **6** 285–292.
- Nussbaum A, Schiessler G, Hammon HM & Blum JW 2002 Growth performance and metabolic and endocrine traits in calves pair-fed by bucket or by automate starting in the neonatal period. *Journal of Animal Science* **80** 1545–1555.
- Pfaffl M, Meyer HHD & Sauerwein H 1998 Quantification of insulin-like growth factor-I (IGF-I) mRNA: development and validation of an internally standardised competitive reverse transcription-polymerase chain reaction. *Experimental and Clinical Endocrinology and Diabetes* **106** 506–513.
- Renaville R, Van Einaime C, Breier BH, Vleurick L, Bertozzi C, Gengler N, Hornick JL, Parmentier I, Istasse L, Haezebroeck V, Massart S & Portetelle D 2000 Feed restriction in young bulls alters the onset of puberty in relationship with plasma insulin-like growth factor-I (IGF-I) and IGF-binding proteins. *Domestic Animal Endocrinology* **18** 165–176.
- Renaville R, Hammadi M & Portetelle D 2002 Role of the somatotrophic axis in the mammalian metabolism. *Domestic Animal Endocrinology* **23** 351–360.
- SAS 1995 SAS/STAT User Guide (release 6.11). SAS Inst. Inc., Cary, NC.
- Sauerwein H, Breier BH, Bass JJ & Gluckman PD 1991 Chronic treatment with bovine growth hormone upregulates high-affinity hepatic somatotrophic receptors in sheep. *Acta Endocrinologica* **124** 307–313.
- Sauter SN, Ontsouka E, Roffler B, Zbinden Y, Philipona C, Pfaffl M, Breier BH, Blum JW & Hammon HM 2003 Effects of

- dexamethasone and colostrum intake on the somatotrophic axis in neonatal calves. *American Journal of Physiology* **285** E252–E261.
- Smith JM, Van Amburgh ME, Díaz MC, Lucy MC & Bauman DE 2002 Effects of nutrient intake on the development of the somatotrophic axis and its responsiveness to GH in Holstein bull calves. *Journal of Animal Science* **80** 1528–1537.
- Spencer GSG 1985 Hormonal systems regulating growth. *Livestock Production Science* **12** 31–46.
- Unterman TG & Phillips LS 1985 Glucocorticoid effects on somatomedins and somatomedin inhibitors. *Journal of Clinical Endocrinology and Metabolism* **61** 618–626.
- Vandehaar MJ, Sharma BK & Fogwell RL 1995 Effects of dietary energy restriction on expression of insulin-like growth factor-I in liver and corpus luteum of heifers. *Journal of Dairy Science* **78** 832–841.
- Vottero A, Kimchi-Sarfaty C, Kratzsch J, Chrousos GP & Hochberg Z 2003 Transcriptional and translational regulation of the splicing isoforms of the growth hormone receptor by glucocorticoids. *Hormone and Metabolic Research* **35** 7–12.

Received in final form 7 August 2003

Accepted 20 August 2003

Made available online as an

Accepted Preprint 26 August 2003