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Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation

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ABSTRACT

Blood flow and net nutrient fluxes for portal-drained viscera (PDV) and liver (total splanchnic tissues) were measured at 19 and 9 d prepartum and at 11, 21, 33, and 83 d in milk (DIM) in 5 multiparous Holstein-Friesian cows. Cows were fed a grass silage-based gestation ration initially and a corn silage-based lactation ration peripartum and postpartum. Meals were fed at 8-h intervals and hourly (n = 8) measures of splanchnic metabolism were started before (0730 h and 0830 h) feeding at 0830 h. Dry matter intakes (DMI) at 19 and 9 d prepartum were not different. Metabolism changes measured from 19 to 9 d prepartum were lower arterial insulin and acetate, higher arterial nonesterified fatty acids and increased net liver removal of glycerol. After calving, PDV and liver blood flow and oxygen consumption more than doubled as DMI and milk yield increased, but 85 and 93% of the respective increases in PDV and liver blood flow at 83 DIM had occurred by 11 DIM. Therefore, factors additional to DMI must also contribute to increased blood flow in early lactation. Most postpartum changes in net PDV and liver metabolism could be attributed to increases in DMI and digestion or increased milk yield and tissue energy loss. Glucose release was increasingly greater than calculated requirements as DIM increased, presumably as tissue energy balance increased. Potential contributions of lactate, alanine, and glycerol to liver glucose synthesis were greatest at 11 DIM but decreased by 83 DIM. Excluding alanine, there was no evidence of an increased contribution of amino acids to liver glucose synthesis is required in early lactation. Increased net liver removal of propionate (69%), lactate (20%), alanine (8%), and glycerol (4%) can account for increased liver glucose release in transition cows from 9 d before to 11 d after calving.

(**Key words:** portal-drained viscera, liver, transition)

Abbreviation key: ECD = expected calving date, **ME** = metabolizable energy, **PAH** = ρ-aminohippurate, **PCV** = packed-cell volume, **PDV** = portal-drained viscera, **TAG** = triacylglycerol.

INTRODUCTION

The transition from late gestation to early lactation is a period of dramatic physiological and metabolic adaptation for the dairy cow. During late gestation the nutritional demands of the fetus and uterus increase exponentially, while intake is often reduced by the endocrine changes that induce parturition, parturition itself, and other factors impacting on intake (Ingvarsten and Andersen, 2000). After calving, increases in nutrient requirements for milk synthesis outpace increases in intake, while hypertrophy of visceral tissues adds to nutrient demands (Bell, 1995). For the dairy cow, the most severe nutritional imbalances typically occur during transition (Grummer, 1995). The implications of transition metabolism and management for the well-being, reproduction, longevity, and production of the cow and the profitability of the dairy enterprise, are intuitive. The last decade has seen a considerable increase in basic and applied research concerning the nutritional physiology and metabolism of the transition dairy cow, and transition metabolism and nutrition has been the subject of numerous reviews (e.g., Bell, 1995; Grummer, 1995; Drackley, 1999; Drackley et al., 2001). However, as emphasized in many of these reviews, there is a paucity of information on the quantitative changes in nutrient absorption and the metabolism of splanchnic tissues (the portal-drained viscera [**PDV**; the gastrointestinal tract, pancreas, spleen, and associated mesenteric and omental fat] and liver) that occur in vivo during transition.

Available data describing the metabolism of splanchnic tissues during transition in dairy cows is largely based on measurements obtained in vitro, using tissue biopsy, or in vivo measurements of blood-borne metabolite concentration or turnover (e.g., Bell, 1995; Drackley et al., 2001). The hormonal changes initiating parturi-

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tion and lactation are known to induce changes in fatty acid metabolism as early as 10 d before calving (Grummer, 1995). Increases in liver protein synthesis have also been reported 10 d before parturition in dairy cows, suggesting adaptations of protein metabolism required for lactation and perhaps liver hypertrophy, may precede parturition (Bell, 1995). In sheep, liver blood flow, glucose production, and lactate and NEFA removal increased markedly in late gestation (Freetly and Ferrell, 1997, 1998, 2000). In dairy cows, glucose turnover increases after calving (Bell, 1995), but it is assumed that propionate availability limits glucose production, increasing glucogenic demands on amino acids (Drackley et al., 2001). However, quantitative effects of transition on liver glucose production and glucose precursor utilization have not been described in dairy cows in vivo. The objective of our study was to measure the effects of transition on the quantitative metabolism of the PDV and liver of dairy cows.

MATERIALS AND METHODS

Cows, Diets, and Treatments

All procedures used were licensed and regulated by the UK Home Office under the Animals (Scientific Procedures) Act of 1986. Five Holstein-Friesian cows fitted with ruminal cannulas and catheters enabling measurements of splanchnic metabolism (Huntington et al., 1989) in the portal vein, a hepatic vein, two mesenteric veins, and the caudal aorta via the mesenteric artery were used. In addition, each cow's right carotid artery was elevated to a subcutaneous position for temporary catheterization if the aortal catheter lost patency. The cows were approaching their third (three cows), fourth, or seventh lactation at the start of the study. Three cows were cannulated and catheterized for previous studies and had completed from one to four full lactations since catheterization surgery (402 to 1402 d before sampling for the present experiment). Two of these cows had duodenal and ileal cannulas that were established during abdominal surgery for catheterization of splanchnic blood vessels. The other two cows were surgically prepared for the present study. They had rumen and catheterization surgery during and at the end of, respectively, their previous lactation (at least 108 d before sampling for the present experiment). Cows were housed in an unheated tie-stall barn with forced air ventilation and translucent roofing. Yokes or head halters were used for restraint and wood-shavings on rubber mats were used for bedding. Cows had constant access to water, and the barn was artificially lit from approximately 0600 to 1800 h. Cows were moved to straw-bedded box stalls for calving and returned to tie stalls within 48 h of parturition.

Table 1. Composition of the total mixed rations (g/kg DM) fed during gestation and lactation.

Composition	Gestation	Lactation
Ingredient		
Chopped barley straw	250	
Maize silage	150	350
Grass silage	450	150
Soybean meal, 54% CP	60	150
Dry rolled barley	80	
Dry rolled wheat		105
Rapeseed meal		75
Ground corn		50
Molassed sugar beet shreds		50
Wheat mids		49
Fat (Megalac)		10
Mineral mix	10[1]	11[2]
Chemical Analysis		
CP	118	186
NDF	526	325
Ash	80	72
Oil	34	41
Starch	98	241
Sugars	31	57
Estimated ME, MJ/kg DM	9.82	12.06

[1]Containing 138 g sodium, 100 g phosphorus, 100 g magnesium, 15 g calcium, 4.5 g manganese, 2.5 g zinc, 1.5 g copper, 300 mg iodine, 100 mg cobalt, 15 mg selenium, 250 IU vitamin E, 60,000 IU vitamin D3, and 300,000 IU vitamin A per kilogram.

[2]Containing 270 g calcium, 60 g magnesium, 40 g phosphorus, 40 g sodium, 5 g zinc, 4 g manganese, 1.5 g copper, 500 mg iodine, 50 mg cobalt, 15 mg selenium, 500 IU vitamin E, 100,000 IU vitamin D3, and 500,000 IU vitamin A per kilogram.

Beginning at least 6 wk before expected calving date (**ECD**), cows were offered a grass silage-based gestation TMR (Table 1) to meet estimated requirements for metabolizable energy (**ME**) and CP (NRC, 1989). The amount offered was then held constant until calving. A corn silage-based TMR (Table 1) was fed at 2 kg DM/d beginning 10 d before ECD and for ad libitum intake after calving. Cows were milked at approximately 0600 and 1700 h, and daily rations were fed as 3 equal meals provided at 0830, 1630, and 2230 h. Orts were removed at 0815 h. Ration composition was adjusted weekly for the average DM content of components during the preceding week.

