Abundance of message for insulin-like growth factors-I and -II and for receptors for growth hormone, insulin-like growth factors-I and -II, and insulin in the intestine and liver of pre- and full-term calves[1]

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ABSTRACT: The somatotropic axis and insulin are involved in pre- and postnatal development. In pre- and full-term calves (GrP_o and GrN_o; born after 277 and 290 d of pregnancy, respectively) and in preterm calves on d 8 of life after being fed for 7 d (GrP_8), we studied whether there are differences in the abundance of messenger RNA (mRNA) of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin among different intestinal sites (duodenum, jejunum, ileum, and colon) and whether there are ontogenetic differences during the perinatal period in intestine and liver. Intestinal site differences ($P < 0.05$) existed in mRNA levels of IGF-I and IGF-II and receptors for GH, IGF-I, IGF-II, and insulin. Abundance of mRNA of IGF-I and -II and of receptors for IGF-I and GH was highest ($P < 0.05$) in the colon, abundance of the receptor for IGF-II was comparably high in the colon and ileum, and that of the receptor for insulin was similarly high in colon, ileum, and jejunum. Among GrP_o, GrN_o, and GrP_8 groups, there were differences ($P < 0.05$) in mRNA levels of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II and insulin. Abundance of mRNA of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II and insulin was highest ($P < 0.05$) in GrP_o calves immediately after birth and was primarily seen in the ileum. In liver, the mRNA levels differed ($P < 0.05$) among groups for IGF-II and receptors for IGF-I, IGF-II, and insulin, and were highest ($P < 0.05$) for IGF-II in GrP_o, for receptors of IGF-I in GrN_o, and were higher ($P < 0.05$) in GrP_o than GrP_8 for receptors of IGF-II. In conclusion, mRNA levels of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin were different at different intestinal sites and in intestine and liver and changed during the perinatal period.

Key Words: Calves, Insulin-Like Growth Factor, Intestine, Liver, Messenger Ribonucleic Acid, Somatotropin

 J. Anim. Sci. 2003. 81:2294–2300

Introduction

During the postnatal period, there are marked morphological and functional changes of the gastrointestinal tract, but in preterm calves, the proliferation and morphological reactions and changes of brush border enzyme activities of the intestinal epithelium to ingestion of colostrum and milk replacer are in part reduced when compared with full-term calves (Bittrich et al., 2001).

Whereas IGF-II exhibits growth-promoting activity primarily during prenatal stages, GH, IGF-I, and insulin are especially important for postnatal development (D'Ercole, 1996; Breier et al., 2000; Butler and LeRoith, 2001b). The GH-IGF axis of calves in postnatal life is basically functional and affected by nutrition (Hammon and Blum, 1997). IGF-I and IGF-II messenger RNA (**mRNA**) are present in the ileum and liver of full-term neonatal calves (Cordano et al., 2000; Pfaffl et al., 2002). The mRNA for these receptors of

[1]This study was supported by Swiss National Science Foundation (grant 32-56823.99). We respect the in vivo work of Sabine Bittrich, who received a grant from H.W. Schaumann-Stiftung, D-Hamburg, Germany. We thank M. Iburg, Lohmann Animal Health, Cuxhaven, Germany, for providing the chicken-derived immunoglobulin.

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Received February 24, 2003.

Accepted May 5, 2003.

GH, IGF-I, IGF-II, and insulin are present in the ileum and liver of full-term neonatal calves (Pfaffl et al., 2002) and binding capacities of receptors for IGF-I, IGF-II, and insulin in the mucosa of the small and large intestine of neonatal calves are modified by nutrition (Baumrucker et al., 1994; Hammon and Blum, 2002) and ontogenetic state (Georgiev et al., 2003). Data on mRNA abundance of IGF-I and IGF-II, and on the GH receptor in different regions of the intestine and in liver in pre- and full-term calves on d 1 and in preterm calves on d 8 of life have not been reported.

Based on these premises, we have tested the hypotheses that there are differences in mRNA abundance of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin 1) among duodenum, jejunum, ileum and colon, 2) between preterm calves (born 14 d before normal term) and unfed full-term calves (born after normal pregnancy lengths of approximately 290 d) in intestine and liver on d 1 of life, and 3) between preterm calves on d 1 and preterm calves on d 8.

