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Effects of parturition and feed restriction on concentrations and distribution of the insulin-like growth factor-binding proteins in plasma and cerebrospinal fluid of dairy cows

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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,

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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 NE_L/kg of DM for the last 25 d of gestation (close-up period) and 7.2 MJ of NE_L/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 NE_L/(cow × d)] antepartum (EBap) and postpartum (EBpp) was calculated as follows: EBap = NE_L intake (MJ) − 0.46 × kg of $BW^{0.75}$ and EBpp = NE_L intake (MJ) − (ECM × 3.14 + 0.293 × kg of $BW^{0.75}$) (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 NE_L/(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