Protein turnover—what does it mean for animal production?

G. E. Lobley

Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK (e-mail: gel@rri.sari.ac.uk). *Received 17 February 2003, accepted 26 May 2003.*

Lobley, G. E. 2003. **Protein turnover—what does it mean for animal production?** Can. J. Anim. Sci. **83**: 327–340. The dynamics of protein turnover confer great advantages for homeothermy, plasticity and metabolic function in mammals. The different roles played by the various organs have led to aspects of protein synthesis and degradation that aid the various functions performed. The so-called "non-productive" organs such as the gastro-intestinal tract and liver produce large quantities of export proteins that perform vital functions. Not all these proteins are recovered, however, and thus function can result in lowered net conversion of plant protein to animal products. The splanchnic tissues also oxidize essential amino acids (AA). For example, the gut catabolizes leucine, lysine and methionine, but not threonine and phenylalanine, as part of a complex interaction between AA supply and tissue metabolic activity. Losses by oxidation and endogenous secretions can markedly alter the pattern of absorbed AA. The fractional rates of extraction of total AA inflow to the liver are low and this allows short-term flexibility in controlling supply to peripheral tissues. Recent evidence suggests that the role of the liver in AA catabolism is more a response to non-use by other tissues rather than an immediate regulation of supply to the periphery. Neither arterial supply of AA nor the rate of transport into peripheral tissues limits protein gain, except when supply is very limited. Rather, control is probably exerted via hormone-nutrient interactions.

Key words: Protein synthesis, amino acid, gastro-intestinal tract, liver, muscle, mammary gland

Lobley, G. E. 2003. **Le taux de renouvellement des protéines—quelle est sa signification en production animale ?** Can. J. Anim. Sci. **83**: 327–340. La dynamique reliée au renouvellement des protéines confère une grande flexibilité aux mammifères pour maintenir leur homéothermie et leurs fonctions métaboliques. Les divers rôles joués par les organes les ont conduit à des niveaux différents de la synthèse et de la dégradation protéique en fonction des rôles métaboliques à accomplir. Les organes communément appelés « non-productifs » tels le système gastro-intestinal et le foie produisent des quantités importantes de protéines exportées qui accomplissent des fonctions vitales. Cependant, la récupération des ces protéines n'est pas complète et ces fonctions peuvent ainsi résulter en une diminution de la conversion nette des protéines végétales en produits animaux. De plus, le tissu splanchnique oxyde aussi les acides aminés (AA) essentiels. Par exemple, le système digestif catabolise la leucine, la lysine et la méthionine, mais non pas la thréonine et la phénylalanine. Les pertes causées par l'oxydation et les sécrétions endogènes peuvent profondément altérer le profil des AA absorbés. Le taux d'extraction hépatique du flux afférent des AA totaux est faible et ceci permet une flexibilité à court-terme pour contrôler l'apport aux tissus périphériques. Des évidence récentes suggèrent que le rôle du foie dans le catabolisme des AA soit plus une réponse à la non-utilisation des autres tissus qu'un régulation immédiate de l'apport pour les tissus périphériques. Ni l'apport artériel en acide aminé ou le taux de transport aux tissus périphériques ne limite le gain protéique, à l'exception d'un apport très limité. Plutôt, le contrôle est probablement exercé via une interaction hormone-nutriment.

Mots clés: Synthèse protéique, acides aminés, système digestif, foie, muscle, glande mammaire.

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A primary aim of animal scientists is to enhance the rate of conversion of plant foodstuffs into saleable animal products. The efficiencies of such conversions into protein products, such as meat, milk, fiber or the fetus, are often low, averaging 45–60% in growing pigs (Lenis et al. 1999), but only 20–30% in growing cattle (Wessels and Titgemeyer 1997) and lactating cows (Moorby and Theobald 1999). So what factors lead to poor conversion efficiencies? Obviously, digestibility is important because not all dietary N is absorbed in either "apparent" or "real" (when endogenous flows are included) terms. Another complication arises from imbalances in the dietary amino acid (AA) supply compared with animal needs. Adjustment of the dietary AA profile forms the basis for the long-established "ideal protein" concept used for nonruminants, based on judicious use of feed ingredients coupled with any necessary supplementation with individual AA, e.g., lysine in the case of pigs offered corn-based rations (Fuller et al. 1989) or methionine for poultry (Jackson and Potter 1987). Such an approach is now recommended for ruminants (National Research Council 2001), with the delay in introduction caused by the challenges of predicting the effects of rumen metabolism on protein (AA) supply coupled with inadequate definition of animal needs. Nonetheless, even when the absolute amounts and the profile of AA supply are optimized,