Materials and Methods

Animals, Husbandry, and Experimental Procedures

The experiments were performed according to the Swiss Law on Animal Protection; the experimental procedures were approved by the Committee for Animal Experimentation of the Canton of Freiburg (Granges-Paccot, Switzerland) and approvals were supervised by the Federal Veterinary Office in Berne, Switzerland.

Single-born calves (5 females and 16 males) from different dairy breeds (14 Simmental × Red Holstein, 4 Holstein Friesian, and 3 Brown Swiss), originating from the Swiss Federal Research Station for Animal Production in Posieux, were separated from their dams immediately after birth and were assigned to three groups. Preterm calves were born on d 277 of gestation within 42 ± 3 h after cows were injected with 500 μg of prostaglandin $F_{2\alpha}$ (Estrumate; Essex Pharma GmbH, Friesoythe, Germany) and 5 mg of Flumethason (Flumilar; Veterinaria AG, Zürich, Switzerland). Preterm calves were killed immediately after birth, before colostrum intake (**GrP_o**; n = 7), or on d 8 of life, after being fed for 7 d (**GrP_8**; n = 7). Fullterm calves were spontaneously born after normal lengths of pregnancy (290 ± 2 d) and were killed immediately after birth, before being fed (**GrN_o**; n = 7).

Calves of GrP_8 were held on straw in individual boxes for 7 d. They received first-milked colostrum during the first 3 d of life, and on d 4, 5, 6, and 7, they were fed first-milked colostrum that was diluted with 25, 50, 75 and 75 parts of milk replacer, respectively. Calves received 60g of colostrum/kg of BW on d 1, 80g/kg BW on d 2, and 100g/kg BW from d 3 to 7. Calves received their first meal on average within 2 h after birth. They were bottle-fed four times daily on d 1 and 2, and then twice daily. From d 3 on, they were fed at 0800 and 1600. Colostrum from first milking was a pool from cows of the Federal Research Station for Animal Production and from farms in the neighborhood of the research station. Colostrum was frozen in plastic bottles at –20°C. Before being fed, colostrum was warmed to 40°C. The milk replacer (UFA-200-Natura, without antibiotics; Union Futter AG, Sursee, Switzerland) was prepared as a 100-g/L solution. Composition of colostrum and milk replacer are presented in Table 1.

To protect against infections, calves were injected s.c. with 20 mL of an immunoglobulin preparation (Gammaserin, 100 g of immunoglobulin G/L; Gräub AG, Bern, Switzerland) after the first blood sample, and between d 2 and 5, they were injected s.c. with antibiotics once daily (2.5 mg of Baytril 5%/kg of BW, Bayer AG, Leverkusen, Germany and 15 mg of Betamox LA/kg of BW, Norbrook Laboratories, Neddry, U.K.).

Intestinal and Liver Tissue Sampling

After calves were killed immediately after birth (GrN_o and GrP_o) or on d 8 of life (GrP_8), the abdominal cavity was opened and the gastrointestinal tract was immediately removed. Sections of 0.5- to 1-cm pieces from different regions of the intestine (duodenum, jejunum, ileum, and colon) and liver were dissected using sterilized instruments. The removed tissue samples were snap-frozen in liquid nitrogen and then stored at –80°C until analyzed for mRNA of IGF-I and IGF-II and for receptors for GH, IGF-I, IGF-II, and insulin.

Ribonucleic Acid Extraction and mRNA Determinations

The total RNA extraction was performed in tissue samples from duodenum, jejunum, ileum, colon, and liver of all the animals using TRIzol reagent (Gibco BRL, Basel, Switzerland) according to the instructions of the manufacturer and was resuspended in Rnase-free water, treated with diethyl pyrocarbonate (Sigma-Aldrich Vertriebs GmbH, Deisenhofen, Germany). Integrity and purity of RNA were determined by the ratio of optical density measurements at 260 and 280 nm (OD 260 nm/280 nm) being greater than 1.9 and by electrophoresis using ethidium bromide staining. Reverse transcription reactions were performed using random hexamer primers (Pfaffl et al., 2002). Primers for PCR of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II and insulin were as described by Pfaffl et al. (2002). The real-time PCR (**RT-PCR**) quantification was performed with the LightCycler System (Roche Molecular Biochemicals, Rotkreuz, Switzerland) using software package 3.3 (Roche Molecular Biochemicals). The detailed procedures in RT-PCR assays, including generation of the external recombinant DNA standards used for the real-time mRNA absolute quantification, were recently published (Pfaffl et al. 2002).

