Effects of Abomasal Vegetable Oil Infusion on Splanchnic Nutrient Metabolism in Lactating Dairy Cows

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ABSTRACT

Changes in the metabolism of nutrients by the portal-drained viscera (PDV) and liver may contribute to the reduction in dry matter intake (DMI) and other production responses generally observed in lactating dairy cows fed supplemental long-chain fatty acids (LCFA). In the present study, effects of a 7-d abomasal infusion of vegetable oil on arterial concentration and splanchnic (PDV and liver) metabolism of nutrients were measured in six cows at 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum. Cows were fed *ad libitum* DMI at 8-h intervals, and blood samples for measurement of splanchnic metabolism were obtained over 8 h beginning 2 h before feeding at 0830 h. Blood flow for the PDV and liver was increased by oil infusion and was greater in ELAC, despite similar-feed DMI during blood sampling. Increased blood flow in ELAC was associated with greater liver oxygen removal and glucose release that accompanied greater milk yield. In contrast, oil infusion had no effect on splanchnic oxygen use. Greater blood flow during oil infusion may have been due to specific effects of intestinal LCFA supply on PDV blood flow. Net PDV release and liver removal of branched-chain volatile fatty acids (VFA) and ammonia were increased by oil infusion. Net PDV release of longer-chain (4 and 5 C) VFA and NEFA was greater in ELAC, but net PDV flux of other nutrients was not affected by lactation stage, possibly due to the similarity of feed DMI. Oil infusion increased arterial concentration and net PDV release and liver removal of NEFA, and it decreased net liver release and arterial concentration of glucose. Effects of oil infusion on liver glucose release were associated with decreased daily DMI. In ELAC, arterial concentration and net liver removal of NEFA were also increased,

but liver release of glucose was greater than in MLAC. Oil infusion and ELAC both increased net liver removal of L-lactate. The resulting decrease in net total splanchnic release of L-lactate in ELAC reflects decreased tissue energy balance of the cows. Generally, stage of lactation and relative milk yield had greater effects on metabolism of the liver than the PDV, in which metabolism was largely dictated by DMI. In the present study, there was little evidence to suggest an effect of stage of lactation on the metabolic response of the PDV and liver to postprandial LCFA supply.

(Key words: long-chain fatty acids, portal-drained viscera, liver, metabolism)

Abbreviation key: ELAC = early lactation, LCFA = long-chain fatty acids, ME = metabolizable energy, MLAC = midlactation, PCV = packed-cell volume, PDV = portal-drained viscera, RQ = respiratory quotient, TSP = total splanchnic.

INTRODUCTION

The diet of lactating dairy cows is often supplemented with rumen-inert fat in an attempt to increase energy intake and milk production, while minimizing inhibitory effects of fatty acids on ruminal microbes (Palmquist, 1994). However, depression in DMI and effects on blood concentrations of VFA, glucose and other products of digestion have been observed, even when long-chain fatty acids (LCFA) are infused postprandially to preclude direct rumen effects (Grummer and Carroll, 1991). Although effects of dietary fat on *in vivo* liver lipid metabolism in preruminant calves are reported (Leplaix-Charlat et al., 1996), we are currently aware of no other published studies in ruminants that have examined the effects of postprandial LCFA supply on portal-drained viscera (PDV) and liver (total splanchnic [TSP]) tissue metabolism. Splanchnic metabolism is important in determining the availability of precursors for milk and body tissue synthesis, and thus production responses to supplemental fat. In addition, the liver is considered an important source of signals that may have a role controlling intake (Friedman, 1998). Increased liver oxidation

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of long-chain fatty acids reduces intake in rats (Scharrer and Langhans, 1986) and may be responsible for decreased DMI in lactating cows fed fat. Therefore, changes in splanchnic nutrient metabolism may be indicative of the metabolic responses and mechanisms underlying intake and production responses to dietary fat supplementation. The objective of the present experiment was to measure the effects of postruminal infusion of vegetable oil and stage of lactation on net flux of nutrients across the PDV and liver of dairy cows.

MATERIALS AND METHODS

Cows, Diet, and Treatments

Details of cows, their diet, treatments, and lactation performance were described by Benson et al. (2001). Briefly, six rumen-fistulated, multicatheterized Holstein $\times$ British Friesian cows were fed a TMR (20% grass silage, 30% dehydrated alfalfa, and 50% concentrates on a DM basis) for ad libitum intake. Daily rations were offered in equal meals provided at 0830, 1630, and 2230 h. Treatments consisted of 7-d abomasal infusions of 400 g/d water or a 50:50 mixture (by weight) of sunflower and rapeseed oils (oil) providing mostly (91%) unsaturated LCFA as triglycerides, which immediately followed water infusions. Abomasal infusion lines were established via a rumen fistula and the reticulo-omasal orifice. Infusions began at an average of 55 d (early lactation [ELAC]) and 111 d postpartum (mid lactation [MLAC]).

