

Economics of visceral energy metabolism in ruminants: Toll keeping or internal revenue service?

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ABSTRACT: Measurements across a range of productive states show that the portal-drained viscera and liver, or the total splanchnic tissues, account for 40 to 50% of body oxygen consumption or heat. This high rate of metabolism is in part attributable to high rates of protein turnover and thus, AA utilization, as well as other “service” functions supporting nutrient assimilation and “waste management.” This metabolic intensity and the anatomic position of absorptive and liver tissues have led to the assumption that the tissues that assimilate and process incoming nutrients from the diet exact a toll in payment for their entry. This “toll” is believed to reduce the extent to which absorbed nutrients gain admission to the arterial blood pool and reach “productive” organs such as the mammary gland or skeletal muscle. Measurements of net nutrient flux generally support this concept of splanchnic metabolism “restricting entry” and, thus, dictating supply. On a

net basis the appearance of the major carbon-based nutrients absorbed into the portal vein is typically low compared to their rate of disappearance from the gut lumen. An alternative interpretation is that this low net recovery of absorbed nutrients across splanchnic tissues is attributable to extensive metabolism of nutrients from the arterial pool, which masks true rates of absorption. In this scenario, any tax to support community services is paid using internal funds. Measurements of nutrient kinetics, based on isotopic labeling, support the latter scenario. In the case of the liver, catabolism of AA is driven in part by supply and demand, with overpopulation dealt with by deportation, restructuring, or the metabolic equivalent of cremation. Similarly, relative rates of AA metabolism by the gut and mammary gland vary with requirement. Metabolism of many energy-yielding nutrients varies with supply, demand, and the need for community services such as waste management.

Key Words: Splanchnic Metabolism, Energy, Amino Acids

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Introduction

In balancing energy inputs and outputs, energy expenditure, or heat, represents a substantial component of the energy budget of ruminant livestock. The fraction of total metabolizable energy spent as heat generally ranges from 1 at maintenance (by definition), to about 0.7 in growing heifers (e.g., Reynolds et al., 1991) and 0.5 to 0.6 in lactating dairy cows (e.g., Andrew et al., 1991). The metabolic sources of this expenditure at both the cellular and organ level have been reviewed (e.g., Webster, 1980; McBride and Kelly, 1990) and include the maintenance of cell structure and functional integrity through processes such as ion transport and protein turnover. Because protein synthesis and degradation

are energetically expensive processes, tissues with high rates of protein turnover typically have a high rate of energy expenditure and AA requirement, as well as a high rate of blood flow to provide oxygen and remove carbon dioxide. High rates of metabolism typify most of the visceral tissues, such as the heart, kidney, gastrointestinal tract, and liver, whose many support and service functions contribute substantially to their high rate of metabolism. The kidneys receive more blood flow per unit mass than any other tissue in the ruminant (Hales, 1973), but in this case, a high rate of blood flow is in part attributable to their function in the excretion of waste products and the maintenance of fluid balance, thus, their fractional extraction of oxygen is lower than for tissues such as the liver (Reynolds et al., 1991). In growing heifers, the kidneys accounted for roughly 6% of body oxygen consumption, but their blood flow and oxygen consumption were relatively constant across two diet forage:concentrate ratios and two levels of intake, suggesting that their metabolic functions represent a true maintenance cost (Reynolds et al., 1991).

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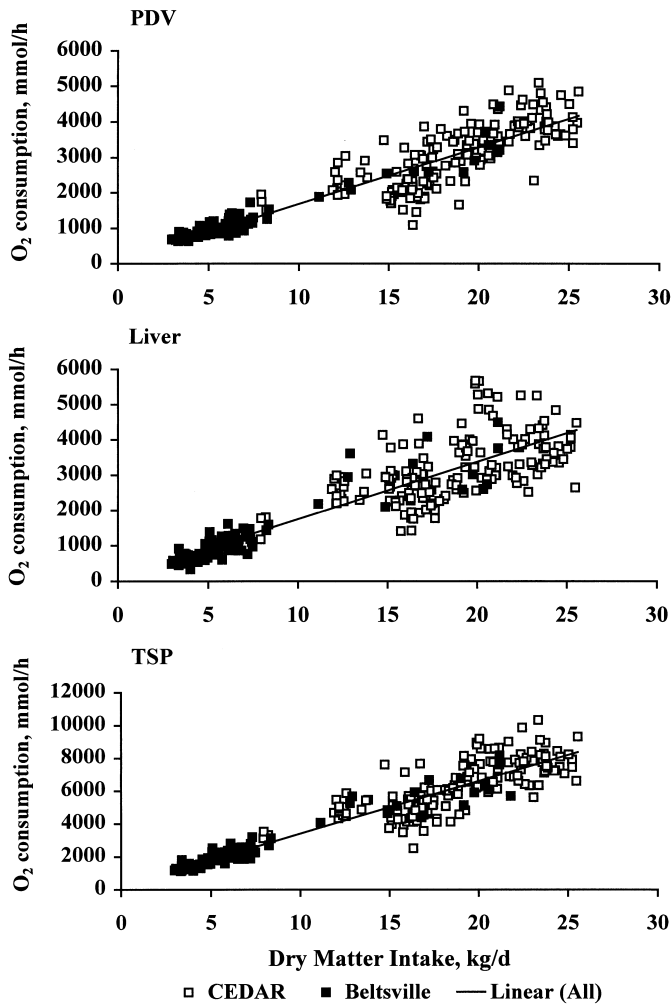


Figure 1. Relationship between dry matter intake and portal-drained viscera (PDV), liver, and total splanchnic (TSP) oxygen consumption in cattle. See text for references.