Table 1. Composition of colostrum and milk replacer fed for 7 d to preterm calves (GrP_8)

Trait	Colostrum, first milking	Milk replacer[a]
Water, g/kg milk	760	905
DM, g/kg milk	240	95
Gross energy, MJ/kg of milk (MJ/kg of DM)	6.0 (24.9)	1.7 (18.3)
Crude protein, g/kg of milk (g/kg of DM)	133 (555)	21 (220)
Crude fat, g/kg of milk (g/kg of DM)	64 (265)	20 (210)
Nitrogen free extract, g/of milk (g/kg of DM)	25 (104)	27 (311)
Ash, g/kg milk (g/kg of DM)	18 (75)	25 (263)
IGF-I, μg/kg of milk	383	5

[a]The milk replacer contained (per kilogram): skim milk powder (550 g), whey (40 g), corn-derived products (dextrose, glucose, oat cream, starch; 172 g), tallow (145 g), lard (44 g), lecithin (as emulsifier; 19 g), calcium (12 g), phosphorus (7.5 g), magnesium (1.6 g), sodium (4.9 g), zinc (80 mg), manganese (60 mg), iron (20 mg), copper (8 mg), iodine (3 mg), selenium (0.5 mg), cobalt (0.5 mg), vitamin A (26.25 μmol of retinol equivalent), cholecalciferol (195 nmol), vitamin E (360 μmol of α-tocopherol equivalent), thiamine (57 μmol), riboflavin (21 μmol), vitamin B_6 (59 μmol), and vitamin B_{12} (37 nmol).

Feed Analyses

Aliquots of 50 mL of the first-milked colostrum pools and of the milk replacer were lyophilized. Dry matter and CP were determined by the Kjeldahl method, crude fat by Soxhlet extraction, and crude ash after combustion at 550°C. All analyses were performed using standard procedures at the Swiss Federal Research Station for Animal Production. Contents of water, nitrogen-free extracts (i.e., sugar and especially lactose) and GE (based on energy equivalents of 36.6, 17.0, and 24.2 MJ/kg of fat, nitrogen-free extracts, and CP, respectively) were evaluated.

Statistical Analyses

The results from real-time RT-PCR quantification on LightCycler are expressed as means ± SEM in fmol/μg of total RNA. Group differences of intestinal mRNA were evaluated using the RANDOM and REPEATED methods of the MIXED procedure with an interanimal random effect of differences between the animals and a correlation structure within animals (SAS Inst., Inc., Cary, NC). Age and gut segments were used as fixed effects within animals. Differences ($P < 0.05$) were evaluated by LSD (*t*-test). Group differences of liver mRNA were evaluated using the GLM procedure of SAS. Differences were evaluated by LSD (*t*-test). Differences were considered significant if $P < 0.05$. Data were logarithmically transformed for normal distribution.

Ubiquitin was selected and tested as control gene in intestine and liver and was measured by real-time PCR. In the liver, but not in the intestine, ubiquitin mRNA levels were modified ($P < 0.05$) by age and/or feeding. In the intestine, ubiquitin mRNA levels were affected ($P < 0.001$) by intestinal sites. Thus, ubiquitin was not a suitable housekeeping gene. The mRNA levels of IGF-I and IGF-II and for receptors of GH, IGF-I, IGF-II and insulin were therefore calculated based on standard curves exclusively (i.e., under these conditions ubiquitin as a housekeeping gene was not necessary).