Blood Sampling Protocol

Benson and Reynolds (2001) described details of the blood sampling protocol and measurement of blood flow. Briefly, 25 sets of blood samples were obtained simultaneously from the mesenteric artery, hepatic portal vein, and hepatic vein on d 7 of water and subsequent oil infusions during an 8-h period (0630 to 1430 h) that included feeding at 0830 h. Continuous mesenteric vein infusion of ρ -aminohippurate for measurement of blood flow began 1 h before blood sampling. Blood for nutrient analysis was collected in syringes treated with potassium EDTA (1.6 mg/ml blood), whereas blood for gas analysis was collected in syringes treated with heparin (125 U/ml of blood). Aliquots of blood were immediately frozen in liquid nitrogen for later analysis of ammonia, urea, and individual VFA concentration. Remaining blood was centrifuged at 3000 rpm and 4°C for 20 min and aliquots of plasma immediately frozen in liquid nitrogen for later analysis of L-lactate, BHBA, and NEFA concentration. Tubes containing blood and plasma were immersed in ice until analyzed or frozen as soon as possible. While 25 sample sets were obtained, for analyses other than ρ -aminohippurate and glucose samples were pooled across

contiguous sampling times to reduce analytical requirements. This pooling reduced the total number of samples analyzed for each sampling site within a cow-sampling period to 17 for ammonia, urea, BHBA, NEFA, and L-lactate; 11 for blood gases; and 8 for VFA.

Sample Analysis

Measurements of blood packed-cell volume (PCV), hemoglobin, partial pressure of oxygen (O_2) and carbon dioxide (CO_2), and pH were obtained as soon as possible after the samples were collected using a blood gas analyzer (Instrumentation Laboratories 1302, Warrington, Cheshire, UK). Hemoglobin concentration of blood samples was measured using a commercial kit (Boehringer Mannheim, Lewes, UK), and blood PCV was determined by microcentrifugation. Plasma glucose and ρ -aminohippurate concentrations were measured as soon as possible on a Cobas Mira analyzer (Roche Diagnostic Products, Welwyn Garden City, UK) using enzymatic (Glucose Trinder Assay; Sigma-Aldrich Ltd., Poole, UK) and colorimetric assays (Benson and Reynolds, 2001), respectively. Blood samples for analysis of ammonia and urea content were thawed and diluted with 0.9 part (vol/vol) ice-cold water and 0.1 part (vol/vol) ice-cold 6 M perchloric acid. Supernatants obtained following centrifugation at $10,000 \times g$ for 40 min at 4°C were neutralized with 2 M potassium bicarbonate and centrifuged for 20 min at $1,800 \times g$ and 4°C. The resulting supernatants were analyzed for ammonia concentration using an enzymatic assay (Kun and Kearney, 1974) or frozen (-20°C) for later analysis of urea concentration using a manual version of an automated colorimetric assay described by Reynolds et al. (1988b).

Concentrations of VFA were measured by capillary GLC using a 30-m $\times$ 0.25-mm Stabilwax-DA column (Thames Restek, Slough, UK). Blood samples for VFA analysis were prepared as described (Reynolds et al., 1986), except that the anion exchange resin was changed from the BioRex 5 resin (BioRad, Hemel Hempstead, UK) used in the original procedure to AG4X4. The manufacturer changed the BioRex 5 resin after the original procedure for sample cleanup was validated (Reynolds et al., 1986). Using the AG4X4 resin, the recoveries of VFA added to blood during deproteinization, and adjusted for recovery of added internal standard, averaged 99, 99, 99, 98, 100, and 96% for acetate, propionate, n-butyrate, i-butyrate, n-valerate, and i-valerate, respectively. L-Lactate (Gutman and Wahlfeld, 1974), BHBA (Reynolds et al., 1988a), and NEFA (Reynolds et al., 1988a) concentrations of thawed plasma samples were measured using enzymatic methods adapted for use on the Cobas Mira.

Table 1. Splanchnic plasma and blood flow and packed-cell volume (PCV) in response to abomasal infusion of water or oil at an average of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum.

	Lactation stage				SEM	Significance, <i>P</i> <		
	ELAC		MLAC			Lactation stage	Infusion	Stage × infusion
	Water	Oil	Water	Oil				
Plasma flow, L/h								
Arterial	463	468	422	380	44	0.18	0.70	0.61
Hepatic	2117	2272	1929	2080	48	0.01	0.01	0.97
Portal	1655	1807	1511	1702	41	0.02	0.01	0.65
Blood flow, L/h								
Arterial	640	629	534	526	77	0.21	0.91	0.99
Hepatic	2932	3118	2591	2887	85	0.01	0.02	0.55
Portal	2296	2492	2060	2389	67	0.03	0.01	0.35
PCV, % ¹	28	27	29	28	0.3	0.01	0.03	0.95
Hemoglobin, g/L								
Arterial	95.4	92.6	95.4	91.4	1.3	0.68	0.02	0.65
Mean ¹	94.6	92.1	94.2	90.7	1.4	0.54	0.05	0.75

¹Average for arterial, portal vein, and hepatic vein samples.

Calculations and Statistical Analysis

Plasma flow for the PDV and liver was calculated from ρ -aminohippurate dilution as described previously (Reynolds et al., 1988b), and blood flow was calculated from plasma flow and PCV. Concentrations of O₂ and CO₂ in blood were calculated using measured partial pressures of O₂ and hemoglobin concentrations or partial pressure of CO₂ and pH, respectively (Oddy et al., 1984). Net flux of nutrients and gases across the PDV, liver, and TSP tissues was calculated as the product of venous-arterial concentration difference multiplied by plasma flow (Reynolds et al., 1988b). The fluxes calculated are net values, often the result of both metabolite release into and uptake from blood. Using venous-arterial differences, a negative flux represents a net removal from blood and a positive flux represents a net production or release.