Splanchnic Metabolism

In contrast to the kidneys, the portal-drained viscera (**PDV**; gastrointestinal tract, pancreas, spleen, and associated omental and mesenteric fat) and liver (together the total splanchnic tissues) account for substantial portions of body oxygen consumption and their metabolism varies with intake. Across a variety of experimental conditions, the total splanchnic tissues account for from 36 to 54% of body oxygen use in growing and lactating cattle (e.g., Reynolds, 1995). This high rate of metabolism is in part dictated by DM and metabolizable energy intake (Reynolds, 1995), which, to a large extent, determines the mass and functional load of the component splanchnic tissues. Across a series of studies in growing and lactating cattle at USDA in Beltsville (Reynolds, 1995), and more recent studies in lactating and nonlactating dairy cattle at the author's current location (Reynolds et al., 2000; Benson et al., 2002; Caton et al., 2001), total splanchnic oxygen use increased with increasing DMI (Figure 1). More variation

in splanchnic oxygen consumption was observed in studies conducted in dairy cattle at higher intakes, in part due to greater variety in diet composition, but also due to differences in productive state. In this regard, the energy expenditure of the liver responds to nutrient requirements as well as DMI (Reynolds, 1995), and the relationship between DMI and oxygen consumption in dairy cows (Figure 1) was more variable for the liver ($r^2 = 0.33$) than for the PDV ($r^2 = 0.59$). Because the metabolism of the splanchnic tissues is determined by DMI, they also account for a substantial portion of body heat increment. In growing beef heifers fed ground corn- or alfalfa-based diets at two equalized metabolizable energy intakes, the total splanchnic tissues accounted for 44 and 72% of the incremental increase in body oxygen consumption for the two diets, respectively (Reynolds et al., 1991).

The intense metabolism of the PDV and liver is driven largely by their roles in nutrient assimilation and waste management (McBride and Kelly, 1990). Major expenditures of the PDV include the community services these tissues provide in terms of diet digestion, nutrient absorption and metabolism, maintenance of gut epithelial structure and immune functions, and synthetic processes within the pancreas and spleen. The synthesis of numerous compounds and its role in waste and toxin management dictate the liver's metabolic activity. For both the liver and PDV, cell maintenance and nutrient transfer require substantial levels of ion transport. All of these, and other community services, exact a substantial tax in terms of energy expenditure, which is paid through the metabolism of oxidizable substrates. For the ruminant, these taxes are paid largely through the metabolism of acetate, glucose, β -OH-butyrate (**BOHB**), and long-chain fatty acids, which account for much of body carbon dioxide production (Annison and Bryden, 1999). In addition, many nonessential AA are important sources of oxidizable carbon for specific tissues, such as the small intestinal enterocytes (Windmueller and Spaeth, 1980), and provide ATP through specialized interorgan carbon shuttles (Stoll et al., 1999). In addition, all AA are subject to catabolism and oxidation, particularly when absorbed and available in excess of requirement (Elwyn, 1970; Waterlow, 1999).

Because most nutrients are absorbed via gut epithelial cells and enterocytes, they are available for metabolism during absorption. In addition, as a consequence of the anatomical position of the PDV and liver as a portal vascular system, all nutrients absorbed into portal vein blood must "run the gauntlet" of the liver before reaching the vena cava. These anatomical considerations and the intense metabolic activity required to support the community services of the splanchnic tissues has led to the logical assumption that substantial portions of the nutrients absorbed from the gut lumen are metabolized during their absorption and transfer to arterial blood. By analogy, the absorptive tissues of the PDV and subsequently the liver are perceived as exacting a toll in payment for nutrient entry, acting as a

gatekeeper and restricting entry of absorbed nutrients, thereby dictating nutrient availability in arterial blood and, thus, supply to tissues such as the mammary gland or muscle.

Measurement of Splanchnic Metabolism

This concept of metabolic “toll-keeping” is supported by comparisons of net nutrient release by the PDV with estimates or measurements of rate of absorption of those nutrients from the lumen of the digestive tract. With the exception of long-chain fatty acids absorbed into lymph, measurements of blood-borne nutrient absorption and metabolism by the PDV and liver can be obtained using multicatheterization procedures. These procedures enable simultaneous sampling of arterial and appropriate venous blood draining the liver and PDV, or sections of the PDV, and measurement of blood flow. Net rates of nutrient removal or release by the tissues of interest can then be calculated as the product of blood flow and venous-arterial difference (Huntington et al., 1989). However, these measurements represent net flux, which equals the combined rates of nutrient release into venous blood and removal from arterial blood. For example, net PDV flux of glucose in ruminants is often zero or slightly negative. This does not necessarily mean that there is no absorption of glucose, but that the rate of glucose release from the small intestinal enterocytes into mesenteric vein blood is equal to or less than the rate of removal of glucose from arterial blood by PDV tissues. The PDV represents an anatomical and vascular aggregation of heterogeneous tissue types. In the case of glucose and AA, whereas some absorptive metabolism may occur in the small intestinal enterocytes, those cells represent a small proportion of the total PDV. Many other PDV tissues such as the rumen and hindgut epithelium, gut muscle, pancreas, and adipose have substantial glucose and AA requirements. As discussed in more detail later, any use of these nutrients from arterial blood will mask an equivalent release into venous blood.