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Abbreviations: **AA**, amino acid; **BCAA**, branch-chain amino acid; **FSR**, fractional synthesis rate; **GIT**, gastro-intestinal tract; **MDV**, mesenteric-drained viscera; **PDV**, portal-drained viscera

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there is still catabolism (oxidation) of the AA by the animal tissues, with consequent loss of substrate that could otherwise be used to synthesize protein. These losses may be associated with other essential needs of the animal and thus will inevitably "limit" the maximal efficiency that could be achieved. On the other hand, such catabolism may not be obligate and instead may reflect manipulable components of tissue metabolism.

This short review will focus on the latter aspect, the impact of tissue metabolism, and address two questions: "what is the role of whole body and tissue protein turnover?"; and "where in the body, and why, does AA oxidation occur and what regulatory factors may be involved?". The answers to these questions are linked directly to how ingested AA are partitioned between anabolic and catabolic fates.

PROTEIN TURNOVER

Protein "turnover" involves the continual synthesis and breakdown of body proteins. In a growing, or lactating, animal the difference between synthesis and breakdown represents net gain and reflects the partition of AA between anabolic and catabolic fates. So what advantages does protein turnover confer on the animal and what are the metabolic consequences of maintaining such a process?

Energetic Considerations

The survival and evolution of mammals is a consequence of their ability to maintain a near-constant internal temperature. Mechanisms have evolved to generate heat under regulated conditions. Protein turnover, along with many other processes, is one such mechanism and two decades ago it was fashionable to correlate whole body heat production and protein synthesis. Across a wide species range (mice to dairy cows), log-transformed data for adult animals fed to maintenance showed 15–20 MJ of heat produced per kg of protein synthesized (Lobley 1988). This did not represent the actual costs, however, but indicated rather that protein synthesis was one of a number of energy-dependent cellular reactions that, in unison, ensured homeothermy. Indeed, the biochemical stoichiometry of polypeptide synthesis would suggest a minimum cost of 2.8–3.2 MJ kg^{-1}, less than 15% of the correlation value (Lobley 1990). In practice, the few direct measurements of the energy costs of protein turnover have yielded higher values than those predicted theoretically. For example, acute use of protein synthesis inhibitors in chicks in vivo lowered oxygen consumption by more than 30% (Aoyagi et al. 1988). Similar inhibition of protein synthesis in various ovine tissues in vitro reduced energy expenditure by even larger amounts (Early et al. 1988). Such experiments yielded empirical estimates for the cost of protein synthesis of approximately 5.4–7.2 MJ kg^{-1} protein synthesized. Additional, but lower, costs (< 25% of those for protein synthesis) arise from the process of protein degradation (Lobley 1990). Although protein turnover (and thus heat production) does respond to many stimuli (nutrition, physiological state; Lobley 1988) this should be seen a part of the underpinning maintenance of homeothermy and not a mechanism for allowing rapid changes in energy expenditure.

