Effect of feed restriction on metabolites in cerebrospinal fluid and plasma of dairy cows

T. Laeger, S. Görs, C. C. Metges, and B. Kuhla¹

Research Unit Nutritional Physiology "Oskar Kellner," Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

ABSTRACT

Endocrines and metabolites in the circulation act as long-term hunger or satiety signals in the brain during negative energy balance and play an important role in the control of feed intake. These signals also occur in the cerebrospinal fluid (CSF), which surrounds the hypothalamus and brainstem: 2 major centers of feed intake regulation. Thus CSF functions as a transport medium for fuel signals between blood and brain. The CSF metabolite concentrations are mainly under control of the blood-brain barriers, which provide specific carrier molecules facilitating the entry of substances required by the brain and protect the brain from factors that could impair neuronal function. The transport of small molecules such as amino acids (AA) across the blood-brain barriers may be limited by competing AA that share a common transporter for the uptake into brain. Consequently, CSF metabolite concentrations differ from those in blood. Thus it appears likely that central (CSF) rather than peripheral (blood) metabolites act as pivotal signals for the control of feed intake. However, the contribution of putative orexigenic and anorexigenic signals in CSF of cows has not been studied so far. Therefore, the aim of this study was to elucidate associations existing between both plasma and CSF metabolites, each in response to feed restriction-induced negative energy balance. Seven German Holstein dairy cows, between 87 and 96 DIM of the second lactation (milk yield, 27.9 L/d) were fed ad libitum (AL) for 4 d and CSF from the spinal cord and blood from the jugular vein was withdrawn before morning feeding at the fifth day. Subsequently, animals were feed restricted (R) to 50% of the previous AL intake for 4 d and CSF and plasma were collected at the ninth day. Body weight, feed intake, water intake, and milk production were determined. Thirty-one AA, β -hydroxybutyric acid, cholesterol, glucose, lactate, nonesterified fatty acids, urea, and osmolality were measured in both CSF

and plasma, whereas free fatty acids and volatile fatty acids were determined in plasma only. Although plasma arginine (132%), leucine (134%), lysine (117%), non-esterified fatty acids (224%), and cholesterol (112%) increased, tryptophan and carnosine decreased (–33% and –20%, respectively) in R animals as compared with AL animals. In CSF, concentrations of these metabolites were not affected after R feeding, suggesting that these identified plasma metabolites have only little potential to contribute to central feed intake regulatory signaling in cows. By contrast, in CSF, serine, threonine, and tyrosine decreased (–20, –24, and –31%, respectively) after R feeding. Therefore, these 3 AA are potential centrally acting anorexigenic signals in cows.

Key words: cerebrospinal fluid, dairy cow, metabolite, plasma

INTRODUCTION

High-yielding dairy cows often show inadequate feed intake in early lactation and, as a consequence, drop into negative energy balance (NEB). This period is, among others, characterized by an altered pattern of plasma metabolites driven by an increased release of body tissue reserves and efflux toward high milk production. An imbalanced metabolite profile has been suggested to prevent sufficient feed intake. Metabolites in blood such as glucose, NEFA, AA, as well as hormones and cytokines, act as hunger or satiety signals in the brain and play an important role in the control of feed intake in rodents. In ruminants, i.v. injections of metabolites such as NEFA, lactate, and AA (e.g., lysine) may decrease feed intake (Baile and Forbes, 1974). On the contrary, glucose plays a relatively minor role for the control of feed intake of ruminants, as a major portion of ingested carbohydrates is degraded to VFA in the rumen and, as such, absorbed by the ruminal wall. In this context, ruminal or i.v. infusion of VFA depressed feed intake (Baile and Forbes, 1974), whereas infusion of glucose did not affect feed consumption of dairy cattle (Dowden and Jacobson, 1960). However, when an isotonic glucose solution was injected into cerebrospinal fluid (CSF) of the third brain ventricle of sheep, feed intake decreased within the first 2 h (Seoane and

Received May 3, 2011.

Accepted October 29, 2011.

¹Corresponding author: b.kuhla@fbn-dummerstorf.de

Baile, 1972). This finding suggests that less peripheral but rather central mechanisms are important for feed intake control by glucose in ruminants. Recent studies confirm that central, rather than less peripheral, glucose-sensing mechanisms mediate neuroendocrine functions in ruminants (Ohkura et al., 2000).

Due to the blood-brain barrier (BBB) and the blood-CSF barrier (choroid plexus epithelium) the passage of molecules between the blood and the neural tissue or its fluid spaces is limited and regulated. The transport of small molecules such as AA across the BBB may be limited by the competition among AA sharing a common transporter for the uptake into the brain, which usually prevents appreciable fluctuations in brain AA concentrations. For example, branched-chain AA (BCAA), together with tryptophan and tyrosine, share a competitive transport carrier across the BBB (Bassil et al., 2007). Therefore, variations in blood BCAA levels may indirectly influence brain tryptophan and tyrosine levels and, thus, the synthesis rate of neurotransmitters and hormones involved in feed intake control. Consequently, fuels that cross the BBB may affect feeding behavior.

The CSF functions as a transport medium for metabolites and hormones between blood and brain. Interestingly, CSF surrounds the hypothalamus and brainstem, 2 major centers of feed intake regulation. Contributions of signals from CSF to the control of feed intake are demonstrated in ruminants (Seoane and Baile, 1972). However, the role of orexigenic and anorexigenic signals in CSF of lactating dairy cows has not been studied so far.

Feed deprivation or fasting represent models to induce NEB to identify long-term signals controlling energy balance (Havel, 2001). Thus, the aim of this study was to elucidate associations existing between both plasma and CSF metabolites each in response to feed restriction. We expect to identify metabolites potentially involved in the control of feed intake. The thus identified metabolites may either serve as candidates for metabolite infusion studies but also provide knowledge about the role of metabolites in influencing feed intake. Such knowledge is the prerequisite for developing feeding strategies and recommendations aligned to prevent insufficient feed intake in high-yielding dairy cows.