To obtain measurements of gross or “unidirectional” rates of nutrient removal or release by splanchnic tissues measurements of net flux are combined with isotopic labeling to trace metabolism of a specific nutrient (Bergman, 1975). By labeling the blood pool, usually under steady-state conditions, removal of the labeled nutrient from arterial blood can be measured and, by difference, gross rates of nutrient release calculated. However, for the PDV, these measurements of arterial nutrient extraction do not account for any use or “sequestration” during absorption. To measure this absorptive use during passage from the gut lumen to venous blood, the labeled nutrient can be introduced into the lumen of the gut and recovery in portal blood measured. However, to obtain true rates of sequestration during “first-pass” absorption, the extraction of labeled nutrient recirculated to the PDV in arterial blood must be accounted for by simultaneously enriching the blood

pool using a separate label. The concepts underlying this methodology are illustrated in Figure 2 for measurements of arterial and absorptive use of Leu and Phe by the PDV of dairy cows (Reynolds et al., 2001; Caton et al., 2001) using approaches based on a model developed in sheep (MacRae et al., 1997a; Yu et al., 2000). Alternatively, absorptive use of nutrients can be estimated by comparing known or estimated rates of absorption from the lumen of the gut, with gross release by the PDV, or in the case of glucose and AA, the mesenteric-drained viscera (MDV). Depending on the site of sampling relative to the confluence of the ileocecal vein, the MDV represents the small intestine and associated mesenteric fat, with or without the large intestine and cecum (Reynolds and Huntington, 1988a; MacRae et al., 1997b).

Volatile Fatty Acid Metabolism

In ruminants, the VFA represent the principal form of energy assimilation, accounting for roughly two-thirds of digestible energy absorption (Sutton, 1985) and representing an important source of carbon for oxidation and fat synthesis. However, *in vitro* studies (Stevens and Stettler, 1966) and pioneering *in vivo* measurements of Bergman and Wolff (1971) suggested substantial losses of the principal VFA acetate, propionate, and n-butyrate during their absorption across rumen epithelial tissue. Using multicatheterization procedures, Bergman and Wolff (1971) measured net flux of VFA in sheep fed maintenance levels of dehydrated alfalfa pellets at Cornell University. Because arterial concentrations of propionate and n-butyrate were very low, they assumed little arterial use of these VFA by the PDV and that net flux equated with gross release. Arterial concentrations of acetate are substantial, therefore removal of arterial acetate by the PDV was measured using ^{14}C -acetate extraction, enabling the calculation of gross acetate release into the portal vein. These measurements of PDV release of VFA were then compared to previously published measurements of ruminal VFA production obtained by isotope dilution in sheep fed similar amounts of dehydrated lucerne at the Rowett Research Institute in Scotland (Bergman et al., 1965). Assuming that these measurements of ruminal production equated with amounts truly absorbed, they estimated that 30, 50, and 92% of acetate, propionate, and n-butyrate are subjected to “first-pass” absorptive metabolism and never reach venous blood. On a net basis, PDV release of acetate accounted for only 50% of ruminal acetate production. These extensive proportions of “absorptive use” of VFA by the rumen have since become dogma. Other measurements of net increases in PDV release of VFA during ruminal infusions into fed cattle (Huntington et al., 1983; Krehbiel et al., 1992) and sheep (Weekes and Webster, 1975; Kristensen et al., 2000b,c), or sheep maintained totally by intragastric infusions (Gross et al., 1990a,b), have supported the concept of extensive absorptive use of VFA. However,