Measurements

Feed refusals, DM content of feed and refusals, and DMI were measured daily throughout the study. Daily feed samples were immediately frozen and composited on a weekly basis. Composite samples were dried at 60°C, ground, and analyzed as described previously (Benson et al., 2001). Milk yield was measured daily and daily milk composition measured for samples obtained on Monday, Wednesday, and Friday of each week as described by Benson et al. (2001). Measurements of net splanchnic metabolism were planned to be obtained

approximately 20 and 10 d before ECD and 10, 20, 30, and 84 d after calving. The day relative to calving on which sampling occurred varied with actual calving date and because samplings falling on Saturday and Sunday were rescheduled for Friday and Monday, respectively. Measurements during gestation were repeated at 10-d intervals until parturition. On average, cows were sampled at –19, –9, 11, 21, 33, and 83 d relative to calving, which was close to our intended schedule of sampling relative to calving. However, the actual day of sampling relative to calving varied among cows, such that some cows were sampled closer to their calving date than others. Blood sample sets representing one 8-h feeding period were obtained simultaneously from the artery and portal and hepatic veins at hourly intervals, beginning at 0730 h and ending at 1430 h. Cows were fed after the second set of samples was obtained at 0830 h. At each sampling time, blood for metabolite analyses was collected into syringes treated with heparin (25 U/ml of blood) and immediately placed in crushed ice until processed. Blood for gas analysis was collected under anaerobic conditions in syringes treated with heparin (Benson et al., 2002), which were immediately sealed and kept on ice until analyzed, normally within 30 min of sampling. Blood flow was determined by measuring dilution of ρ-aminohippurate (**PAH;** 100 g/L) infused continuously into a mesenteric vein at approximately 12 g/h following a priming dose (4.5 g) at 0630 h. Preparation of PAH and sterile mesenteric vein infusions were as described by Huntington et al. (1989) but using a Harvard 22 infusion pump (Harvard Apparatus, Edenbridge, Kent, UK) and 140-ml syringes (Sherwood Medical, Crawley, Sussex, UK). In addition, a jugular vein catheter (178 mm outside diameter Tygon tubing, Fisher Scientific, Loughborough, UK) was established on the day before sampling. This catheter was used for infusion of 1-[^{13}C]-leucine for measurement of leucine metabolism, which will not be reported in the present paper. Continuous, sterile infusion of 1-[^{13}C]-leucine (200 mg in 60 ml/h) was generally as described previously (Bequette et al., 1996), followed a priming dose (200 mg) at 0630 h, and continued throughout blood sampling.

Sample analyses. Blood samples were kept on crushed ice until processed. Blood for metabolite analysis was added to a composite sample for each sampling site that was immediately flash-frozen using liquid N, then mixed when thawed for analysis. Plasma was harvested from a subsample of blood after centrifugation at 1800 $\times g$ for 20 min at 2°C. Plasma was flash-frozen after the addition to a composite sample or analyzed immediately for PAH, glucose and L-lactate as described by Benson et al. (2002). Blood pH, hemoglobin, and PCV and partial pressures of CO_2 and O_2 were measured as described by Benson et al. (2002). Frozen blood and plasma were stored at –85°C until analyzed. After thawing, composite samples for each cow sampling and sampling site were analyzed for blood concentrations of ammonia, urea, BHBA, and VFA and plasma concentrations of NEFA and insulin as described by Benson et al. (2002) and Benson and Reynolds (2001). In addition, composite blood samples were deproteinized and neutralized as described by Benson et al. (2002) and analyzed for alanine as described by Reynolds and Tyrrell (1991). Composite plasma samples were analyzed for triacylglycerol (**TAG**) and glycerol using an enzymatic assay (Assay 337-B, Sigma-Aldrich Ltd., Poole, UK) adapted for use on a Cobas Mira analyzer (Roche Diagnostic Products, Welwyn Garden City, UK).

Calculations. Calculation of blood O_2 and CO_2 concentrations, blood and plasma flows, and net flux rates of metabolites for the PDV, liver, and total splanchnic tissues were as described previously (Benson et al., 2002). With venous-arterial differences, positive rates denote net release of a metabolite into venous blood, while negative rates denote net removal from blood supply. For metabolites removed by the liver, extraction rate was calculated as net removal divided by total blood supply times 100. For metabolites released by the PDV and removed by the liver, the percentage of net PDV release removed by the liver on a net basis was also calculated as net liver removal divided by net PDV release times 100. In addition, the maximal possible net contribution of glucose precursors removed by the liver to glucose released by the liver, on a net basis, was calculated as 0.5 times net precursor removal divided by net glucose release times 100. A similar calculation was made for the maximal contribution of NEFA and n-butyrate removed by the liver to net BHBA release by the liver, assuming an average of 16 carbons per mole of NEFA removed. This is a conservative estimate of the carbon content of plasma NEFA and could underestimate NEFA carbon removal by as much as 9% (Drackley et al., 2001).

Statistical analyses. Average rates of metabolism for each cow sampling were analyzed statistically for effects of average day relative to calving using the mixed procedure of SAS (2001). Data were analyzed as repeated measures within cow using either a compound symmetry or spatial power covariance structure, depending on goodness of fit criteria (Littell et al., 1996). In addition, the probability of linear, quadratic, cubic, or quartic effects of average day relative to calving were tested using sequential sums of squares and the mixed procedure of SAS (2001) as described by Littell et al. (1996). To aid interpretation of changes as cows progressed through transition, means for measurements at

Table 2. Dry matter intake, milk yield and composition, and body weight in transition dairy cows.

	Average day relative to calving								$P <$[2]			
Item	−19	−9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
DMI, kg/d	9.6	9.6	14.7***	17.1***	19.5***	22.1***	0.7	0.001	0.001	0.001	0.010	0.143
Milk yield, kg/d			36.4	41.3***	43.1***	40.7**	1.2	0.001	0.037	0.001	0.244	—
4% FCM, kg/d			41.2	41.9	44.2†	42.4	2.4	0.335	0.984	0.086	0.687	—
Milk composition, g/kg												
Protein			35.1	31.2**	31.2**	31.5**	0.8	0.009	0.062	0.006	0.063	—
Fat			43.3	38.2	38.9	37.9	2.7	0.224	0.217	0.191	0.232	—
Lactose			45.8	46.4*	46.8**	47.1*	0.4	0.029	0.049	0.031	0.889	—
Component yield, kg/d												
Protein			1283	1290	1344	1289	36	0.378	0.977	0.166	0.286	—
Fat			1598	1570	1677	1547	129	0.715	0.686	0.445	0.469	—
Lactose			1671	1916***	2018***	1911**	57	0.001	0.034	0.001	0.313	—
BW, kg	768	766	676***	662***	660***	647***	31	0.001	0.001	0.001	0.987	0.001

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d −19 or 11 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

19 d before calving (11 d after calving for milk variables) were compared to each subsequent mean using the Dunnet's adjustment for LS means comparisons within the mixed procedure. One cow was not sampled at 12 wk postpartum, therefore least squares means are presented, and the SEM for n = 5 is given. There were no missing blood samples for any sampling day included in the statistical analysis of the data. Blood flow and net flux rates are presented as the average hourly rate for the 8-h sampling period, which may differ from average rates of metabolism for the entire day. As the number of animals used was limited, differences are discussed as significant at $P < 0.10$. Three additional measurements, obtained before calving in cows that calved later than, or on, their expected calving date, were not included in the statistical analysis. These samplings occurred on d −33 and −1 relative to calving in one cow, and during calving in another cow. This meant that one measurement included for 9 d before calving (11 d for that cow) was obtained from a cow fed 2 kg of lactation ration DM for 10 d.

RESULTS

There were relatively few transition problems or veterinary treatments during the course of the study. One cow (6132) suffered from milk fever and was "down" for 24 h after calving but responded to treatment. Another cow had excessive uterine discharge and received a medicated uterine "flush" at 15 d postpartum. Two cows were inseminated before their measurement at 12 wk postpartum.

Dry Matter Intake, Milk Yield, and Body Weight

Dry matter intake was the same on the two sampling days before calving (Table 2), only declining, on average, on d −1, 0, 1, and 2 relative to parturition (Figure 1). This slight dip in daily DMI coincided with the period that cows were in box stalls for calving, so may in part reflect consumption of straw bedding not included in measurements of DMI. After returning to tie stalls and the change to the lactation ration, DMI increased (cubic, $P < 0.01$) and was highest during sampling at 12 wk postpartum (Table 2 and Figure 1). Milk (quadratic, $P < 0.001$) and 4% FCM (quadratic, $P < 0.09$) yield on sampling days was highest 33 d after calving (Table 2). Milk fat and protein yield were not affected by day postpartum. Milk protein concentration was higher during sampling at 11 d postpartum than on subsequent sampling days (quadratic, $P < 0.006$), while milk lactose concentration and yield increased (quadratic, $P < 0.04$) as lactation progressed (Table 2). The BW of cows decreased 90 kg at calving, then continued to decline gradually through wk 12 postpartum (quartic, $P < 0.001$; Table 2).

Blood Flow, Packed Cell Volume, and pH

Portal vein and liver plasma and total blood flow (Table 3) increased (quartic, $P < 0.01$ or less) after parturition and were greatest at 83 d postpartum. Hepatic artery blood and plasma flow also increased after calving (quadratic, $P < 0.01$), but hepatic artery flow rates were greatest at 21 d postpartum. Arterial packed cell volume and hemoglobin concentration were not affected by transition, but arterial pH increased linearly ($P < 0.08$) over the course of the study.