MATERIALS AND METHODS

Animals, Husbandry, Feeding, Measurement of Zootechnical Data, and Calculation of Energy Balance

Seven German Holstein dairy cows between 87 and 96 d of the second lactation 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 (registration no. LALLF M-V/TSD/7221.3-2.1-002/09). All cows were not pregnant, in healthy condition, and 42 to 50 mo old. They were fed twice daily (0700 and 1600 h) for 4 d ad libitum (AL) a TMR consisting of corn and grass silage, hay, grain feed, minerals, and vitamins, providing adequate nourishment to meet the needs of dairy cows (see Table 1; 7.2 MJ of NE_L/kg of DM) and sampled for CSF and plasma before morning feeding at the fifth day. Subsequently, animals were feed restricted (R) to 50% of the previous AL intake for 4 d and subsequently sampled again for CSF and plasma at the ninth day before morning feeding. Cows had free access to water and were milked twice daily (0630 and 1530 h); both were recorded daily. The daily feed intake was measured individually. Body weight was measured after AL and R feeding each day after the morning milking. To calculate energy balance, milk was analyzed for fat, protein, and lactose content by an infrared spectrophotometric method (Milkoscan; Foss GmbH, Rellingen, Germany) at the State Institute for Agriculture, Forestry and Horticulture (Iden, 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 (EB) was calculated thus: EB = NE_L intake (MJ) – (ECM × 3.14 + 0.293 × kg of BW^{0.75}) and was expressed in MJ of NE_L/(cow × d). Additionally, rectal temperature during sampling was recorded to assess a potentially detrimental effect of lumbar puncture. Sampling of CSF by lumbar puncture did not affect clinical health status and rectal temperature measured every day was in the normal range.

Blood and CSF Sampling

After local anesthesia with 10 mL of isocaine (20 mg/mL procainhydrochloride and 0.025 mg/mL epinephrine; Selectavet Dr. Otto Fischer GmbH, Weyarn-Holzolling, Germany), CSF from spinal cord was obtained by lumbar puncture between the sixth lumbar vertebra and sacrum with a needle with fitted stylet (120-mm length, 1.2-mm diameter) and blood was withdrawn into EDTA tubes from the jugular vein before morning feeding. Samples of 9 mL of blood and 4 mL of CSF were immediately treated with 50 or 30 µL of aprotinin [Carl Roth GmbH & Co. KG, Karlsruhe, Germany; 0.19% (wt/vol) in 0.9% (wt/vol) NaCl], respectively. An aliquot of 50 µL of CSF was taken for cell count analysis using a cell counter (Multisizer II; Beckman-Coulter GmbH, Krefeld, Germany). Blood was centrifuged at 1,573 × *g* for 20 min and CSF at 4,566 × *g* for 5 min (each at 4°C). Plasma and CSF aliquots were

Table 1. Ingredients and chemical composition of the lactation diet fed to cows after parturition

Diet	Amount
Ingredient	
Corn silage, g/kg of DM	310
Grass silage, g/kg of DM	200
Straw, g/kg of DM	20
Sugar-beet pulp, g/kg of DM	130
Barley meal, g/kg of DM	120
Soy, protected, g/kg of DM	40
Extracted soybean meal, g/kg of DM	150
Minerals, g/kg of DM	20
Propylene glycol, g/kg of DM	10
Chemical composition	
Utilizable protein, g/kg of DM	170
NE _L , MJ/kg of DM	7.2
Crude fiber, g/kg of DM	152
Starch and sugar, g/kg of DM	240
Crude fat, g/kg of DM	30

shock-frosted in liquid nitrogen, and stored at -80°C for no longer than 12 mo until analyses. Due to the limited volume of CSF available, analyses of VFA and FFA concentrations could not be performed.

Determination of AA, BHBA, Cholesterol, Glucose, Lactate, NEFA and FFA Profile, Urea, VFA, and Osmolality

Concentrations of BHBA, cholesterol, lactate, NEFA, and urea were determined by routine analyses (Cobas Mira, Clinic for Cattle, Stiftung Tierärztliche Hochschule Hannover, Hannover, Germany) using the following commercial kits: BHBA (no. RB 1008) from Randox Laboratories Ltd. (Crumlin, UK); cholesterol (no. 553–124) from MTI Diagnostics GmbH (Idstein, Germany); lactate (no. A11A01721) from Horiba Europe (Montpellier, France); NEFA (no. 434-91795) from Wako Chemicals GmbH (Neuss, Germany); and urea (no. LT-UR 0050) from Lehmann (Berlin, Germany). Concentrations of glucose were immediately determined after sampling by an automated YSI 1500-L unit (YSI Inc., Yellow Springs, OH). The concentrations of plasma VFA were determined by gas chromatography-flame ionization detector (GC-FID; Series 17A; Shimadzu Corp., Kyoto, Japan) on an FFAP (free fatty acid phase) column (25 m, 0.25 i.d., 0.25 μm) according to Ryan (1980). Amino acids were analyzed by HPLC (Series 1200; Agilent Technologies Deutschland GmbH, Böblingen, Germany; 250 $\times$ 4 mm HyperClone ODS (C18) 120 Å column protected by a 4 $\times$ 3 mm C18 pre-column) with automated pre-column derivatization and fluorescence detection (Kuhla et al., 2010). To this end, pre-column derivatization was performed by an injector program with ortho-phthalaldehyde/3-mercaptopropionic acid for primary and 9-fluorenylmethoxycarbonyl

chloride for secondary AA using 3-mercaptopropionic acid as reducing agent and iodoacetic acid to block sulfhydryl groups. Plasma FFA were derivatized to their methyl esters (FAME) using trimethylsulfonium hydroxide and analyzed by GC-MS-QP 2010 (Shimadzu Corp.) on an HP-ULTRA 2 column (50 m, 0.32 i.d.; 0.52 μm), according to Costa et al. (1998). The osmolality was determined by using an automatic cryoscopic osmometer (Osmomat 030; Gonotec GmbH, Berlin, Germany).