The effects of cow, abomasal infusion (water or oil), stage of lactation (ELAC or MLAC), and the interaction between infusion and stage were tested using residual variance with the GLM procedure of SAS (1999). Only 23 of 24 sets of blood samples were obtained due to the loss of one cow's carotid artery catheter immediately before blood sampling for oil infusion in ELAC. Least squares means reported were all obtained using the full statistical model for ANOVA, and any standard error of the means presented is for $n = 6$. Due to the small number of cows used ($n = 6$), differences were considered significant at $P < 0.10$.

RESULTS

Blood Flow and Packed-cell Volume

Portal vein and liver plasma and total blood flow (Table 1) were significantly increased by oil infusion ($P < 0.02$)

and were greater in ELAC than MLAC ($P < 0.03$). There was no effect of oil infusion or stage of lactation on hepatic artery blood flow. Mean PCV for the three blood vessels sampled (Table 1) was decreased in response to oil infusion ($P < 0.03$) and was greater in MLAC than ELAC ($P < 0.01$). Both arterial ($P < 0.02$) and mean ($P < 0.05$) hemoglobin concentration (Table 1) were decreased by oil infusion.

Volatile Fatty Acids

In ELAC, arterial concentration of total VFA (Table 2) was decreased by oil infusion, whereas in MLAC oil infusion increased arterial concentration of total VFA (lactation stage by infusion interaction [$P < 0.09$]). This response was largely due to changes in arterial acetate concentration (Table 2), which accounted for 95% of total VFA concentration in arterial blood and had a similar lactation stage by infusion interaction ($P < 0.04$). Arterial propionate concentration was greater in MLAC than in ELAC ($P < 0.01$) and was decreased by oil infusion ($P < 0.01$). Arterial n-butyrate concentration also showed a stage of lactation $\times$ infusion interaction ($P < 0.03$), but the response was inverse to that of acetate; it was increased by oil infusion in ELAC but decreased by oil infusion in MLAC (Table 2). Arterial concentration of i-butyrate, i-valerate, and n-valerate were much lower than the three major VFA, but arterial concentration of i-butyrate ($P < 0.07$) and i-valerate ($P < 0.01$) were greater in ELAC than in MLAC and were not affected by oil infusion ($P > 0.25$). In contrast, arterial n-valerate concentration was greater in MLAC than ELAC ($P < 0.02$) and increased by oil infusion ($P < 0.01$ [Table 2]).

Overall, there were no significant effects ($P > 0.16$) of either stage of lactation or oil infusion on net PDV, liver, or TSP fluxes of total VFA (Table 2), but in ELAC, net

Table 2. Arterial concentrations and net flux of VFA across portal-drained viscera (PDV), liver, and total splanchnic (TSP) tissues during abomasal infusion of water or oil at an average of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum.

	Lactation stage				SEM	Significance, <i>P</i> <		
	ELAC		MLAC			Lactation stage	Infusion	Stage × infusion
	Water	Oil	Water	Oil				
Arterial concentration, μM								
Acetate	2225	2116	2164	2259	47	0.39	0.89	0.04
Propionate	67.6	58.5	71.9	68.1	2.10	0.01	0.01	0.24
n-Butyrate	28.4	32.9	26.7	25.0	1.30	0.01	0.33	0.03
n-Valerate	6.3	7.5	6.5	8.1	0.20	0.02	0.01	0.35
i-Butyrate	5.4	5.7	5.0	5.1	0.30	0.07	0.46	0.85
i-Valerate	8.7	9.4	7.2	7.6	0.50	0.01	0.25	0.82
Total ¹	2331	2237	2287	2373	4.90	0.37	0.94	0.09
Net PDV flux, mmol/h								
Acetate	3331	3142	3185	3449	96.0	0.42	0.71	0.03
Propionate	1162	1196	1177	1174	35.0	0.92	0.67	0.61
n-Butyrate	235	240	209	213	9.0	0.01	0.66	0.95
n-Valerate	52.9	51.4	46.1	47.7	2.70	0.07	0.95	0.62
i-Butyrate	33.6	39.4	31.3	34.3	1.00	0.01	0.01	0.17
i-Valerate	56.2	64.0	52.9	57.6	2.00	0.02	0.01	0.46
Total ¹	4871	4740	4703	4975	135.0	0.82	0.62	0.16
Net liver flux, mmol/h								
Acetate	1074	988	911	751	101.0	0.06	0.25	0.73
Propionate	−1089	−1110	−1093	−1100	33.0	0.93	0.70	0.85
n-Butyrate	−189	−192	−168	−179	8.0	0.03	0.38	0.62
n-Valerate	−57.1	−56.2	−50.9	−52.7	2.70	0.10	0.88	0.64
i-Butyrate	−31.4	−36.4	−28.8	−32.3	0.90	0.01	0.01	0.47
i-Valerate	−52.0	−56.7	−48.3	−53.5	1.80	0.07	0.01	0.90
Total	−345	−519	−464	−658	112.0	0.29	0.13	0.94
Net total splanchnic flux, mmol/h								
Acetate	4404	4037	4079	4144	124	0.41	0.25	0.10
Propionate	80	76	84	74	4.4	0.90	0.16	0.47
n-Butyrate	47	51	42	36	3.5	0.01	0.74	0.20
n-Valerate	−4.2	−5.2	−4.8	−5.1	2.70	0.10	0.88	0.64
i-Butyrate	2.2	3.6	2.5	1.9	0.54	0.23	0.47	0.08
i-Valerate	4.3	7.0	4.6	4.1	0.82	0.13	0.19	0.06
Total	4531	4156	4207	4281	150.0	0.47	0.29	0.11

¹Total is the sum of acetate, propionate, n-butyrate, n-valerate, i-butyrate, and i-valerate.