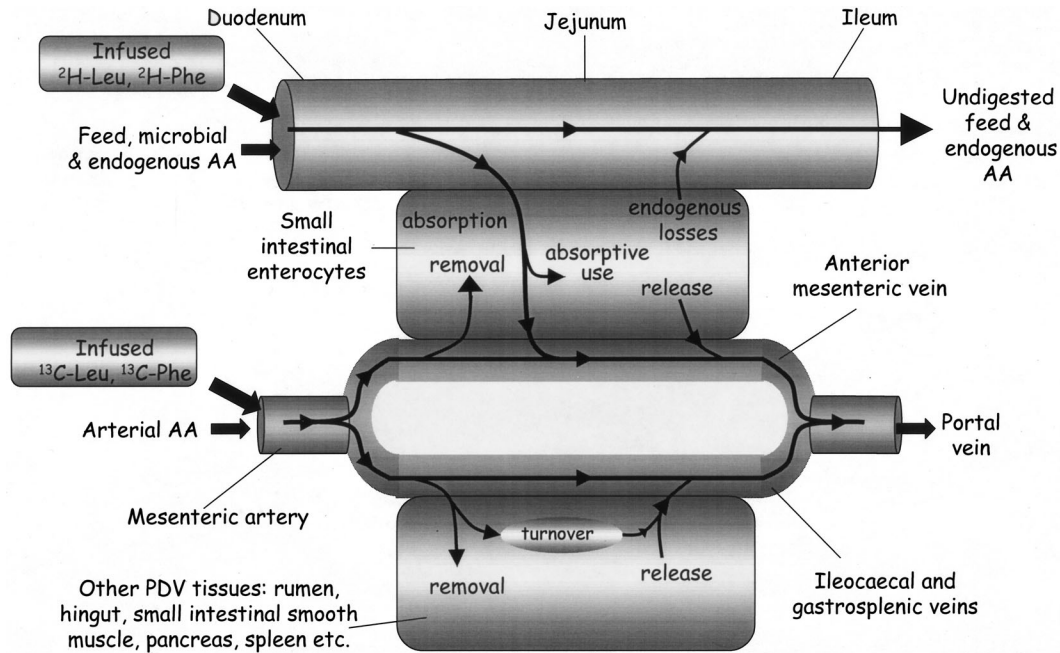


Figure 2. Flow of leucine and phenylalanine within the portal-drained viscera as measured using multicatheterization, intestinal cannulation and dual isotope labelling procedures (Caton et al, 2001; Reynolds et al., 2001) based on the approach of MacRae et al. (1997a).

proportions of absorptive VFA use vary across studies, presumably due to differences in basal diet, level of feeding, and the composition and amount of VFA infused. On a net basis, increased net PDV release accounts for 70 to 47% of acetate, 85 to 60% of propionate, and 32 to 20% of n-butyrate infused into the rumen. A logical explanation for these shortfalls is that, as suggested by the comparisons of Bergman and Wolff (1971), absorptive loss occurs as a consequence of extensive metabolic “toll-keeping.”

More recently an alternative interpretation has developed. Like Bergman and Wolff (1971), Kristensen et al. (2000a) used multicatheterization techniques and isotopically labeled acetate to measure gross PDV release of VFA in sheep, but a washed-rumen approach was used to simultaneously measure VFA absorption from the rumen. Gross PDV release of acetate, propionate, and n-butyrate account for 109, 95, and 23% of their absorption from the rumen, suggesting considerably less absorptive use of acetate and propionate than observed previously. The PDV still metabolized considerable amounts of arterial acetate, but there was very little “first-pass” absorptive use of acetate and propionate, suggesting that measurements of ruminal production by isotope dilution may include a substantial amount of microbial utilization. Microbial utilization may also be a consideration when VFA are infused into the rumen of fed animals, but there would be little microbial activity in a washed rumen. Although a microbial population was maintained in the rumen of sheep maintained totally by intragastric infusion, their

capacity for VFA utilization is uncertain (Gross et al., 1990a,b).

Alimentary Ketogenesis

As in the hindgut of nonruminants, there is extensive absorptive use of n-butyrate in the ruminal epithelium. The fate of this butyrate includes both oxidation and conversion to the ketone bodies, BOHB and acetoacetate, though in vitro studies suggest the predominant (> 90%) fate of n-butyrate in ruminal epithelium is the conversion to ketone bodies (Gross et al., 1990a). β -Hydroxybutyrate represents the principal product of alimentary ketogenesis, generally accounting for 75% or more of total PDV release of ketone bodies (Lomax and Baird, 1983; Heitmann et al., 1987; Reynolds et al., 1992). However, under conditions of increased n-butyrate absorption, less acetoacetate is reduced to BOHB and as much as 55% of total alimentary ketogenesis in the PDV is accounted for by acetoacetate (Krehbiel et al., 1992). Other ketogenic VFA in epithelium include i-butyrate and n-valerate, and recent evidence confirms there is also considerable in vivo metabolism of these longer-chain VFA during their absorption (Kristensen et al., 2000b,c). Based largely on in vitro evidence, acetate metabolized during absorption has historically been considered ketogenic in ruminal epithelium in vivo, but the observations of Kristensen et al. (2000a) challenge that assumption.

On a net basis, estimates of the maximal amount of n-butyrate utilized during absorption and subsequently released into the portal vein as BOHB or BOHB plus

acetoacetate range from 14 to 48% in sheep (Gross et al., 1990a,b; Kristensen et al., 2000c) and cattle (Krehbiel et al., 1992). Like acetate, the ketone bodies represent an important source of oxidizable substrate in arterial blood, so measurements of net PDV release underestimate their gross release to the extent of their use from arterial supply. Recent measurements in multicatheterized sheep obtained using ^{13}C -BOHB indicate that arterial BOHB use by the PDV was substantial, equaling net PDV release, and accounting for 40% of body irreversible loss rate (ILR; Kristensen et al., 2000c). The effect of ruminal n-butyrate infusion on BOHB metabolism was also measured. Release of BOHB by the PDV accounted for 48 and 62% on a net and gross basis, respectively, of the infused n-butyrate not recovered in the portal vein. Presumably, conversion to acetoacetate, oxidation during absorption, and microbial utilization account for the remaining 38% of ruminally infused n-butyrate.

Based on recent evidence, it appears that the absorptive use of acetate and propionate is less than suggested previously. The ketogenic VFA are subjected to considerable absorptive metabolism, but this largely represents a repackaging as ketone bodies that are subsequently released into venous blood and transferred to the hepatic circulation. The low net recovery of absorbed acetate and BOHB from metabolized n-butyrate across the PDV is in part a consequence of the use of these substrates from arterial blood, which negates substantial amounts of gross release. In the PDV and other body tissues, acetate and BOHB account for substantial amounts of carbon dioxide production and fatty acid synthesis (Annison and Bryden, 1999).