Results

Amounts of mRNA of IGF-I and -II and Receptors for GH, IGF-I, IGF-II and Insulin in the Duodenum, Jejunum, Ileum, and Colon of Preterm Calves on d 1, of Full-Term Calves on d 1, and of Preterm Calves on d 8 of Life

Levels of IGF-I mRNA (Table 2) were different among intestinal sites (colon > duodenum, jejunum, ileum [$P < 0.001$]; duodenum, ileum > jejunum [$P < 0.05$]) and between groups ($GrP_o > GrN_o$ in ileum [$P < 0.001$]). In addition, there was a significant ($P < 0.05$) group × gut site interaction. Levels of IGF-II mRNA (Table 2) were different among intestinal sites (colon > duodenum, jejunum and ileum [$P < 0.001$]) and among groups ($GrP_o > GrN_o$ and GrP_8 in ileum [$P < 0.01$]). There was a significant ($P < 0.01$) group × gut site interaction.

Levels of IGF-I receptor mRNA (Table 3) were different among intestinal sites (colon > duodenum, jejunum and ileum [$P < 0.001$]; ileum > duodenum and jejunum [$P < 0.01$]) and between groups ($GrP_o > GrN_o$ in jejunum [$P < 0.05$], ileum [$P < 0.001$]; $GrP_o > GrP_8$ in ileum [$P < 0.001$]). There was a significant ($P < 0.01$) group × intestinal site interaction. Levels of IGF-II receptor mRNA (Table 3) were different among intestinal sites (colon > jejunum [$P < 0.05$]; ileum > duodenum [$P < 0.05$], and jejunum [$P < 0.01$]) and between groups [$GrP_o > GrN_o$ in ileum ($P < 0.01$); $GrP_o > GrP_8$ in ileum ($P < 0.01$)]. Levels of insulin receptor mRNA (Table 3) were different among intestinal sites (jejunum, ileum, colon > duodenum [$P < 0.001$]) and between groups ($GrP_o > GrN_o$ in ileum [$P < 0.01$]; $GrP_8 > GrP_o$ in duodenum [$P < 0.05$]). Levels of GH receptor mRNA (Table 3) were different among intestinal sites (colon > duode-

Table 2. Intestinal messenger RNA (mRNA) levels of IGF-I and IGF-II of preterm calves at birth (GrP_o), of full-term calves at birth (GrN_o), and in preterm calves on d 8 of life (GrP_8)

Trait	Groups[a]			Pooled SEM	Differences (*P*-values) between groups	
	GrP_o	GrN_o	GrP_8		GrP_o vs. GrN_o	GrP_8 vs. GrP_o
IGF-I mRNA, $\times 10^{-3}$ fmol/μg of total RNA						
Duodenum[cd]	38.12	13.54	17.53	8.64	NS	NS
Jejunum[e]	10.13	5.44	18.52	3.37	NS	NS
Ileum[d]	24.23	5.96	16.23	3.01	0.001	NS
Colon[b]	103.68	45.06	64.37	15.04	NS	NS
IGF-II mRNA, $\times 10^{-3}$ fmol/μg of total RNA						
Duodenum[c]	42.97	30.14	46.88	7.31	NS	NS
Jejunum[c]	47.24	29.27	74.83	12.85	NS	NS
Ileum[c]	146.13	21.38	54.97	16.70	0.001	0.01
Colon[b]	659.44	518.99	532.49	134.79	NS	NS

[a]Calves of GrP_o (n = 7) were born 2 wk preterm (after 277 d of pregnancy); those of GrN_o (n = 7) were born full-term (after 290 d of pregnancy) and were killed immediately after birth. Calves of GrC_8 (n = 7) were born preterm, fed first-milked colostrum for 3 d, and then first-milked colostrum, which was diluted with 25, 50, 75, and 75 parts of a milk replacer (whose nutrient content was similar to that of mature milk) on d 4, 5, 6, and 7, respectively, and were killed on d 8 of life.
[b,c,d,e]Within a column, means at the different intestinal sites for IGF-I and IGF-II with different superscript letters are different ($P < 0.05$). NS: $P > 0.05$.

num, jejunum and ileum [$P < 0.001$]; ileum > duodenum and jejunum [$P < 0.05$]; jejunum > duodenum [$P < 0.001$] and between groups ($GrP_o > GrN_o$ in jejunum [$P < 0.05$], ileum [$P < 0.001$]; $GrP_o > GrP_8$ [$P < 0.05$] in ileum and colon ; $GrP_8 > GrP_o$ in jejunum [$P < 0.001$]). In addition, there was a significant ($P < 0.001$) group × gut site interaction.

