ABSTRACT

Hormones and metabolites act as satiety signals in the brain and play an important role in the control of feed intake (FI). These signals can reach the hypothalamus and brainstem, 2 major centers of FI regulation, via the blood stream or the cerebrospinal fluid (CSF). During the early lactation period of high-yielding dairy cows, the increase of FI is often insufficient. Recently, it has been demonstrated that insulin-like growth factors (IGF) may control FI. Thus, we asked in the present study if IGF-binding proteins (IGFBP) are regulated during the periparturient period and in response to feed restriction and therefore might affect FI as well. In addition, we specifically addressed conditional distribution of IGFBP in plasma and CSF. In one experiment, 10 multiparous German Holstein dairy cows were fed ad libitum and samples of CSF and plasma were obtained before morning feeding on d −20, −10, +1, +10, +20, and +40 relative to calving. In a second experiment, 7 cows in second mid-lactation were sampled for CSF and plasma after ad libitum feeding and again after feeding 50% of the previous ad libitum intake for 4 d. Intact IGFBP-2, IGFBP-3, and IGFBP-4 were detected in plasma by quantitative Western ligand blot analysis. In CSF, we were able to predominantly identify intact IGFBP-2 and a specific IGFBP-2 fragment containing detectable binding affinities for biotinylated IGF-II. Whereas plasma concentrations of IGFBP-2 and IGFBP-4 increased during the periparturient period, IGFBP-3 was unaffected over time. In CSF, concentrations of IGFBP-2, both intact and fragmented, were not affected during the periparturient period. Plasma IGF-I continuously decreased until calving but remained at a lower concentration in early lactation than in late pregnancy. Food restriction did not affect concentrations of IGF components present in plasma or CSF. We could show that the IGFBP profiles in plasma and CSF are clearly distinct and that changes in IGFBP in plasma do not simply correspond in the brain. We thus assume independent control of IGFBP distribution between plasma and CSF. Due to the known anorexic effect of IGF-I, elevated plasma concentrations of IGFBP-2 and IGFBP-4 during the postpartum period in conjunction with reduced plasma IGF-I concentrations may be interpreted as an endocrine response against negative energy balance in early lactation in dairy cows.

Key words: insulin-like growth factor, insulin-like growth factor binding proteins, cerebrospinal fluid, transition period, feed restriction

INTRODUCTION

The increase in feed intake (FI) during the early lactation period of high-yielding dairy is often insufficient to meet the energy requirements for milk production and, as a consequence, cows go into negative energy balance (EB). Negative EB is characterized by altered circulating concentrations of numerous metabolites and hormones (Andersson, 1988; LeBlanc, 2010), such as increased plasma NEFA and BHBA and reduced glucose concentrations. Some of these metabolites and hormones may act as satiety signals directly in the hypothalamus (Sartin et al., 2010; Relling et al., 2012), one of the major centers controlling FI in ruminants. The blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier control the concentrations of metabolites and hormones in the brain and in CSF and, thus, CSF concentrations differ from those in blood during the transition period or after feed restriction (Laeger et al., 2012, 2013). It has been demonstrated that IGF may also influence FI. At least in rodents,
administration of IGF into either circulation or CSF was shown to decrease FI (Tannenbaum et al., 1983; Vickers et al., 2001). However, the potential role of IGF-binding proteins (IGFBP) in CSF as signals affecting FI during the transition period of dairy cows has not been studied.

Insulin-like growth factor I is primarily produced by the liver and plays an important role in growth and metabolic processes (Górecki et al., 2007). Its production is affected by the diet and reduced after fasting and in early lactation (Sander et al., 2011; Piechotta et al., 2013). Insulin-like growth factor I exists almost entirely bound to binding proteins (IGFBP), which modulate its ligand-receptor interactions (Shimasaki and Ling, 1991; Górecki et al., 2007). Furthermore, IGFBP serve as carrier proteins for IGF-I to cross the BBB (Riikonen, 2006). Currently, 6 different IGFBP (IGFBP-1 to IGFBP-6) are known, which are not present simultaneously in circulation and bind almost all of the circulating IGF; hence, very little (<1%) unbound IGF is present (Rajaram et al., 1997). In humans, IGFBP-3, primarily produced by the liver (Ferry et al., 1999), is the most abundant IGFBP, accounting for 75 to 80% of all IGF binding. Circulating IGFBP-3 together with the acid-labile α-subunit and IGF form a 150- to 200-kDa complex that prolongs the half-life of IGF and alters its interaction with cell surface receptors (Rajaram et al., 1997). Accordingly, 20 to 25% of the IGF are bound to one of the remaining IGFBP (Guler et al., 1989). Besides in plasma, several different IGFBP have been identified in CSF, in which IGFBP-2 is the major form in humans (Binoux et al., 1991). The IGFBP found in the CSF are suggested to be synthesized locally by glial cells and neurons rather than derived from plasma crossing the BBB (Ocrant et al., 1990).