PDV release of n-butyrate ($P < 0.01$), i-butyrate ($P < 0.01$), i-valerate ($P < 0.02$), and n-valerate ($P < 0.07$) were all greater than in MLAC. Net release of acetate and net removal of n-butyrate, i-butyrate, i-valerate, and n-valerate by the liver were also greater in ELAC than in MLAC ($P < 0.10$ [Table 2]). There was a net TSP release for all VFA except n-valerate, for which there was a slight net uptake, but net release of acetate accounted for 97% of the net TSP release of total VFA. Net TSP removal of n-valerate was greater in MLAC than in ELAC ($P < 0.10$), whereas net TSP release of n-butyrate was greater in ELAC than MLAC ($P < 0.01$).

Net PDV release of i-butyrate and i-valerate and their net removal by the liver were increased in response to oil infusion ($P < 0.01$ [Table 2]). There also were stage of lactation × infusion interactions for net acetate flux across both PDV and TSP tissues ($P < 0.10$). For both tissue beds, net acetate flux was decreased by oil infusion in ELAC and increased by oil infusion in MLAC (Table

3). In contrast, net TSP release of i-butyrate ($P < 0.08$) and i-valerate ($P < 0.06$) was increased by oil infusion in ELAC and decreased by oil infusion in MLAC.

Ammonia and Urea

There were no effects of infusion or stage of lactation on arterial concentration of ammonia or urea ($P > 0.46$ [Table 3]). On a net basis, PDV urea removal averaged 33% of urea released by the liver, whereas ammonia N removal by the liver accounted for 70% of liver release of urea. There was no effect of stage of lactation on net PDV, liver, or TSP flux of ammonia or urea ($P > 0.28$ [Table 3]). Infusion of oil increased net PDV release ($P < 0.01$) and liver uptake ($P < 0.02$) of ammonia, while net TSP flux was unchanged ($P > 0.92$). Numerical increases in net liver release of urea (Table 3) accounted for 69% of the increase in ammonia nitrogen removal by the liver when oil was infused.

Table 3. Arterial concentration and net flux of blood metabolites and gases across portal-drained viscera (PDV), liver and total splanchnic (TSP) tissues, and PDV and liver respiratory quotient (RQ) during abomasal infusion of water or oil at an average of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum.

	Lactation stage				SEM	Significance, <i>P</i> <		
	ELAC		MLAC			Lactation stage	Infusion	Stage × infusion
	Water	Oil	Water	Oil				
Arterial concentration, mM								
Ammonia	0.346	0.328	0.315	0.334	0.015	0.46	0.99	0.27
Urea	5.47	5.72	5.56	5.61	0.216	0.97	0.51	0.65
O ₂	5.41	5.24	5.38	5.16	0.078	0.53	0.03	0.75
CO ₂	27.65	27.48	27.71	27.68	0.377	0.75	0.81	0.85
Net PDV flux, mmol/h								
Ammonia	891	1029	878	986	38	0.50	0.01	0.71
Urea	-218	-212	-271	-267	46	0.28	0.92	0.99
O ₂	-4345	-4316	-4095	-4220	182	0.38	0.81	0.69
CO ₂	1683	1436	1580	1012	283	0.38	0.19	0.60
PDV RQ	0.42	0.40	0.45	0.28	0.085	0.60	0.31	0.44
Net liver flux, mmol/h								
Ammonia	-937	-1083	-941	-1029	43	0.58	0.02	0.53
Urea	686	731	703	739	35	0.73	0.28	0.91
O ₂	-4401	-4398	-3988	-3770	229	0.05	0.65	0.66
CO ₂	2674	2641	2854	2560	336	0.89	0.65	0.72
Liver RQ	0.59	0.63	0.69	0.67	91	0.45	0.86	0.76
Net TSP flux, mmol/h								
Ammonia	-49	-52	-63	-56	15	0.57	0.92	0.76
Urea	473	526	419	479	50	0.35	0.31	0.95
O ₂	-8646	-8735	-8073	-7969	333	0.08	0.99	0.79
CO ₂	4338	4073	4338	3471	346	0.41	0.14	0.42

Blood Gases

Arterial blood O₂ concentration was decreased ($P < 0.03$) by oil infusion but was unaffected by stage of lactation ($P > 0.53$ [Table 3]). Infusion of oil had no effect on net PDV, liver, or TSP removal of O₂ ($P > 0.65$ [Table 3]). Oxygen removal by the TSP was greater in ELAC than MLAC ($P < 0.08$) due to an increase in liver removal ($P < 0.05$), as PDV uptake did not differ ($P > 0.38$). Arterial CO₂ concentrations were unaffected by oil infusion ($P > 0.81$) and stage of lactation ($P > 0.75$ [Table 3]). Similarly, net flux of CO₂ across PDV, liver, and TSP tissues and respiratory quotient (RQ [net CO₂ release/net O₂ removal]) for the PDV and liver were not affected by stage of lactation or infusion ($P > 0.14$ [Table 3]).