Glucose Metabolism

Previous estimates of the recovery of starch digested in the small intestine as increased net PDV glucose release also indicate a considerable shortfall, suggesting substantial absorptive use of glucose for metabolic fates such as oxidation, support of fat synthesis, mucin synthesis, and glycolysis to lactate and glycerol-3-phosphate. In ruminants, in which long-chain fatty acids are largely absorbed as NEFA, glycerol is required for triglyceride and subsequently chylomicron synthesis and long-chain fatty acid absorption into lymph (Harrison and Leat, 1975). However, this potential loss of absorbed glucose does not entirely explain the shortfall in net PDV glucose release in ruminants, because similar shortfalls occur in pigs (Rerat et al., 1988), in which long-chain fatty acids are absorbed primarily as acyl-glycerides. In cattle, the net recovery of starch infused into the abomasum as increased net PDV glucose release has ranged from 25 to 51% (Huntington and Reynolds, 1986; Reynolds et al., 1998a,b; Harmon et al., 2001). This low recovery in part reflects incomplete digestion in the small intestine, but in studies in which ileal flow of starch has been accounted for, the shortfall is still substantial (Kreikemeier et al., 1991; Kreike-

meier and Harmon, 1995). Other explanations for the low recovery include fermentation of starch in the ileum or use of arterial glucose by the PDV. As for acetate and BOHB, use of arterial glucose by the PDV is considerable and can account for 20 to 43% of body IRL (Bergman, 1975; Piccioli Cappelli, 1997; Harmon et al., 2001). This use in part reflects use by adipose tissue, as well as the stomach tissues. In beef steers fed lucerne, the MDV utilized acetate, BOHB, and glucose on a net basis, and net MDV use of glucose was equal to 69% of total net PDV glucose use (Reynolds and Huntington, 1988a,b). When the steers were changed to a diet containing large amounts of cornstarch, a switch to net MDV glucose release was measured. However, the increase in net MDV glucose absorption measured was in part countered by an increase in net stomach use, which may in part reflect increased glucose use by omental adipose drained by the gastrosplenic vein.

More recent studies confirm that increased glucose absorption from the PDV, resulting from postruminal infusion of starch or glucose, is accompanied by increased PDV use of arterial glucose. In a recent study in growing cattle (Harmon et al., 2001), increased net PDV release of glucose accounted for 51% of the starch equivalents in starch hydrolyzate infused into the abomasum. This low net recovery was in part due to a 132% increase in PDV use of arterial glucose measured using ^{14}C -glucose. The increase in PDV use of arterial glucose accounted for 20% of the glucose in starch infused and 52% of the increase in body IRL of glucose. Correction for the PDV use of arterial glucose revealed that 71% of the glucose infused appeared in the portal blood. The remaining 29% was either not digested completely to glucose, was fermented, or was used during absorption. Absorptive use of glucose was not measured in the studies of Harmon et al. (2001), but in young pigs, absorptive use of glucose infused into the duodenum was only 6% (Stoll et al., 1999). In the same pigs, absorptive and arterial glucose use accounted for 6 and 27% of total PDV production of carbon dioxide, respectively.

In contrast to the studies of Harmon et al. (2001), numerical increases (48%) in PDV use of arterial glucose during duodenal glucose infusions in sheep were not significant (Piccioli Capelli et al., 1997). This may relate to previous findings that the net portal recovery of glucose infused into the abomasum is greater than when similar amounts of glucose are infused as starch (Huntington and Reynolds, 1986; Kreikemeier et al., 1991). Possible explanations for a greater portal recovery of glucose compared to starch include differences in regulatory signals arising during the entry of glucose via the duodenum vs the ileum, which have varied effects on the use of glucose in fat and other PDV tissues (Reynolds et al., 1997).

Amino Acid Metabolism

As for VFA and glucose, comparison of net PDV release of AA with amounts absorbed from or infused into

Table 1. Ratio of net portal-drained visceral to net mesenteric-drained visceral release of amino acids (PDV/MDV) in sheep and cattle

Amino acid	Sheep ^a	Dairy cows ^b
Leu	0.64	0.68
Val	0.57	0.46
Lys	0.56	0.72
Thr	0.69	0.38
Ile	0.55	0.61
Phe	0.68	0.76
EAA ^c	—	0.62
NEAA ^d	—	0.50
TAA ^e	—	0.56

^aMacRae et al., 1997b.^bBerthiaume et al., 2001.^cEssential amino acids measured.^dNonessential amino acids measured.^eTotal amino acids measured.

the small intestine of ruminants have suggested that a substantial loss occurs during absorption. Tagari and Bergman (1978) compared rates of disappearance of AA from the small intestine of two sheep, measured using re-entrant cannulas, with simultaneous measurements of their net PDV release and found a considerable (36 to 100%) shortfall in the recovery of many AA (Bergman, 1986). The shortfall was more than 100% for glutamate and aspartate, which undoubtedly relates to their preferential use as energy substrates for intestinal tissue (Windmueller and Spaeth, 1980). In the study of Stoll et al. (1999), absorptive use accounted for 94% of glutamate infused into the duodenum of young pigs, although this measurement was not corrected for any potential use of absorbed glutamate trace recirculated to the PDV in arterial blood. In addition to absorptive use, the large shortfall in glutamate and aspartate absorption in the study of Tagari and Bergman (1978) is also attributable to the hydrolysis of glutamine to glutamate and asparagine to aspartate during preparation of digesta for analysis. In addition, measurements of net AA flow underestimate intestinal disappearance unless endogenous secretions in the ileum are accounted for. Finally, and perhaps most importantly for many AA, PDV use of arterial AA is not accounted for in net flux measurements.