Different physiological conditions, such as diurnal rhythm, nutrition, exercise, and pregnancy have been reported to regulate IGFBP (Rajaram et al., 1997), but how the transition from late pregnancy to early lactation affects the concentration of IGFBP in dairy cows has not yet been studied. Under conditions of negative EB, altered levels of IGFBP are found in different compartments and thereby IGFBP may block anabolic or anorexic effects of IGF-I. Therefore, the main objective of this study was to investigate the expression and distribution of IGFBP in plasma and CSF during the periparturient period and in response to feed restriction-induced negative EB.

**MATERIALS AND METHODS**

**Animals, Husbandry, Feeding, and Sampling**

For the first experiment, 10 German Holstein dairy cows in second (n = 9) and third (n = 1) parturition were kept in tie-stalls in accordance with the guidelines for the use of animals as experimental subjects of the State Government in Mecklenburg-West Pomerania (Germany; registration no. LALLF M-V/TSD/7221.3-2.1-001/10). All cows were healthy and 44 to 52 mo old. They were fed twice daily (0700 and 1600 h) a TMR consisting of corn and grass silage, grass hay, grain feed, minerals, and vitamins, to meet the energy and nutrient recommendations of dairy cows calculated according to the German Society of Nutrition Physiology [2001; 6.4 MJ of NEL/kg of DM for the last 25 d of gestation (close-up period) and 7.2 MJ of NEL/kg of DM for lactation]. Feed was available ad libitum at all times. Cows were sampled for CSF from the spinal cord and for blood EDTA plasma from the jugular vein before morning feeding on d −20 (−23.1 ± 4.8; mean ± SD), −10 (−11.8 ± 4.2), +1, +10, +20, and +40 relative to calving, as described previously (Laeger et al., 2013). Cows had free access to water and were milked twice daily (0630 and 1530 h). The daily milk yield and daily FI were measured individually. Body weight was measured once per week.

To calculate EB, milk was analyzed for fat, protein, and lactose content by an infrared spectrophotometric method (MilkoScan; Foss GmbH, Rellingen, Germany) at the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V. (Güstrow, Germany). Energy-corrected milk was calculated as follows: ECM (kg) = (0.038 × g of fat + 0.024 × g of protein + 0.017 × g of lactose) × kg of milk/3.14. Energy balance [MJ of NEL/(cow × d)] antepartum (EBap) and postpartum (EBpp) was calculated as follows: EBap = NEI intakes (MJ) − 0.46 × kg of BW⁰.⁷⁵ and EBpp = NEI intake (MJ) − (ECM × 3.14 + 0.293 × kg of BW⁰.⁷⁵) (Reist et al., 2002). All cows were in positive EB prepartum and in negative EB (Table 1) until the end of the sampling period, as described previously (Laeger et al., 2013).

For the second experiment, 7 German Holstein dairy cows (42 to 50 mo old) between 87 and 96 d of the second lactation were fed ad libitum, as described above. After local anesthesia, CSF and blood EDTA plasma was withdrawn before morning feeding. Afterward, animals were feed restricted to 50% of the previous ad libitum intake for 4 d to induce a negative EB [−28.7 MJ of NEI/(cow × d)], as described previously (Laeger et al., 2012), and subsequently sampled again for CSF and plasma.