Metabolic Flexibility

So, if contribution to bio-energetics is one function of protein turnover then what are the others? Well, clearly "plasticity" is also vital. Continual, extensive remodeling of tissue proteins to alter tissue structure, metabolism or activity occurs as part of normal physiological mechanisms. For example: bone extension and associated muscle fiber elongation during normal growth; development (and subsequent involution) of the uterus plus fetal growth during pregnancy; development, function and later regression of the mammary gland during the lactation cycle. These are examples of relatively slow, chronic events that, nonetheless, require increases and decreases in both cell number and size within specific tissues and that may require opposing events in other organs. For example, in early lactation, development and function of the mammary gland is supported by diversion of nutrients away from muscle that then enters a net catabolic phase (Bell et al. 2000). All these various changes are aided by the differences in half-life of proteins (Goldberg and St John 1976), with those in more responsive (or malleable) tissues (e.g., digestive tract, liver) being more rapid than those tissues that play a more structural role (e.g., bone collagen). Not all responses take place over weeks or years, however. For example, enzymes secreted into the lumen of the gut to aid digestion are themselves degraded, with their constituent AA reabsorbed and used to synthesize new proteins (Ball 2002). Flexibility is also necessary for unpredicted events, with metabolic contingency plans in place to combat infection or support wound repair and, for these, rapid diversion of body resources is essential. Indeed, even just maintaining the immune system in a state of readiness in order to respond rapidly to a challenge may necessitate extensive nutrient diversion (Klasing and Calvert 1999; Obled et al. 2002; Obled 2003). Similarly, during undernutrition (including famine), mobilization of less important storage proteins (e.g., muscle) can be used to maintain the structure and function of vital organs (e.g., kidneys, liver, the cardiovascular system). Thus the ability of protein metabolism to respond to both "planned" and "unplanned" events is vital to aid animal survival in both normal and challenged environments. One downside is that the AA needs of a particular tissue or system may not match exactly (or even well) those released from other organs (Obled 2002) and, consequently, some become in temporary excess and are oxidized. Thus, metabolic flexibility has a cost.

Magnitude of Protein Turnover

With such a wide range of physiological and non-physiological factors that can affect protein metabolism it is impossible to answer the question "what is the rate of protein turnover?" in a particular species. Nonetheless some idea of *minimum* estimates can be gained from the "speedometer" shown in Fig. 1. Several features are immediately obvious.

First, the rate of protein synthesis greatly exceeds normal protein intake. For example, the daily digestible protein intake of the human is usually 50–100 g d^{-1}, while protein synthesis exceeds 300 g d^{-1} (Clugston and Garlick 1982). Similarly, a dairy cow may consume approximately 2 kg

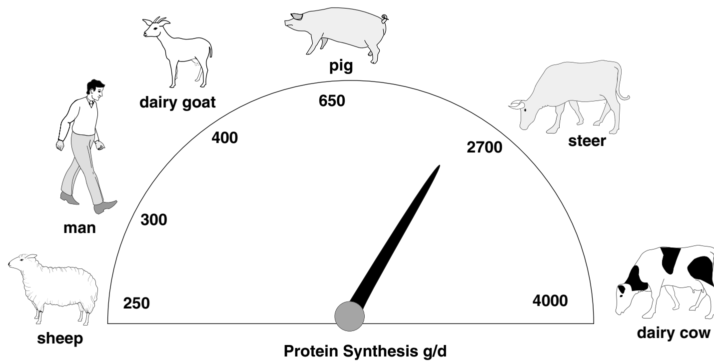

Fig. 1. Rates of whole body protein synthesis in various species at height of productive performance (from Lobley 1998).

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apparent digestible crude protein (N × 6.25) but synthesizes in excess of 4 kg d^{-1} (Bequette et al. 1996b; Lapierre et al. 2002). Corresponding values for the young, rapidly growing pig are 250 g d^{-1} digestible protein intake and daily protein synthesis of 500 g (Reeds et al. 1980). These daily rates of synthesis represent 2–8% of total body protein and so the equivalent of the protein mass of the animal is replaced every 8, 18 and 30 d for young pigs, dairy cows and adult humans, respectively.

Second, the difference between protein retained (or secreted as milk or fibre) and protein synthesis reflects the amount of tissue protein degraded daily. Again, these values for breakdown usually exceed daily protein intake i.e., 300 and 350 g for the human and pig examples and 3.5 kg for the lactating cow. Thus, at least 60% of the body remodeling that occurs involves AA mobilized from tissue, with a smaller contribution from the "new" dietary inputs. This is an important adaptive feature as it maintains concentrations of "free" AA in plasma and tissue fluids within finite limits, even when intake (absorption) is zero.

Third, it then follows that the amount of protein retained, or secreted, is small compared with turnover. The ratio of accretion:synthesis is 30% in rapidly growing pigs (Reeds et al. 1980), 33% in lactating dairy cows (Bequette et al. 1996b; Lapierre et al. 2002), 6% in finishing beef steers (Lobley et al. 1987; Lobley et al. 2000) and zero in "non-productive", weight-stable adults (Clugston and Garlick 1982).