Arterial Concentrations

With the exception of ammonia, O_2, and n-valerate, arterial concentrations of metabolites, gasses, insulin,

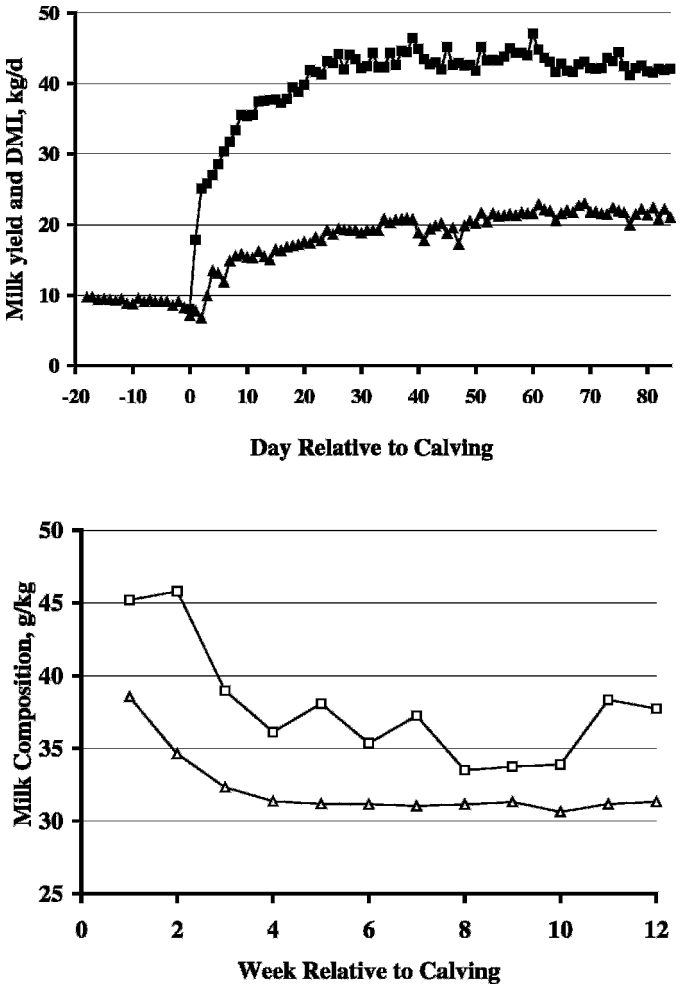

Figure 1. Dry matter intake (▲) and milk yield (■), and milk fat (□) and protein (△) concentration in transition dairy cows.

and VFA measured were affected by transition (Table 4). Concentrations of glucose, lactate, and insulin decreased in a quadratic manner ($P < 0.003$) after calving, whereas TAG concentration decreased in a quartic pattern ($P < 0.002$). Arterial concentrations of BHBA (quadratic, $P < 0.04$), NEFA (cubic, $P < 0.001$), alanine (linear, $P < 0.04$), urea (cubic, $P < 0.03$), CO_2 (linear, $P < 0.001$), acetate (cubic, $P < 0.02$), propionate (quartic, $P < 0.007$), i-butyrate (quadratic, $P < 0.03$), n-butyrate (quadratic, $P < 0.001$) and i-valerate (quadratic, $P < 0.03$) all changed with successive samplings. Except for an increase in NEFA concentration and a decrease in insulin and acetate concentration, arterial concentrations measured 9 d before calving were not different from measurements 19 d before calving, based on Dunnet's comparisons. Therefore, for the majority of compounds measured, significant changes in arterial concentrations only occurred after calving. For most

Table 3. Blood flow and arterial packed cell volume, hemoglobin, and pH in transition dairy cows.

	Average day relative to calving							$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
Plasma flow, L/h												
Portal vein	695	716	1287***	1277***	1340***	1505***	118	0.001	0.001	0.005	0.848	0.006
Liver	807	821	1560***	1601***	1623***	1747***	77	0.001	0.001	0.001	0.623	0.001
Hepatic artery	113	112	272*	314**	283**	239†	57	0.029	0.027	0.007	0.987	0.184
Blood flow, L/h												
Portal vein	964	1000	1809***	1780***	1870***	2093***	170	0.001	0.001	0.007	0.794	0.009
Liver	1121	1147	2187***	2222***	2247***	2437***	119	0.001	0.001	0.001	0.569	0.001
Hepatic artery	157	157	380*	433**	388*	335	78	0.027	0.024	0.007	0.938	0.177
Arterial packed-cell volume, %	28.1	28.5	28.7	27.8	27.9	28.0	1.0	0.707	0.607	0.886	0.303	0.743
Arterial Hb[3], g/L	96.9	97.8	95.9	96.0	95.0	100.7	4.2	0.820	0.492	0.237	0.639	0.889
Arterial pH	7.40	7.42*	7.42†	7.41	7.42†	7.43*	0.01	0.113	0.072	0.871	0.267	0.104

[1]Overall effect of average day relative to calving.
[2]Polynomial effects of day relative to calving.
Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.
[3]Hemoglobin.

Table 4. Arterial concentration of metabolites (mmol/L), gasses (mmol/L), insulin (mmol/L), and volatile fatty acids (μmol/L) in transition dairy cows.

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
Glucose	3.53	3.51	3.03***	3.23**	3.24**	3.45	0.11	0.001	0.383	0.001	0.122	0.081
Lactate	0.372	0.361	0.280*	0.259*	0.286†	0.375	0.029	0.042	0.802	0.001	0.856	0.282
BHBA	0.499	0.475	0.838**	0.662	0.760*	0.656	0.096	0.007	0.127	0.040	0.844	0.354
NEFA	0.115	0.173*	0.361***	0.236**	0.185*	0.088	0.027	0.001	0.109	0.001	0.001	0.063
Triacylglycerol	0.160	0.177	0.039***	0.055***	0.065***	0.071***	0.014	0.001	0.001	0.001	0.475	0.002
Glycerol	0.041	0.044	0.050†	0.043	0.037	0.029*	0.003	0.005	0.002	0.038	0.043	0.417
Alanine	0.200	0.189	0.180	0.203	0.215	0.243†	0.015	0.069	0.031	0.318	0.071	0.874
Ammonia	0.180	0.169	0.156	0.158	0.138	0.169	0.020	0.530	0.618	0.166	0.508	0.430
Urea	2.648	2.923	4.538***	5.485***	6.295***	7.413***	0.467	0.000	0.001	0.006	0.024	0.301
Oxygen	5.46	5.51	5.39	5.41	5.36	5.69	0.23	0.811	0.461	0.277	0.667	0.846
Carbon dioxide	24.7	25.3	26.8**	26.9**	27.6***	30.4***	0.5	0.001	0.001	0.825	0.594	0.738
Insulin	0.094	0.076†	0.037***	0.044**	0.059*	0.081	0.011	0.015	0.587	0.002	0.049	0.146
Acetate	1707	1437*	1585	1915	1985	2043 †	127	0.014	0.007	0.608	0.013	0.126
Propionate	60.5	52.5	96.1***	94.7***	92.6***	84.9**	14.9	0.001	0.001	0.001	0.955	0.007
i-Butyrate	4.0	4.2	6.5**	5.3†	6.0*	5.5†	0.9	0.022	0.048	0.023	0.473	0.587
n-Butyrate	14.7	13.3	43.8***	30.2**	35.4***	31.6**	4.3	0.001	0.005	0.001	0.310	0.110
i-Valerate	4.4	4.4	12.3***	7.6*	8.5*	9.5**	1.3	0.001	0.013	0.030	0.099	0.173
n-Valerate	5.9	6.4	6.8	6.8	7.9*	6.8	1.0	0.272	0.173	0.081	0.436	0.371
Total VFA	1796	1518*	1751	2060	2136 †	2185*	134	0.009	0.003	0.389	0.014	0.076
Total VFA carbon	3721	3155 †	3756	4328 †	4496*	4621*	283	0.002	0.001	0.199	0.025	0.111

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

compounds, these changes were greatest numerically at 11 (glucose, BHBA, NEFA, TAG, glycerol, insulin, propionate, i-butyrate, n-butyrate, i-valerate) or 21 d (lactate) postpartum. The increase in arterial alanine concentration also exhibited a cubic pattern ($P < 0.08$), as the lowest average concentration was measured 11 d postpartum. For urea, CO_2, and acetate, changes in their arterial concentration were greatest numerically at 83 d after calving.