**Quantitative Western Ligand Blot Analysis of IGFBP**

Insulin-like growth factor-binding proteins were analyzed in plasma and CSF by quantitative Western ligand blot analysis, as described previously (Hossenlopp...
et al., 1986; Metzger et al., 2011). Briefly, plasma and CSF samples and serial dilutions of recombinant human IGFBP standards (R&D Systems GmbH, Wiesbaden, Germany) were diluted 1:20 and 1:1.25, respectively, boiled in sample buffer [312.5 mM Tris (pH 6.8), 50% (wt/vol) glycerol, 5 mM EDTA (pH 8), 1% (wt/vol) SDS, and 0.02% bromophenol blue] for 5 min. Proteins were separated by SDS-PAGE (Laemmli, 1970) and then transferred onto a polyvinylidene fluoride membrane (Millipore Corp., Bedford, MA). The blots were blocked and then incubated with biotin-labeled human IGFBP-2 (1:500; BioIGF2-10; Ibt GmbH, Binzwangen, Germany). The membrane was then washed 5 times and incubated with horseradish peroxidase conjugated streptavidin (1:2,500; Ibt Systems GmbH). All incubation and washing steps were performed at room temperature. Finally, the membrane was washed 5 times and the binding proteins were detected by enhanced chemiluminescence using the reagent Luminata Forte (Millipore, Danvers, MA) at room temperature. Finally, the membrane was washed 5 times and IGFBP-2 was detected as described above.

**Immunoblot Determination of IGF-I**

For the determination of total plasma IGF-I concentration, an ACTIVE IGF-I coated tube immunoradiometric assay (DSL-5600; Diagnostic Systems Laboratories Inc., Webster, TX) was used according to Sander et al. (2011). Separation of IGF-I from its binding proteins was done by an acid-ethanol extraction procedure and IGF-I concentrations were determined with a 2-site immunoradiometric assay as described previously (Sander et al., 2011). The intra- and interassay coefficients of variation were 1.5 to 3.5 and 1.5 to 8.5%, respectively. Due to the limited volume of CSF available, analyses of IGF-I concentration could not be performed.

**Statistical Analyses**

Statistical evaluation was performed using SAS (version 9.3; SAS Institute Inc., Cary, NC). For the first experiment, all response variables were analyzed by repeated-measures ANOVA, applying PROC GLIMMIX. Repeated measures on the same animal were taken into account by the residual option in the random statement of PROC GLIMMIX to construct the block diagonal structure of the residual covariance matrix for each animal. The 2-way ANOVA model for analysis of IGFBP contained the fixed effects body fluid (levels: plasma and CSF), day relative to parturition (levels: −20, −10, +1, +10, +20, and +40), and their interaction. Multiple pairwise comparisons were performed using the Tukey-Kramer procedure. In addition, a model with the fixed effect body fluid (levels: plasma and CSF), the covariate day relative to parturition (d−20, −10, +1, +10, +20, and +40), and the interaction body fluid × day was used to estimate, test, and compare the intercepts of both body fluids. Data originating from the second experiment (ad libitum vs. restrictive feeding) was statistically analyzed using the Kolmogorov-Smirnov test for normal distri-

### Table 1. Energy Balance (EB) of dairy cows at the days relative to parturition of cerebrospinal fluid (CSF) and plasma sampling

<table>
<thead>
<tr>
<th>Day</th>
<th>EB [MJ of NE(_d)/ (cow × d)]</th>
</tr>
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<tbody>
<tr>
<td>−20</td>
<td>28.4 ± 6.0</td>
</tr>
<tr>
<td>−10</td>
<td>17.2 ± 5.8</td>
</tr>
<tr>
<td>+1</td>
<td>−7.0 ± 10.8</td>
</tr>
<tr>
<td>+10</td>
<td>−61.0 ± 8.9</td>
</tr>
<tr>
<td>+20</td>
<td>−57.2 ± 14.9</td>
</tr>
<tr>
<td>+40</td>
<td>−42.1 ± 4.8</td>
</tr>
</tbody>
</table>

\(1\) Data are presented as the mean ± SE (n = 10). The EB significantly differed over time at \(P < 0.05\) (ANOVA).

**Western Immunoblot Analysis**

Insulin-like growth factor-binding protein 2 was analyzed in CSF by Western immunoblot analysis as described previously in Hoeflich et al. (2004). For sample preparation, SDS PAGE, and protein transfer, the same procedure was used as described for Western ligand blotting. Then, the blots were blocked and incubated with a rabbit anti-bovine IGFBP-2 antibody (1:1,000; 06–107; Merck-Millipore, Darmstadt, Germany) overnight at 4°C. The membrane was washed 5 times and then incubated with HRP conjugated goat anti-rabbit IgG (1:2,500; 7074; Cell Signaling Technology Inc., Danvers, MA) at room temperature. Finally, the membrane was washed 5 times and IGFBP-2 was detected as described above.
bution with subsequent paired t-test to compare means between feeding states in plasma and CSF separately. A statistical difference was considered as significant if $P \leq 0.05$ and as a trend if $0.05 < P < 0.10$.