TISSUE PROTEIN SYNTHESIS

With such high rates of synthesis, why is there not more effective conversion of plant nutrients to animal products? In part, the answer lies in how whole body protein synthesis is distributed between the various tissues and organs (Fig. 2). For example, the gastro-intestinal tract (GIT) and liver together comprise approximately 8–14% of body protein mass in both ruminants and non-ruminants (Lobley et al. 1980; Burrin et al. 1990; Seve and Ponter 1997) and make a minor contribution in terms of saleable product. Nonetheless, they account for 25–45% of whole body protein synthesis (Attaix et al. 1988; Lobley 1993; Lobley et al. 1994; Seve and Ponter 1997; Lapierre et al. 2002). Thus, the rate of protein synthesis per 100 g protein [the fractional synthesis rate (FSR)] is much higher for splanchnic organs (e.g., liver, 44% d^{-1} pigs, 22% d^{-1} sheep; duodenum, 117 % d^{-1} pigs, 50% d^{-1} sheep; Seve et al. 1993; Lobley et al. 1994) than peripheral tissues such as muscle (3–12% d^{-1} pigs, 3–4% d^{-1} sheep; Lobley 1993; Seve and Ponter 1997). While the splanchnic tissues also account up to 50% of total oxygen consumption (Reynolds et al. 1991), the relationship between energy expenditure and protein turnover discussed for the whole animal does not appear to hold at the tissue level. The GIT has either a similar [pigs (Ponter et al. 1994)] or higher [sheep (Lobley et al. 1994)] FSR than the liver, but hepatic oxygen consumption (per unit tissue mass) is much greater (Lobley 1991).

For growing animals, muscle has lower rates of protein FSR; 10% young pigs (Ponter et al. 1994), 3% fattening lambs (Lobley et al. 1992) and 2% fattening steers (Lobley et al. 2000). When compared with the corresponding fractional rates of gain (5, 0.9 and 0.3%) this indicates that the rate of protein synthesis per se does not limit lean tissue growth.

For the mammary gland, milk protein synthesis is generally assumed to represent 93% of milk protein synthesis (precasein loses 15 AA residues because of a signal peptide sequence during secretion; Blackburn et al. 1982). In practice, rates of protein synthesis for the whole mammary gland exceed protein output by 25–38% (Bequette et al. 1996b), but how this additional synthesis is partitioned between turnover of milk and constitutive proteins in the udder is unknown. Furthermore, although the mammary gland may contribute 40–42% of whole body protein flux (Bequette et al. 1996b), the gland only consumes 7–14% of whole body oxygen (from Tyrrell et al. 1988; Guinard and Rulquin 1995; Thivierge et al. 2002) so, again, the link between protein turnover and energy expenditure is not strong at the tissue level. This is not too surprising as it would be "unwise", in evolutionary terms, for homeothermic reliance on an organ the metabolic activity of which varies considerably with both stage of lactation and nutrient supply.

Export versus Constitutive Protein Synthesis

So why do tissues have such widely different rates of turnover? At first sight it does not appear to be linked to the protein deposition or secretion. Thus, the ratio of net product formation to protein synthesis appears much greater in peripheral tissues [10–50%, muscle (Ponter et al. 1994; Lobley et al. 2000); 21–28% fiber (Nash et al. 1994; Liu et al. 1998); 70–75%, mammary gland (Bequette et al. 1996b; Thivierge et al. 2002)] than for the liver and GIT, which have high rates of turnover and low rates of net gain.