Statistical Analyses

The data were statistically analyzed using the Kolmogorov-Smirnov test for normal distribution with subsequent paired *t*-test to compare means between feeding states in plasma and CSF separately. A statistically significant difference was considered when $P \leq 0.05$ and trends if $0.05 < P < 0.10$.

RESULTS

Zootechnical Parameters

As compared with the AL feeding (mean $\pm$ SD, 123.8 $\pm$ 10.2 MJ/d, DM 32.7 $\pm$ 7 kg/d) period, R feeding for 4 d (69.1 $\pm$ 5.4 MJ/d, DM 18.6 $\pm$ 1.9 kg/d) resulted in an NEB, a significantly decreased milk yield and loss of BW (the majority presumably due to loss of gut fill) and decreased water intake [AL vs. 50% R: energy balance, 12.2 vs. -28.7 MJ of NE_L (cow $\times$ d); BW, 535.3 vs. 480.7 kg; water intake, 63.5 vs. 43.0 L/d; milk yield, 27.9 vs. 24.1 L/d; all $P < 0.05$; Figure 1].

Plasma and CSF Concentrations of Glucose, NEFA, BHBA, Cholesterol, Lactate, VFA, FFA, and Osmolality

Plasma glucose concentrations did not change in response to feed restriction but in CSF, glucose tended to be reduced ($P = 0.08$; Figure 2A). After feed restriction, plasma NEFA concentrations were 2.2-fold that of AL feeding ($P < 0.01$; Figure 2B) but decreased in CSF of R cows. At the lower intake level, plasma, but not CSF BHBA and cholesterol concentrations, were elevated when compared with AL intake ($P = 0.09$ and $P < 0.01$; respectively Figure 2C and 2D). Lactate concentrations did not change in response to feed restriction, in CSF or in plasma, but surprisingly, the mean of CSF lactate was approximately 190% of plasma lactate (Figure 2E).

Total VFA plasma concentrations, in particular acetic acid, tended to decrease after feed restriction ($P = 0.06$; Table 2). Other plasma VFA such as propionic

Figure 1. Energy intake, energy balance, water intake, BW, and milk yield of cows during ad libitum feeding and during 50% feed restriction. Data are presented as mean $\pm$ SD. Mean values with different lowercase letters differ with $P < 0.05$ (paired t -test).

acid, n-butyric acid, n-caproic acid, and i-valeric acid were not detectable in the R group (Table 2). Plasma free palmitic acid (C16:0) was significantly increased (105%; $P < 0.05$; Table 3), whereas myristic acid (C14:0) and palmitoleic acid (C16:1) did not change in response to feed restriction. Plasma stearic acid (C18:0) significantly decreased (89%), whereas that of oleic acid (C18:1) increased to 111% after energy restriction (each $P < 0.05$; Table 3). Osmolality was unaffected after feed restriction in plasma, but tended to decrease in CSF of R cows ($P = 0.07$; Figure 3).

Plasma and CSF Concentrations of AA and Urea

In plasma, the BCAA leucine and isoleucine accumulated to 134% after feed restriction ($P < 0.05$; $P = 0.07$, respectively). The basic plasma AA arginine and

lysine but also 1- and 3-methylhistidine were elevated after R (132, 117, and 141%, respectively; each $P \leq 0.05$). Plasma α -aminobutyric acid ($P = 0.08$) and α -aminoadipic acid levels ($P = 0.07$) showed a tendency to increase in R animals. Plasma tryptophan (–33%) and carnosine (–20%) were significantly decreased, whereas tyrosine tended to decrease (–25%; $P = 0.07$) after R feeding. However, with the exception of tyrosine and 1- and 3-methylhistidine, all AA that responded to feed restriction in plasma did not show parallel changes in CSF.

In CSF, tyrosine, serine, and threonine were significantly lower in the feed-restricted group (–31, –20, and –24%, respectively) but they did not change in plasma. Also, CSF cysteine tended to decrease in R cows ($P = 0.08$). On the contrary, CSF 1- and 3-methylhistidine were significantly increased (157%) after feed restric-

Figure 2. Plasma and cerebrospinal fluid (CSF) concentrations of glucose (A), NEFA (B), BHBA (C), cholesterol (D), and lactate (E) in mmol/L of cows after ad libitum feeding and after 50% feed restriction. Data are presented as mean $\pm$ SD and numbers in bars indicate numbers of animals (* $P < 0.1$, *** $P < 0.01$; paired t -test).

Table 2. Concentrations of plasma VFA ($\mu\text{mol/L}$) after ad libitum feeding and 50% feed restriction (mean $\pm$ SD; $n = 5$)

Item	Feeding		<i>P</i> -value
	Ad libitum	Restriction	
Acetic acid	662.7 $\pm$ 135.8	458.1 $\pm$ 133.5	0.057
Propionic acid	ND ¹	ND	
n-Butyric acid	13.2 $\pm$ 16.5	0.0	0.149
n-Caproic acid	4.7 $\pm$ 8.3	0.0	0.277
i-Valeric acid	9.8 $\pm$ 16.0	0.0	0.240
Total	690.4 $\pm$ 157.0	458.1 $\pm$ 133.5	0.056

¹Not detectable.

tion. Also, the total AA concentration was significantly decreased in CSF (-8%) after feed restriction, but remained unchanged in plasma ($P < 0.05$; Table 4). Urea concentrations were not affected by R feeding, in plasma or in CSF (Table 4).

