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Circadian Changes in Net Nutrient Fluxes Across the Portal-Drained Viscera, the Liver, and the Hindquarters in Preruminant Calves

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ABSTRACT: The objective of this study was to describe in preruminant calves the circadian patterns in net nutrient fluxes across the portal-drained viscera, the liver, and the hindquarters and to relate them to previously published variations in tissue energy expenditure. In vivo arterio-venous techniques were used, and animals were fed a conventional milk replacer. In the portal-drained viscera, net glucose absorption occurred 1 to 5 h postprandially with some lactate release. Arterial plasma non-esterified fatty acid (NEFA) concentrations decreased subsequently, as well as net NEFA uptake by the portal-drained viscera. Triglyceride absorption that occurred 1 as well as 7 or 8 h postprandi ally did not take place via the portal vein. Changes in energy expenditure of the portal-drained viscera did not correlate with changes in net nutrient fluxes. In the liver, the maximum contribution of lactate to hepatic glucose production was 20%. The NEFA and triglycerides were taken up by the liver, whereas urea was released. Only increases in NEFA uptake could partly explain the postprandial rise in hepatic energy expenditure. In the hindquarters, up to 48% of the glucose taken up could be converted into lactate; the remaining balance was possibly oxidized, thereby contributing as much as 33% to energy expenditure. No hourly patterns were noted in net nutrient fluxes through hindquarters. In conclusion, in preruminants, contribution of nutrients to oxidation differed from that noted in ruminants and seemed to vary with nutrient absorption and availability patterns, especially in the portal-drained viscera.

Key Words: Calves, Portal Circulation, Liver, Hindquarters, Nutrient Flux

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Introduction

Energy requirements of preruminant calves have been clearly established, and if diet composition does not greatly influence the efficiency of ME utilization for growth, it does modify the composition of gain (INRA, 1978, 1988). In calves, as in older ruminants, a large proportion of ME consumed is used by splanchnic tissues that have high specific requirements in energy (170 to 214 cal·d⁻¹·g⁻¹ of fresh portal-drained visceral and 254 to 331 cal·d⁻¹·g⁻¹ of hepatic tissue; Ortigues et al., 1995a,b). Indeed, portal-drained viscera (PDV) and liver contribute approximately 17 and 13%, respectively, to total energy expenditure (EE) but represent only 6 and 2% of BW. These tissues are also responsible for 33 to 54% and 29 to 32%, respectively, of the rise in whole-animal EE noted over the 6 to 7 h after the meals (Ortigues et al., 1995b). Such postprandial periods are characterized by specific patterns of nutrient absorption and metabolism, as indicated by circadian changes in blood metabolites (Durand and Bauchart, 1986). However, no clear direct relationship has yet been established between changes in the pattern of nutrients available to splanchnic tissues and their energy metabolism.

In contrast, the carcass has much smaller energy requirements (39 cal·d⁻¹·g⁻¹ of boneless). In calves, hindquarters contribute only 18% to total EE and present no clear changes in EE over the day (Ortigues et al., 1995b). No data are available on net nutrient fluxes through hindquarters in preruminant calves.

Consequently, the objective of the present study was to describe in preruminant veal calves the changes in net energy-yielding nutrient fluxes across PDV, liver, and hindquarters with time of the day, and to relate them to known variations in tissue EE measured on the same animals (Ortigues et al., 1995b). The circadian rhythm under consideration

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correlated to that of usual management practices whereby veal calves are fed two meals a day, spaced at unequal time intervals.

Materials and Methods

Animals. Four male Holstein × Friesian calves were used. They were housed in individual pens on wood shavings. At the age of 14.5 ± 1.04 d and at an average BW of 46.6 ± 2.45 kg, they were fitted with chronic blood catheters in the portal vein (PV), a hepatic vein (HV), a mesenteric vein (MV), a mesenteric artery (MA), and the posterior vena cava (VC). Blood flow probes were also implanted around the PV (electromagnetic probe), the posterior aorta, and the left branch of the hepatic artery (ultrasonic probes), as fully described in Ortigues et al. (1995b). The experiment was conducted in a manner compatible with national legislation on animal care (Certificate of Authorization to Experiment on Living Animals, no. 004495, Ministry of Agriculture).

Feeding. All calves were fed a commercial milk replacer containing milk powder and tallow as the main fat source (239 g of CP, 210 g of fat, 4.78 Mcal of ME/kg of DM) in two equal daily feedings at 0800 and 1600. Feed allowances were calculated for a gain of 1 kg/d, assuming that maintenance ME requirements amounted to 100 kcal/kg BW.75, that the energy content of body gain averaged 2.500 kcal/kg of gain for 3- to 5-wk-old calves, and that the efficiency of ME utilization for growth equaled .7 (van Es, 1966). After appetite returned to normal (approximately 7 d after surgery), feed allowances were determined (on what was considered as d 1) and left unchanged thereafter.

