Differences in splanchnic metabolism between late gestation and early lactation dairy cows

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ABSTRACT

In the transition from the pre- to postcalving state, the demands on the cow increase from support of gestation to high rates of milk production. This extra demand is met partly by increased intake but may also involve altered metabolism of major nutrients. Six multiparous Holstein cows were used to monitor changes in net fluxes of nutrients across the portal-drained viscera and liver (splanchnic tissues) between late gestation and early lactation. Blood samples were obtained simultaneously from the portal, hepatic, and subcutaneous abdominal veins and the caudal aorta 18 d before expected calving and 21 or 42 d after calving. On the day of blood sampling and the 3 d preceding sampling, cows were fed every 2 h. The precalving (1.63 Mcal of net energy for lactation/kg and 1,326 g of metabolizable protein/d) and postcalving (1.72 Mcal of net energy for lactation/kg and 2,136 g of metabolizable protein/d) diets were based on corn silage, alfalfa hay, and corn grain. Dry matter intake increased postcalving. Net splanchnic release of glucose increased postpartum because of tendencies for both increased portal absorption and net liver release. Increased removal of lactate, rather than AA, contributed to the additional hepatic gluconeogenesis. Although portal absorption of AA increased with intake at the onset of lactation, hepatic removal of total AA-N tended to decline. This clearly indicates that liver removal of AA is not linked to portal absorption. Furthermore, net liver removal relative to total liver inflow even decreased for Gly, His, Met, Phe, and Tyr. Together, these data indicate that in early lactation, metabolic priority is given to direct AA toward milk protein production rather than gluconeogenesis, in cows fed a corn-based ration.

Key words: splanchnic, amino acid, glucose, dairy cow

INTRODUCTION

Nutritional management and metabolism of the transition dairy cow have been extensively studied in recent years (see reviews by Drackley, 1999, and Overton and Waldron, 2004). It is widely recognized that intake in the immediate postpartum period lags behind that needed to support milk production such that the cow experiences negative energy and protein balance for several weeks following the initiation of lactation. To cope with the large increase in nutrient demand associated with milk production during this time, the cow experiences a multitude of metabolic adaptations (Bell, 1995).

At the initiation of lactation, the demand for glucose for lactose production increases markedly and is partially met by an increase in gluconeogenesis, as well as a decrease in glucose oxidation (Bennink et al., 1972; Bell, 1995). The contribution of AA to gluconeogenesis has been considered important during early lactation in the dairy cow (Bell, 1995), but supportive evidence has come from observations either *ex vivo* or *in vitro* (Overton et al., 1999; Drackley et al., 2001). The other important demand for AA is to support milk protein synthesis and this requirement increases greatly at the onset of lactation. Therefore, despite an increased supply of MP through increased DMI and rations formulated for lactation, these 2 demands create a negative protein balance for cows in early lactation.

Few studies have examined *in vivo* the hepatic removal of glucose precursors in relation to glucose net release by the liver in dairy cows in transition. Reynolds et al. (2003) reported net fluxes of several nutrients from 19 d before to 83 d after calving. Those authors concluded that there were minimal changes in splanchnic metabolism during the prepartum period, but that in early lactation there were substantial increases in liver net release of glucose concomitant with increased hepatic

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removal of propionate, lactate, glycerol, and alanine compared with that observed 19 d precalving. The only AA measured was alanine, but the contribution of AA to liver glucose synthesis was estimated by difference between hepatic removal of other glucose precursors and glucose release. These estimates suggested that although the absolute amount of AA used to support gluconeogenesis was increased, the proportion of glucose derived from AA remained unchanged, except for alanine on d 11 postcalving.

Although it has been observed that circulating AA concentrations decrease in dairy cows in early lactation (Meijer et al., 1995; Doepel et al., 2002), it is not known to what extent this decrease is due to the pressure on available AA to support gluconeogenesis, milk protein production, or both. Recent studies, however, have shown that when protein supply is limited in dairy cows, the liver significantly reduces removal of most AA (Raggio et al., 2004), indicating a fundamental need to preserve AA to support milk protein output.

Therefore, we hypothesized that the metabolic priority of the dairy cow would direct AA toward milk protein synthesis rather than gluconeogenesis. Therefore, despite an increased absorption of AA, liver removal of AA would not increase in early lactation and the contribution of AA to glucose net release across the liver would not increase relative to late gestation. The objective of the current study was to examine the change in portal and liver net fluxes of AA, glucose, and lactate from late gestation to early lactation.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures were approved by the Institutional Committee for Animal Care of the Lennoxville Research Centre and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). At a minimum of 6 wk before calving, 8 multiparous Holstein cows were surgically implanted with abomasal catheters and chronic indwelling catheters in the mesenteric, portal, and hepatic veins and the caudal aorta via a mesenteric artery as described previously (Huntington et al., 1989; Doepel et al., 2006). In addition, the right carotid artery was surgically raised to a subcutaneous position to allow access to arterial blood in the event that the aorta catheter failed. Before calving, cows all received the same close-up diet and no abomasal infusion. The current comparisons form part of a larger study in which cows received abomasal infusions immediately after calving of either water or 300 g/d glutamine in a crossover design with 21-d periods (Doepel et al., 2007). To avoid the