DISCUSSION

Earlier studies deduced the orexigenic and anorexic effects of metabolites primarily from i.v. infusion studies and from plasma concentrations measured after feed deprivation or restriction (Havel, 2001). The latter intervention strategies may induce NEB, allowing the investigation of metabolic adaptational processes but also long-term hunger and satiety signals (Havel, 2001). Our study demonstrated that feed restriction to 50% resulted in a NEB, which is characterized by different concentration responses of numerous metabolites in plasma and CSF as compared with AL feeding, probably because of the different utilization of metabolites by the brain and a different transport rate at the BBB.

Glucose

Duodenal glucose infusion in dairy cows had no effect on feed intake unless non-physiologically high blood concentrations were achieved (Rigout et al., 2002) and i.v.-administered glucose did not depress feed intake in

dairy cattle (Al-Trad et al., 2009). This is in accordance with our finding showing that plasma glucose levels did not change in response to feed restriction. We rather observed a trend toward decreased glucose concentrations in CSF after feed restriction. After injection of isotonic 2-deoxy-D-glucose (**2-DG**), an inhibitor for glucose utilization, into the third brain ventricle of sheep, increased feed intake was observed within the first 30 min (Seoane and Baile, 1972). Additionally, central application of 2-DG leads to increased expression of orexigenic neuropeptides in rodents (Lee et al., 2005). Because we observed decreased CSF glucose concentrations after feed restriction—at least in tendency—our findings support the notion that central (not peripheral) glucose might exert an anorexic role in ruminants.

BHBA

Increased plasma BHBA concentrations observed after feed restriction point to an incomplete hepatic β -oxidation upon NEFA mobilization. Investigations with African pygmy goats (fasted for 2h) revealed that hypophagia is related to the amount of i.p.-injected BHBA (Rossi et al., 2000). Their study, however, could not clarify whether BHBA acts peripherally, centrally, or both. Under prolonged starving conditions, the brain metabolism shifts from glucose toward ketone body

Table 3. Proportion (%) of saturated and unsaturated FFA in plasma after ad libitum feeding and 50% feed restriction (mean $\pm$ SD, $n = 5$)

Item	Feeding		Change after restriction, %	<i>P</i> -value ¹
	Ad libitum	Restriction		
C14:0	4.3 $\pm$ 0.8	4.7 $\pm$ 0.5	+9.3	0.245
C16:0	40.5 $\pm$ 0.9	42.5 $\pm$ 1.1	+4.9	0.002
C16:1	3.5 $\pm$ 1.0	4.1 $\pm$ 1.0	+17.1	0.387
C18:0	40.1 $\pm$ 2.3	35.8 $\pm$ 2.0	−10.7	0.010
C18:1	11.6 $\pm$ 1.2	12.9 $\pm$ 1.1	+11.2	0.008
Total	100	100		

¹Values in bold font are significant ($P < 0.05$).

Table 4. Concentrations of plasma (PL) and cerebrospinal fluid (CSF) amino acids, organic acids, and dipeptides ($\mu\text{mol/L}$) and urea (mmol/L) after ad libitum feeding and 50% feed restriction (mean $\pm$ SD)