Plasma Metabolites

Arterial glucose concentration ($P < 0.01$) and net liver and TSP glucose release were decreased by the oil infusion ($P < 0.06$ [Table 4]). Arterial glucose concentration was greater in MLAC than ELAC ($P < 0.01$), but both liver and TSP glucose production were greater in ELAC ($P < 0.01$). The slight net removal of glucose by the PDV tissues was unaffected by oil infusion ($P > 0.6$) or stage of lactation ($P > 0.2$).

Net removal of L-lactate by liver was increased ($P < 0.05$) by oil infusion and, as net PDV release was unaffected

($P > 0.68$), net TSP release of L-lactate was reduced ($P < 0.08$) by oil infusion. Arterial concentration of L-lactate was unaffected by oil infusion but was lower in ELAC than in MLAC ($P < 0.01$). Net removal of L-lactate by the liver was greater ($P < 0.01$) in ELAC than in MLAC (Table 4) and, as there was no effect of stage of lactation on net PDV release of L-lactate ($P > 0.64$), net TSP release of L-lactate was lower in ELAC than in MLAC ($P < 0.01$ [Table 4]). There were no effects of either stage of lactation or oil infusion on arterial concentration ($P > 0.13$) or net PDV, liver, or TSP flux of BHBA ($P > 0.40$ [Table 4]).

Arterial NEFA concentrations were increased by oil infusion ($P < 0.02$) and were greater in ELAC than in MLAC ($P < 0.01$ [Table 4]). Greater arterial concentrations of NEFA during oil infusion were associated with increased net uptake of NEFA by liver ($P < 0.02$). Overall there was a net TSP uptake of NEFA that was increased by oil infusion ($P < 0.02$). Net PDV release and liver removal of NEFA were greater in ELAC than in MLAC ($P < 0.02$), such that net TSP removal of NEFA was not affected by lactation stage ($P > 0.99$).

DISCUSSION

Packed-cell Volume, Blood Flow, and Blood Gas Exchange

Possible explanations for the decrease in PCV and hemoglobin during abomasal oil infusion include the

Table 4. Arterial concentrations (μM) and net flux of plasma metabolites across portal-drained viscera (PDV), liver, and total splanchnic (TSP) tissues during abomasal infusion of water or oil at an average of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum.

	Lactation stage				SEM	Significance, $P <$		
	ELAC		MLAC			Lactation stage	Infusion	Stage $\times$ infusion
	Water	Oil	Water	Oil				
Arterial concentration, μM								
Glucose	3520	3440	3670	3540	31	0.01	0.01	0.37
L-Lactate	344	312	448	417	30	0.01	0.32	0.99
BHBA	800	807	745	749	33	0.13	0.87	0.97
NEFA	121	159	73	113	13	0.01	0.02	0.95
Net PDV flux, mmol/h								
Glucose	-2.9	-10.6	-19.2	-22.8	8.95	0.15	0.56	0.83
L-Lactate	205	209	219	206	11.3	0.64	0.68	0.50
BHBA	264	269	261	264	11.9	0.74	0.77	0.95
NEFA	23	26	10	17	3.2	0.01	0.17	0.62
Net liver flux, mmol/h								
Glucose	932	873	832	768	28	0.01	0.06	0.90
L-Lactate	-181	-230	-42	-89	21	0.01	0.05	0.97
BHBA	382	368	362	331	32	0.40	0.51	0.80
NEFA	-48	-61	-36	-48	4.3	0.02	0.02	0.92
Net TSP flux, mmol/h								
Glucose	929	865	811	745	30	0.01	0.06	0.97
L-Lactate	25	19	178	117	27	0.01	0.08	0.77
BHBA	644	636	621	591	39	0.42	0.65	0.79
NEFA	-25	-33	-26	-32	2.4	0.99	0.02	0.63

blood sampling schedule and volume of blood removed, as sampling during oil infusion always followed a control sampling. Portal and liver plasma and blood flows were increased by oil infusion and were greater in ELAC than in MLAC. Overall, the hepatic artery accounted for 20% of liver blood flow, which is similar to the proportion reported for cows in early and midlactation (Reynolds, 1995), but higher than the contribution observed in cows in late lactation (Reynolds et al., 1994) or growing cattle (Reynolds, 1995). A greater proportional contribution of the hepatic artery to liver blood flow has been suggested to be a reflection of the increased metabolic activity and O_2 requirements of the liver during early lactation (Reynolds, 1995). The high rates of liver blood and plasma flow observed in the present study are comparable to measurements obtained in early lactation cows (Bach et al., 2000) at DMI similar to the present study. Bach et al. (2000) also observed a ratio of portal vein to total liver plasma flow of 0.8.