confounding effects of the glutamine treatment (Doepel et al., 2007), only the data from the water treatments were used for the current comparison; that is, data were obtained either at d 21 ($n = 2$) or d 42 ($n = 4$) postcalving. Preliminary statistical analysis revealed that there were minimal differences in metabolic parameters between these 2 days and, therefore, data postcalving were aggregated as one time. Initially, treatment distribution was equal between the two 21-d periods, but one cow calved before the precalving blood samples could be obtained, and one cow developed mastitis shortly after calving. Overall, cows were sampled 18 ± 8 d precalving and 36 ± 11 d postcalving and $n = 6$ for all data reported.

For 4 wk precalving and 6 wk postcalving, cows were fed diets as outlined in Doepel et al. (2006). Briefly, the precalving TMR contained 1.63 Mcal of NE_L /kg and supplied 1,326 g of MP/d (14.1% CP), whereas the postcalving TMR contained 1.72 Mcal of NE_L /kg and supplied 2,136 g of MP/d (16.8% CP). Both TMR were based on corn silage, alfalfa hay, and corn grain. The precalving diet was fed ad libitum twice daily at 0800 and 1600 h except for d 18 to 21 before expected calving, when it was offered in 12 equal meals per day by automatic feeders. The postcalving diet was also offered ad libitum twice daily except for d 18 to 21 of each period, when it was supplied 12 times daily. Orts were recorded daily. Moisture content of the silages was determined weekly and used to make ration adjustments. Cows were milked twice daily at 0830 and 1930 h, and milk yield was recorded at each milking. Milk was sampled at each milking from d 19 to 21 of each period.

Blood Sampling

On d 18 ($n = 6$) precalving and d 21 ($n = 2$) or d 42 ($n = 4$) postcalving, blood samples were simultaneously collected into heparinized tubes from the arterial, hepatic venous, and portal catheters every 45 min for 4 h (6 samples), covering 2 cycles of feeding. In the postcalving periods, blood samples were also obtained by venipuncture from the subcutaneous abdominal vein following the same 45-min sampling schedule. To determine plasma flow across the splanchnic tissues, *para*-amino hippuric acid (**pAH**; 10% wt/vol) was infused into a mesenteric vein catheter (Huntington et al., 1989). A priming dose of 2 g was given a minimum of 40 min before the first blood sample was obtained, followed by a continuous infusion of 14.4 g/h. Blood was placed on ice immediately after collection. All laboratory analyses were conducted on individual samples (i.e., samples were not pooled). Packed cell volume was determined by the microhematocrit method. For

lactate analyses, 1 mL of fresh blood was mixed with 0.9 mL of water and 0.1 mL of 6 *N* perchloric acid and left on ice for 1 h before centrifugation and collection of the supernatant. The remainder of the blood was centrifuged (15 min, $1,800 \times g$ at 4°C) within 30 min of collection. Urea-N and pAH were analyzed on fresh plasma samples. For AA analysis, 1 g of plasma was added to 0.2 g of an internal standard of AA labeled with stable isotopes and the processed samples frozen at -80°C. The internal standard solution was prepared with labeled AA diluted in water as outlined in Doepel et al. (2007). The labeled AA (95 to 99 atom %) were supplied by CDN isotopes (Montreal, QC, Canada) for His, Leu, Lys, Met, and Phe, and by Cambridge Isotope Lab (Andover, MA) for others. The remainder of the plasma was stored at -20°C for subsequent analysis of glucose and ammonia. Additional samples of arterial, portal, hepatic, and mammary blood (2 mL) were collected into a blood-gas collection device (Monovette, Sarstedt, Aktiegesellschaft and Co., Germany) for the determination of pH and partial pressure of oxygen.

Laboratory Analyses

Milk nitrogen content ($CP = N \times 6.38$) was determined by combustion (Nitrogen Determinator, model FP-428, Leco, St. Joseph, MI). Milk true protein, whey, NPN, and casein contents were determined as described by Raggio et al. (2004). Phenylalanine and Tyr concentrations in milk were measured by the isotopic dilution method (Calder et al., 1999) after hydrolysis as described by Borucki Castro et al. (2007). Milk fat was measured according to the Röse-Gottlieb method (AOAC, 1996), whereas milk lactose was calculated as milk DM% - (fat % + protein % + ash %).

Partial pressure of oxygen (O_2) and pH were determined in fresh blood using a blood gas analyzer (model IL 1306, Instrument Laboratory, Lexington, MA). A spectrophotometric method using lactate dehydrogenase was used to measure blood L-lactate concentration (Benson et al., 2002). Blood hemoglobin was determined colorimetrically using cyanmethemoglobin as the standard. Plasma concentrations of urea-N and pAH were determined on the day of sampling with an automatic analyzer (Technicon Autoanalyser II, Technicon Instruments Corp., Tarrytown, NY) as described previously (Eisemann et al., 1987). An enzymatic reaction (glutamate dehydrogenase) as described by Bergmeyer and Beutler (1985) was used to determine ammonia concentrations no more than 1 wk after blood sampling. Glucose concentrations were determined colorimetrically using an enzymatic (glucose oxidase/peroxidase) reaction (Boehringer Mannheim, Dorval, QC, Canada).