A substantial utilization of arterial AA by the PDV is confirmed by comparison of net measurements of MDV and PDV flux of AA in sheep and cattle (Reynolds et al., 1988a; MacRae et al., 1997b; Berthiaume et al., 2001). For essential AA, the ratio of net PDV release to net MDV release in sheep and cattle ranges from 0.55 to 0.66 (Table 1). The higher net release of these AA by the MDV indicates a substantial use of arterial AA by the stomach and other tissues of the PDV that do not have metabolic access to AA during their absorption into the mesenteric veins (Figure 2). In both sheep (MacRae et al., 1997b) and dairy cattle (Berthiaume et al., 2001), comparison of net rates of small intestinal disappearance and net MDV release of AA suggests very

Table 2. Sequestration of essential amino acids by the portal-drained viscera of sheep (MacRae et al., 1997a)

Amino acid	% of IRL ^a	Arterial/total ^b
Leu	46	0.82
Val	65	0.86
Lys	53	0.84
Thr	48	0.83
Ile	56	0.79
His	32	0.76
Phe	47	0.49

^aTotal portal-drained visceral sequestration as a percentage of total body irreversible loss (IRL). Mean of values for sheep fed 800 or 1,200 g (as-fed basis) alfalfa pellets daily.^bProportion of total PDV sequestration (arterial and absorptive use) accounted for by removal from arterial blood.

little or no absorptive use of most essential AA but confirms substantial net losses of the glutamate-glutamine and aspartate-asparagine pairs and other nonessential AA (Berthiaume et al., 2001). Nitrogen from the catabolism of these and other AA is used in the synthesis of Ala using pyruvate, in part arising from the glycolysis of glucose. Alanine is typically the AA released across the PDV of ruminants in the largest amount on a net basis, transferring nitrogen and carbon to the liver for urea and glucose synthesis (Reynolds et al., 1994a).

Direct measurements of absorptive use of essential AA in sheep have been obtained using a dual isotope method reported by MacRae et al. (1997a; Figure 2). Using simultaneous differential labeling of AA in the small intestine lumen and blood, the absorptive and arterial use of AA by the PDV can be measured as discussed previously. Measurement of essential AA metabolism (Table 2) indicates that utilization by the PDV of sheep accounts for substantial amounts of body IRL, but that the predominant source of the AA utilized is arterial blood. For most of the essential AA measured, arterial use accounted for 75% or more of total PDV use, the only exception being Phe, for which a lower arterial use indicated that total PDV use was equally divided between absorptive and arterial supply. Generally, the rate of absorptive use was comparable for the essential AA, whereas the proportional rate of total PDV use was similar to the proportional composition of PDV proteins. Further use of these methods for measurements of PDV and MDV use of Leu in sheep (Yu et al., 2000) indicate that the MDV accounted for only 12% of arterial Leu use by the PDV. In addition, only 1% of Leu utilized during absorption was oxidized to carbon dioxide, suggesting that most of the Leu sequestered was used for enzyme and constitutive protein synthesis. In contrast, 49% of Leu sequestered from arterial blood by the MDV was oxidized.

A dual isotope approach was also used to describe the effects of intake level on absorptive and arterial Leu and Phe utilization by the PDV of lactating and nonlactating dairy cows (Reynolds et al., 2001). These measurements confirm observations in sheep that arterial use accounts for a substantial portion of total Leu

and Phe absorption and IRL, but the proportion of absorption accounted for was considerably lower when the cows were lactating. As in sheep, absorptive use was a higher proportion of total PDV use for Phe than for Leu. Absorptive use was negligible at low intakes but increased by greater intake within both the nonlactating and lactating state, suggesting an increase in enterocyte mass, or perhaps differences in the absorptive use of these AA caused by increasing supply relative to requirement.

Using the same approach, measurements of PDV metabolism of Leu and Phe, in response to abomasal infusions of casein or an equivalent mixture of free essential AA over a 6-d period, were obtained in late-lactation cows (Caton et al., 2001). Absorptive use of both AA was again negligible during control samplings. Both absorptive and arterial PDV use of Leu and Phe were increased by infusion of the essential AA mixture, but not casein. During free AA infusion the increase in total PDV use was equal to the increase in their true absorption from the lumen of the small intestine. This confirms and explains previous results from studies at this location in lactating dairy cows, in which increases in net PDV release of AA accounted for most (75%) of the AA infused into the abomasum as casein, but only 22% when an equivalent supply of free essential AA was infused (Reynolds et al., 1999). The low recovery in the later case appears due to an increase in both absorptive and arterial essential AA use. Reasons for the response are not certain, but as for glucose metabolism, in response to abomasal starch vs glucose infusion, differences in the metabolic response of the PDV observed may relate to the site of absorption of the AA. Alternatively, the lack of nonessential AA in the mixture of free essential AA supplied may have caused an imbalance in AA availability that stimulated a catabolic response.