Portal-Drained Visceral Metabolism

Of the compounds measured in the present study, only net PDV metabolism of TAG ($P > 0.36$) and CO_2 ($P > 0.29$) were not significantly affected as cows progressed through transition (Table 5). Net PDV release of lactate (cubic, $P < 0.001$), BHBA (linear, $P < 0.001$), NEFA (quadratic, $P < 0.03$), glycerol ($P < 0.03$), alanine (quadratic, $P < 0.001$), ammonia (linear, $P < 0.001$), insulin (linear, $P < 0.06$), and all the VFA measured (linear, $P < 0.001$ for all; n-valerate quadratic, $P < 0.007$) and removal of urea (linear, $P < 0.05$) and O_2 (linear, $P < 0.001$) increased after calving. For many of the compounds affected (lactate, BHBA, ammonia, urea, O_2, insulin, and all the VFA), the greatest numerical increase occurred at 83 d postpartum, but net PDV release of NEFA, glycerol, and alanine were greatest numerically at 11, 21, and 33 d postpartum, respectively. Net PDV flux of glucose varied across sampling times ($P < 0.05$) but only tended to increase linearly ($P < 0.11$). Based on Dunnet's comparisons, there were no significant differences between measurements of net PDV flux of any compounds measured 19 and 9 d before calving.

Liver Metabolism

Of the compounds measured, only net liver release of acetate ($P > 0.21$) was not significantly different across sampling days (Table 6). Net liver release of glucose (quartic, $P < 0.001$), BHBA (quadratic, $P < 0.001$), urea (linear, $P < .007$) and CO_2 (quadratic, $P < 0.001$) and removal of lactate (quadratic, $P < 0.001$), NEFA (quartic, $P < 0.02$), glycerol (quartic, $P < 0.008$), alanine (quartic, $P < 0.001$), ammonia (linear, $P < 0.001$), O_2 (quartic, $P < 0.02$), and all of the VFA except acetate (linear, $P < 0.001$ for all; n-valerate quadratic, $P < 0.007$) increased after calving. Net liver flux of TAG varied as well (quartic, $P < 0.02$), with the only substantial numerical change being an increase in net removal at 9 d before calving. Net liver removal of insulin also varied (quartic, $P < 0.08$), with the least removal measured 9 d before calving. Otherwise, Dunnet's comparisons indicated there were no differences ($P > 0.10$) in net liver metabolism between 19 and 11 d prepartum.

Liver extraction as a percentage of PDV release and total supply. As a percentage of net PDV release, net liver removal of lactate (cubic, $P < 0.02$), alanine (quartic, $P < 0.02$), NEFA (cubic, $P < 0.07$), and insulin (quartic, $P < 0.03$) varied with transition (Table 7). Liver removal accounted for the largest percentage of lactate, alanine, and ammonia released by the PDV at 11 d postpartum, after which the proportion of PDV lactate and ammonia release removed by the liver declined. Net liver removal of O_2 (linear, $P < 0.05$) and ammonia (linear, $P < 0.02$) as a percentage of total supply increased as transition and lactation progressed. Net liver removal of lactate (quadratic, $P < 0.01$), alanine (quartic, $P < 0.02$), NEFA (quadratic, $P < 0.05$), glycerol (cubic, $P < 0.007$), and insulin (quartic, $P < 0.01$) as a percentage of total supply varied through transition, with the greatest numerical extraction of lactate, alanine, and glycerol measured at 11 d postpartum. As a percentage of net PDV release, liver removal of propionate (cubic, $P < 0.01$) and n-butyrate (cubic, $P < 0.04$) varied slightly across sampling days, and was numerically lowest on d –9 and 11 relative to calving (Table 8). Liver removal of propionate (cubic, $P < 0.02$), i-butyrate (quadratic, $P < 0.08$), n-butyrate (cubic, $P < 0.03$), i-valerate (cubic, $P < 0.04$), and n-valerate (linear, $P < 0.05$) as a percentage of total supply also varied across samplings (Table 8). Based on Dunnet's comparisons to measurements at 19 d before calving, liver removal of propionate ($P < 0.01$), i-butyrate ($P < 0.05$), n-butyrate ($P < 0.10$), and i-valerate ($P < 0.01$), as a percentage of total supply, was lower at 11 d postpartum. Liver removal of propionate as a percentage of net PDV release was also lower ($P < 0.01$) 11 d after calving than on the first day of sampling.

Liver removal of glucose and BHBA precursors. In terms of their maximal potential net contribution to net glucose release by the liver (Table 9), the contribution of propionate increased (linear, $P < 0.06$) and lactate decreased (linear, $P < 0.01$) as lactation progressed. The maximal potential contribution of alanine (quartic, $P < 0.02$), glycerol (cubic, $P < 0.003$), and TAG (quartic, $P < 0.03$) but not total precursors measured, varied across days relative to calving. Numerically, the greatest net liver removal of alanine, glycerol, and total glucose precursors, relative to net liver glucose release, was measured at 11 d postpartum. In contrast, the greatest potential contribution of propionate and lowest potential contribution of lactate to glucose released was measured at 83 d postpartum. For BHBA (Table 9), the maximal potential contribution of NEFA (cubic, $P < 0.001$), n-butyrate (quadratic, $P < 0.06$), and total measured precursors (cubic, $P < 0.01$) also varied across sampling days with the greatest potential contribution of NEFA and thus total BHBA precursors at 11 d post-

Table 5. Net portal-drained visceral flux of metabolites, gasses, insulin, and volatile fatty acids in transition dairy cows (mmol/h).

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
Glucose	–24.6	–30	3.6*	–25.8	–1.3†	–4.7	12.1	0.042	0.103	0.344	0.977	0.917
Lactate	96.6	94.7	134**	182.3***	203.1***	208.1***	21.5	0.001	0.001	0.002	0.001	0.089
BHBA	127	133	192	216*	284***	299***	31	0.001	0.001	0.096	0.153	0.767
NEFA	16.0	24.0	66.0**	45.0†	36.0	21.0	14.0	0.029	0.953	0.023	0.099	0.184
Triacylglycerol	16.8	–3.7	0.1	–1.3	–1.0	–1.1	9.2	0.650	0.390	0.357	0.357	0.375
Glycerol	2.9	6.9	14.0*	16.0*	10.7	4.2	4.3	0.175	0.903	0.024	0.217	0.212
Alanine	23.3	24.0	74.1**	73.3***	91.8***	80.5***	8.6	0.001	0.001	0.001	0.384	0.386
Ammonia	292	324	494**	601***	688***	983***	54	0.001	0.001	0.368	0.381	0.613
Urea	–57	–156	–134	–183	–44	–355*	90	0.175	0.048	0.269	0.129	0.837
Oxygen	–1633	–1527	–2653**	–2996***	–3355***	–4217***	384	0.001	0.001	0.112	0.229	0.080
Carbon dioxide	1143	1023	925	720	1465	1297	343	0.686	0.531	0.679	0.285	0.380
Insulin	29.4	20.8	32.4	29.9	33.4	49.5†	8.2	0.278	0.054	0.581	0.648	0.334
Acetate	1446	1330	2015*	1976*	2674***	3374***	262	0.001	0.001	0.674	0.111	0.977
Propionate	343	306	795***	834**	1009***	1220***	203	0.001	0.001	0.075	0.228	0.078
i-Butyrate	11.1	8.9	17.3†	18.7*	23.3**	29.9***	2.8	0.001	0.001	0.337	0.252	0.403
n-Butyrate	46	40.1	170.5***	134.2**	185.9***	222.7***	20.6	0.001	0.001	0.017	0.988	0.296
i-Valerate	18.8	17.0	42.1*	40.4*	57.3**	77.0***	11.5	0.003	0.001	0.531	0.299	0.668
n-Valerate	10.7	7.9	40.3***	32.6***	40.7***	49.5***	5.6	0.001	0.001	0.007	0.783	0.091
Total VFA	1876	1710	3080**	3035**	3990***	4872***	496	0.001	0.001	0.217	0.147	0.572
Total VFA carbon	4297	3898	7578***	7429**	9701***	11800***	1275	0.001	0.001	0.255	0.118	0.396

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

Table 6. Net liver flux of metabolites, gasses, insulin, and volatile fatty acids in transition dairy cows (mmol/h).