Measurements. Feeds and live weight measurements were already described in Ortigues et al. (1995b). Twenty-four-hour measurements of nutrient fluxes through the PDV, liver, and hindquarters were performed 10 d (d 10) after calves had resumed their normal eating habits. Calves had previously been accustomed to handling and to the blood sampling procedures. Twelve hours before the start of sampling and throughout d 10, Na2EDTA (6%) was used as the anticoagulant for catheter maintenance to avoid the risk of activation of the lipoprotein lipase by heparin. On d 10, blood samples were collected from the MA, PV, HV, and VC 15 min before and 30 min after the morning meal, and every 60 min thereafter until the evening meal. One-hour sampling resumed 30 min after the evening meal until the next morning meal. To ensure uniformity in sample collection, all samples were taken while the calves were standing quietly. Samples were analyzed for hematocrit, blood glucose (glucose oxidase method, kit 6.127.1, Biomérieux, 69280 Marcy l'Etoile, France) and L-lactate (Bergmeyer, 1974), and plasma non-esterified fatty acids (NEFA; kit 46551 Unipath SA, 69572 Dardilly, France), triglycerides (TG; kit 6.123.8, Biomérieux), and urea (kit 6.198.2, Biomérieux). For the determination of NEFA concentrations, samples were pooled across the following hours: 3 to 4, 5 to 6, and 7 to 8 after the morning meal and 3 to 4, 5 to 6, 7 to 8, 9 to 10, 11 to 13, and 14 to 15 after the evening meal on the basis of results obtained by Durand and Bauchart (1986). Plasma urea concentrations were not measured in samples from VC. Blood flows were determined as described by Ortigues et al. (1995b). Plasma flows were calculated from measured blood flows and hematocrit values. Net nutrient fluxes were calculated as the product of blood or plasma flow rate and blood or plasma venous-arterial concentration differences (VA). For the determination of net NEFA fluxes, calculations were performed for each hourly interval using the hourly plasma flows and the corresponding hourly or pooled NEFA concentrations. Positive fluxes denote net release of a metabolite or net portal uptake. Extraction rates were calculated as the ratio between the net flux of a metabolite and the supply of this metabolite to the tissue considered.

Statistical Analyses. Results were analyzed according to the univariate repeated measures analysis (SAS, 1987) using hour as the repeated measures factor. Separate analyses were conducted for each of the intervals between meals (from 1 h before to 8 h after the morning meal, and from 1 h before to 15 h after the evening meal). The Huynh Feld epsilon (HF Eps) adjustment factor of the F-test was used to account for possible unequal correlations between repeated measures (Homer, 1989). This factor is reported in addition to the standard error of treatment means (SEM = √[residual mean square/number of observations per treatment]). The significance levels presented are the actual probabilities, considering that hour differences are nonsignificant when P > .10. Hours were compared by orthogonal contrasts.

Results

Animal Health and Catheter Patency. Postsurgical recovery of calves was good; BW gain averaged 749 ± 66.9 g/d for a ME intake of 5.5 ± .09 Mcal/d, and animals weighed 60.2 ± 4.49 kg at the time of measurements. Most catheters remained functional throughout the experiment except for one catheter in posterior VC. Additionally, posterior aortal blood flows could be recorded in two calves only because of technical mishaps. All blood flow results have been presented in Ortigues et al. (1995b).

Glucose and L-lactate. Significant postprandial changes in blood glucose and lactate concentrations were noted in all vessels, but synchronization of the changes and level of significance differed slightly between vessels and metabolites (Tables 1 and 2; Figure 1). Generally, concentrations increased immediately after the meals and remained elevated for 4
Table 1. Changes in arterial, portal, hepatic, and vena cava concentrations of blood glucose (mM) and L-lactate (mM) as well as of plasma non-esterified fatty acids (NEFA, \( \mu M \)), triglycerides (TG, \( \mu M \)), and urea (mM) after the morning meal in preruminant calves