The effect of oil infusion on liver plasma flow in the present study was attributed to metabolizable energy (ME) intake during the blood sampling period or to neural or hormonal responses to postprandial oil infusion (Benson and Reynolds, 2001). While oil infusion decreased daily DMI in our study, the reduction occurred late in the day and before the morning milking, and thus feed DMI during the period of sampling was not affected (Benson et al., 2001). In addition, decreases in blood PCV and hemoglobin and arterial O_2 concentration also may

have contributed to the increase in blood flow during oil infusion. Assuming a constant O_2 extraction efficiency, splanchnic blood flow may have increased during oil infusion to compensate for decreased blood O_2 level and restore O_2 supply. However, this theory is not supported by work elsewhere. Experimentally induced anemia in pregnant sheep had no effect on blood flow to tissues comprising the TSP (Edelstone et al., 1987), or PDV blood flow and O_2 removal in preruminant lambs, where PDV O_2 extraction efficiency varied inversely with PCV and O_2 supply (Holzman et al., 1985). In humans, increased splanchnic blood flow following ingestion of oil was accompanied by an increase in blood PCV, suggesting other factors are responsible for the increased flow observed (Brundin, 1998). In this regard, increased absorption of oleic acid increases blood flow to the mucosa and muscularis of the jejunum of dogs via signals independent of changes in intestinal metabolism (Chou et al., 1985). These authors theorized that oleate effects on intestinal blood flow might facilitate increased lymph flow, aiding any oleate absorption into mesenteric veins. In the present study, increased liver blood flow during oil infusion was due totally to increased portal vein flow and not accompanied by changes in PDV O_2 removal, implying a specific effect on PDV blood flow.

The RQ for the PDV (0.39) was low compared to other studies in lactating cows. A mean value of 0.56 (0.71 to 0.43) was reported for the PDV of lactating cows (Reynolds et al., 1988a), and a value of 2.3 was calculated from

the data of Baird et al. (1975). The variability of these values may be due in part to the variability of sampling and measurement technique, as well as variations in gut lumen and blood CO₂ exchange. The net release of CO₂ by the PDV tissues can be attributable to a number of sources, including gut fermentation, respiratory metabolism in gut tissues, and bicarbonate in saliva. In addition, it is required for absorption of VFA and can be transferred directly from arterial blood to the lumen of the gut (Reynolds et al., 1988a). For these reasons, "conclusions concerning the metabolism by tissue cannot be made" from net PDV flux of CO₂ (Pell et al., 1986).

The liver, like the PDV, is highly metabolically active and in the present study consumed amounts of O₂ similar to the PDV. The RQ for the liver was also low (0.64) compared with previous studies (0.72 for Reynolds et al., 1988a; 0.90 for Baird et al., 1975) in lactating cows. Liver release of CO₂ underestimates total production to the extent that CO₂ is used for metabolic processes, such as urea synthesis from ammonia (Stryer, 1981). Neither O₂ consumption nor net CO₂ release by the liver or TSP tissues was affected by oil infusion. Brundin (1998) also noted no difference in TSP O₂ consumption after oral or intravenous administration of vegetable oil in humans, despite an increase in blood flow.

Removal of O₂ by the PDV was not affected by stage of lactation, but the liver removed more O₂ in ELAC. This in part reflects increased liver glucose synthesis, NEFA removal, and perhaps oxidation. This increase in liver O₂ use may also explain the increase in liver blood flow observed in early lactation. Although blood flow to the PDV and liver is highly correlated with DMI, liver blood flow was elevated in ELAC despite similar DMI compared with MLAC. This implies that the increase in liver blood flow in ELAC was a result of the metabolic activity and O₂ requirement when milk yield and metabolic demands on the liver were increased.

Effects of LCFA Infusion

PDV nutrient metabolism. Although daily DMI was reduced by oil infusion, net PDV absorption of total VFA was unaffected. As discussed previously, DMI during the 8-h sampling period was unaffected (Benson et al., 2001), and therefore, the supply of fermentable substrates to the rumen, and subsequently total VFA absorption, would be expected to be similar. However, net PDV absorption of branched-chain VFA and ammonia were increased by oil infusion. These results are difficult to explain, as net PDV release of the other VFA was largely unaffected. Possible explanations for increased net PDV absorption of branched-chain VFA and ammonia attributable to oil infusion include an inhibition of gut motility caused by increased intestinal oil supply (Nicholson and

Omer, 1983), which may have altered the extent and pattern of fermentation, as well as oxidative metabolism of the gut tissues. Alternatively, hormonal and metabolic effects of oil infusion may have altered the metabolism of PDV tissues, and thereby branched-chain VFA and ammonia release. In addition, inhibitory effects of any unabsorbed oil on hindgut fermentation and microbial growth, and thus ammonia utilization, may have increased net ammonia absorption.

In spite of greater portal vein blood flow during oil infusion, net PDV removal of O₂ was unchanged. In addition, oil infusion had no effect on the net PDV release of CO₂, BHBA, and L-lactate, which are all products of PDV metabolism of VFA and glucose. These observations suggest that during sampling the metabolic activity of the PDV was dictated more by feed DMI and fermentable energy supply than by total ME and oil infusion, and that increases in blood flow during oil infusion were not a consequence of changes in the metabolic activity of the gut.