Liver Metabolism

Recent studies now suggest alternative explanations for the low net PDV recovery of many nutrients absorbed from the lumen of the ruminant gut. For acetate, propionate, glucose, and most essential AA, there appears to be little absorptive use and their low net recovery appears due to overestimates of their absorption or extensive removal from arterial blood. For the ketogenic VFA, there is extensive absorptive use, but they are essentially repackaged and released into the portal blood as ketone bodies. Nonessential amino acids are also subject to extensive absorptive use and their N used in the synthesis of Ala released by the PDV. Before reaching the peripheral circulation, these absorbed nutrients must negotiate the liver, which is metabolically active in the extreme. Representing about 2% of empty BW in dairy cows, the liver receives about 40% of cardiac output and is responsible for 20 to 25% of body energy expenditure in cattle (Reynolds, 1995). General aspects of liver nutrient metabolism, in relation to amounts absorbed across the PDV, have been addressed

in numerous previous reviews (e.g., Elwyn, 1970; Reynolds, 1995; Annison and Bryden, 1999). General relationships between net PDV release and liver removal of major nutrients are shown for lactating dairy cattle in Table 3. On a net basis, there is normally little liver removal of acetate, and in most studies in ruminants, a small net release of acetate is reported. Thus, the liver has little impact on the supply of absorbed acetate to the rest of the body. In contrast, nearly all the propionate, n-butyrate, and longer-chain VFA absorbed are removed, such that their supply in arterial blood is negligible. Similarly, on a net basis, virtually all acetoacetate released by the PDV is removed by the liver (Reynolds et al., 1992; Krehbiel et al., 1992). n-Butyrate and acetoacetate are largely converted to BOHB in the liver, which is also produced from the oxidation of long-chain fatty acids. Like acetate, there is normally a substantial supply of BOHB released to the periphery on a net basis (Table 3), providing substrate for energy expenditure and fat synthesis.

Most propionate removed by the liver is repackaged as glucose, which is also released to the periphery for productive and maintenance functions (Table 3). In the fed state, the other principal precursors for glucose synthesis include lactate and AA, with the glucogenic nonessential AA considered the AA of greatest importance for glucose synthesis. Ruminant nutritionists have often questioned the extent to which glucose precursor supply limits liver glucose synthesis. However, across a number of studies in which propionate or nonessential AA have been infused into fed ruminants to mimic an increase in their absorption, liver glucose production has been unchanged and liver lactate removal reduced (see Reynolds, 1995). However, in early-lactation dairy cows (Table 3) abomasal infusion of casein for 6 d increased liver glucose production, but infusion of a mixture of free AA supplying the same amount of essential AA evoked a similar increase in liver glucose release (Reynolds et al., 1999). This suggests the response to casein infusion was not attributable to the nonessential AA infused. Liver glucose production may have been increased through direct effects of essential AA on liver metabolism, or indirectly through an increased glucose requirement in other tissues resulting from the provision of a balanced mixture of essential AA. In this regard, liver urea production was decreased when the essential AA mixture was infused, indicating an improved overall utilization of AA. In contrast, mesenteric vein infusion of a mixture of free nonessential AA equal in composition to milk protein caused a large increase in liver urea production and a decrease in milk yield and protein composition in cows fed a low-protein diet in early lactation (Reynolds et al., 1995). In the same cows, infusions of AA providing the essential or total AA in milk protein increased milk protein content and yield. None of the infusions increased liver glucose production significantly. Although nonessential AA are undoubtedly important carbon sources for liver glucose

Table 3. Net splanchnic (portal-drained viscera and liver) metabolism (mmol/h) of nutrients in lactating dairy cows consuming 23 kg DM and producing 37 kg milk daily during abomasal infusions of water (Reynolds et al., 1999 and unpublished observations)

Item	PDV ^a	Liver	Total splanchnic
Acetate	2,409	452	2,860
Propionate	1,012	-942	70
n-Butyrate	214	-180	34
i-Butyrate	28	-27	1
i-Valerate	48	-47	1
n-Valerate	40	-42	-2
β -OH-Butyrate	210	408	618
L-Lactate	175	-24	151
Glucose	-7	781	775
EAA ^b	268	-21	247
NEAA ^c	363	-224	139
TAA ^d	631	-245	386
Oxygen	-3,897	-3,686	-7,583

^aPortal-drained viscera.

^bEssential amino acids measured.

^cNonessential amino acids measured.

^dTotal amino acids measured.

synthesis, their utilization is dependent on an adequate and balanced supply of essential AA.