Plasma amino acid concentrations were measured by isotope dilution using GC-MS (Calder et al., 1999).

Calculations

Milk AA output in protein was calculated using crude milk protein yield measured during the last 3 d of each period, with a 3.5% correction for bloodborne proteins, and reported AA composition (Swaigood, 1995), with the exception of Phe and Tyr, which were analyzed. Mammary plasma flow was estimated according to the Fick principle using Phe and Tyr as internal markers, again with allowance for a 3.5% contribution from bloodborne proteins: mammary plasma flow = (milk [Phe + Tyr] $\times$ 0.965) $\div$ (arterial - mammary [Phe + Tyr]), as described by Cant et al. (1993). Plasma flow across the splanchnic tissues was calculated from downstream dilution of the infused pAH (Katz and Bergman, 1969). The net fluxes of AA and glucose across the portal-drained viscera (PDV), liver, total splanchnic tissues (TSP), and mammary gland were calculated as the product of the average plasma venous-arterial concentration difference and the average plasma flow. Ammonia and lactate net fluxes were calculated using plasma and blood concentrations, respectively, and blood flow. Blood flow was calculated as plasma flow divided by (1 - packed cell volume). Urea fluxes were calculated using plasma water concentrations [plasma concentration / (1 - DM of plasma)] and blood water flows, which were calculated as blood flow multiplied by (1 - DM of blood) (Milano et al., 2000). A negative flux indicates utilization or removal, whereas a positive flux indicates net production or release of the nutrient across the tissue. Concentrations of O_2 in blood were calculated using measured partial pressure of O_2 , pH, and hemoglobin concentration (Bartels and Harms, 1959). Hepatic removal of AA as a percentage of PDV release was calculated as hepatic net flux/portal net flux $\times$ 100. Hepatic extraction as a percentage of total supply was calculated as hepatic net flux/liver total inflow $\times$ 100, with total inflow calculated as the product of portal concentration and portal plasma flow plus the product of hepatic arterial plasma flow and arterial concentration.

Statistical Analysis

Metabolite concentrations and net flux data were averaged over the 6 sampling times on each sampling day for statistical analysis. Dry matter intake, milk yield, and milk composition were averaged over the last 3 d of each period. All data were statistically analyzed using the GLM procedure (SAS Institute, 1999) with time

Table 1. Dry matter intake and milk production during the pre- and postcalving periods

Item	Time		SEM/SD ¹	P-value
	Precalving	Postcalving		
DMI, kg/d	14.7	18.5	0.44	0.002
Yield				
Milk, kg/d		40.2	5.4	
CP, g/d		1,214	152	
True protein, g/d		1,161	144	
Casein, g/d		1,001	101	
Whey, g/d		159	46	
Fat, g/d		1,493	224	
Lactose, g/d		1,876	224	
Milk composition				
CP, %		3.03	0.13	
True protein, % of CP		95.6	0.6	
Casein, % of CP		82.7	2.7	
Whey, % of CP		12.9	2.5	
Fat, %		3.71	0.19	
Lactose, %		4.69	0.34	

¹DMI reported as least squares means $\pm$ SEM; all other data reported as means $\pm$ SD; n = 6.

(precalving vs. postcalving) as the main effect and cow as a blocking factor. Differences were considered significant if $P \leq 0.10$ and as a trend for $0.10 < P \leq 0.15$. Data are reported as least squares means with pooled standard errors (SEM), unless otherwise stated.

RESULTS AND DISCUSSION

DMI and Plasma Flow

The exact time of sampling averaged 18.3 ± 8.4 d before calving and 36.3 ± 11.2 after calving. Milk yield and DMI are shown in Table 1. Dry matter intake increased by 3.8 kg/d from d 18 precalving to d 36 postcalving. The intake at d 36 is typical for this period (Doepel et al., 2002; Reynolds et al., 2003), whereas the precalving intake is higher than that reported by Reynolds et al. (2003) but similar to that observed by Doepel et al. (2002). This difference in precalving intake between the 2 studies may be related to the high NDF content and composition (high lignin due to straw inclusion) of the diet used by Reynolds et al. (2003), which may have limited intake as a result of slow passage rate through the rumen.

Portal plasma flow increased from 1,119 L/h precalving to 1,522 L/h postcalving (SEM: 74.8, $P = 0.01$), whereas hepatic flow increased from 1,323 to 1,896 L/h (SEM: 96.4, $P = 0.02$). Likewise, portal and hepatic blood flows, calculated using packed cell volumes of 29.1 and 28.4% pre- and postcalving, respectively, also increased. Reynolds et al. (2003) reported portal plasma flows of 695 L/h at 19 d precalving and 1,340 L/h at 33 d postcalving. This equates to a 93% increase pre- to postcalving, whereas in the current study the

increase was 36%. This discrepancy probably relates to differences in energy intake between the 2 studies, as positive correlations between blood flow and energy intake have been reported (Huntington, 1990; Lescoat et al., 1996). In the current study, precalving ME intake was 37.8 Mcal/d and increased to 49.2 Mcal/d (a 30% increase), whereas in the study of Reynolds et al. (2003) ME intake increased 150% from 22.5 Mcal/d precalving to 56.2 Mcal/d postcalving.