Amounts of mRNA of IGF-I and -II and for Receptors of GH, IGF-I, IGF-II and Insulin in the liver of Preterm Calves on d 1, of Full-Term Calves on d 1, and of Preterm Calves on d 8 of Life

Levels of mRNA of IGF-II and of receptors for IGF-I, IGF-II and insulin (Table 4) differed significantly

Table 3. Intestinal messenger RNA (mRNA) levels of receptors for IGF-I, IGF-II, insulin, and GH of preterm calves at birth (GrP_o), of full-term calves at birth, (GrN_o) and in preterm calves on d 8 of life (GrP_8)

Trait	Groups[a]			Pooled SEM	Differences (*P*-values) between groups	
	GrP_o	GrN_o	GrP_8		GrP_o vs. GrN_o	GrP_8 vs. GrP_o
IGF-I receptor mRNA, $\times 10^{-3}$ fmol/μg of total RNA						
Duodenum[d]	0.28	0.16	0.24	0.05	NS	NS
Jejunum[d]	0.32	0.25	0.45	0.11	0.05	NS
Ileum[c]	1.08	0.28	0.33	0.08	0.001	0.01
Colon[b]	4.28	4.63	4.25	1.27	NS	NS
IGF-II receptor mRNA, $\times 10^{-6}$ fmol/μg of total RNA						
Duodenum[cd]	2.96	1.64	1.79	0.44	NS	NS
Jejunum[d]	2.26	1.91	2.08	0.68	NS	NS
Ileum[b]	7.92	2.19	2.50	0.72	0.01	0.01
Colon[bc]	6.99	2.48	3.98	1.65	NS	NS
Insulin receptor mRNA, $\times 10^{-3}$ fmol/μg of total RNA						
Duodenum[c]	4.17	4.91	9.66	1.75	NS	0.05
Jejunum[b]	21.34	11.87	23.28	4.82	NS	NS
Ileum[b]	19.90	6.32	15.87	2.82	0.01	NS
Colon[b]	12.95	14.17	18.74	3.05	NS	NS
GH receptor mRNA, $\times 10^{-3}$ fmol/μg of total RNA						
Duodenum[e]	0.35	0.10	0.40	0.14	NS	NS
Jejunum[d]	0.57	0.31	2.42	0.34	0.05	0.001
Ileum[c]	2.91	0.25	1.17	0.21	0.001	0.05
Colon[b]	7.31	5.10	3.80	1.37	NS	0.05

[a]Calves of GrP_o (n = 7) were born 2 wk preterm (after 277 d of pregnancy), and those of GrN_o (n = 7) were born full-term (after 290 d of pregnancy) and were killed immediately after birth. Calves of GrC_8 (n = 7) were born preterm, fed first-milked colostrum for 3 d, and then first-milked colostrums that was diluted with 25, 50, 75, and 75 parts of a milk replacer (whose nutrient content was similar to that of mature milk) on d 4, 5, 6, and 7, respectively, and were killed on d 8 of life.
[b,c,d,e]Within a column, means at the different intestinal sites for IGF-I, IGF-II, insulin, and GH receptors with different superscript letters are different ($P < 0.05$). NS: $P > 0.05$.