RESULTS

Both in plasma and CSF, IGFBP-2, IGFBP-3, and IGFBP-4 were detectable. However, the comparison between both body fluids revealed that during the periparturient period, IGFBP concentrations differed significantly between CSF and plasma, with the exception that exclusively IGFBP-2 was at comparable concentrations both in plasma and CSF at distinct time points (Figure 2). In plasma, concentrations of IGFBP-2 and IGFBP-4 increased starting from late pregnancy until d 10 postpartum ($P < 0.05$; Tukey test: $-20$ and $-10$ vs. 10; Figure 2) and declined thereafter. The IGFBP-2 and IGFBP-4 concentrations in CSF did not change during the periparturient period. Thus, the courses of IGFBP-2 and IGFBP-4 concentrations in plasma and CSF did not run parallel to each other over time (Figure 2). In contrast, the plasma IGFBP-3 concentration was lowest on the first day after calving ($P < 0.05$;
Tukey test: −20, −10, 10, and 20 vs. 1; Figure 2). In CSF, IGFBP-3 was below the limit of quantification. The band below the 24-kDa-molecular-weight marker (approximately 20 kDa), which contained detectable affinities for biotinylated IGF-II was identified as an IGFBP-2 fragment by use of specific antibodies directed against the carboxyl-terminal part of IGFBP-2. Plasma IGF-I concentration continuously decreased after calving and remained lower in early lactation than in late pregnancy (P < 0.05; Tukey test: −20 and −10 vs. 10, 20, and 40; Figure 2). Also during feed restriction between 87 and 96 d of lactation, intact IGFBP-2, IGFBP-3, and IGFBP-4 were detected in plasma and CSF. Again, IGFBP-2 was the dominant IGFBP within CSF and present in an intact and fragmented form. In plasma, IGFBP-4 was present at the highest concentrations, whereas IGFBP-2 and IGFBP-3 concentrations were comparable to each other. Feed restriction did not affect plasma concentrations of IGFBP or IGF-I (Figure 3). Within the CSF, IGFBP-2 and IGFBP-4 concentrations were unaffected after feed restriction in CSF (Figure 3), whereas concentrations of IGFBP-3 were below the limit of quantification in 6 of 7 samples from the CSF. The IGFBP-2 and IGFBP-4 concentrations differed significantly between plasma and CSF (Figure 3).

DISCUSSION

IGFBP-2

An important role of the IGF system in producing the anabolic effects on maternal and fetal tissues was suggested (Rajaram et al., 1997). In human serum, IGFBP-2 concentration steadily decreased throughout gestation due to the presence of IGFBP-2-specific proteases (Giudice et al., 1990), which is in accordance with the present finding with a lower plasma IGFBP-2 concentration before calving. On the other hand, studies in humans revealed that protein restriction (Smith et al., 1995) and prolonged fasting for more than 1 wk (Thissen et al., 1994) increased the serum IGFBP-2 concentration. Also, in Holstein steers fed a low-protein diet, an increased plasma IGFBP-2 concentration was observed (Lee et al., 2005). Thus, we cannot exclude that negative EB during a 4-d period of restricted feeding may not be sufficient for an increase in IGFBP-2 concentration in plasma, especially as increased plasma IGFBP-2 concentrations were present during negative EB in early lactation. Nutrition-induced changes in serum IGFBP-2 concentration appear to be the direct effect of dietary protein on IGFBP-2 expression in the liver (Rajaram et al., 1997), whereas pregnancy-related differences are due to active degradation, as discussed earlier.

In humans, age-related changes in the BBB function are supposed to account for increased CSF IGFBP-2 concentrations originating from blood (Pirttilä et al., 2004). Based on the observation that the plasma IGFBP-2 concentration increases after parturition, whereas the CSF IGFBP-2 concentration is unaffected during the periparturient period, it may be possible that IGFBP-2 does not cross the BBB in dairy cows. Astrocytic glial cells as the dominant cell type of the brain are known to produce IGFBP-2 and, thus, are an abundant source of local IGFBP-2 (Olson et al., 1991). In addition, the choroid plexus epithelium is capable of synthesizing and secreting IGFBP-2, as shown in sheep (Chen et al., 2008). In both conditions of negative EB, central IGFBP-2 concentrations were unaffected and, thus, may not be involved in acute control of energy homeostasis. In CSF, we were able to identify a fragment of IGFBP-2 characterized by a molecular weight of approximately 20 kDa. An IGFBP-2 fragment with a corresponding size was also identified in the perinuclear fraction isolated from mouse brains (Hoeflich et al., 2004). Both in mice and cows, the IGFBP-2 fragment still has detectable affinities for IGF-II, which is probably due to the presence of at least 1 of the 2 known IGF-binding domains in the intact protein. Moreover, this IGFBP-2 fragment contains the carboxyl-terminal part of IGFBP-2, which carries a nuclear localization signal (Azar et al., 2013). The presence of this structural element, therefore, may also allow for biological effects in the brain that are independent of the presence of bound IGF-II.