| Item               | Hours after the morning meal | SEM | HF Eps* | Hour effect |
|--------------------|-----------------------------|-----|---------|-------------|
|                    | n   | 1   | 2     | 3     | 4     | 5     | 6     | 7     | 8     |       |       |       |
| **Blood glucose**  |     |     |       |       |       |       |       |       |       |       |       |
| Arterial           | 4   | 4.89a | 6.33b | 6.11b | 6.50b | 6.22b | 5.89b | 5.17a | 5.39a | 5.33a | .246  | .44   | P < .01 |
| Portal             | 4   | 4.94a | 7.11b | 6.67b | 6.94b | 6.67b | 5.78a | 5.50a | 5.39a | 5.11a | .346  | .30   | P < .03 |
| Hepatic            | 4   | 5.17a | 7.33b | 6.78b | 7.11b | 6.78b | 6.33b | 5.61a | 5.56a | 5.44a | .275  | .42   | P < .01 |
| Vena cava          | 3   | 5.17  | 6.33  | 5.83  | 6.28  | 5.94  | 5.89  | 5.44  | 5.50  | 5.11  | .383  | .20   | NS**   |
| **Blood L-lactate**|     |     |       |       |       |       |       |       |       |       |       |       |
| Arterial           | 4   | .72a  | 1.02b | 1.04b | 1.10b | .91b  | .92b  | .71a  | .82a  | .86a  | .064  | 1.00  | P < .01 |
| Portal             | 4   | .90   | 1.20  | 1.24  | 1.18  | .98   | .92   | .86   | .89   | .93   | .115  | .80   | NS     |
| Hepatic            | 4   | .79   | 1.03  | .95   | .95   | .87   | .96   | .72   | .75   | .70   | .111  | 1.00  | NS     |
| Vena cava          | 3   | 1.01  | 1.25  | 1.38  | 1.32  | 1.29  | 1.33  | 1.29  | 1.92  | .95   | .118  | .27   | NS     |
| **Plasma NEFA**    |     |     |       |       |       |       |       |       |       |       |       |       |
| Arterial           | 4   | 377a  | 228a  | 362a  | 253a  | 238a  | 549b  | 74.6  | 1.00  | P < .02 |
| Portal             | 4   | 342   | 207   | 282   | 228   | 167   | 390   | 64.4  | .37   | NS     |
| Hepatic            | 4   | 188a  | 77b   | 106b  | 87b   | 88b   | 219a  | 17.2  | .18   | P < .02 |
| Vena cava          | 3   | 357a  | 253b  | 314ab | 208b  | 155b  | 441a  | 65.0  | 1.00  | P < .03 |
| **Plasma TG**      |     |     |       |       |       |       |       |       |       |       |       |       |
| Arterial           | 4   | 367a  | 476a  | 771ab | 473a  | 356a  | 300a  | 432a  | 590a  | 842b  | 90.5  | .31   | P < .05 |
| Portal             | 4   | 399a  | 476a  | 734ab | 439a  | 351a  | 270a  | 402a  | 597a  | 839b  | 93.3  | .22   | P < .09 |
| Hepatic            | 4   | 350a  | 487a  | 721ab | 509a  | 366b  | 291a  | 386a  | 511a  | 879b  | 95.8  | .19   | P < .10 |
| Vena cava          | 3   | 443   | 546   | 742   | 545   | 407   | 315   | 417   | 561   | 940   | 119.4 | .25   | NS     |
| **Plasma urea**    |     |     |       |       |       |       |       |       |       |       |       |       |
| Arterial           | 4   | 2.08  | 2.25  | 2.20  | 2.20  | 2.31  | 2.30  | 2.08  | 2.18  | 2.16  | .08   | .54   | NS     |
| Portal             | 4   | 2.10  | 2.05  | 2.15  | 2.06  | 2.16  | 2.25  | 2.00  | 2.03  | 2.01  | .09   | .40   | NS     |
| Hepatic            | 4   | 2.11  | 2.18  | 2.06  | 2.20  | 2.10  | 2.28  | 2.11  | 2.18  | 2.13  | .06   | .33   | NS     |

*Huynh Feld epsilon.
**NS = not significant (P > .10).
*Within a line, means with different superscripts are statistically different.
Table 2. Changes in arterial, portal, hepatic, and vena cava concentrations of blood glucose (mM) and L-lactate (mM) as well as of plasma non-esterified fatty acids (NEFA, μM), triglycerides (TG, μM), and urea (mM) after the evening meal in preruminant calves

| Item                      | Hours after the evening meal | SEM | HF Eps* | Hour effect |
|---------------------------|------------------------------|-----|---------|-------------|
| **Blood glucose**         |                              |     |         |             |
| Arterial                  |                              |     |         |             |
| 4                         | 5.33a, 6.28b, 6.94b, 7.17b, 6.20b, 6.17b, 5.83b, 5.94b, 5.83b, 5.83b, 5.67ad, 5.56ad, 5.50a, 5.50a, 5.39a, 5.50a, 5.22a | 0.15 | .10     |             |
| Portal                    |                              |     |         |             |
| 4                         | 5.11a, 6.72b, 7.39b, 7.50b, 6.61b, 6.33b, 5.72bc, 5.61bc, 5.89bc, 5.78bc, 5.67bc, 5.50bc, 5.28b, 5.39a, 5.17a, 4.94a | 0.47 | .01     |             |
| Hepatic                   |                              |     |         |             |
| 4                         | 5.44a, 6.89b, 7.56b, 7.78b, 7.06b, 6.39b, 6.00c, 6.26c, 5.94c, 5.89c, 6.00c, 5.56c, 5.50c, 5.50c, 5.50c | 0.08 | .01     |             |
| Vena cava                 |                              |     |         |             |
| 3                         | 5.11a, 5.83a, 7.00b, 7.00b, 6.11a, 6.00a, 5.61a, 5.83a, 5.72a, 5.89a, 5.61a, 5.50a, 5.50a, 5.50a | 0.11 | .01     |             |
| **Blood L-lactate**       |                              |     |         |             |
| Arterial                  |                              |     |         |             |
| 4                         | .86a, 1.15b, 1.12b, 1.09b, 1.14b, .99ab, 1.00ab, .90ab, .90ab, .90ab, .90ab, .82a, .85a, .80a, .76a, .76a, 1.00 | .01 | .01     |             |
| Portal                    |                              |     |         |             |
| 4                         | .93a, 1.22b, 1.24b, 1.20b, 1.21b, 1.16b, 1.18b, 1.00ab, .99ab, .90ab, .90ab, .90ab, .80ab, .70c, .76c, .006 | .01 | .01     |             |
| Hepatic                   |                              |     |         |             |
| 4                         | .70, 1.10, 1.02, 1.00, 1.04, .90, 1.03, 1.00, .96, .88, .76, .76, .80, .82, .86, .088 | .24 | .01     |             |
| Vena cava                 |                              |     |         |             |
| 3                         | .95a, 1.17a, 1.35a, 1.31b, 1.25b, 1.04a, 1.11a, .99a, .91a, .90ab, .94a, 1.25b, 1.03a, 1.00a | .07 | .11     |             |
| **Plasma NEFA**           |                              |     |         |             |
| Arterial                  |                              |     |         |             |
| 4                         | 549a, 601a, 484a, 217b, 413a, 465a, 464a, 464a, 464a, 464a, 464a | 0.86 | .01     |             |
| Portal                    |                              |     |         |             |
| 4                         | 390a, 446b, 392ab, 183c, 268b, 246a, 253a, 269a, 243a | 0.42 | .01     |             |
| Hepatic                   |                              |     |         |             |
| 4                         | 219a, 241a, 146b, 101c, 127c, 169b, 186b, 173c, 171c | 0.62 | .01     |             |
| Vena cava                 |                              |     |         |             |
| 3                         | 441b, 486b, 395ab, 164b, 258bc, 323d, 393c, 421a, 415a | 0.01 | .01     |             |
| **Plasma TG**             |                              |     |         |             |
| Arterial                  |                              |     |         |             |
| 4                         | 842a, 853a, 563c, 336c, 316c, 347c, 454c, 630c, 678b, 592b, 614b, 705bc, 668b, 582b, 614b, 564b | 0.55 | .01     |             |
| Portal                    |                              |     |         |             |
| 4                         | 839a, 1055a, 548c, 316c, 305b, 372b, 443b, 605b, 664c, 588c, 574c, 640c, 664c, 591c, 562c, 521c | 0.40 | .01     |             |
| Hepatic                   |                              |     |         |             |
| 4                         | 879a, 1015a, 582bc, 338b, 325b, 365b, 426bc, 555c, 585c, 559c, 580c, 634c, 670c, 562c, 576c, 540c | 0.38 | .01     |             |
| Vena cava                 |                              |     |         |             |
| 3                         | 940c, 1094a, 604b, 334c, 314c, 356c, 486bc, 599c, 641b, 559b, 618b, 700b, 789b, 630b, 610b, 652b | 0.97 | .01     |             |
| **Plasma urea**           |                              |     |         |             |
| Arterial                  |                              |     |         |             |
| 4                         | 2.16, 2.18, 2.38, 2.36, 2.30, 2.30, 2.31, 2.16, 2.16, 2.11, 2.16, 2.21, 2.30, 2.25 | 0.61 | .01     |             |
| Portal                    |                              |     |         |             |
| 4                         | 2.01, 2.05, 2.31, 2.26, 2.13, 2.11, 2.10, 1.96, 1.96, 2.00, 1.95, 2.10, 2.03, 2.11, 2.08 | 0.38 | .01     |             |
| Hepatic                   |                              |     |         |             |
| 4                         | 2.13, 2.13, 2.32, 2.35, 2.35, 2.23, 2.16, 2.18, 2.18, 2.00, 2.10, 2.18, 2.30, 2.16, 2.25 | 0.55 | .01     |             |