Table 4. Hepatic messenger RNA (mRNA) levels of IGF-I and IGF-II and of receptors for IGF-I, IGF-II, insulin, and GH of preterm calves at birth (GrP_o), of full-term calves at birth (GrN_o), and of preterm calves on d 8 of life (GrP_8)

	Groups[a]			Pooled	Group differences (*P*-values)	
Trait	GrP_o	GrN_o	GrP_8	SEM	GrP_o vs. GrN_o	GrP_8 vs. GrP_o
IGF-I[b]	113	152	56	34	NS[d]	NS
IGF-II[c]	8,171	1,197	1,195	1,712	0.001	0.001
IGF-I receptor[b]	0.33	1.28	0.19	0.07	0.001	0.05
IGF-II receptor[b]	81	98	46	9	NS	0.05
Insulin receptor[b]	36.6	22.4	29.4	3.1	0.01	NS
GH receptor[b]	24.7	16.3	13.9	3.3	NS	NS

[a]Calves of GrP_o (n = 7) were born 2 wk preterm (after 277 d of pregnancy), and those of GrN_o (n = 7) were born full-term (after 290 d of pregnancy) and were killed immediately after birth. Calves of GrC_8 (n = 7) were born preterm, fed first-milked colostrum for 3 d, and then first-milked colostrums that was diluted with 25, 50, 75, and 75 parts of a milk replacer (whose nutrient content was similar to that of mature milk) on d 4, 5, 6, and 7, respectively, and were euthanized on d 8 of life.
[b]$\times 10^{-3}$ fmol/μg of total RNA.
[c]$\times 10^{-6}$ fmol/μg of total RNA.
[d]NS: $P > 0.05$.

between groups (IGF-II: $GrP_o > GrN_o$ [$P < 0.01$] and GrP_8 [$P < 0.001$]; IGF-IR: $GrN_o > GrP_o$ [$P < 0.001$] and $GrP_o > GrP_8$ [$P < 0.05$]; IGF-II receptor: $GrP_o > GrP_8$ [$P < 0.05$]; insulin receptor: $GrP_o > GrN_o$ [$P < 0.01$]). There were no significant group differences in the mRNA levels of receptors for GH and IGF-I.

Discussion

This study shows that there is abundance of mRNA of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin at all intestinal sites in preterm and full-term calves at birth. Interestingly, mRNA levels of all traits increased from proximal to distal regions of the intestine, such that the colon had numerically or significantly higher mRNA levels of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin than the small intestine. A similar pattern of distribution of the different members of the GH-IGF family has been described in rat intestine (MacDonald, 1999). Among the different parts of the small intestine, the ileum exhibited the highest mRNA levels of all receptors. The different mRNA levels of receptors suggest site-specific differences in actions of GH, IGF-I, IGF-II, and insulin. Differences in mRNA levels of receptors may also be the expression of differences in mRNA turnover rates (Georgiev et al., 2003).

This study also demonstrates differences dependent on the ontogenetic state of intestinal (especially ileum) and hepatic mRNA levels of IGF-I and IGF-II and of receptors for GH, IGF-I, IGF-II, and insulin. Thus, on d 1 of life in the ileum, the mRNA levels of all factors in preterm calves were consistently higher than in full-term calves. Ontogenetic changes of different members of the GH-IGF system in the gastrointestinal tract and liver have been described in other species, such as pigs, rats, and humans (Peng et al., 1996; Shoba et al., 1999; Goodyer et al., 2001). Differences seen were possibly also an expression of differences in mRNA turnover rates.

The higher mRNA levels of IGF-I in intestine, but not in liver, of preterm than in full-term calves at birth supports evidence for autocrine and/or paracrine roles of IGF-I for the regulation of intestinal growth. It underlines that IGF-I is important for preterm intestinal activities. The finding may be related to enhanced proliferation and/or decreased apoptosis of intestinal cells. This would be in line with studies in mice that lack hepatic IGF-I production but are normally growing, showing that nonhepatic IGF-I may be sufficient for normal development (Yakar et al., 1999; 2000; Butler and LeRoith, 2001a). However, circulating IGF-I concentrations, which are thought to depend mainly on hepatic production (LeRoith et al., 2001) in cattle, are closely correlated with hepatic IGF-I mRNA levels and increase with body mass (Ronge and Blum, 1989; Cordano et al., 2000). Differences in abundance of IGF-I mRNA levels may have been also associated with differences of mRNA stability. That mRNA levels of IGF-II were highest in both intestine and liver is in accordance with previous data in rats, humans, and calves (MacDonald, 1999; Goodyer et al., 2001; Pfaffl et al., 2002). The data are also in agreement with data showing that IGF-II is primarily responsible for the regulation of intra-uterine tissue growth and that its role decreases after birth (D'Ercole, 1996; Wolf et al., 1998; Breier et al., 2000; Butler and LeRoith, 2001b).