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
Glucose	294	317	627***	777***	810***	840***	45	0.001	0.001	0.001	0.050	0.001
Lactate	–112	–145	–266***	–265***	–254**	–140	32	0.004	0.443	0.001	0.520	0.116
BHBA	151	172	396**	354*	449***	275	57	0.003	0.030	0.001	0.617	0.666
NEFA	–21.0	–35.6	–115.8***	–79.9***	–53.5*	–40.2	12.8	0.001	0.551	0.001	0.002	0.011
Triacylglycerol	0.7	–9.6**	–2.2	0.0	–1.2	1.2	3.0	0.066	0.132	0.865	0.091	0.013
Glycerol	–13.8	–18.7	–44.8***	–39.0**	–25.0	–9.3	7.8	0.002	0.492	0.001	0.018	0.008
Alanine	–18.3	–14.6	–60.9***	–45.4**	–24.9	–28.5	6.1	0.001	0.291	0.008	0.017	0.001
Ammonia	–310	–330	–555***	–674***	–723***	–998***	51	0.001	0.001	0.047	0.466	0.156
Urea	132	307	444†	395	468†	704**	117	0.083	0.007	0.638	0.343	0.641
Oxygen	–1505	–1682	–3108***	–3296***	–3425***	–3911***	214	0.001	0.001	0.001	0.720	0.015
Carbon dioxide	587	957	1638***	1680***	1723***	1105†	204	0.001	0.028	0.001	0.530	0.693
Insulin	–19.8	–6.6	–16.3	–24.2	–16.7	–25.6	7.7	0.428	0.312	0.914	0.569	0.076
Acetate	313	424	647	647	314	490	244	0.717	0.742	0.511	0.208	0.402
Propionate	–322	–282	–711**	–768**	–954***	–1156***	192	0.001	0.001	0.093	0.123	0.120
i-Butyrate	–10.1	–9.1	–16.3*	–18.1**	–22.8***	–27.4***	2.3	0.001	0.001	0.117	0.163	0.542
n-Butyrate	–37.7	–31.7	–127.4**	–105**	–159.6***	–185.1***	18.2	0.001	0.001	0.036	0.558	0.556
i-Valerate	–16.8	–15.5	–38.9*	–37.1*	–52.6**	–71.4**	11	0.005	0.001	0.552	0.347	0.696
n-Valerate	–11.3	–9.4	–41.4***	–35.2***	–45.3***	–50.7***	4.6	0.001	0.001	0.001	0.926	0.116
Total VFA	–85	76	–288	–317	–921*	–987*	364	0.048	0.004	0.565	0.167	0.829
Total VFA carbon	–672	–286	–1816	–1865	–3455**	–3920**	912	0.004	0.001	0.305	0.188	0.960

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

Table 7. Net liver removal of compounds as a percentage of net portal-drained visceral release or total supply.

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
% of net PDV release												
Lactate	118.8	153.0*	198.9**	145.7	127.0	71.4	21.4	0.003	0.055	0.039	0.018	0.732
Alanine	78.1	57.8	83.0	65.5	26.3**	36.1*	10.3	0.012	0.006	0.616	0.084	0.014
Ammonia	107.8	101.1	114.5	112.1	106.4	102.3	5.4	0.498	0.708	0.263	0.820	0.094
NEFA	54.8	168.6†	259.4*	224.0†	201.0	342.3*	68.6	0.110	0.033	0.580	0.070	0.984
Glycerol	983.5	357.8	506.7	323.6	596.8	588.1	279.8	0.682	0.666	0.333	0.281	0.617
Insulin	70.4	22.4†	59.6	75.6	39.8	47.8	19.4	0.373	0.864	0.849	0.699	0.027
% of total supply												
Oxygen	33.4	34.7	34.0	36.9	39.5*	39.5†	2.1	0.165	0.043	0.544	0.202	0.262
Lactate	32.4	38.6	45.9*	44.1	38.2	15.9†	6.6	0.041	0.066	0.006	0.189	0.507
Alanine	7.6	6.6	13.8*	9.1	4.2	4.3	1.7	0.015	0.136	0.281	0.028	0.017
Ammonia	62.4	62.9	66.9	71.2†	72.0*	71.2†	3.2	0.118	0.017	0.099	0.513	0.725
NEFA	19.3	22.3	18.3	18.4	16.6	23.0	2.3	0.218	0.596	0.045	0.179	0.413
Glycerol	35.1	42.8†	45.2†	40.5	30.9	18.5**	6.7	0.002	0.001	0.036	0.007	0.655
Insulin	17.5	5.9†	16.8	20.5	9.4	12.5	4.7	0.221	0.876	0.744	0.971	0.010

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

partum. The lowest potential contribution of n-butyrate to BHBA release was measured at 33 d postpartum.

Total Splanchnic Flux

Reflecting combined effects of changes in PDV and liver metabolism, only net total splanchnic flux of TAG, insulin, i-butyrate, and i-valerate were not significantly different across sampling days (Table 10). Net splanchnic release of glucose (quartic, $P < 0.001$), BHBA (quadratic, $P < 0.004$), alanine (cubic, $P < 0.002$), urea (linear, $P < 0.05$), CO_2 (quadratic, $P < 0.03$), acetate (linear, $P < 0.001$), propionate (quadratic, $P < 0.01$), and n-butyrate (quartic, $P < 0.02$) and net removal of NEFA (quartic, $P < 0.02$), glycerol (quartic, $P < 0.004$), O_2 (quartic, $P < 0.002$), and n-valerate (quadratic, $P < 0.07$) increased after calving. Net total splanchnic removal of lactate was greatest at 11 d postpartum (quadratic, $P < 0.004$), then decreased such that a net release was measured at 83 d postpartum. Similar to lactate, the greatest net total splanchnic removal of NEFA and glycerol was measured 11 d after calving. The greatest net total splanchnic release of glucose, urea, and acetate and removal of O_2 was measured at 83 d postpartum, while the largest release of BHBA and alanine was measured at 33 d postpartum. In contrast, the greatest net total splanchnic release of propionate and n-butyrate was measured at 11 d postpartum, reflecting the decrease in the percentage of net PDV release and/or total supply of these VFA removed by the liver.

Table 8. Net liver removal of volatile fatty acids as a percentage of net portal-drained visceral release or total supply.

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
% of net PDV release												
Propionate	94.0	90.9	89.2*	92.2	94.9	95.1	1.5	0.081	0.115	0.294	0.010	0.864
i-Butyrate	91.7	102.5	93.9	97.1	106.2	92.3	7.1	0.510	0.937	0.244	0.599	0.112
n-Butyrate	81.3	71.5	73.4	78.1	85.6	83.3	4.7	0.289	0.187	0.868	0.036	0.768
i-Valerate	89.8	90.5	91.0	91.7	91.7	92.8	3.6	0.996	0.560	0.898	0.961	0.989
n-Valerate	104.2	55.7	103.2	108.2	114.9	102.9	27.4	0.690	0.492	0.580	0.353	0.280
% of total supply												
Propionate	78.5	75.4	70.6**	74.0†	77.7	79.6	3.0	0.032	0.231	0.024	0.014	0.382
i-Butyrate	65.6	65.2	53.4*	59.0	61.9	63.2	4.3	0.179	0.709	0.078	0.184	0.231
n-Butyrate	59.4	51.5	48.0†	52.1	59.6	61.2	5.1	0.167	0.228	0.168	0.027	0.807
i-Valerate	70.8	68.9	56.7**	64.9	67.9	70.2	3.6	0.022	0.773	0.028	0.039	0.217
n-Valerate	63.6	53.7	74.8	74.2	77.3	75.8	7.2	0.131	0.047	0.154	0.506	0.188

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

Table 9. Net liver removal of precursors as a maximal percentage of net liver release of glucose or β-hydroxybutyrate in transition dairy cows.

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
% of net glucose release												
Propionate	55.2	43.5	55.8	49.0	57.6	66.4	7.0	0.187	0.055	0.466	0.396	0.444
Lactate	18.5	22.7*	21.1	16.9	15.6	8.0*	2.7	0.010	0.003	0.273	0.039	0.143
Alanine	3.1	2.3	5.5*	3.0	1.5†	1.7	0.6	0.005	0.080	0.400	0.028	0.019
Glycerol	2.3	3.0	3.6*	2.4	1.5	0.4**	0.7	0.001	0.001	0.032	0.003	0.521
Triacylglycerol	–0.2	1.5†	0.2	0.0	0.1	–0.1	0.5	0.135	0.322	0.985	0.116	0.026
Total	78.9	73.0	84.3	71.3	76.3	77.0	7.5	0.723	0.956	0.822	0.958	0.665
% of net BHBA release												
NEFA	54.0	85.3†	115.4**	86.5†	56.2	62.6	13.1	0.010	0.444	0.060	0.001	0.428
n-Butyrate	42.2	47.9	33.3	26.8	26.6	54.2	11.3	0.487	0.781	0.060	0.502	0.573
Total	96.3	133.2	148.7†	113.3	82.8	119.8	18.1	0.202	0.841	0.966	0.010	0.878

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

DISCUSSION

Gestation, Transition, and Parturition

In the present study, there were no changes in DMI, and subsequently little evidence of changes in net splanchnic metabolism as cows approached parturition. In pregnant sheep, substantial increases in liver flux of glucose, lactate, glycerol, O_2, and NEFA were measured as gestation progressed, and increases were less for single than for twin pregnancies (Freetley and Ferrell, 2000). This suggests that the energy requirements of the gravid uterus dictated the responses of the liver, rather than any metabolic adaptations preceding parturition and lactation per se. Relative to lactation, the ewe is more prone to nutritional deficiency and metabolic diseases, such as ketosis, during gestation (Baird et al., 1983). In this regard, the nutritional demands of gestation relative to maintenance requirements of the ewe (McDonald et al., 1995) must be applied to the cow with caution. In the present study there was little evidence of any major changes in liver metabolism from 19 to 9 d before parturition. However, sampling may have been initiated too late in gestation to measure any alterations in liver metabolism to support fetal growth.