*a*Huynh Feld epsilon.
**NS = not significant (P > .10).
a,b,c,dWithin a line, means with different superscripts are significantly different.
Figure 1. Circadian changes in arterial concentrations (mM) in blood glucose and L-lactate and in plasma non-esterified fatty acids (NEFA), triglycerides (TG), and urea in preruminant calves offered a milk replacer diet. Arrows represent feeding times. Sample standard errors are also shown (n = 4). For NEFA concentrations, the pooled plasma samples are indicated on the graph even though individual hourly data points have been represented for uniformity of presentation.
to 5 h. Concentrations then returned to preprandial levels 6 h onward after the morning meal. The return was more progressive at night because it occurred 11 to 12 h after the evening meal.

Net PDV uptake of glucose (Figure 2) in the morning period averaged −4.7 mmol/h preprandially and switched (P < .01) to a net portal appearance of glucose in the 4 h after the morning meal (84.5 mmol/h) before returning (P < .01) to preprandial net uptake thereafter (−8.9 mmol/h, SEM 37.6, HF Eps .62). In the evening period, a net portal appearance of glucose was also measured from h 1 to 5 postprandially (61.7 mmol/h; P < .01), whereas net uptake averaged −18.1 mmol/h (SEM 22.7, HF Eps .81) thereafter. No simple relationship was noted between this pattern of net PDV fluxes of glucose and arterial glucose concentrations because glucose extraction rates changed with time after the meals. It averaged .6% before the morning meal, increased (P < .10) to an average of 6.6% between h 1 to 6 postprandially, and decreased subsequently (P < .10) to −2.5% in h 7 to 8 postprandially (SEM 4.02, HF Eps 1.00). Similarly in the evening period, glucose extraction rate increased (P < .001) to an average of 5.4% between h 1 to 5 postprandially, before decreasing (P < .001) again to −1.4% (h 6 to 13) and to −5.9% (h 14 to 15; SEM 2.11, HF Eps .88).

Net portal appearance of lactate (Figure 2) averaged 19.1 mmol/h over the whole morning period (SEM 15.8, HF Eps .69) and 9.5 mmol/h over the evening period (SEM 11.3, HF Eps .35) with no significant changes over time. There were only nonsignificant trends for net portal appearance of lactate to be enhanced during periods of net portal glucose appearance (to 30.4 mmol/h during h 1 and 2 after the morning meal and to 39.4 mmol/h during h 3 to 7 after the evening meal). Additionally, net PDV lactate fluxes tended to be lower at night (−.4 mmol/h between h 8 to 15 after the evening meal).