Item	Fluid	Feeding		<i>P</i> -value ¹
		Ad libitum	50% Restriction	
Alanine	PL	271.1 $\pm$ 28.0	236.3 $\pm$ 78.7	0.405
	CSF	39.9 $\pm$ 5.4	47.7 $\pm$ 28.9	0.670
α -Aminobutyric acid	PL	24.0 $\pm$ 9.4	29.1 $\pm$ 4.4	0.081
	CSF	1.7 $\pm$ 0.6	1.9 $\pm$ 0.7	0.898
Arginine	PL	63.7 $\pm$ 12.7	84.0 $\pm$ 12.8	0.033
	CSF	27.6 $\pm$ 4.9	28.5 $\pm$ 5.5	0.266
Asparagine	PL	37.4 $\pm$ 5.9	30.1 $\pm$ 10.7	0.258
	CSF	4.1 $\pm$ 0.6	4.2 $\pm$ 1.7	0.918
Aspartic acid	PL	4.4 $\pm$ 1.6	4.5 $\pm$ 1.9	0.888
	CSF	2.5 $\pm$ 2.3	1.4 $\pm$ 1.0	0.226
β -Alanine	PL	4.2 $\pm$ 1.8	4.4 $\pm$ 2.0	0.782
	CSF	2.1 $\pm$ 0.1	1.5 $\pm$ 0.9	0.397
Citrulline	PL	95.2 $\pm$ 26.7	102.5 $\pm$ 19.4	0.544
	CSF	1.8 $\pm$ 0.9	2.2 $\pm$ 0.3	0.588
Cysteine	PL	31.9 $\pm$ 6.9	35.5 $\pm$ 6.1	0.249
	CSF	0.9 $\pm$ 0.6	0.8 $\pm$ 0.5	0.078
γ -Aminobutyric acid	PL	1.5 $\pm$ 1.0	1.1 $\pm$ 0.7	0.517
	CSF	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.173
Glutamic acid	PL	35.8 $\pm$ 6.7	31.2 $\pm$ 4.7	0.176
	CSF	5.5 $\pm$ 3.0	4.3 $\pm$ 3.3	0.294
Glutamine	PL	250.5 $\pm$ 42.9	284.6 $\pm$ 41.7	0.186
	CSF	451.2 $\pm$ 49.4	409.4 $\pm$ 63.4	0.163
Glycine	PL	359.5 $\pm$ 45.6	357.0 $\pm$ 44.2	0.912
	CSF	12.4 $\pm$ 2.5	13.9 $\pm$ 10.2	0.690
Histidine	PL	37.0 $\pm$ 5.3	38.1 $\pm$ 5.8	0.770
	CSF	5.0 $\pm$ 1.2	5.5 $\pm$ 2.5	0.681
Hydroxyproline	PL	19.3 $\pm$ 11.8	23.0 $\pm$ 3.4	0.470
	CSF	ND ²	ND	
Isoleucine	PL	97.7 $\pm$ 13.5	127.9 $\pm$ 36.9	0.067
	CSF	2.7 $\pm$ 0.9	2.9 $\pm$ 0.8	0.740
Leucine	PL	110.3 $\pm$ 11.2	147.8 $\pm$ 26.7	0.014
	CSF	5.4 $\pm$ 2.8	5.7 $\pm$ 3.1	0.803
Lysine	PL	53.5 $\pm$ 13.8	62.6 $\pm$ 15.4	0.051
	CSF	11.7 $\pm$ 5.3	13.6 $\pm$ 3.4	0.928
Methionine	PL	18.4 $\pm$ 1.8	17.5 $\pm$ 4.1	0.703
	CSF	2.2 $\pm$ 0.4	2.3 $\pm$ 0.6	0.817
Ornithine	PL	36.8 $\pm$ 9.2	35.7 $\pm$ 7.6	0.765
	CSF	5.0 $\pm$ 1.3	4.4 $\pm$ 1.0	0.143
Phenylalanine	PL	36.5 $\pm$ 2.3	41.0 $\pm$ 7.6	0.211
	CSF	2.3 $\pm$ 0.6	2.3 $\pm$ 0.5	0.608
Proline	PL	97.7 $\pm$ 58.1	97.8 $\pm$ 49.6	0.568
	CSF	19.4 $\pm$ 20.7	8.7 $\pm$ 9.4	0.214
Serine	PL	75.1 $\pm$ 10.6	66.5 $\pm$ 22.7	0.532
	CSF	53.3 $\pm$ 7.8	42.8 $\pm$ 7.4	0.031
Threonine	PL	86.3 $\pm$ 13.2	76.3 $\pm$ 28.1	0.353
	CSF	23.1 $\pm$ 4.5	17.5 $\pm$ 3.6	0.019
Tryptophan	PL	32.8 $\pm$ 9.6	22.1 $\pm$ 7.5	0.022
	CSF	1.0 $\pm$ 0.6	0.9 $\pm$ 0.3	0.183
Tyrosine	PL	33.5 $\pm$ 5.4	25.0 $\pm$ 6.3	0.073
	CSF	2.9 $\pm$ 0.5	2.0 $\pm$ 0.6	0.042
Valine	PL	213.8 $\pm$ 28.6	230.1 $\pm$ 54.8	0.302
	CSF	6.4 $\pm$ 1.1	6.3 $\pm$ 2.3	0.815
1- and 3-Methylhistidine	PL	9.6 $\pm$ 3.1	13.5 $\pm$ 5.0	0.019
	CSF	1.4 $\pm$ 1.1	2.2 $\pm$ 1.3	0.011
α -Aminoadipic acid	PL	5.3 $\pm$ 1.5	6.4 $\pm$ 1.2	0.068
	CSF	ND	ND	
Anserine	PL	1.2 $\pm$ 0.5	1.3 $\pm$ 0.2	0.747
	CSF	ND	ND	
Carnosine	PL	11.4 $\pm$ 3.7	9.1 $\pm$ 3.2	0.003
	CSF	1.7 $\pm$ 0.5	1.7 $\pm$ 0.4	0.265
Taurine	PL	37.3 $\pm$ 6.5	43.1 $\pm$ 7.9	0.231
	CSF	1.0 $\pm$ 0.5	1.6 $\pm$ 0.7	0.287

Continued

Table 4 (Continued). Concentrations of plasma (PL) and cerebrospinal fluid (CSF) amino acids, organic acids, and dipeptides ($\mu\text{mol/L}$) and urea (mmol/L) after ad libitum feeding and 50% feed restriction (mean $\pm$ SD)

Item	Fluid	Feeding		<i>P</i> -value ¹
		Ad libitum	50% Restriction	
Total [$\mu\text{mol/L}$]	PL	2,175.0 $\pm$ 175.0	2,284.0 $\pm$ 207.0	0.447
	CSF	690.8 $\pm$ 68.9	634.1 $\pm$ 36.9	0.025
Urea [mmol/L]	PL	4.36 $\pm$ 0.6	4.51 $\pm$ 0.8	0.221
	CSF	4.43 $\pm$ 0.8	4.13 $\pm$ 1.3	0.822

¹Values in bold font are significant ($P < 0.05$).²Not detectable.

utilization and BHBA may act as energy-providing substrate and central anorexigenic signal in the circuits controlling feed intake (Laeger et al., 2010). This physiological role was also confirmed for the sheep brain in which BHBA is primarily utilized under hypoglycemic, but less under normoglycemic, conditions (Kammula, 1976). Changing plasma BHBA levels show proportional changes in CSF, which are about 30% of plasma BHBA in cattle (Sato et al., 2002). Our own analyses support this earlier finding. Because CSF BHBA levels do not rise after R feeding, it seems that BHBA is utilized by the brain under these conditions. Provided that during early lactation, increased plasma BHBA levels are associated with increased CSF BHBA levels, BHBA may be increasingly utilized by the brain and potentially inhibits feed intake of early-lactating cows.

Lactate

Whereas intraruminal injections of lactate during feeding decreased feed intake of cattle (Montgomery et al., 1963), i.v. injections did not (Dowden and Jacobson, 1960). However, in our study, lactate concentrations did not change after feed restriction, which supports the assumption that circulating lactate does not play a major role in feed intake regulatory circuits of cows. The higher CSF lactate level as compared with plasma is presumably due to lactate production of astrocytes, which in turn, serves as fuel for neurons.