The abundance of mRNA of the receptor for GH in the intestine and liver of pre- and full-term calves suggests a role of GH in the perinatal development of

these organ, as described in rats, pigs and humans (Peng et al., 1996; Zogopulos et al., 1996; Phornphutkul et al., 2000; Goodyer et al., 2001). This is supported by studies in neonatal calves, in which GH administration enhanced small intestinal crypt sizes (Bühler et al., 1998). Because mRNA levels of receptors for IGF-I and IGF-II in the intestine of preterm calves were higher than in full-term calves on d 1, whereas in the liver, the level of mRNA for the IGF-I receptor increased from pre- to full-term calves, but for IGF-II, did not change, different roles of both receptors in liver and intestine, dependent on the ontogenetic state, are likely. Insulin is required for normal tissue growth and mediates its effects through the insulin receptor. However, an important function of the insulin receptor during prenatal, and possibly perinatal, stages is to mediate the action(s) of IGF-II (Wolf et al., 1998; Rother and Accili, 2000; Nakae et al., 2001). Interestingly, higher intestinal and hepatic mRNA levels for the insulin receptor in preterm vs. full-term calves were accompanied by corresponding changes of IGF-II mRNA levels, suggesting that there may be functional interactions between IGF-II and the insulin receptor in the intestine of neonatal calves (Georgiev et al., 2003).

Data on d 8 vs. d 1 of life of preterm calves show a general decrease in the abundance of mRNA of IGF-II and of receptors for IGF-I and IGF-II in the intestine and liver, suggesting a decreasing role of IGF-II in these organs. Intestinal mRNA levels of the receptor for GH showed different patterns during the first 8 d of life (i.e., an increase in jejunum and a decrease in ileum and colon, possibly indicating a variable action of GH in the upper and lower intestinal tract). A similar decrease in the abundance of mRNA of the GH receptor in ileum during the perinatal period has been described in rats (Walker et al., 1992). Interestingly, no ontogenetic changes in hepatic mRNA levels for the GH receptor and for IGF-I were found, showing that the hepatic GH-IGF-I axis during the perinatal period was not yet mature, as demonstrated for full-term calves (Hammon and Blum, 1997). The lack of differences between hepatic mRNA levels for the GH receptor on d 1 and d 8 in preterm calves is supported by data in rats (Phornphutkul et al., 2000).

The ontogenetic differences in the mRNA levels of receptors for GH, IGF-I, IGF-II, and insulin could be in part due to the differences in the hormonal status in pre- and full-term calves since the marked rise in the concentrations of cortisol and triiodothyronine close to term has been shown to play an important role in the maturation of the GH-IGF system and to activate tissue expressions of growth factors and their receptors (Forhead et al., 1998; Li et al., 1999; 2002; Sangild, 2001; Sauter et al., 2003). In the present study, obvious differences in preterm calves compared with full-term calves have, however, not been demonstrated (Bittrich et al., 2002). Nevertheless, the proliferation and morphological reactions and changes of brush border enzyme activities of the intestinal epithelium to ingestion of colostrum and milk replacer during the first week of life are in part reduced when compared with full-term calves (Bittrich et al., 2001).

Implications

This study confirms the hypothesis that there are differences in the abundance of messenger ribonucleic acid of insulin-like growth factor-I and -II and of receptors for growth hormone, insulin-like growth factor-I, insulin-like growth factor-II, and insulin at the different intestinal sites and liver (especially in the ileum) in the perinatal period that depend on the ontogenetic state. Additional studies are needed that include turnover rates of messenger ribonucleic acid, how messenger ribonucleic acid levels are related to insulin-like growth factor-I and -II and receptors for growth hormone, insulin-like growth factor-I, insulin-like growth factor-II, and insulin at the protein level, how they are associated with biological functions, and how nutrition modifies the abundance of messenger ribonucleic acid of the growth hormone– insulin-like growth factor axis in the postnatal period of pre- and full-term calves.

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