In the liver, net glucose release was 53.5 mmol/h in the morning period (SEM 31.27, HF Eps 1.00) and 60.9 mmol/h after the evening meal (SEM 26.88, HF Eps .39). The latter remained stable throughout the day (Figure 3), regardless of net portal glucose or lactate appearance. On the other hand, lactate was taken up by the liver: −27.0 mmol/h in the morning period (SEM 25.61, HF Eps .30) and −19.5 mmol/h after the evening meal (SEM 19.56, HF Eps .15; Figure 3). This uptake, however, tended to be slightly lower (nonsignificant [NS]) at night (−5.9 mmol/h between h 7 and 15 after the evening meal) when no net portal appearance of either glucose or lactate took place.

As a consequence of the net PDV and hepatic fluxes, there was no net splanchnic release of glucose at all times (86.6 mmol/h in the morning period, SEM 43.87, HF Eps .98; 71.8 mmol/h after the evening meal, SEM 27.07, HF Eps .53), which was slightly enhanced in the first 4 to 5 h postprandially (115.5 and 136.6 mmol/h in the morning and evening periods, respectively). In contrast, net splanchnic lactate flux was never significantly different from zero (−9.3 mmol/h in the morning period [SEM 24.12, HF Eps = .61] and −10.1 mmol/h after the evening meal [SEM 15.87, HF Eps .71]).

The hindquarters showed net uptake of glucose (−21.1 mmol/h in the morning period [SEM 23.97, HF Eps 1.00] and −27.3 mmol/h after the evening meal [SEM 20.63, HF Eps .23]) and net release of lactate (32.4 mmol/h in the morning period [SEM 15.98, HF Eps .25] and 19.2 mmol/h after the evening meal [SEM 7.13, HF Eps 1.00]) without any significant changes with time during the day (Figure 4).

Non-Esterified Fatty Acids and Triglycerides. Plasma NEFA concentrations followed similar and significant postprandial changes in all vessels (Tables 1 and 2, Figure 1). Generally concentrations decreased immediately after the morning meal to remain at low levels over a total of 6 h, and rose again in the 2 h preceding the evening meal. Concentrations remained elevated during the 2 h that followed the evening meal, they decreased transiently (h 3 to 4 after the evening meal) before rising back to preprandial levels (h 5 to 15 after the evening meal).

Plasma TG concentrations followed the same pattern in all vessels with time, which is representative of the kinetics of lipid absorption (Tables 1 and 2, Figure 1). Concentrations tended to increase (NS) immediately after the morning meal by an increment of 37 to 64% (+151 to +223 µM) and to remain elevated for 3 h with a peak in the 2nd h after the meal. They tended to drop (NS) then to low preprandial levels over h 4 to 6 after the morning meal and to increase again (NS) from h 7 after the morning meal to reach significantly higher (P < .05) levels 8 h after the morning meal. Levels remained elevated 2 h following the evening meal, with a peak at h 1 after the evening meal (P < .001). Average levels were 96 to 123% (i.e., +349 to 420 µM) higher than the low preprandial levels. Low preprandial concentrations were measured again between h 3 to 6 after the evening meal (P < .001), whereas the night period (starting from h 7 after the evening meal) was characterized by significantly (P < .001) elevated concentrations throughout (+61 to +73%).

Net uptake of NEFA by the PDV was noted throughout the day (Figure 2). This uptake was enhanced (P < .05) when arterial NEFA concentrations were elevated, as shown by the positive correlation (r² = .52, P < .001) between net PDV NEFA uptake and arterial NEFA concentrations (NEFA extraction rates by PDV remained stable throughout the day at mean values of −17.2% in the morning [SEM 9.64, HF Eps .98] and −30.2% in the evening [SEM 9.07, HF Eps .23]). Net PDV NEFA uptake averaged −4.59 mmol/h from h 1 to 6 after the morning meal and increased (P < .05) to −14.86 mmol/h in the following 2 h (SEM 3.74, HF Eps .18).
Figure 2. Circadian changes in net fluxes across the portal-drained viscera (mmol/h) of blood glucose and L-lactate and of plasma non-esterified fatty acids (NEFA), triglycerides (TG), and urea in preruminant calves offered a milk replacer diet. Positive net fluxes denote net portal appearance, and negative net fluxes denote net uptake. Arrows represent feeding times. Sample standard errors are also shown (n = 4).

**Hours after the meals**
Figure 3. Circadian changes in net fluxes across the liver (mmol/h) of blood glucose and L-lactate and of plasma non-esterified fatty acids (NEFA), triglycerides (TG), and urea in preruminant calves offered a milk replacer diet. Positive net fluxes denote net release, and negative net fluxes denote net uptake. Arrows represent feeding times. Sample standard errors are also shown (n = 4).
Figure 4. Circadian changes in net fluxes across the hindquarters (mmol/h) of blood glucose and L-lactate and of plasma non-esterified fatty acids (NEFA), and triglycerides (TG) in preruminant calves offered a milk replacer diet. Positive net fluxes denote net release, and negative net fluxes denote net uptake. Arrows represent feeding times. Sample standard errors are also shown (n = 2).

Uptake remained elevated in the first 2 h after the evening meal (−12.27 mmol/h) and then decreased (P < .05) to −3.17 mmol/h during h 3 to 4 before increasing (P < .05) again to an average of −18.76 mmol/h over h 5 to 15 after the evening meal (SEM 4.97, HF Eps 1.00). The net PDV uptake of TG
(Figure 2) was not significantly different from zero at any time of the day (−1.47 mmol/h on average over the morning period [SEM 3.16, HF Eps .95] and −1.60 mmol/h after the evening meal [SEM 4.01, HF Eps .23]).