Net Fluxes of Nitrogen Compounds

AA. Arterial concentrations of all AA decreased ($P < 0.05$) with the onset of lactation with the exception of the branched-chain AA, Ala, Thr, and Trp, which were not affected ($P > 0.15$), and Gly, which increased ($P = 0.02$; Table 2). This decrease in AA concentrations occurred despite the increase in both MP supply and net portal absorption of AA ($P < 0.07$) in early lactation versus late gestation cows (Table 3). Only Cys and Gln net portal absorptions were not affected by time before or after parturition, but net portal absorption of these AA is relatively small. Despite this increased net absorption of AA, net liver removal did not increase for most AA. Net liver flux of Ala, Cys, Glu, Ile, Leu, Met, Ser, Thr, and Val was not affected by physiological state (i.e., gestation vs. lactation), whereas liver net removal of Gln, His, Lys, and Tyr decreased ($P < 0.10$) or tended to decrease ($P = 0.12$) for Phe. Only net liver removal of Gly and Trp increased ($P < 0.05$).

These observations raise several issues. First, it has been suggested that liver removal of AA is related to net portal supply (Lescoat et al., 1996). In practice, increased AA portal absorption is usually associated with

Table 2. Arterial concentrations of amino acids (μM) in Holstein cows pre- and postcalving¹

Amino acid	Precalving	Postcalving	SEM	P-value
Ala	221.8	194.0	13.8	0.21
Cys	130.7	96.2	4.7	0.003
Gln	311.0	216.7	14.8	0.006
Glu	68.7	50.4	3.6	0.02
Gly	194.5	319.1	25.5	0.02
His	71.3	52.3	3.8	0.02
Ile	149.0	180.1	17.0	0.25
Leu	215.4	175.9	18.2	0.19
Lys	99.0	74.8	5.3	0.02
Met	26.6	22.1	1.0	0.03
Phe	58.0	43.3	2.1	0.004
Ser	79.1	62.5	3.7	0.03
Thr	93.0	80.1	6.1	0.20
Trp	47.3	48.5	1.6	0.62
Tyr	64.4	44.0	1.3	0.0001
Val	277.2	269.8	26.0	0.85
TAA-N ²	2,695	2,353	124	0.11
TEAA-N ²	1,312	1,166	73	0.22
TNEAA-N ²	1,382	1,195	61	0.08
Group 1 AA-N ²	535.1	426.7	20.1	0.01
Group 2 AA-N ²	839.1	779.1	64.0	0.54

¹Least squares means $\pm$ SEM; $n = 6$.

²TAA-N = total AA-N; TEAA-N = total essential AA-N (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); TNEAA-N = total nonessential AA-N (Ala, Cys, Gln, Glu, Gly, Ser, and Tyr); group 1 AA-N = His, Met, Phe, Tyr, and Trp; group 2 AA-N = Ile, Leu, Lys, and Val.

greater plasma concentrations and in such situations, we cannot determine if either or both are regulating factors of hepatic removal. However, when AA supply was increased via jugular infusion resulting in increased concentrations but with no change in portal absorption, the relationship between hepatic removal and net portal absorption no longer existed (Berthiaume et al., 2002). Similarly, dissociation between these 2 parameters has been established under the more physiological experimental conditions in the current study: lactation increased intake and, thus, net portal absorption of AA, whereas the high demand to support milk protein secretion led to reduced concentrations of AA. These 2 experimental approaches lead to the same conclusion, however, that net liver removal is not driven by net portal absorption. Rather, in the current study, the ratio of liver removal to net absorption even decreased ($P < 0.10$) when net portal absorption increased between late gestation and early lactation for all AA except Cys, Gly, Thr, and Trp (Table 4). It has been suggested that liver removal may be better correlated with total inflow rather than with portal absorption (Lobley and Lapierre, 2003; Hanigan, 2005; Lapierre et al., 2005), because total inflow involves integration of both portal absorption and utilization by peripheral tissues. Such a concept implies that hepatic extraction is not exclusively due to first-pass removal but rather that a constant fraction of the total liver inflow is extracted per pass, with the amount returned to the liver driven

by peripheral tissue utilization. Higher rates of removal by peripheral tissues such as the mammary gland return a smaller quantity of AA to the liver where less is then extracted and catabolized. In the current study, although the physiological state of the cow had a lesser effect on the ratio of net liver removal to total inflow than when expressed relative to portal absorption, the fraction of liver removal relative to total inflow for Gly, His, Phe, and Tyr decreased ($P < 0.10$) postcalving compared with precalving and tended to decrease ($P = 0.11$) for Met. This indicates that fractional extraction by the liver varies with physiological state.