As for many tissues of the PDV, the high rate of metabolism of the liver is associated with a high rate of protein turnover, and, thus, the liver has a considerable AA requirement for the synthesis of constitutive and export proteins. In addition, the liver serves a "waste management" role in the management of N balance, removing AA absorbed in excess of requirement and consequently producing urea and oxidizing or repackaging their carbon skeletons (Elwyn, 1970; Waterlow, 1999). For many AA, depending on protein status of the animal, net liver removal represents a substantial portion of amounts absorbed by the PDV on a net basis (Elwyn, 1970; Loblely and Milano, 1997; Loblely et al., 2001). For example, the ratio of net liver removal to net PDV release of individual AA removed by the liver can vary from 0 to over 100%, implying that in many cases only small proportions of the AA absorbed reach the periphery. However, the total liver extraction ratio may range from less than 1 to only 20%. This extraction ratio represents the amount removed as a percentage of the total supply in portal and arterial blood. On a net or gross basis, amounts of AA absorbed are relatively small compared to the total arterial supply to the PDV; therefore, the majority of the AA present in portal vein blood were recirculated to the PDV and liver in arterial blood. Thus, whereas the net amount of an AA such as Ala removed by the liver may be equal to or greater than the amount absorbed, only a small fraction of absorbed Ala is removed in the "first-pass" through the liver and the amount removed is highly correlated with arterial concentration. After a 24-h mesenteric vein infusion mimicking a doubling of net Ala absorption across the PDV, the increase in net liver removal of Ala equaled 82% of the Ala infused (Reynolds

and Tyrrell, 1991). This increase in Ala removal was accompanied by a doubling of arterial Ala concentration, but no change in liver extraction ratio (Reynolds et al., 1994b). The response to short-term supplementation of AA may not reflect the longer-term response, because the urea cycle can take days to adapt to alterations in nitrogen input or output (Waterlow, 1999). In lactating dairy cows, abomasal infusion of casein for 6 d increased liver removal of most of the AA measured and for many AA the increase in liver removal was greater than the amount infused, but arterial concentrations of all AA measured were increased (Reynolds et al., 1999). In this case, both the ratio of net liver removal to net PDV release and total liver extraction ratio were increased for most AA measured by providing supplemental AA to the blood pool in excess of requirement (Table 4).

Tissue Supply vs Requirement

In the liver and other body tissues, amount and pattern of supply relative to requirements determine the partition of AA utilization between synthetic and catabolic processes. In lactating dairy cows fed three levels of rumen-protected soybean meal (Metcalf et al., 1996), increasing protein supply increased arterial concentration of AA linearly, but milk protein yield and net mammary gland removal of essential AA responded curvilinearly, reaching a plateau at the intermediate level of protein fed. Milk protein response, and thus removal of AA by the mammary gland, was not determined solely by AA supply. In addition, the utilization of AA within the mammary gland also varies with supply relative to requirement. This is illustrated by the response of mammary gland Leu oxidation in goats. As a fraction of gross Leu removal, mammary Leu oxidation was in-

Table 4. Arterial concentration (μM) and net liver removal of amino acids as a percentage of net PDV release or total supply in early-lactation dairy cows (Reynolds et al., 1999) after 6-d abomasal infusion of water or 800 g casein protein/d

Amino acid	Arterial concentration		Net liver removal			
	Water	Casein	% of PDV		% of Supply	
			Water	Casein	Water	Casein
Ala	235	247	62	98	10	20
Lys	94	116	30	62	10	21
Met	15	21	42	69	11	20
Phe	51	58	39	76	5	12
EAA ^a	1,132	1,443	-21 ^d	41	-2 ^d	5
NEAA ^b	1,166	1,315	51	87	7	15
TAA ^c	2,298	2,758	22	68	3	10

^aEssential amino acids measured.

^bNonessential amino acids measured.

^cTotal amino acids measured.

^dNegative value reflects net liver release of total EAA as a consequence of the net release of branched-chain AA.

creased when diet protein level was increased, and decreased when a mixture of the essential AA devoid of Leu was infused intravenously, creating a Leu deficiency (Bequette et al., 1996a,b). Nutrient supply to the mammary gland is determined both by blood flow and nutrient concentration, but mammary use of the AA supplied is determined by the propensity of the gland for protein synthesis. Blood flow is determined by the metabolic activity of the gland, but also increases in response to a nutrient deficit. In other work in lactating goats, the creation of a His deficiency increased blood flow to the gland as well as the extraction ratio for His to the extent that mammary vein blood was virtually devoid of His (Bequette et al., 2000).

Conclusions

In spite of their numerous service functions and intense metabolic activity, the tax exacted by the splanchnic tissues of ruminants does not dictate the supply of nutrients to peripheral tissues to the extent suggested by measurements of net nutrient absorption. Measurements of unidirectional metabolism reveal that the nutrient requirements of these tissues are largely met through payments made from internal funds provided by arterial blood. This removal of nutrients from arterial blood masks the true rate of absorption of many nutrients. This is especially true for acetate, BOHB, glucose, and most of the essential AA. The ketogenic VFA and propionate are metabolized during their absorptive passage through gut epithelia and the liver but are essentially repackaged into BOHB or glucose made available to the periphery. Rather than dictating the supply of nutrients to peripheral tissues, like other body tissues, the splanchnic tissues draw on reserves available from the blood pool.

Implications

Appropriate representation of the distribution of nutrients via blood flow and the regulation of blood nutrient concentration is crucial for the development of models of absorbed nutrient utilization. Blood flow, and ultimately nutrient utilization, are determined primarily by the propensity for production and resulting tissue requirements, whereas the metabolic fate of nutrients absorbed is determined by tissue requirement relative to supply from the diet. Therefore, animal-specific indicators of productive capacity are needed to accurately predict absorbed nutrient metabolism.

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