A large among-animal variability was noted in the net hepatic NEFA uptake with time (Figure 3). The hepatic uptake tended to be more elevated (NS) between h −1 to 4 (−21.98 mmol/h) and h 7 to 8 (−25.46 mmol/h) after the morning meal than during h 5 to 6 (−10.07 mmol/h; SEM 9.35, HF Eps .34). Similarly, uptake was higher (P < .06) in the 2 h after the evening meal (−35.47 mmol/h) than during the night (−12.11 mmol/h on average; SEM 6.01, HF EPS .67). No clear and significant changes were noted in NEFA extraction rate by the liver throughout the day (−50.8% on average in the morning period [SEM 9.14, HF Eps .20] and −41.6% in the evening period [SEM 5.77, HF Eps .48]). Overall splanchnic tissues showed a relatively constant net uptake of NEFA throughout the day (−31.03 mmol/h). Net hepatic uptake of TG (Figure 3) was not significantly different from zero throughout the day (−13 mmol/h in the morning period [SEM 5.20, HF Eps .78] and −1.01 mmol/h after the evening meal [SEM 5.28, HF Eps .18]).

The hindquarters (Figure 4) showed a net release of NEFA immediately before the morning meal (3.83 mmol/h) and switched (NS) to a net uptake there-after (−8.73 mmol/h from h 1 to 8 after the morning meal [SEM 2.79, HF Eps .30] and −11.93 mmol/h after the evening meal [SEM 4.46, HF Eps .90]). No significant changes were noted in NEFA uptake by the liver throughout the day (−19.1 mmol/h on average; SEM 11.06, HF Eps .78). It increased by 44% on average after the evening meal (−25.46 mmol/h) after the morning meal than during the 1 h after the evening meal (−35.47 mmol/h) than during the night (−12.11 mmol/h on average; SEM 6.01, HF EPS .67). No clear and significant changes were noted in NEFA extraction rate by the liver throughout the day (−50.8% on average in the morning period [SEM 9.14, HF Eps .20] and −41.6% in the evening period [SEM 5.77, HF Eps .48]). Overall splanchnic tissues showed a relatively constant net uptake of NEFA throughout the day (−31.03 mmol/h). Net hepatic uptake of TG (Figure 3) was not significantly different from zero throughout the day (−13 mmol/h in the morning period [SEM 5.20, HF Eps .78] and −1.01 mmol/h after the evening meal [SEM 5.28, HF Eps .18]).

Plasma Urea. Plasma urea concentrations were low in all vessels and remained totally unaffected by time of the day (Tables 1 and 2, Figure 1). Disappearance of urea measured at PDV was −12.7 mmol/h in the morning period (SEM 14.20, HF Eps .34) and −16.4 mmol/h after the evening meal (SEM 8.6, HF Eps .96). On the other hand, the liver showed a net release of urea (9.1 mmol/h in the morning period [SEM 16.70, HF Eps .17] and 12.4 mmol/h after the evening meal [SEM 10.54, HF Eps .22]; Figures 2 and 3).

Discussion

The present experiment was set up with the objectives first of describing the circadian patterns of nutrient fluxes across major body tissues and the patterns of nutrient exchanges among these tissues, and second of determining relationships between net nutrient fluxes and tissue energy expenditure in order to integrate these results into the whole animal energy metabolism (Ortigues et al., 1995b). Results indicate that metabolism of PDV tissues is highly responsive to the kinetics of digestion, previously described by Guilloteau et al. (1975) and Toullec et al. (1979) and that it showed definite circadian patterns of nutrient fluxes. However, the further subsequent tissues are from the gut, the less responsive they are in terms of circadian patterns. This attenuation of response is probably partly related to the dilution of the directly absorbed nutrients into the large flux of circulating arterial metabolites, and consequently to the small arterial-venous differences to be measured (Ortigues et al., 1994). For example, net portal glucose appearance represents only 2% of the total glucose supply to the liver. As far as the hindquarters are concerned, the lack of clear hourly changes in nutrient fluxes can also be ascribed to the reduced number of animals (n = 2) for which blood flow data were available.

In preruminant calves, digestion kinetics are characteristic of the ingredient and chemical composition of the milk replacer (Guilloteau et al., 1975; Toullec et al., 1979). The present experiment was performed using a clotting milk replacer containing milk powder and tallow as the main fat source. Because the basic composition of this milk was close to that used in studies of gastric emptying (Guilloteau et al., 1975), peripheral (Toullec et al., 1979) and splanchnic (Durand and Bauchart, 1986) blood metabolites, net hepatic fluxes (Durand et al., 1984, 1990; Houlier et al., 1991; Chilliard et al., 1994) and insulinemia (Grizard et al., 1982), results obtained here will then be preferentially compared with those reported in the above-mentioned studies. Each tissue bed will be successively discussed.

Portal-Drained Viscera. The PDV energy expenditure was measured in the same animals by Ortigues et al. (1995b). It increased by 44% on average after the morning meal and remained at that level during the 1 to 8 postprandial hours. After the evening meal, it increased by a further 33% over the 7 h postprandially before decreasing back to initial levels. Net nutrient fluxes across the PDV, however, did not follow similar patterns, and comparisons between net O2 and nutrient fluxes across the PDV raised several assumptions on the metabolic fate of nutrients (oxidation vs. other pathways) over the day.