This hypothesis further predicts that the liver would remove AA not utilized for milk protein production. The impact of such a mechanism would be enhanced if there was a concomitant reduction in utilization of AA by the liver, for either energy (oxidative) or anabolic (e.g., gluconeogenesis) purposes. Although such regulation is not fully defined, how the AA are partitioned between the liver for gluconeogenic purposes will depend on a combination of nutritional, physiological, and genetic factors, and aspects of these will be discussed later in association with glucose net flux. Indeed, changes in hormone concentrations and sensitivity occurring at the onset of lactation (Kunz et al., 1985; Gerloff et al., 1986; VandeHaar et al., 1999) may contribute to the differences in hepatic metabolism observed between gestating and lactating cows. For example, growth hormone is elevated at the initiation of lactation (Ko-

Table 3. Splanchnic and mammary net fluxes of amino acids (mmol/h) in Holstein cows pre- and postcalving¹

Amino acid	Tissue ²	Precalving	Postcalving	SEM ³	P-value
Ala	PDV	60.5	83.2	4.2	0.01
	Liver	-59.4	-62.0	4.9	0.72
	TSP	1.1*	21.2	1.4	0.0002
	MG		-31.8	(10.4)	—
	Milk		18.1	(2.3)	—
Cys	PDV	2.9*	4.3	1.7	0.58
	Liver	-2.7†	-5.2	1.3	0.23
	TSP	0.2*	-0.9*	0.5	0.20
	MG		-5.6	(5.4)	—
	Milk		2.8	(0.4)	—
Gln	PDV	3.8*	4.2*	4.6	0.95
	Liver	-31.0	4.8*	5.9	0.008
	TSP	-27.3	9.0†	4.0	0.001
	MG		-26.1	(17.1)	—
	Milk		31.4	(3.9)	—
Glu	PDV	-0.8*	10.7	1.9	0.008
	Liver	36.2	30.6	5.2	0.48
	TSP	35.4	41.3	5.1	0.45
	MG		-21.8	(4.6)	—
	Milk		40.1	(5.0)	—
Gly	PDV	25.8	44.0	2.3	0.003
	Liver	-38.4	-62.5	2.6	0.001
	TSP	-12.7	-18.5	2.5	0.16
	MG		-8.0	(12.4)	—
	Milk		11.7	(1.5)	—
His	PDV	7.2	9.8	0.4	0.007
	Liver	-4.7	-3.0	0.6	0.09
	TSP	2.5	6.8	0.4	0.0006
	MG		-10.0	(2.5)	—
	Milk		8.5	(1.1)	—
Ile	PDV	19.0	29.2	1.4	0.004
	Liver	1.7*	2.5†	1.3	0.64
	TSP	20.6	31.8	2.2	0.02
	MG		-26.5	(3.6)	—
	Milk		22.0	(2.7)	—
Leu	PDV	31.8	45.2	2.2	0.008
	Liver	3.9	2.6†	1.1	0.45
	TSP	35.7	47.8	2.8	0.03
	MG		-39.4	(3.5)	—
	Milk		36.5	(4.6)	—
Lys	PDV	29.2	37.1	1.7	0.02
	Liver	-8.7*	5.2*	4.6	0.09
	TSP	20.4	42.2	3.3	0.006
	MG		-37.2	(7.9)	—
	Milk		27.7	(3.5)	—
Met	PDV	8.0	14.8	2.1	0.07
	Liver	-4.9	-4.7	0.9	0.92
	TSP	3.2†	10.1	1.3	0.01
	MG		-8.8	(0.4)	—
	Milk		9.2	(1.1)	—
Phe	PDV	18.6	26.1	1.0	0.003
	Liver	-13.8	-10.7	1.2	0.12
	TSP	4.8	15.4	0.7	0.0001
	MG		-15.2	(1.8)	—
	Milk		14.6	(1.8)	—
Ser	PDV	29.7	42.2	0.8	0.0001
	Liver	-26.9	-30.6	1.9	0.23
	TSP	2.8†	11.6	1.2	0.003
	MG		-20.2	(4.5)	—
	Milk		29.3	(3.7)	—
Thr	PDV	18.2	27.6	0.7	0.003
	Liver	-7.5	-7.6	1.1	0.99
	TSP	10.6	20.0	1.1	0.002
	MG		-20.1	(3.0)	—
	Milk		17.6	(2.2)	—

Continued

Table 3 (Continued). Splanchnic and mammary net fluxes of amino acids (mmol/h) in Holstein cows pre- and postcalving¹