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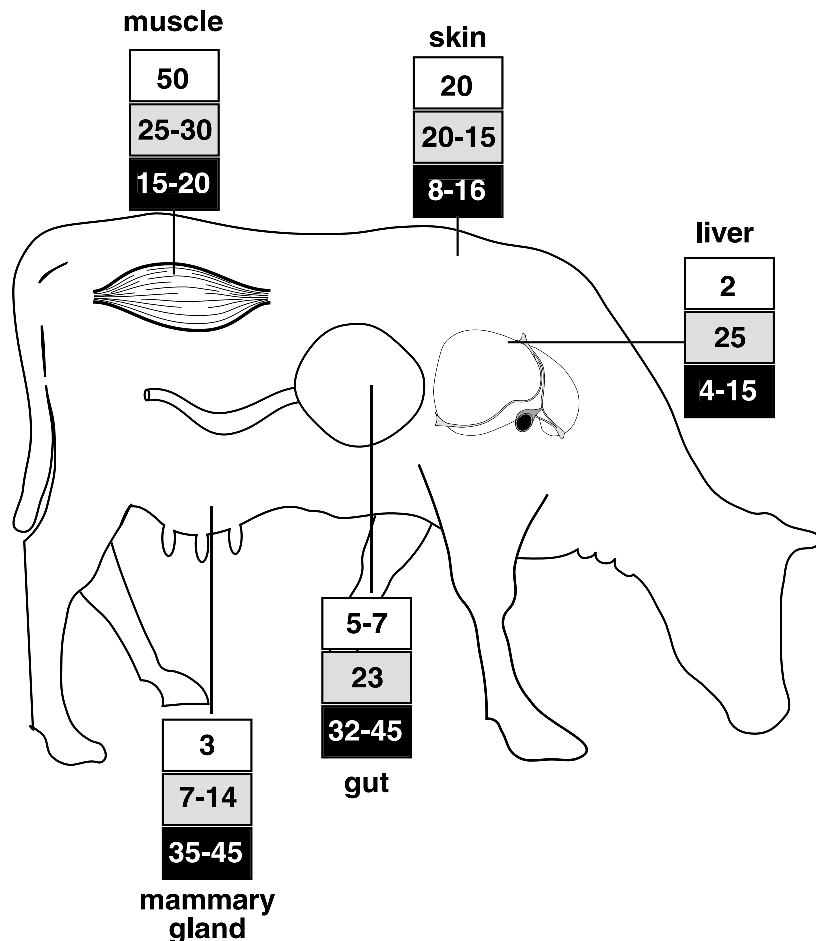

Fig. 2. Percentage contributions of tissue protein mass (□), energy expenditure (■) and protein synthesis (■) to the whole body in steers [data based on Lobley et al. (1980), Lobley (1988), Tyrrell et al. (1988), Reynolds et al. (1991), Guinard and Rulquin (1995) and Thivierge et al. (2002)].

These comparisons are deceptive, however, as both cell replacement and production of export proteins are important "products" of protein metabolism within the splanchnic tissues. In the mucosa of the ovine small intestine, migration of cells from the base to tip of the villi takes approximately 3 d (Attaix and Meslin 1991). Just simply replacing the resultant cellular desquamation would require a FSR of 33% d^{-1} [from (ln2)/half-life], approximately half that measured (Lobley et al. 1994). To this must be added synthesis of secreted proteins, necessary to support digestion and the innate defense system. In total, such endogenous losses account for more than 30% of protein synthesis across the total GIT of dairy cows (Demers et al. 1999; Ouellet et al. 2002). Similarly, upper values for the production of export proteins (e.g., albumin, transferrin, apolipoproteins) as a function of total hepatic protein synthesis are 30–43% in sheep (Connell et al. 1997), with approximately 20% of hepatic phenylalanine removal in dairy cows used to support production of these proteins (Raggio et al. 2002). Thus, both liver and the GIT may not be any less "efficient" than other "export" tissues, such as the mammary gland and skin, but the proteins are secreted and used within the body rather than yielding a product, such as milk or fiber, harvested by humans. Furthermore, the liver export proteins, such as albumin, may be used as anabolic sources by peripheral tissues (Maxwell et al. 1990) while GIT secretions are digested and the AA re-absorbed for further use.