Net portal appearance of glucose took place in the first 4 to 5 postprandial hours, as in Toullec et al. (1979) and Durand and Bauchart (1986). Present results suggest that the balance between PDV energy requirements and glucose and NEFA availability probably determines the metabolic fate of these metabolites. It may be assumed that in periods of elevated energy requirements and of high glucose availability (as in the 4 to 5 postprandial hours), glucose might be the preferred fuel for oxidation in PDV tissues (e.g., 1 to 4 after the morning meal). If glucose absorption from the gut is high relative to PDV requirements, lactate synthesis and release may
be enhanced (e.g., h 1 to 2 after the morning meal). Indeed lactate is probably derived from glucose metabolism (Kaempf et al., 1988), because lactose is hydrolyzed into glucose and galactose in the gut (Coombe and Smith, 1973). Conversely, when energy requirements of PDV tissues are further enhanced, even in periods of high glucose absorption, a greater proportion of glucose may be directed towards oxidation, at the expense of further lactate release (e.g., h 1 to 2 after the evening meal).

Beyond the first 4 to 5 postprandial hours, Guilloteau et al. (1975) showed that approximately 40% of dietary carbohydrates still remained in the abomasum after the meal. This suggests, in conjunction with the measured net glucose and lactate fluxes, that some glucose was still absorbed from the lumen of the intestines probably until h 7 or h 8 after the morning and evening meals, respectively, and metabolized, thereby contributing to maintaining net lactate release and to covering to some extent gut oxidative requirements.

The end of net portal appearance of glucose is concurrent with decreasing blood glucose concentrations, which in turn should modify the insulin status of the animal. According to Kamalu and Trenkle (1978) and Grizard et al. (1982), blood insulin concentrations changed simultaneously with blood glucose concentrations but with no immediate response in NEFA levels. In the present experiment, changes in blood NEFA concentrations did not always coincide with opposite changes in net portal glucose appearance and glycemia. Indeed, arterial plasma NEFA concentrations followed with a 2-h delay the drop in glycemia that occurred 4 to 5 h after the morning meal. A similar delay was noted after the evening meal whereby NEFA concentrations remained elevated in the first 2 h postprandially. In fact, NEFA levels were more highly correlated to plasma TG levels (r = .60, P < .01) than to glycemia (NS).

Peaks of arterial plasma TG reveal lipid absorption from the PDV after gastric emptying (Durand and Bauchart, 1986). Some lipid absorption occurred in the first 2 h after the meals, then ceased in the 4 subsequent hours in relation with milk curdling in the abomasum and a limited gastric emptying of lipids (Guilloteau et al., 1975; Durand and Bauchart, 1986). Most of lipid absorption took place 7 h onward after the meal, in agreement with Bauchart and Aurousseau (1981), who showed that the fatty acid composition of TG was then close to that of the diet. The two phenomena occurred simultaneously after the evening meal, and elevation of arterial plasma TG concentrations was indeed more pronounced in the evening than in the morning. Although lipid absorption across the gut was obvious from circulating TG levels, this absorption could not be discerned from net PDV fluxes of TG, contrary to the results of Durand et al. (1990), who showed that at peak lipid absorption the portal route contributed approximately 55% to total intestinal TG absorption as indicated by net fluxes of chylomicrons and very-low-density lipoproteins.

The simultaneous increases in circulating levels of NEFA and TG were also observed by Auboiron et al. (1994) in preruminant calves fed a high-fat diet. The lipoprotein lipase that is located on the surface of the capillary endothelium (Bauchart, 1993) is known to respond to the postprandial transport of TG and to hydrolyse plasma TG into NEFA (Olivecrona et al., 1991). In ruminants, a majority of plasma NEFA originates from plasma TG hydrolysis (Pethick and Dunshea, 1993). Sites of TG hydrolysis would include gut, liver, and hindquarters (Bergman et al., 1971). Liberated NEFA could be immediately taken up at those sites, resulting in no measurable net NEFA release by any of the tissue beds studied, as noted here. On the contrary, in the present experiment, a net uptake of NEFA was always measured in the PDV, and this uptake was elevated in periods of high circulating NEFA levels. In sheep, 15% of total gross utilization of NEFA was taking place in the gut (Pethick and Dunshea, 1993). Possible metabolic fates of NEFA in PDV include mesenteric fat deposition, re-esterification and secretion as TG in lymph, or oxidation depending on the insulin status of the animal. When circulating NEFA levels were low, NEFA taken up by PDV might be oriented toward mesenteric fat deposition. On the other hand, in the 4 h around the evening meal and in h 6 to 15 after the evening meal, NEFA uptake probably contributed to TG synthesis and release in lymph (Durand and Bauchart, 1986). Additionally when glucose availability was lowest as in the night period, it may be postulated that a greater proportion of PDV oxidative requirements could be covered by NEFA rather than glucose.

In conclusion, elevated PDV energy expenditure corresponded to periods of glucose and lipid as well as probably amino acid (Houlier et al., 1991) absorption. Additive effects were noted after the evening meal, in response to the delayed lipid absorption. However, no specific cost could be assigned to the different absorption phases, because changes in PDV O2 consumption did not correlate with changes in net nutrient fluxes. Information on net PDV fluxes of other metabolites such as ketone bodies and amino acids, as well as on simultaneous insulin patterns, is obviously lacking in preruminant calves, and further interpretation of the data is not possible.