IGFBP-3

In growing Holstein steers (Thissen et al., 1994) or in prepubertal Friesian heifer calves (Vestergaard et al., 2003) adapted to a low FI, it has been demonstrated that chronic dietary restriction decreases serum IGFBP-3 concentration. While an acute setting of feed restriction over a period of 4 d did not result in significant differences in plasma IGFBP-3 concentrations, the nadir of plasma IGFBP-3 concentration coincides with the nadir of IGF-I concentration around parturition and may reflect inadequate FI and negative EB 1 d after calving. However, altered expression of IGFBP-3 by different tissues may also explain the fluctuations of IGFBP-3 concentrations in the serum. Interestingly, hepatic IGFBP-3 mRNA expression (Loor et al., 2005) and protein expression of IGFBP-3 in the mammary gland (Skaar et al., 1991) in dairy cows also reached a nadir around parturition and may, thus, be related
Figure 2. Periparturient plasma and cerebrospinal fluid (CSF) IGF-binding protein (IGFBP) and IGF-I concentrations of dairy cows (n = 10). Insulin-like growth factor-binding protein 3 concentrations in the CSF were below the limit of quantitation for the majority of samples. Data are presented as the mean ± SE; * indicates a significant slope ($P \leq 0.05$) of the trend line; # indicates $0.05 < P < 0.1$; ‡ indicates significantly different slopes of plasma versus CSF trend lines. The IGFBP protein expression was analyzed by quantitative Western ligand blot (A). Protein expression of functional IGFBP in plasma and CSF of 1 representative animal on different days relative to parturition is shown (B). Identification of intact and fragmented IGFBP-2 in the CSF of periparturient dairy cows by combined Western ligand (left panel) and immunoblotting (right panel) is also shown (C). Insulin-like growth factor-binding protein 2 was identified by specific IGFBP-2 antibodies, as described in the Materials and Methods section. rh = recombinant human.
to altered serum levels of IGFBP-3, as discussed later. Antepartum, IGFBP-3 serum concentrations are progressively reduced between d 244 and 275 of gestation (Piechotta et al., 2013). Therefore, the low periparturient concentrations of IGFBP-3 at about 1 μg/mL within the circulation as observed in the present study may be caused by the decline in IGFBP-3 concentrations in the last trimester of pregnancy (Piechotta et al., 2013). During pregnancy, IGFBP-3 is proteolytically cleaved (Giudice et al., 1990; Hossenlopp et al., 1990). The low concentrations of functionally intact IGFBP-3 in cow serum or plasma around parturition may, thus, be due to pregnancy-associated proteases, which have been identified in dominant follicles derived from cattle (Santiago et al., 2005; Luo et al., 2011). Insulin-like growth factor-binding protein 3 is detectable by Western ligand blotting in human CSF (Arnold et al., 1999), and also is a component of the CSF from dairy cows in mid lactation after feed restriction.

**IGFBP-4**

A study in growing Holstein steers revealed a reduction in plasma IGFBP-4 concentration under a chroni-
IGF-I

To specifically address the potential effects of altered IGFBP profiles in circulation, we also studied plasma IGF-I concentrations in negative-EB cows. In lactating dairy cows, lower levels of IGF-I have been detected compared with dry cows (Ronge and Blum, 1989). Notably, undernutrition in beef cattle impaired the IGF-I response to exogenous growth hormone (GH) injection (Elsasser et al., 1989). Under negative EB, the dissociation of the GH/IGF regulatory network was also observed in humans (Merimee et al., 1982). As also found in studies with periparturient and preparturient dairy cows by Skaar et al. (1991), Sander et al. (2011), and Piechotta et al. (2012), IGF-I plasma concentration was higher antepartum and remained lower in early lactation than in late pregnancy. However, the baseline of IGF-I concentration was higher in our study, which may be the result of different ages of the animals (Konigsson et al., 2008) or crossbreds used in other studies. The present study is in accordance with the revealed negative correlation between IGF-I and IGFBP-2 concentrations (Rajaram et al., 1997). When energy or protein intake is restricted, IGF-I concentration in serum is decreased in nonruminants (Thissen et al., 1994). The latter study is in accordance with the persistent lower plasma IGF-I concentration during the negative EB after parturition observed here, whereas no changes after feed restriction for 4 d could be shown.