Osmolality

The salt concentration and, consequently, the osmolality affects feed intake in ruminants. An infusion of hypertonic NaCl solution into the lateral cerebral ventricle numerically decreased the feed intake of cattle (McKinley et al., 1987). This finding is in accordance with our result that CSF osmolality tended to decrease in R cows.

NEFA

Palmitic (C16:0) and stearic acids (C18:0) constitute the predominant portion of plasma NEFA. When C16:0 was continuously i.v. infused over a period of 4 d, feed intake of sheep decreased, whereas administration of C18:0 resulted in a smaller insignificant decrease in feed intake (Vandermeerschen-Doizé and Paquay, 1984). Our results support this earlier finding showing that proportions of plasma C16:0 increase, whereas C18:0 decrease after feed restriction. It was shown that especially unsaturated FA depress DMI with negative effects on milk yield and long-chain FA act as signals of satiety and energy status to the brain in ruminants (Allen, 2000). Thus, i.v. infusion of oleic acid (C18:1) but not of C18:0 resulted in a significant decrease of feed intake in sheep (Vandermeerschen-Doizé and Paquay, 1984). The increased plasma NEFA concentration determined in our study is mainly due to an increased portion of

Figure 3. Plasma and cerebrospinal fluid (CSF) osmolality (Osm/kg) of cows after ad libitum feeding and after 50% feed restriction. Data are presented as mean $\pm$ SD and numbers in bars indicate numbers of animals (* $P < 0.1$; paired *t*-test).

C14:0, C16:0, C16:1 and C18:1 after feed restriction. The lower portion of C18:0 as compared with C16:0 in R animals is likely reflected by the fatty acid composition of adipose tissue, which contains more C16:0 than C18:0. Curiously, the disproportionately higher content of monounsaturated fatty acids after feed restriction would favor anorexigenic signaling (Allen, 2000); however, R cows are expected to be hungry. Whether the higher portion of monounsaturated fatty acids is also present in CSF remains to be elucidated.

Amino Acids

Abomasal infusion of branched AA did not affect feed intake of lactating cows fed AL (Korhonen et al., 2002), indicating that leucine, isoleucine, and valine are not important regulators for feed intake of cows. However, fasting for 6 d provoked a significant increase in plasma leucine of dairy cows (Baird et al., 1972). In our study, leucine did not change in response to feed restriction in CSF but was significantly increased in plasma after feed restriction, too, presumably because of an increased tissue protein mobilization. In rodents, leucine is supposed to decrease food intake after intracerebroventricular administration via decreased expression of orexigenic neuropeptide Y and increased abundance of anorexigenic pro-opiomelanocortin in the hypothalamus (Ropelle et al., 2008).

Plasma arginine, a precursor for NO production and, thus, potentially involved in hypothalamic feed intake regulation was elevated after feed restriction. This finding corresponds with the observation that arginine subcutaneously administered in mice resulted in NO synthesis and increased short-term feed intake, whereas inhibition of NO synthesis decreased feed intake (Morley and Flood, 1991). In contrast, abomasal and i.v.

infusions of arginine did not alter feed intake in dairy cows (Vicini et al., 1988). Thus, it seems that arginine and NO are less critical signals for feed intake control in cows.

Elevated plasma lysine after R originates possibly from protein breakdown in splanchnic tissue, skin, and skeletal muscle. Oral application of lysine did not change feed intake in cows (Robinson et al., 2005). The authors suggested that rumen bacteria would adapt with time to lysine feeding and become more efficient at degrading it, but unfortunately the authors did not determine plasma lysine levels (Robinson et al., 2005). Unphysiologically high plasma levels of lysine after i.v. injection in sheep depressed the intake of concentrate feed (Baile and Martin, 1971). However, whether lysine plays a role in the control of feed intake in cows remains to be evaluated in further studies.

Carnosine, a dipeptide of β -alanine and histidine, inhibited feed intake after intracerebroventricular injection in chicks (Tomonaga et al., 2004). The observed plasma carnosine decrease after feed restriction suggests that carnosine might have anorexigenic properties in cows, too.

In accordance with the observation of an increased serum methylhistidine level after starving of rats (Nagasawa et al., 1998), which occurs as a result of a muscle protein breakdown, we found increased plasma and CSF methylhistidine levels in cows after feed restriction. Whether methylhistidine is able to cross the BBB is not known, but accumulation of methylhistidine in CSF may be reflected by an increased catabolism of neurostenin, a contractile actomyosin-like protein containing methylhistidine (Enwonwu and Worthington, 1973). Again, whether methylhistidine indeed acts as an orexigenic signal remains to determine in future studies.

Table 5. Summary of changed metabolites after feed restriction and comparison of their potential role in the central control of feed intake between nonruminants and ruminants

Item	Concentration after restriction in cows ¹		Potential central anorexic in cows	Central anorexic in nonruminants	Reference
	PL	CSF			
NEFA	↑	=		+	Schwinkendorf et al. (2011)
Cholesterol	↑	=			
Arginine	↑	=			
Leucine	↑	=		+	Ropelle et al. (2008)
Lysine	↑	=			
Serine	=	↓	+		
Threonine	=	↓	+		Tomonaga et al. (2004)
Tryptophan	↓	=			
Tyrosine	=	↓	+		
Carnosine	↓	=		+	

¹PL = plasma; CSF = cerebrospinal fluid. Arrows pointing upward represent an increase; arrows pointing downward represent a decrease.

Serine and threonine in the CSF decrease after feed restriction, suggesting that these AA may act as potential anorexigenic signals in the bovine (Table 5). In humans, ingestion of serine and threonine was shown to decrease food intake (Veldhorst et al., 2009). Conversely, the response to an AA-imbalanced diet lacking the most limiting AA threonine depressed feed intake in pigs, whereas both feed intake and plasma threonine values were restored by dietary supplementation of threonine (Robinson, 1975). Interestingly, in dairy cows, plasma serine concentrations increased after calving and remained elevated within the first few weeks postpartum. (Meijer et al., 1995). As feed intake is often insufficient to meet the energy requirements for enormous milk production, peaking serine concentrations might be one central anorexigenic signal (Table 5).