Lactate metabolism. One of the major changes in liver metabolism observed in gestating ewes was an increase in liver lactate removal and glucose release as gestation progressed (Freetly and Ferrell, 1998), presumably as a consequence of Cori cycling between the liver and uterus. In contrast, we observed no significant change in net liver flux of these compounds between 19 and 9 d before calving. In the cow sampled 4 times over the last 30 d of gestation in the present study (6698), there was little evidence of any change in liver lactate or glucose metabolism until the day before parturition (Figure 2). In the cow sampled on the day of calving (6132), no outward signs of parturition were evident as the cow approached her seventh calving. The cow was eating and ruminating as normal. Sampling was terminated after five hourly samples were obtained because excessively high levels of plasma lactate (>1.5 m*M*) and glucose (>6 m*M*) were measured on repeated samplings. A rectal palpation was performed and confirmed the calf was engaged in the pelvis. She calved within an hour of the cessation of blood sampling. The most striking observation in this cow was the increase in liver lactate removal and glucose release measured (Figure 2), which likely represented cycling of glucose and lactate carbon between the uterus and liver to provide energy for uterine contractions. In this regard, the increase in liver lactate removal measured in the cow sampled 1 d before calving (6698; Figure 2) may have been attributable to an increase in the number and magnitude of preliminary (Braxton Hicks) uterine contractions preceding parturition. Alternatively, these changes in lactate and glucose metabolism may have been associated with an increase in milk synthesis before calving. However, glucose use for prepartum milk synthesis would not give rise to lactate and thus could not explain the increase in arterial lactate concentration observed at calving.

Liver lipid metabolism. At 9 d before calving, arterial concentrations of insulin and acetate were lower and arterial concentrations of NEFA were higher than when cows were sampled at 19 d prepartum (Table 4). A decrease in insulin and increase in NEFA concentration of jugular vein plasma has been noted previously in dairy cows during the last 2 wk of gestation (Grummer, 1995). Parturition in dairy cows is associated with a dramatic shift in adipose tissue metabolism from an overall state of net lipogenesis to one of net and overriding lipolysis and release of NEFA to blood (Bell, 1995).

Table 10. Net total splanchnic flux of metabolites, gasses, insulin, and volatile fatty acids in transition dairy cows (mmol/h).

	Average day relative to calving								$P <$[2]			
Item	–19	–9	11	21	33	83	SEM	$P <$[1]	Linear	Quad	Cubic	Quartic
Glucose	269	284	631***	758***	808***	835***	38	0.001	0.001	0.001	0.025	0.001
Lactate	–15.6	–50.3	–132**	–84.3†	–51.1	73.1†	31.9	0.004	0.048	0.004	0.070	0.293
BHBA	278	303	587***	573**	731***	583**	61	0.001	0.001	0.001	0.192	0.754
NEFA	–5.0	–11.4	–49.8***	–35.5**	–17.5	–18.9	6.1	0.001	0.266	0.001	0.003	0.014
Triacylglycerol	17.5	–13.3	–2.2	–1.3	–2.2	0.4	11.4	0.558	0.727	0.416	0.252	0.187
Glycerol	–10.8	–11.7	–30.8***	–23.0**	–14.3	–5.7	4.3	0.001	0.159	0.001	0.008	0.004
Alanine	5.0	9.3	11.8	28.0†	66.5***	48.6**	7.2	0.001	0.001	0.026	0.002	0.065
Ammonia	–18.6	–6.2	–60.6	–74.0	–34.9	–21.5	28.6	0.596	0.786	0.159	0.737	0.218
Urea	75	150	309	234	424	477†	141	0.440	0.048	0.530	0.915	0.753
Oxygen	–3137	–3161	–5760***	–6323***	–6746***	–8101***	434	0.001	0.001	0.001	0.454	0.002
Carbon dioxide	1727	1913	2562†	2416	3173**	2368	411	0.094	0.083	0.025	0.423	0.612
Insulin	9.7	14.1	16.1	5.8	16.7	24.6	7.1	0.539	0.178	0.545	0.781	0.426
Acetate	1759	1736	2661**	2633**	2973***	3872***	206	0.001	0.001	0.339	0.953	0.360
Propionate	20.5	23.9	83.8***	65.6**	54.2*	60.7*	12.3	0.003	0.017	0.008	0.081	0.066
i-Butyrate	0.9	–0.1	0.9	0.7	0.5	2.4	1.1	0.580	0.152	0.374	0.981	0.379
n-Butyrate	8.3	8.4	43***	29.4***	26.2**	36.5***	3.3	0.001	0.001	0.005	0.019	0.016
i-Valerate	2	1.5	3.2	3.4	4.6	5.6	1.5	0.562	0.073	0.746	0.603	0.837
n-Valerate	–0.6	–1.5	–1.1	–2.7	–4.6*	–1	1.4	0.278	0.507	0.061	0.203	0.360
Total VFA	1790	1768	2790**	2730**	3054***	3978***	211	0.001	0.001	0.261	0.925	0.293
Total VFA carbon	3624	3576	5759**	5587**	6215***	8109***	429	0.001	0.001	0.225	0.845	0.254

[1]Overall effect of average day relative to calving.

[2]Polynomial effects of day relative to calving.

Superscripts signify means within rows differ from means for d –19 relative to calving: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, †$P < 0.10$.

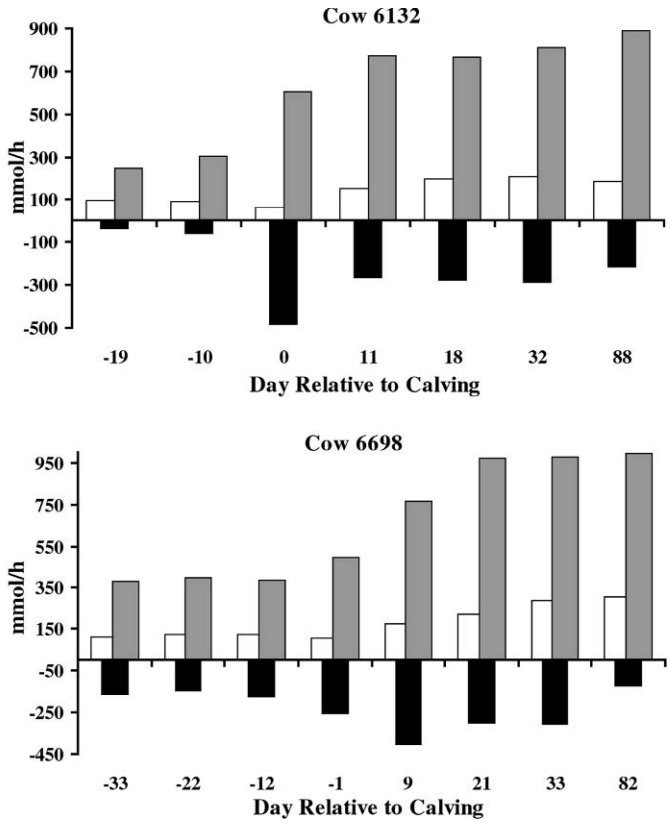

Figure 2. Net portal-drained viscera release (open bars) and liver removal (solid bars) of L-lactate and net liver release of glucose (gray bars) in 2 cows during transition.

The changes in plasma NEFA levels in the present and other studies suggest that decreases in insulin and other hormonal changes not measured in the present study (Grummer, 1993, 1995; Bell, 1995) initiate this shift in body lipid metabolism in the final 14 to 7 d before calving. These apparent changes in body lipid metabolism were accompanied in the present study by an increase in net liver removal of TAG (Table 6), as well as numerical increases in net liver removal of lactate and NEFA 9 d before calving.