Amino acid	Tissue ²	Precalving	Postcalving	SEM ³	P-value
Trp	PDV	3.7	7.6	0.9	0.04
	Liver	-1.7	-3.6	0.5	0.05
	TSP	2.1	4.0	0.5	0.04
	MG		-5.0	(2.0)	—
	Milk		3.6	(0.5)	—
Tyr	PDV	15.7	22.1	0.9	0.004
	Liver	-12.8	-9.3	1.0	0.051
	TSP	2.9	12.8	0.8	0.0004
	MG		-13.8	(2.0)	—
	Milk		14.3	(1.8)	—
Val	PDV	20.2	33.6	1.1	0.0004
	Liver	2.3*	1.5*	1.9	0.79
	TSP	22.6	35.1	2.1	0.009
	MG		-33.0	(5.0)	—
	Milk		27.5	(3.4)	—
TAA-N ⁴	PDV	317.5	524.0	19.6	0.0007
	Liver	-209.0	-147.7	25.2	0.15
	TSP	108.5	376.3	16.8	<0.0001
	MG		-401.5	(85.5)	—
	Milk		394.5	(49.3)	—
TEAA-N ⁴	PDV	187.0	310.0	11.3	0.0006
	Liver	-41.5	-26.0	5.6	0.11
	TSP	145.5	283.9	13.0	0.0007
	MG		-252.4	(34.1)	—
	Milk		215.4	(26.9)	—
TNEAA-N ⁴	PDV	139.9	208.5	15.5	0.03
	Liver	-170.0	-129.2	21.0	0.23
	TSP	-30.0	79.3	7.0	0.0001
	MG		-151.4	(55.3)	—
	Milk		179.1	(22.4)	—
Group 1 AA-N ⁴	PDV	82.7	138.9	5.1	0.0006
	Liver	-52.7	-48.9	3.9	0.52
	TSP	30.0	90.1	2.6	<0.0001
	MG		-94.8	(14.8)	—
	Milk		88.4	(11.0)	—
Group 2 AA-N ⁴	PDV	128.9	186.1	6.4	0.002
	Liver	-9.5*	17.4†	8.8	0.08
	TSP	119.4	203.5	8.1	0.0007
	MG		-172.8	(25.5)	—
	Milk		141.3	(17.7)	—

¹Least squares means $\pm$ SEM; n = 6.²PDV = portal-drained viscera; TSP = total splanchnic tissue; MG = mammary gland.³Values in parentheses are SD, not SEM.⁴TAA-N = total AA-N; TEAA-N = total essential AA-N (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); TNEAA-N = total nonessential AA-N (Ala, Cys, Gln, Glu, Gly, Ser, and Tyr); group 1 AA-N = His, Met, Phe, Tyr, and Trp; group 2 AA-N = Ile, Leu, Lys, and Val.* $P < 0.05$; † $P < 0.10$: not significantly different from zero.

prowski and Tucker, 1973) and induction of growth hormone release with growth hormone-releasing factor reduced liver removal of α -amino N (Reynolds et al., 1992).

The TSP net flux of all AA increased ($P < 0.05$) with the onset of lactation and the associated increase in MP supply, except for Cys and Glu release that were not affected and for Gly, for which there was net splanchnic uptake (Table 3). Glycine is unusual as the only AA for which post-liver supply is usually negative, indicating elevated utilization by the liver for functions such as hippurate detoxification as well as glutathione

synthesis. For the essential AA (EAA), post-liver supply always met or exceeded mammary uptake, except for His, although according to NRC (2001), these cows would be fed only 90% of MP requirements. The MP requirements are estimated using a fixed conversion factor of absorbed protein to metabolic functions of 67%, but previous studies have shown that dairy cows at restricted intakes are more efficient in the transfer of absorbed AA into milk protein (Doepel et al., 2004; Raggio et al., 2004). The increase in efficiency under situations of limited supply is also supported, in part, by the current observations because no mobilization of

Table 4. Net liver flux of amino acids as a percentage of net portal-drained visceral release or total liver inflow in Holstein cows pre- and postcalving¹

Amino acid	Parameter ²	Precalving	Postcalving	SEM	P-value
Ala	Removal	96.5	74.0	5.6	0.04
	Extraction	16.1	14.1	1.7	0.47
Cys	Removal	87.8	152.2	18.4	0.06
	Extraction	1.5	2.8	0.7	0.25
Gly	Removal	348.8	144.2	147.2	0.37
	Extraction	13.3	10.1	1.0	0.08
His	Removal	58.2	27.2	4.7	0.005
	Extraction	4.4	2.4	0.6	0.07
Met	Removal	55.1	29.1	4.4	0.009
	Extraction	10.6	7.6	1.0	0.11
Phe	Removal	70.8	38.3	3.7	0.002
	Extraction	13.8	9.3	0.7	0.008
Ser	Removal	88.1	70.3	4.0	0.03
	Extraction	19.3	19.3	1.2	0.97
Thr	Removal	33.4	24.5	5.6	0.31
	Extraction	4.8	3.8	0.8	0.40
Trp	Removal	32.4	43.0	6.3	0.30
	Extraction	2.2	3.4	0.8	0.35
Tyr	Removal	79.8	37.9	7.3	0.008
	Extraction	12.2	7.9	0.9	0.02
TAA-N ³	Removal	58.5	24.0	4.9	0.004
	Extraction	4.9	2.7	0.7	0.06
TEAA-N ³	Removal	14.5	4.8	6.6	0.34
	Extraction	1.6	0.7	0.5	0.21
TNEAA-N ³	Removal	138.8	56.4	17.2	0.02
	Extraction	8.3	5.2	1.0	0.07
Group 1 AA-N ³	Removal	60.4	30.6	4.2	0.004
	Extraction	6.3	4.6	0.7	0.14