When comparing the two major agricultural products, milk and meat, the gain:synthesis ratio in muscle is much lower than for the mammary gland. Two reasons may account for this. First, extensive selection pressure within the dairy industry has tended to produce animals with high genetic potential and these have been used experimentally (Bequette et al. 1996b; Thivierge et al. 2002). In contrast, studies with ruminant meat animals (both beef and lamb) have either been on moderate quality animals or under limited intakes (Lobley et al. 2000), where the proportion of protein turnover needed to maintain N equilibrium "dilutes" the apparent efficiency ratio. When the incremental efficiency above basal metabolism is examined, however, then the ratio of gain to synthesis increases markedly, even in fattening lambs (25%; Fig. 3). Furthermore, in the pig industry, where strong selection pressure has been applied, muscle protein gain:synthesis can be higher (23–47%; from Seve et al. 1993), particularly in young animals. In general, the efficiency of protein synthesis declines with age (Lobley 1993) and it is a peculiar feature of protein turnover studies that experiments with pigs tend to use younger animals, while those with ruminants have focussed rather on older (fattening) animals. Thus, trans-species comparisons need to

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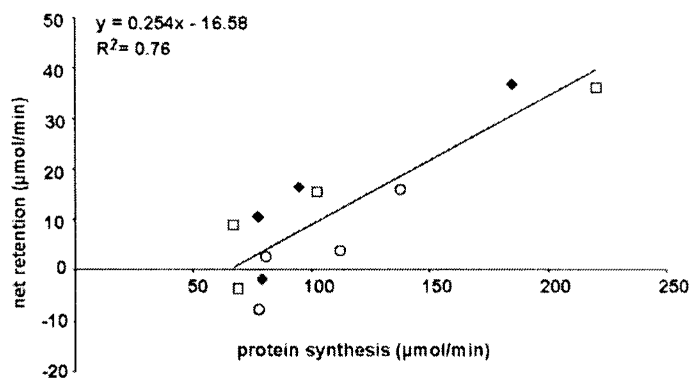

Fig. 3. Response of net retention of AA to changes in protein synthesis across the hind-quarters of sheep as intake is altered from 0.5 maintenance through to 2.5 maintenance. Values are for leucine (◆), threonine (○) and valine (□) [from Hoskin et al. (2001, 2003)].

be carefully matched for both intake and physiological age. The second consideration relates to the difference between export and constitutive proteins. Export proteins, such as fiber or casein, do not undergo intra-cellular protein degradation, except for removal of signal peptides involved in secretion, and, thus, net production is driven by changes in synthesis. For constitutive proteins, including those that comprise muscle, net accretion reflects the balance between synthesis and degradation and thus changes in either, or both, processes can increase (or decrease) gain. Although a number of factors, both nutritional (Seve and Ponter 1997; Lobley 1998) and hormonal (Davis et al. 2003), are known to stimulate protein synthesis in muscle, degradation can also be manipulated. Indeed, the rapid lean growth of certain genotypes (e.g., callipyge) has been linked to suppression of protein breakdown (Lorenzen et al. 2000).

Are there advantages in seeking improvements in lean tissue gain that are independent of protein synthesis changes? The answer is probably a qualified "yes". Increased muscle gain through reduced degradation with unchanged (or even lowered) synthesis would improve the energetic efficiency of growth. In addition, high rates of protein turnover (driven by synthesis) are associated with elevated rates of AA oxidation (Reeds et al. 1980; Lobley et al. 1987; Harris et al. 1992), so increased muscle growth through reduced protein degradation may confer general advantages in efficiency. On the downside, however, most mechanisms that link to protein degradation tend to adjust slowly (hours or days) while control of synthesis can be near instantaneous (Kimball and Jefferson 2002). Thus, an important aspect of metabolic control may be lost. Furthermore, meat tenderness has been linked to the capacity for post-mortem proteolysis and this may be influenced by the rate of turnover (and thus protein degradation) in vivo (Thomson et al. 1997). Thus, reduced protein degradation may lower final meat quality, although this effect appears minimal if moderate rates of gain are achieved in the pre-slaughter period (Lobley et al. 2000).

So maintenance of high rates of protein turnover in animals performs a number of key functions essential to general well-being and the ability to adapt and respond to a number of physiological and environmental challenges. Inevitably, this overall flexibility will involve costs, one of which appears to be oxidation of dietary (and endogenous) AA.