In pregnant and nonpregnant sheep a net release of TAG was measured across the liver using multicatheterization procedures (Freetly and Ferrell, 2000), whereas a gross removal of labeled TAG by the liver of catheterized sheep was measured by Bergman et al. (1971). Reid et al. (1979) measured a net release of TAG by the liver of two dry and two lactating cows fed to requirements and a net removal of TAG when the same cows were fasted for 6 d. In the present study, net liver removal of TAG was near 0, suggesting equal rates of gross removal and release, except 9 d before calving, when a significant net removal was measured (Table 6). Reasons for this increase in liver removal of TAG by the liver are not certain. Reid et al. (1979) suggested that the increase in net liver removal of TAG by the liver of dairy cows during fasting was a consequence of decreased gross release, due to an increase in NEFA oxidation, rather than an increase in gross removal. If the simultaneous increase in arterial NEFA concentration observed 9 d before calving reflects a decrease in lipogenesis within adipose tissue and associated reductions in adipose lipoprotein lipase activity, then the increase in net liver removal of TAG may represent an alternative disposal of absorbed TAG. The potential role of liver lipoprotein lipase in the uptake of plasma TAG by the bovine liver was considered previously (Drackley, 1999).

Changes in lactate and NEFA removal by the liver between 19 and 9 d before calving were significant when considered relative to their net PDV release (Table 7) and liver release of glucose and BHBA, respectively (Table 8). In support of this observation, isotopic measurements of glucose and lactate turnover, and interconversion, obtained in one cow at 28, 21, and 5 d prepartum, showed an increased proportional conversion of lactate to glucose at 5 d prepartum (Baird et al., 1983). Liver removal of NEFA is dictated by the concentration of NEFA in plasma reaching the liver, as liver extraction of NEFA supply is constant across a range of plasma NEFA levels (Bell, 1995). In support of this generality, liver extraction of total NEFA supply was not significantly different across sampling times in the present study. Although liver NEFA extraction was slightly lower in early lactation (Table 7), when arterial concentration was highest (Table 4), overall changes in liver extraction of NEFA supply were relatively small. As the liver receives as much as 38% of cardiac output (Huntington et al., 1990), it is particularly susceptible to an oversupply of NEFA when arterial concentrations increase during transition.

Splanchnic Blood Flow and Oxygen Consumption

Portal vein and liver blood flow increased during lactation to more than double the average rates before calving by d 83 postpartum (213 and 215%, respectively), but the majority of this increase (85 and 93%, respectively) occurred in the first 11 d after calving (Figure 3). Previous studies have suggested that PDV and liver blood flow are largely determined by ME intake, but that other factors, such as total DMI and the fiber content of the diet and resulting effects on gut fill, mass, and work load, modulate this overriding relationship between splanchnic blood flow and ME (Reynolds, 1995). In the present study, increases in ME intake after calving were greater than for DMI, as the ME concentration of the lactation ration was greater (Fig-

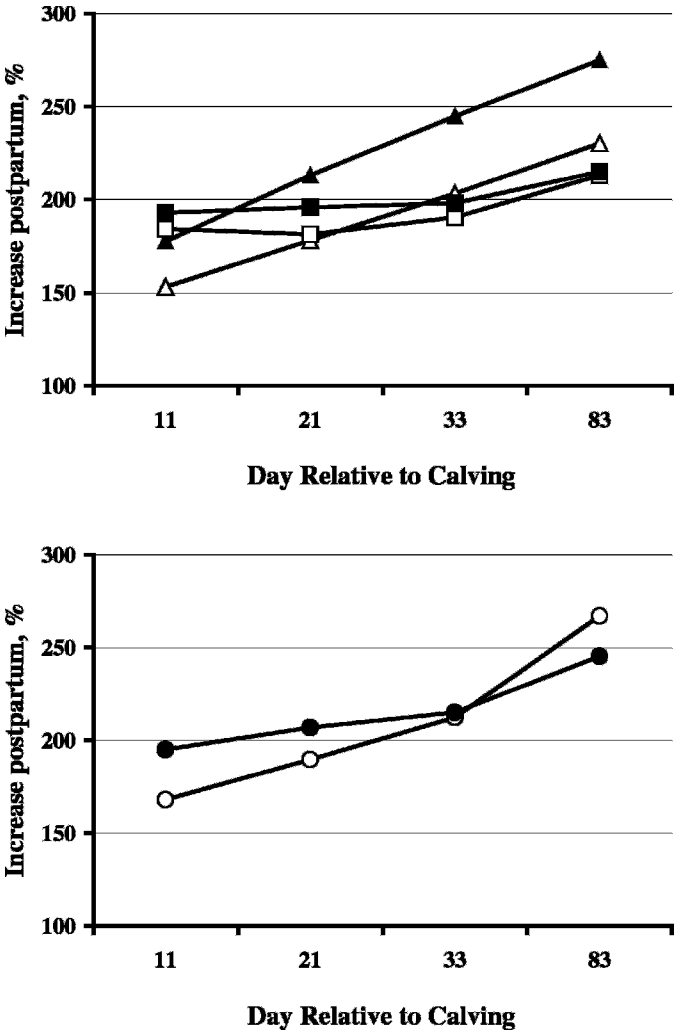

Figure 3. Postpartum increase in DM (open triangle) and metabolizable energy intake (solid triangle), PDV (open square) or liver (solid square) blood flow, and PDV (open circle) or liver (solid circle) oxygen consumption relative to average rates prepartum in transition dairy cows.

ure 3). While the relative increase in ME intake was similar to the relative increase in portal vein blood flow at 11 d postpartum (Figure 3), ME intake continued to increase with successive samplings and at a greater rate than blood flow. These comparisons illustrate that the relationship between ME intake and blood flow changed as cows progressed through transition. This may in part reflect the influence of the fiber content of the diet on PDV blood flow during gestation, as well as additive effects of liver O_2 requirements, and PDV and liver growth, on splanchnic blood flow. Reynolds (1995) noted that liver blood flow is not determined solely by ME intake but also influenced by oxidative metabolism, which is in part reflected by variations in hepatic artery blood flow to meet liver O_2 demands.

In the present study increases in liver and PDV blood flow were associated with similar relative increases in PDV and liver O_2 consumption during early lactation (Figure 3). In early lactation, the increase in liver O_2 consumption was associated with greater hepatic artery flow and a numerical increase in the proportion of total hepatic blood flow attributable to the hepatic artery. This contribution was greatest at 21 d postpartum. Previous studies have shown that the splanchnic tissues of the dairy cow undergo a substantial increase in mass during the first 8 wk postpartum (Gibb et al., 1992). These increases in tissue mass are dictated by DMI and likely require an increased nutritive blood flow, which may have contributed to the increases in splanchnic blood flow observed in early lactation in the present study. Thus, in early lactation increases in DM and ME intake, and resulting increases in PDV and liver tissue mass, the work of digestion, and oxidative metabolism, as well as additive effects of lactation on liver oxidative metabolism, are associated with dramatic increases in splanchnic blood flow within the first 2 wk of lactation. At 12 wk postpartum, O_2 consumption by the splanchnic tissues had increased to a greater extent than blood flow (Figure 3), which was reflected by an increase in liver extraction of O_2 supply (Table 7).

Nutrient Metabolism by the Portal-Drained Viscera

The transitional response of net PDV nutrient release was generally characterized by two patterns of response, reflecting the origin of the compounds released. For compounds whose supply was determined primarily by the absorption of products of diet digestion and fermentation, increases in their net PDV release appeared to be driven by relative increases in diet intake (e.g., VFA, lactate, BHBA, and ammonia). A linear relationship between diet or diet component intake and net PDV release of these nutrients has been reported previously in a number of studies (e.g., Nozière et al., 2000). For compounds released from PDV tissues as a consequence of adipose turnover (NEFA and glycerol), maximum rates of release were measured in early lactation, presumably as a consequence of negative energy balance and the mobilization of portal-drained adipose tissue. Glycerol and NEFA released into the portal vein predominantly reflect release from omental and mesenteric fat, which can account for up to 28% of body adipose tissue in postpartum dairy cows (Gibb et al., 1992). For alanine, which is both absorbed from the small intestine and synthesized from degraded amino acids and pyruvate, increases in early lactation reflect both increased protein digestion in the small intestine and protein turnover within PDV tissues. In addition, interpretation of net PDV flux rates for amino acids and

other absorbed nutrients not subject to immediate clearance by the liver is complicated by simultaneous absorption into portal blood and extraction from arterial blood by PDV tissues. In this regard, net PDV flux of glucose, urea, and CO_2 showed considerable variation in the present study, reflecting the small venous-arterial concentration differences for these compounds and their potential bi-directional transfer across PDV tissues. Although insulin concentration was reduced during transition, this reduction was not attributable to significant differences in net PDV or liver flux. However, circulating insulin concentrations are also determined by kidney clearance, and measurement of secretion ideally employs more frequent sampling than possible in the present study (Benson and Reynolds, 2001).