Furthermore, CSF tyrosine was decreased after feed restriction. Tyrosine acts a precursor of many neurotransmitters, including adrenalin, noradrenalin, and dopamine, all of them known to exert an appetite-suppressing effect. Thus, our results let us assume that tyrosine—although indirectly—may participate in central anorexigenic signaling in cows (Table 5).

The decrease in plasma tryptophan concentration after feed restriction could be explained by the fact that tryptophan is limiting for milk protein synthesis and was preferentially used to maintain milk yield, which was only decreased by about 15%, whereas feed intake was 50% lower. Nevertheless, tryptophan may indirectly serve as an anorexigenic metabolite because tryptophan is a precursor of serotonin, which, in turn, induces anorexia in chicks (Pinchasov et al., 1989).

Further relationships between brain histamine, a metabolite of histidine, and feed intake were reported. When histidine levels rise, decarboxylation of histidine and production of histamine will also increase (Haq et al., 1996). Feeding of histidine, intracerebral or intraperitoneal injection of histidine may all elevate brain histamine and depress feed intake in rodents (Haq et al., 1996). In our study, histidine did not change after feed restriction in plasma or in CSF, suggesting that histidine plays a rather unimportant role in feed intake control of cows.

CONCLUSIONS

To summarize, we investigated a broad range of metabolites in plasma and CSF of cows after AL and R feeding. Earlier studies deduced the orexigenic and anorexigenic effects of metabolites primarily from plasma data. Here, we demonstrated that many metabolites (NEFA, cholesterol, arginine, leucine, lysine, serine, threonine, tryptophan, tyrosine, and carnosine)

revealed different responses after feed restriction, either in plasma or CSF. This is presumably due to a different permeability of the BBB bordering the peripheral from brain metabolism. Although R feeding led to drastic concentration responses of numerous metabolites in plasma, these metabolites were not changed in CSF and, therefore, should not have potential to exert central feed intake signaling in cows. Hence, studies assigning plasma metabolites a feed intake regulatory role per se should be interpreted with more caution and better differentiate between centrally and peripherally induced signaling. In contrast, due to decreased CSF concentration of serine, threonine, and tyrosine after feed restriction, we propose that this adaptational response is aligned to overcome NEB by diminishing satiety.

ACKNOWLEDGMENTS

We thank Olaf Bellmann, Kirsten Karpati, Ute Lüdtke, Marlies Althaus, Claudia Arlt, Birgit Sobczak, and Torsten Viergutz and the staff from the Tiertechnikum [all from the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany] for their skillful assistance and excellent technical support. Sebastian Bühler (University of Rostock) is acknowledged for support in osmolality measurement. This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. This work was supported by the core budget of the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany).

REFERENCES

- Al-Trad, B., K. Reisberg, T. Wittek, G. B. Penner, A. Alkaassem, G. Gäbel, M. Füll, and J. R. Aschenbach. 2009. Increasing intravenous infusions of glucose improve body condition but not lactation performance in midlactation dairy cows. *J. Dairy Sci.* 92:5645–5658.
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- Baile, C. A., and J. M. Forbes. 1974. Control of feed intake and regulation of energy balance in ruminants. *Physiol. Rev.* 54:160–214.
- Baile, C. A., and F. H. Martin. 1971. Hormones and amino acids as possible factors in control of hunger and satiety in sheep. *J. Dairy Sci.* 54:897–905.
- Baird, G. D., R. J. Heitzman, and K. G. Hibbitt. 1972. Effects of starvation on intermediary metabolism in the lactating cow. A comparison with metabolic changes occurring during bovine ketosis. *Biochem. J.* 128:1311–1318.
- Bassil, M. S., N. Hwalla, and O. A. Obeid. 2007. Meal pattern of male rats maintained on histidine-, leucine-, or tyrosine-supplemented diet. *Obesity (Silver Spring)* 15:616–623.
- Costa, C. G., L. Dorland, U. Holwerda, I. T. de Almeida, B. T. Poll-The, C. Jakobs, and M. Duran. 1998. Simultaneous analysis of plasma free fatty acids and their 3-hydroxy analogs in fatty acid β -oxidation disorders. *Clin. Chem.* 44:463–471.
- Dowden, D. R., and D. R. Jacobson. 1960. Inhibition of appetite in dairy cattle by certain intermediate metabolites. *Nature* 188:148–149.