Net PDV glucose flux was also not affected by oil infusion and was negative, indicating greater use of arterial glucose than glucose absorption into the portal vein. However, during the course of blood sampling, net PDV glucose flux increased such that there was a shift from net use of glucose to net release approximately 2 h after feeding (Figure 1). This may reflect a postprandial increase in rumen outflow of undigested starch to the small intestine. There was no evidence of a postprandial response of net L-lactate release by the PDV (data not shown), but there was a marked increase in net PDV release of propionate after feeding (Figure 2), suggesting postprandial responses of propionate, but not L-lactate, were dictated by rumen fermentation. This implies that much of the L-lactate absorbed into the portal vein was a product of glycolysis and not silage intake, fermentation, or propionate metabolism during absorption.

Net PDV NEFA flux was increased by oil infusion, which may be due to increased release of NEFA from PDV-associated fat. Only a small proportion of dietary NEFA are absorbed directly into the portal vein, as the majority of NEFA with carbon chain length greater than 12 are absorbed into lymph. Gagliostro and Chilliard (1991) observed increased lipolytic activity of adipose tissue sampled from lactating cows given a duodenal infusion of rapeseed oil, which may explain the increase in net PDV release of NEFA during oil infusion in our study.

Liver metabolism. Grummer and Carroll (1991) proposed that fat supplementation spares glucose from oxidation and that this glucose may be utilized for lactose synthesis and milk production. In the present study, milk production was unaffected by oil infusion despite a decrease in daily DMI (Benson et al., 2001) and liver

Figure 1. Patterns of net portal-drained viscera glucose flux (mmol/h) during an 8-h sampling period at the end of 7-d abomasal infusions of water (solid line) or oil (dashed line) in lactating dairy cows at a mean of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum. Cows were fed after sampling at 0830 h.

glucose production; therefore, a larger proportion of liver glucose release was used for milk synthesis. Oil infusion decreased liver glucose output without affecting DMI and liver VFA removal during the 8-h sampling period (Table 2). This suggests that abomasal oil infusion had inhibitory effects on liver glucose production independent of precursor supply during sampling.

Abomasal oil infusion increased the maximum possible contribution of propionate and L-lactate to liver glucose output. This reflects the decrease in liver glucose output and the increase in L-lactate uptake by the liver and suggests a decrease in the contribution of other precursors not measured in the current study (amino acids, glycerol, or pyruvate [Lomax and Baird, 1983]). Alternatively, oil infusion may have caused an increase in the nonglucogenic use of propionate, lactate, or other potential sources of glucose carbon. Increases in insulin and decreases in glucagon could also cause a reduction in liver glucose production (Brockman and Laarveld, 1986), but, in the present study, arterial concentration of insulin was reduced by oil infusion, whereas net liver removal of pancreatic glucagon was increased (Benson and Reynolds, 2001). These changes in pancreatic hormones do not explain the decrease in liver glucose release, but they do provide an explanation for the increase in liver L-lactate removal when oil was infused. L-Lactate is utilized via lactate dehydrogenase and pyruvate carboxylase, and the later enzyme is stimulated by pancreatic glucagon (Brockman and Laarveld, 1986).

In vitro work using hepatocytes from sheep, goats, and calves has shown that under certain conditions specific LCFA (oleate and palmitate) can stimulate gluconeogenesis from propionate (Faulkner and Pollock, 1986; Aiello and Armentano, 1988), without altering the glucogenic use of L-lactate (Aiello and Armentano, 1988). However, in the present study, oil infusion decreased liver glucose release and plasma glucose concentration, perhaps via decreased daily DMI or a decreased glucose requirement, rather than a direct effect of infused LCFA on liver metabolism. Drackley et al. (1992) noted a tendency for plasma glucose concentration to linearly decrease with increasing unsaturation and chain length of abomasally infused LCFA, which they attributed to decreased DMI.

There was a net removal of total VFA and release of acetate by the liver, which was unaffected by oil infusion. Net liver removal of branched-chain VFA was increased by oil infusion, but this accompanied an equivalent increase in their net PDV release, and thus net TSP output was unchanged. As often noted (Reynolds, 1995; Nozière et al., 2000), it appeared that the liver removed VFA (with the exception of acetate) in proportion to their supply from the gut. This is illustrated by the patterns of net PDV absorption and liver removal of propionate across the 8-h sampling period (Figure 2). Postprandial increases in net PDV release of propionate were mirrored by increased net liver removal, and, therefore, little variation in net TSP release was observed.

Figure 2. Patterns of net portal-drained viscera (PDV) (a) and liver (b) propionate fluxes (mol/h) during an 8-h sampling period at the end of 7-d abomasal infusions of water (solid line) or oil (dashed line) in lactating dairy cows at a mean of 55 (early lactation [ELAC]) and 111 (midlactation [MLAC]) d postpartum. Cows were fed after sampling at 0830 h.

Net removal of NEFA by the liver was increased by oil infusion, presumably due to increased arterial concentration and supply from the PDV, as NEFA are removed by the liver in proportion to their supply by blood (Emery et al., 1992). BHBA is a product of NEFA and n-butyrate metabolism by the liver, but oil infusion had no effect on BHBA production by the liver. The maximum possible contribution of n-butyrate and NEFA removed by the liver to BHBA released was greater than 100% during oil infusion, perhaps reflecting an increase in-liver utilization of NEFA via oxidation or triglyceride synthesis

(Emery et al., 1992). In the present study, an increase in the oxidation of NEFA and other metabolites may have contributed to the depression in daily DMI observed when oil was infused. In nonruminants, increases in liver ATP levels due to LCFA oxidation depress appetite and intake (Scharrer and Langhans, 1986; Friedman, 1998).