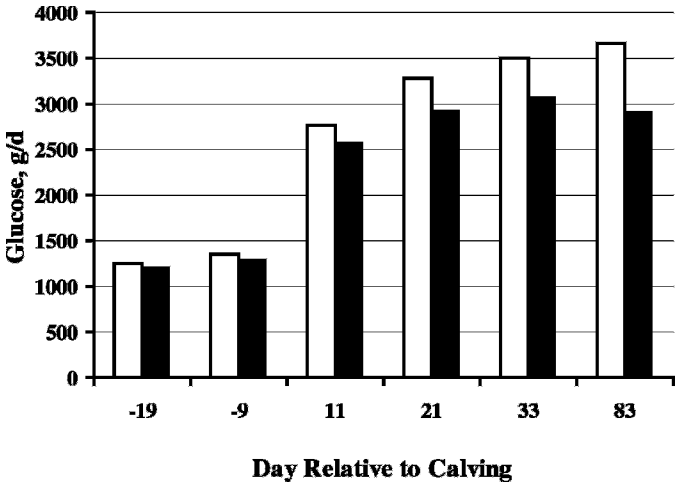

Figure 4. Measured liver glucose release (open bar) and predicted glucose requirement (solid bar) in transition dairy cows.

Liver Metabolism

Glucose metabolism. Whole body glucose and NEFA turnover are greater in lactating than in gestating cattle and sheep (Baird et al., 1983; Bell, 1995; Drackley, 1999). In sheep, glucose turnover is greater during lactation than late gestation (Baird et al., 1983) as a result of greater hepatic production (van der Walt et al., 1983), but the contribution of lactate to glucose synthesis was greater during gestation. In the present study, rates of liver glucose production in late gestation were similar to rates of glucose turnover measured by isotope dilution in two Holstein cows at the same stage of gestation (Baird et al., 1983). After calving, liver glucose production increased as milk yield and ME intake increased, nearly doubling within 11 d of lactation (Table 6). Liver glucose production is highly regulated and coordinated with body requirements, which in a majority of physiological circumstances are determined by, or related to, ME intake (Reynolds, 1995). However, this relationship is challenged in states of undernutrition, such as feed restriction, early lactation in the dairy cow, or late gestation in the ewe, when body requirements are greater than nutrient inputs.

Predicted glucose supply and requirement. In the present study, measured glucose output by the liver, which excludes the contributions of the kidneys, was more than adequate to meet body glucose requirements calculated using estimated requirements for maintenance, gestation, and measured milk lactose synthesis (Elliot, 1976; Drackley et al., 2001; Figure 4). The calculated surplus, relative to estimated requirement, was least in gestation and increased as lactation progressed, perhaps reflecting a shift in ME utilization towards body energy deposition. The estimate of glucose requirement of Elliot (1976) assumed a minimal body glucose requirement for maintenance. At 83 d postpartum, predicted glucose requirement is much lower than measured glucose production, reflecting an underestimate of glucose use, and thus requirement for tissue energy deposition.

Glucose precursor supply. Numerous reviews have emphasized the importance of body reserves for meeting energy requirements, and the potential need for glucose precursors other than propionate in early lactation (Bell, 1995; Drackley et al., 2001). Measurements of liver pyruvate carboxylase mRNA (Greenfield et al., 2000) and utilization of alanine for glucose synthesis (Drackley et al., 2001) in vitro suggest a greater dependence on alanine and, by conjecture, other amino acids in the days following parturition. Perhaps more importantly, an increase in pyruvate carboxylase activity will enable a greater liver removal of lactate. In the present study, net liver removal of all the glucose precursors measured increased after calving, with the exception of TAG, and net liver removal of lactate, alanine, and glycerol were greatest at 11 d postpartum. These changes provide further evidence for an increased contribution of lactate and alanine to glucose synthesis in the first days of lactation. When expressed relative to liver glucose release, the maximal net contribution of alanine and glycerol were measured at 11 DIM, while the contribution of lactate was greatest at 9 d before calving and 11 DIM. This later observation confirms the results of Baird et al. (1983), who found no difference in the proportional contribution of lactate to glucose synthesis between late gestation and early lactation in dairy cows. The data are the first of which we are aware showing an increase in glycerol removal by the liver and potential contribution to liver glucose synthesis immediately after calving in dairy cows, as measured previously in fasted cows (Lomax and Baird,

1983). The potential contribution of lactate to glucose synthesis declined as lactation progressed with a more positive net splanchnic flux of lactate reflecting a decrease in Cori cycling between the liver and extrasplanchnic tissues and a calculated increase in body energy balance (Benson et al., 2002).

With the exception of alanine, data from the present study do not support the concept that a greater relative contribution of amino acids to glucose synthesis is required in early lactation. The removal of other amino acids by the liver was not measured and surely increased after calving. However, based on relative rates of net liver glucose release and measured precursor removal (Table 9), the minimum required amino acid contribution in addition to alanine (100 minus total), was not different across sampling times but numerically lowest (15.7%) at 11 DIM. While there was a doubling of the potential contribution of alanine to liver glucose synthesis, in the present study there were no indications of a shortage of glucose precursors at 11 d postpartum. On an incremental basis, increases in net propionate, lactate, alanine, and glycerol removal by the liver between 9 d before and 11 d after calving can account for 100% (69.2, 19.5, 7.5, and 4.2%, respectively), of the concurrent increase in net liver glucose production. A greater contribution of amino acids to glucose synthesis may have occurred in the first days of lactation, before intake increased (Figure 1). However, considering the importance of essential AA for milk and visceral tissue protein synthesis during this period, the use of their carbon for glucose synthesis may reflect obligatory catabolic processes and ureagenesis rather than a metabolic requirement for lactation.

A greater potential contribution of propionate to glucose synthesis at 83 d postpartum was balanced by a reduction in the potential contribution of lactate, which is the precursor most heavily recruited for glucose synthesis when propionate is in short supply (Lomax and Baird, 1983). However, if lactate is derived from Cori cycling of glucose with other body tissues, less ATP will be available for anabolism of peripheral tissues. Similarly, the alanine removed by the liver may be synthesized using pyruvate derived from glycolysis; thus, an increased contribution of lactate and alanine to glucose synthesis in early lactation reflects a state of energy movement from body tissues to milk. Results of the present study do not refute the concept that propionate availability limits glucose production in early lactation. However, based on other studies, the provision of additional propionate may have simply reduced lactate removal by the liver, without increasing glucose production (Reynolds, 1995).

Metabolism of other nutrients. Aspects of liver lipid and glucose metabolism during transition were discussed previously. While liver metabolism of these nutrients appeared to be driven by increases in milk yield, changes in net liver metabolism of many metabolites after calving were generally associated with, or dictated by, changes in their net PDV release as DMI increased. In the case of the VFA and ammonia removed by the liver, increases in net PDV release were largely matched by increases in net liver removal of these compounds. One exception in this regard was the reduced extraction of propionate, i-butyrate, n-butyrate, and i-valerate as a percentage of total supply at 11 d postpartum (Table 8), when arterial concentrations were increased (Table 4), which may reflect a limitation of liver metabolic capacity during transition. For BHBA, changes in net liver release after calving could be attributed both to changes in PDV release of n-butyrate and thus DMI, as well as changes in liver removal of NEFA, and thus body energy balance. On a net basis, estimates of liver removal of NEFA and n-butyrate carbon were nearly equal to, or exceeded, net BHBA carbon release (Table 9). These measurements excluded acetoacetate released by the PDV, which is normally removed by the liver and converted to BHBA (Reynolds, 1995). Other fates of NEFA carbon include oxidation, TAG synthesis, and release as acetate.

CONCLUSIONS

Apart from an increase in net liver removal of glycerol at 9 d before calving and numerical increases in liver NEFA and lactate removal, there was little evidence in the present study of substantial changes in net splanchnic metabolism as dairy cows approached parturition. This suggests that even in late gestation, diet intake is the primary determinant of splanchnic tissue metabolism. After calving, substantial increases in PDV and liver blood flow were measured by 11 d postpartum, which in part reflect increases in DM and ME intake, as well as liver oxidative metabolism, and perhaps splanchnic tissue growth to support lactation. Changes in net splanchnic metabolism after calving generally reflected changes in DMI (e.g., PDV release of VFA and ammonia), milk yield (e.g., liver glucose release) or body energy status (e.g., liver removal of NEFA, glycerol, lactate, and alanine), depending on the origins of the nutrients measured. Measured net glucose release by the liver was greater than calculated requirements, with an increasing surplus evident as lactation progressed. In the present study, the maximal potential contribution of lactate, alanine, and glycerol to liver glucose synthesis was greatest 11 d after calving, but there was no evidence of an increased contribution of other amino acids to glucose synthesis as milk yield and liver glucose production increased. These data em-

phasize the importance of lactate and alanine conversion to glucose via pyruvate carboxylase in early lactation, but suggest that the gluconeogenic contribution of other amino acids may reflect obligatory catabolic processes and ureagenesis, rather than a metabolic requirement in early lactation.

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