- Enwonwu, C. O., and B. S. Worthington. 1973. Accumulation of histidine, 3-methylhistidine, and homocarnosine in the brains of protein-calorie deficient monkeys. *J. Neurochem.* 21:799–807.
- Haq, A.-U., H. M. Bundrant, and L. P. Mercer. 1996. Food intake is inversely correlated with central nervous system histamine receptor (H_1) concentrations in male Sprague-Dawley rats fed normal, low protein, low energy or poor quality protein diets. *J. Nutr.* 126:3083–3089.
- Havel, P. J. 2001. Peripheral signals conveying metabolic information to the brain: Short-term and long-term regulation of food intake and energy homeostasis. *Exp. Biol. Med.* (Maywood) 226:963–977.
- Kammula, R. G. 1976. Metabolism of ketone bodies by ovine brain in vivo. *Am. J. Physiol.* 231:1490–1494.
- Korhonen, M., A. Vanhatalo, and P. Huhtanen. 2002. Evaluation of isoleucine, leucine, and valine as a second-limiting amino acid for milk production in dairy cows fed grass silage diet. *J. Dairy Sci.* 85:1533–1545.
- Kuhla, B., M. Kucia, S. Görs, D. Albrecht, M. Langhammer, S. Kuhla, and C. C. Metges. 2010. Effect of a high-protein diet on food intake and liver metabolism during pregnancy, lactation and after weaning in mice. *Proteomics* 10:2573–2588.
- Laeger, T., C. C. Metges, and B. Kuhla. 2010. Role of β -hydroxybutyric acid in the central regulation of energy balance. *Appetite* 54:450–455.
- Lee, K., B. Li, X. Xi, Y. Suh, and R. J. Martin. 2005. Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. *Endocrinology* 146:3–10.
- McKinley, M. J., D. A. Denton, D. Gellatly, R. R. Miselis, J. B. Simpson, and R. S. Weisinger. 1987. Water drinking caused by intracerebroventricular infusions of hypertonic solutions in cattle. *Physiol. Behav.* 39:459–464.
- Meijer, G. A., J. Van der Meulen, J. G. Bakker, C. J. Van der Koelen, and A. M. Van Vuuren. 1995. Free amino acids in plasma and muscle of high yielding dairy cows in early lactation. *J. Dairy Sci.* 78:1131–1141.
- Montgomery, M. J., L. H. Schultz, and B. R. Baumgardt. 1963. Effect of intraruminal infusion of volatile fatty acids and lactic acid on voluntary hay intake. *J. Dairy Sci.* 46:1380–1384.
- Morley, J. E., and J. F. Flood. 1991. Evidence that nitric oxide modulates food intake in mice. *Life Sci.* 49:707–711.
- Nagasawa, T., J. Hirano, F. Yoshizawa, and N. Nishizawa. 1998. Myofibrillar protein catabolism is rapidly suppressed following protein feeding. *Biosci. Biotechnol. Biochem.* 62:1932–1937.
- Ohkura, S., T. Tanaka, S. Nagatani, D. C. Bucholtz, H. Tsukamura, K. Maeda, and D. L. Foster. 2000. Central, but not peripheral, glucose-sensing mechanisms mediate glucoprivic suppression of pulsatile luteinizing hormone secretion in the sheep. *Endocrinology* 141:4472–4480.
- Pinchasov, Y., B. I. Fancher, W. H. Burke, and L. S. Jensen. 1989. Glycolic acid and tryptophan effects on feed intake and hypothalamic indoleamines in chicks. *Physiol. Behav.* 45:585–589.
- Rigout, S., S. Lemosquet, A. Bach, J. W. Blum, and H. Rulquin. 2002. Duodenal infusion of glucose decreases milk fat production in grass silage-fed dairy cows. *J. Dairy Sci.* 85:2541–2550.
- Robinson, D. W. 1975. Food-intake regulation in pigs. 4. Influence of dietary threonine imbalance on food-intake, dietary choice and plasma acid patterns. *Br. Vet. J.* 131:595–600.
- Robinson, P. H., E. J. DePeters, I. Shinzato, and H. Sato. 2005. Influence of feeding free lysine to early lactation dairy cows on ruminal lysine escape, rumen fermentation and productivity. *Anim. Feed Sci. Technol.* 118:201–214.
- Ropelle, E. R., J. R. Pauli, M. F. A. Fernandes, S. A. Rocco, R. M. Marin, J. Morari, K. K. Souza, M. M. Dias, M. C. Gomes-Marcondes, J. A. R. Gontijo, K. G. Franchini, L. A. Velloso, M. J. A. Saad, and J. B. Carvalheira. 2008. A central role for neuronal AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) in high-protein diet-induced weight loss. *Diabetes* 57:594–605.
- Rossi, R., S. Dörig, E. Del Prete, and E. Scharrer. 2000. Suppression of feed intake after parenteral administration of D- β -hydroxybutyrate in pygmy goats. *J. Vet. Med. Ser. A - Physiol. Pathol. Clin. Med.* 47:9–16.
- Ryan, J. P. 1980. Determination of volatile fatty acids and some related compounds in ovine rumen fluid, urine, and blood plasma, by gas-liquid chromatography. *Anal. Biochem.* 108:374–384.
- Sato, H., T. Kurosawa, and S. Oikawa. 2002. Acetic acid and 3-hydroxybutyric acid levels in cerebrospinal fluid of cattle. *Anim. Sci. J.* 73:137–141.
- Schwinkendorf, D. R., N. G. Tsatsos, B. A. Gosnell, and D. G. Mashek. 2011. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. *Int. J. Obes. (Lond)* 35:336–344.
- Seoane, J. R., and C. A. Baile. 1972. Effects of intraventricular (3 ventricle) injections of 2-deoxy-D-glucose, glucose and xylose on feeding behavior of sheep. *Physiol. Behav.* 9:423–428.
- Tomonaga, S., T. Tachibana, T. Takagi, E.-S. Saito, R. Zhang, D. M. Denbow, and M. Furuse. 2004. Effect of central administration of carnosine and its constituents on behaviors in chicks. *Brain Res. Bull.* 63:75–82.
- Vandermeersch-Doizé, F., and R. Paquay. 1984. Effects of continuous long-term intravenous infusion of long-chain fatty acids on feeding behaviour and blood components of adult sheep. *Appetite* 5:137–146.
- Veldhorst, M. A. B., A. G. Nieuwenhuizen, A. Hochstenbach-Waelen, K. R. Westerterp, M. P. K. J. Engelen, R.-J. M. Brummer, N. E. P. Deutz, and M. S. Westerterp-Plantenga. 2009. Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite* 52:388–395.
- Vicini, J. L., J. H. Clark, W. L. Hurley, and J. M. Bahr. 1988. Effects of abomasal or intravenous administration of arginine on milk production, milk composition, and concentrations of somatotropin and insulin in plasma of dairy cows. *J. Dairy Sci.* 71:658–665.