Liver. Hepatic O2 consumption increased by 20% on average over the 8 h that followed the morning meal, by a further 23% in h 1 to 4 after the evening meal and returned progressively back to initial levels (Ortigues et al., 1995b). Net nutrient fluxes, on the other hand, were not clearly affected by the patterns of portal nutrient flows. Net hepatic glucose release averaged .97 mmol·h−1·kg BW−1, which compares well with the net hepatic glucose release (.5 mmol·h−1·kg BW−1) measured at 7 h postprandially in 45% feed-
restricted calves (Chilliard et al., 1994). It remained stable throughout the day even at night, contrary to Durand and Bauchart (1986), who measured a net hepatic glucose uptake at night. Consequently, hepatic gluconeogenesis seemed to be unresponsive to changes in the supply of dietary glucose or possibly of glucose precursors, such as lactate. Hepatic lactate uptake tended to be lower at night (i.e., in periods of no net portal appearance of lactate) without affecting gluconeogenesis. Lactate is an important precursor of hepatic glucose synthesis. In the present experiment, it could be calculated from a simple carbon balance that over the whole day up to 20% of glucose could be synthesized from lactate. It is likely, however, that the proportions of the different glucose precursors (e.g., galactose [Coombe and Smith, 1973], amino acids, glycogen, glycerol) varied over the day even if the present results could not clarify this point. Similarly, in ruminant sheep, Annison et al. (1963) measured from tracer studies that 14% of the glucose pool was derived from lactate.

Throughout the day, NEFA were taken up by the liver to a much larger extent than in the PDV. This uptake was greater around meal times (i.e., in periods of elevated circulating plasma NEFA levels) except at night, when hepatic uptake of NEFA was low. The night period is generally characterized by elevated plasma NEFA concentrations. Net release of NEFA by any of the different glucose precursors (e.g., glycogen, glucose) seemed to be unresponsive to changes in the supply of dietary glucose or possibly of glucose precursors, such as lactate. Hepatic lactate uptake tended to be lower at night (i.e., in periods of no net portal appearance of lactate) without affecting gluconeogenesis. Lactate is an important precursor of hepatic glucose synthesis. In the present experiment, it could be calculated from a simple carbon balance that over the whole day up to 20% of glucose could be synthesized from lactate. It is likely, however, that the proportions of the different glucose precursors (e.g., galactose [Coombe and Smith, 1973], amino acids, glycogen, glycerol) varied over the day even if the present results could not clarify this point. Similarly, in ruminant sheep, Annison et al. (1963) measured from tracer studies that 14% of the glucose pool was derived from lactate.

In conclusion, the postprandial rises in hepatic energy expenditure were partly used for oxidative purposes. Indeed, as previously discussed, no NEFA release by hindquarters could partly be recycled through the Cori cycle into lactate. Assuming, based on a simple carbon balance, that up to 2 moles of lactate could be released from the metabolism of 1 mole of glucose, it could be calculated that up to 48% of glucose uptake by hindquarters was released as lactate. Net exchanges of glucose and lactate suggested that the Cori cycle was of greater importance than in ruminants (Ortigues et al., 1994). Assuming also that the remaining balance of the glucose taken up is oxidized, it could contribute a maximum of 33% to hindquarters energy expenditure, which is much higher than in ruminants (Jarrett et al., 1976). The remaining energy requirements could partly be covered by the oxidation of fatty acids that are taken up as NEFA or TG by the hindquarters. Indeed, as previously discussed, no NEFA release by the hindquarters was measured during periods of elevated lipolysis (around the evening meal and overnight).

In conclusion, both the energy expenditure and the net nutrient fluxes in the hindquarters remained stable during the day.

**General Conclusions**

Metabolism of PDV tissues was responsive to the kinetics of digestion and absorption of nutrients through the gut, unlike hepatic or hindquarters metabolism, which did not show any clear circadian changes. Generally, glucose metabolism in gut tissues (glycolysis vs. oxidation) probably depended on the supply of glucose to PDV tissues. On the other hand, hepatic gluconeogenesis seemed independent of the patterns of precursor supply, and a large proportion of the splanchnic glucose output was probably recycled via the Cori cycle in carcass tissues. Triglyceride absorption from the gut, noted from arterial concentrations, never seemed to take place via the portal route. Triglyceride absorption was accompanied by an enhanced plasma hydrolysis and elevated circulating NEFA concentrations. Net release of NEFA by any of
the tissue beds studied was never observed, even during periods of body fat mobilization (night period). Overall, changes in tissue energy expenditure could not be clearly related to changes in the net metabolism of specific nutrients.

Implications

The present work is the first to describe circadian changes in net fluxes of major nutrients across three important tissue beds in preruminants and to relate them to tissue energy expenditure. In the portal-drained viscera, the patterns of net nutrient flux are probably responsible for changes with time in the partition of nutrient use through different metabolic pathways, although no clear correlation could be established here. The possibility of reducing gut energy requirements by manipulating nutrient supply needs to be studied. On the other hand, the hepatic and hindquarters metabolism of the nutrients studied seemed little affected by time during the day. Consequently, relationships between nutrient metabolism and hepatic or hindquarters energy expenditure would need to be clarified in order to determine whether nutrient supply to and utilization by carcass tissues of growing veal calves can be improved.

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