Effects of Stage of Lactation

PDV nutrient metabolism. Lomax and Baird (1983) and Reynolds et al. (1988a) have shown increases in

portal vein blood flow and net PDV absorption of VFA and L-lactate in lactating cows compared to nonlactating cows. This is largely the result of increased DMI, as numerous studies have shown linear relationships between DM or ME intake and net PDV release of VFA and other nutrients (Lomax and Baird, 1983; Nozière et al., 2000). However, there has been little research measuring changes in splanchnic metabolism of dairy cows at differing stages of lactation. In the present experiment, DMI and net PDV absorption of total VFA, acetate, propionate, L-lactate, and BHBA were similar in ELAC (8 wk) and MLAC (16 wk). Net PDV absorption of ammonia is highly correlated with N intake (Reynolds, 1995) and was similarly unaffected by stage of lactation in the present study. In contrast, absorption of n-butyrate, branched-chain VFA, and n-valerate were greater in ELAC. Reasons for this specific increase in the PDV absorption of longer-chain VFA are uncertain. The response may be related to longer-term adaptation of the rumen microflora to higher DMI as lactation progressed, effects of water intake associated with higher milk yield in ELAC on rumen fermentation, or changes in gut tissue mass and subsequent metabolism of absorbed VFA.

Net PDV release of NEFA was greater in ELAC compared to MLAC. Reynolds et al. (1988a) reported a decrease in net PDV release of NEFA between wk 4 and 8 of lactation. These differences in net PDV release of NEFA were probably due to an increase in lipolysis of adipose tissue drained by the portal vein associated with greater milk energy yield (Benson et al., 2001) or lower DMI (Reynolds et al., 1988a) in ELAC, and thus reduced tissue energy balance.

Liver metabolism. Net liver removal of propionate was not affected by stage of lactation, but net liver removal of n-butyrate, branched-chain VFA and n-valerate were greater in ELAC, a consequence of the increase in their net PDV release. Net acetate release by the liver was also greater in ELAC compared with MLAC. This may be attributable to increased NEFA removal and metabolism. However, abomasal oil infusion also increased net liver removal of NEFA but had no effect on net liver release of acetate. In very ELAC, greater liver removal of NEFA would be expected to be associated with more NEFA oxidation and ketone body synthesis, but there was no effect of stage of lactation on net liver release of BHBA in the present study. Net glucose release by the liver was higher in ELAC when milk yield and glucose requirement for milk synthesis were greater (Benson et al., 2001), but arterial glucose concentration was lower, perhaps due to an increased use for milk synthesis. In ELAC, greater glucose release by the liver was associated with more liver removal of L-lactate, and thus a decreased net TSP release. This suggests that the metabolic changes associated with increased milk

production in ELAC result in a requirement for additional L-lactate to restore liver carbon balance (Reynolds, 1995). Greater L-lactate removal by the liver in ELAC was associated with increased net liver removal of pancreatic glucagons (Benson and Reynolds, 2001), but in contrast to the response to abomasal oil infusion, increased glucagon removal by the liver in ELAC was associated with greater net glucose release.

Increased liver removal of L-lactate in ELAC may in part reflect an increase in Cori cycling of glucose and lactate between the liver and glycolysis in other tissues, as occurs during starvation (Stryer, 1981). In MLAC, when the cows were in more positive tissue-energy balance (calculated tissue-energy balance increased by 13.2 MJ/d), an increased liver-carbon balance and TSP release of lactate to peripheral tissues may support more body fat synthesis in lactating dairy cows, as occurs with increased intake level in growing ruminants (Prior, 1978). Similarly, differences in liver lactate removal, and thus net TSP lactate release, have been positively associated with increased tissue energy and fat retention in growing cattle (Reynolds et al., 1991; Reynolds, 1995). In the present study, net TSP flux of NEFA was negative, representing liver removal of NEFA from peripheral tissues, and not affected by stage of lactation. However, tissue exchanges of blood NEFA do not account for LCFA exchange via lipoproteins.

CONCLUSIONS

To our knowledge, this study was the first to measure the effect of postruminal LCFA supply on net flux of nutrients across the PDV and liver in lactating dairy cows. Abomasal infusion of a mixture of sunflower and rapeseed oils had a number of effects on splanchnic metabolism, including increases in PDV and liver blood flow, which were not associated with measurable changes in O₂ removal. Increased abomasal oil supply increased liver removal of L-lactate and NEFA, but liver glucose release was decreased. Comparison of net PDV and liver flux of nutrients between the two stages of lactation were generally consistent with a lack of change in DMI, and thus net PDV metabolism, coupled with an increased metabolic activity of the liver to support greater milk yield in ELAC. In contrast to the effects of oil infusion, greater liver blood flow in ELAC was associated with an increased liver removal of O₂, whereas increases in net liver removal of lactate and NEFA in ELAC accompanied an increase in liver glucose release. With the exception of net PDV release of acetate, the metabolic response to postruminal supply of LCFA did not differ between 8 and 16 wk postpartum.

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