¹Least squares means $\pm$ SEM; n = 6.²Removal = hepatic net flux/portal net flux $\times$ 100; extraction = (hepatic net flux/liver total inflow) $\times$ 100.³TAA-N = total AA-N; TEAA-N = total essential AA-N (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); TNEAA-N = total nonessential AA-N (Ala, Cys, Gln, Glu, Gly, Ser, and Tyr); group 1 AA-N = His, Met, Phe, Tyr, and Trp.**Table 5.** Metabolite and oxygen arterial concentrations (mM) and net fluxes (mmol/h) in Holstein cows pre- and postcalving¹

Metabolite		Tissue ²	Treatment		SEM ³	P-value
			Precalving	Postcalving		
Glucose	Concentration		3.63	2.87	0.09	0.002
		PDV	−30.5	33.5	26.1	0.14
	Flux	Liver	521	669	58.2	0.15
		TSP	487	703	33.4	0.01
		MG		−590	(52)	
Lactate	Concentration		0.53	0.39	0.02	0.008
		PDV	65	166	19.6	0.01
	Flux	Liver	−110	−287	21.8	0.002
		TSP	−45	−121	26.9	0.10
			6.13	5.74	0.18	<0.0001
Oxygen	Concentration		6.13	5.74	0.18	<0.0001
		PDV	−2,328	−2,970	108	0.01
	Flux	Liver	−3,002	−4,346	450	0.09
		TSP	−5,326	−7,351	437	0.02
			0.060	0.041	0.017	0.46
Ammonia	Concentration		0.060	0.041	0.017	0.46
		PDV	522	697	42.9	0.03
	Flux	Liver	−476	−665	58.3	0.07
		TSP	46	32	48.5	0.85
			8.9	10.4	0.46	0.07
Urea-N	Concentration		8.9	10.4	0.46	0.07
		PDV	−402	−444	78.0	0.72
	Flux	Liver	808	1,100	99.3	0.09
		TSP	406	656	87.9	0.10

¹Least squares means $\pm$ SEM; n = 6; negative values indicate net removal, whereas positive values indicate net release across the tissue.²PDV = portal-drained viscera; TSP = total splanchnic tissues; MG = mammary gland.³Values in parentheses are standard deviation, not SEM.

body proteins was required to provide sufficient post-liver supply of EAA to account for mammary uptake, although MP supply was less than predicted requirements (NRC, 2001). The net portal absorption and post-liver supply of the EAA were both in excess of mammary gland uptake and the amounts secreted in milk protein (Table 3), a comparative approach that has been validated in several recent studies (see Lapiere et al., 2005). The exception was His, but this AA can be supplied, without degrading tissue protein, from stores such as the intra-muscle dipeptide carnosine (not measured in this study) or from hemoglobin, as suggested for His-deficient pigs (Heger et al., 2007). In the current study, hemoglobin concentrations decreased numerically from 10.5 to 9.8 ± 0.3 g/100 mL from late gestation to lactation, and that could account for an endogenous supply of His of 0.5 mmol/h.

Regarding the nonessential AA (NEAA), the deficit in NEAA-N at the mammary gland (Clark et al., 1977; Guinard and Rulquin, 1994) is usually met by surplus extraction of the EAA, especially those of group 2 (Ile, Leu, Lys, and Val), allowing N balance to be attained (Raggio et al., 2006). Similarly, in the current study with cows in early lactation, mammary uptake relative to milk output was in excess for EAA-N (252 vs. 215 mmol/h), whereas uptake of NEAA-N was in deficit (152 vs. 179 mmol/h).

Ammonia and Urea. Arterial plasma ammonia concentrations were not different ($P = 0.46$) between the pre- and postcalving periods (Table 5). Although PDV ammonia absorption was higher postpartum than prepartum ($P = 0.03$), liver uptake was also higher ($P = 0.07$) resulting in no net difference in post-hepatic release. The postpartum increase in portal absorption is related to the higher dietary CP intake during this time (3,145 g/d postpartum vs. 2,058 g/d prepartum). Net flux of urea-N across the PDV was negative, averaging 423 mmol/h across both periods, and is representative of urea recycling through the gut wall. The increased ($P = 0.07$) plasma concentrations of urea-N in the postpartum period resulted from elevated hepatic ureagenesis ($P = 0.09$), with increased hepatic removal of ammonia accounting for most of the increased urea net release.