As demonstrated in rats, subcutaneous infusions of recombinant human IGF-I (Vickers et al., 2001) and intracerebroventricular administration of IGF (Tannenbaum et al., 1983) resulted in a reduction in FI. By contrast, in beef calves IGF-I is positively correlated with FI (Blanco et al., 2010). Thus, lower serum IGF-I may passively or actively reflect altered FI around parturition. The specific effect of exogenous IGF-I on FI in cattle has not been assessed to the best of our knowledge. Whether or not the CSF concentration of IGF-I changes during the periparturient period in cows remains to be determined.

IGFBP and IGF-I

It is clear that the relative contribution of the different IGFBP to the global IGFBP profile in plasma is greatly shifted in the periparturient period. While in the circulation, the concentration of the higher-molecular-weight IGFBP-3 is progressively reduced and maintained at a reduced level and the amounts of the smaller IGFBP forms (IGFBP-2 and IGFBP-4) are increased. Corresponding changes in serum concentrations for IGFBP and IGF-I are in line with results of Skaar et al. (1991). In that study, mammary tissue levels of IGFBP also corresponded with those present in the circulation. Particularly in the colostrum, high amounts of IGF-binding activity for iodinated IGF-II have been found. By means of a direct comparison of the different binding activities present in mammary gland secretions, in fact, a shift from higher-molecular-weight (42–46 kDa) IGFBP to lower-molecular-weight (30 and 34 kDa) IGFBP was described around parturition (Skaar et al., 1991). Our data not only are in line with that particular study but also support the idea of Skaar et al. (1991) that negative EB may be causative for the dynamic IGFBP concentrations around parturition. Notably, also in the liver of dairy cows, abundant adaptations of gene networks, including the IGF system, have been observed in the early postpartum period, which is characterized by negative EB (McCarthy et al., 2010). More specifically, a shift from high- to low-molecular-weight IGFBP is observed in the liver of lactating dairy cows with severe negative EB (Fenwick et al., 2008). In the liver of these animals, gene expression was increased for IGFBP-2 but decreased for IGFBP-3. The reduction of IGFBP-3 as a compound from the ternary complex may result in a severe reduction of IGF-I half-life, which could be responsible for the reduced IGF-I concentrations, as found in our study. However, reduced IGF-I serum levels also may derive from reduced gene expression under negative EB in periparturient dairy cows (Fenwick et al., 2008). Lower IGFBP-3 gene expression and increased gene expression of IGFBP-2 in the liver of periparturient dairy cows were also confirmed by other groups (Gross

et al., 2011). However at the same time, Gross et al. (2011) further described decreased expression of GH receptor mRNA expression in the liver, which might nicely explain the molecular basis of altered GH sensitivity during negative EB, characterized by lower levels of IGF-1 and IGFBP-3 on one hand and higher levels of IGFBP-2 on the other. Altogether, the alterations of the IGFBP profile during the periparturient period may result in a reduction of anabolic or anorectic IGF effects, potentially independent of GH. This potential GH-independent mechanism was not disproven by our 4-d feed restriction experiment, as we cannot exclude that the duration of the experiment may have been related to the absence of effects both on IGFBP and IGF-I concentrations.

CONCLUSIONS

We investigated concentrations of IGFBP in plasma and in CSF of dairy cows during the periparturient period and after ad libitum and restrictive feeding of lactating cows. Restrictive feeding for 4 d did not lead to concentration responses of IGF-I and IGFBP in plasma or in CSF. We demonstrated that IGFBP-2 and IGFBP-4 concentrations differ between CSF and plasma and showed different responses during the periparturient period in plasma compared with CSF of dairy cows. This is presumably due to the different permeability of the BBB bordering the periphery and the brain. We propose that increased plasma IGFBP-2 and IGFBP-4 concentrations in conjunction with reduced plasma IGF-1 concentration may serve as antianorectic signals under the condition of negative EB during early lactation in dairy cows.

ACKNOWLEDGMENTS

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