Net Fluxes of Energy Metabolites

Glucose. Total post-liver supply of glucose was 216 mmol/h higher in early lactation compared with late gestation ($P = 0.01$, Table 5), with approximately 30% of this increase resulting from increased net portal flux (shift from utilization to absorption) and 70% from hepatic gluconeogenesis. The difference between pre- and postcalving hepatic glucose output reported by Reynolds

et al. (2003) was larger than that observed in the current study. Their precalving diet, however, supplied less ME than in the current study, and glucose rate of appearance is thought to be closely linked to digestible energy intake in cattle (Wieghart et al., 1986). Postcalving, TSP glucose supply was 703 mmol/h, more than the requirement of 588 mmol/h estimated by Overton (1998) but close to the 737 mmol/h predicted by Danfaer et al. (1995) for lactating cows. The proportion of glucose net splanchnic release required to support lactose synthesis (457 mmol/h) averaged 68%. Lemosquet et al. (2007) reported that lactose synthesis averaged 73% of glucose rate of appearance, which would include, in addition to net splanchnic release of glucose, kidney synthesis of glucose and glycogenolysis. The increase in glucose net flux across the TSP during the transition from gestation to early lactation, however, was only half of that required to cover milk lactose output. This would imply that glucose utilization for other metabolic pathways is altered by lactation. Indeed, glucose oxidation has been reported to decrease at the onset of lactation (Benninck et al., 1972) and, similarly, administration of growth hormone increased lactose output and decreased glucose oxidation (Bauman et al., 1988).

It has been proposed that the contribution of AA to hepatic gluconeogenesis increases at the initiation of lactation in both proportional (Drackley et al., 2001) and absolute (Reynolds et al., 2003) terms. For example, Reynolds et al. (2003) estimated that the minimum contribution of AA to gluconeogenesis did not vary between d -19 to d +83 of calving and averaged 23%. As glucose production increased from 294 to 840 mmol/h over that period, this would lead to an increased net liver removal of AA (from ~60 to 200 mmol glucose equivalents per h). However, for the only AA measured, net removal of Ala was higher at d 11 and 21 but not at d 33 and 83 postcalving compared with precalving values. Therefore, the contribution of alanine to gluconeogenesis more than doubled from 9 d before calving (2.3%) to 11 d after calving (5.5%) but by d 33 this had declined to 1.5%. The current study agrees with the latter observation in that pre- (d -18) and postcalving (d 36) did not differ in the potential contribution of alanine to gluconeogenesis (5.9 and 4.6%, respectively). In contrast to the hypothesis of Reynolds et al. (2003), however, direct measures of removal of glucogenic AA by the liver were similar pre- and postcalving (82 and 80 mmol of glucose equivalents per h, respectively), with maximal potential contributions of 15.7 versus 11.9%, in the pre- and postcalving periods, respectively. These estimates are maximal contributions because part of the hepatic removal of AA is used for the synthesis of export proteins; for example, up to 20% of liver Phe removal is used for this purpose (Raggio et al., 2007).

Two possible explanations may account for the differences observed between the results of Reynolds et al. (2003) and the current study. First, the current study offered 50% more energy precalving, whereas the cows received a similar amount of energy postcalving compared with the latter study. Second, the ration used in the current study contains a higher proportion of corn grain, which may have increased absorption of propionate, thus reducing the need for other glucogenic substrates.

Lactate. From the precalving to the postcalving period, lactate release by the PDV increased by 101 mmol/h ($P = 0.01$), whereas hepatic removal increased by 177 mmol/h ($P = 0.002$; Table 5). If all the lactate extracted by the liver was used for gluconeogenesis, this would contribute 88 mmol/h, or 60%, to the observed increment in hepatic glucose net flux. Net portal absorption of lactate represented 57% of liver removal, and net hepatic removal in excess of the increased portal net absorption may not represent a net input of carbon (and glucose) to the animal, but rather reflects the dynamic exchange between glucose and lactate within the Cori cycle. Sources such as alanine deamination in muscle may also contribute to the post-hepatic lactate supply. Net flows of other glucose precursors, such as propionate (from increased energy intake) and glycerol (derived from net lipolysis), were not measured, but would be anticipated to increase gluconeogenesis.

Oxygen. Net splanchnic removal of O_2 increased by 38% from d 18 precalving to d 36 postcalving ($P = 0.02$), with two-thirds of this additional removal due to liver metabolism (Table 5). The increase in hepatic O_2 removal was similar to that of hepatic blood flow (43%). Although whole-body O_2 consumption and energy intake are positively related, the increment in liver O_2 removal was greater than the increment in ME intake. In addition to the increased splanchnic oxygen demands due to gluconeogenesis, digestion, and other metabolic processes associated with lactation, oxygen usage also increases because of proliferation of the splanchnic tissues in early lactation. For example, Gibb et al. (1992) reported that the gastrointestinal tract, liver, and spleen together increase in weight by 13.4% during the first 5 wk postpartum.

CONCLUSIONS

The greater DMI of early lactation cows versus late gestation cows resulted in an increase in splanchnic release of AA, glucose, and urea. Despite the increased net portal appearance of AA, net liver AA removal did not increase. This confirms that net portal appearance of AA was not a regulating factor for liver AA removal. This also means that the potential contribution of AA

to gluconeogenesis did not increase from pre- to postcalving, although glucose liver net flux increased over that period. Together, these data indicate that in early lactation for cows consuming these diets and producing milk components at the rates measured here, metabolic priority is given to direct AA toward milk protein production rather than gluconeogenesis. Finally, the increase in net splanchnic release of glucose between pre- and postcalving only met half of the requirement for lactose output and therefore other metabolic fates of glucose (e.g., oxidation) must have declined.

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