METABOLIC USE OF DIETARY PROTEIN AND AMINO ACIDS

Improving conversion of dietary protein (or other forms of N) into saleable product requires that less digested AA are catabolized with more available for anabolism. So why and where are AA catabolized within the body? There are several reasons as to "why", but these interact, and unraveling them has proved difficult. First, there is obligate catabolism linked to specific non-protein needs e.g., use of glycine for hippurate biosynthesis (Le Floc'h et al. 1995), transmethylation reactions involving methionine (Lobley et al. 1996a), synthesis of neurotransmitters from aromatic AA (Pogson et al. 1989). Second, there are losses associated with increased metabolic function, or rate of turnover, within tissues. Examples here include incomplete re-absorption of GIT desquamations and secretions associated with digestion and absorption of dietary material. To be effective within the lumen, certain of these proteins need to be resistant to degradation and thus increased secretion (e.g., mucins) or stimulation of the defense system (e.g., defensins) leads to net AA losses. The final component involves removal of those AA in excess of the body needs. This may occur either because supply of AA exceeds capacity for net protein gain (limited by genetic potential or restrictions in other dietary components, such as energy) or an imbalanced pattern of AA supply with a required removal of those in excess. The problem of AA imbalance has been largely solved in the pig industry due to the introduction of the "ideal protein" concept and balanced rations, involving inclusion of individual AA (Kim et al. 2001). This is a more daunting task with the complex digestive physiology of the ruminant but, finally, procedures based on a similar concept are slowly being introduced (Rulquin and Vérité 1993; Schwab 1996; National Research Council 2001).

Some of these fates will not be amenable to manipulation, but others may well be. Therefore, the remainder of this review will focus on the impact of tissue metabolism on the fate of AAs and how this may link with protein and AA dynamics. Data will be taken mainly from ruminant sources as other recent reviews have considered non-ruminant metabolism (Seve and Ponter 1997; Fuller and Reeds 1998). The same general principles will apply across all mammalian species, although there will be differences in degree.

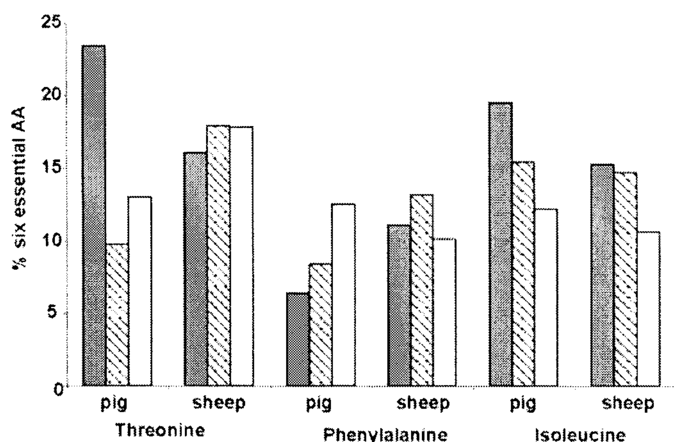

Fig. 4. Individual AA (expressed as a proportion of sum of isoleucine, leucine, lysine, phenylalanine, threonine and valine) in (■) diet (pigs) or small intestine disappearance (sheep); (▧) appearance in portal vein (absorption); and (□) in whole body protein [from van Goudoever et al. (2000), Campbell and Taverner (1988)] and sheep (MacRae et al. 1993, 1997).

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GIT Metabolism

In the pathway from absorption to end product, the first challenge is the GIT, where both non-essential and essential AA can be either catabolized or are needed to support net endogenous secretions and replacement of sloughed cells. The former will be detected as oxidation reactions (Lobley et al.1996b; Reeds et al. 1996; van Goudoever et al. 2000; Lapierre et al. 2002), while the latter contributes to post-ileal N losses (de Lange et al. 1992; Ouellet et al. 2002). If either, or both, of these processes occur, then there will be absolute and, perhaps, relative differences in AA composition between net appearance in the portal vein and either the diet (non-ruminants) or disappearance from the small intestine (ruminants). In pigs, between 5 and 100% of dietary essential AA intake does not reach the liver (van Goudoever et al. 2000; Burrin et al. 2001), indicating extensive and differential use by the GIT. In consequence, the pattern of available AA in the portal vein can be very different from that needed to support peripheral tissue gain (Fig. 4) and this will inevitably reduce the efficiency of dietary protein use. In both sheep and dairy cows, the corresponding losses across the GIT appear to be both quantitatively smaller and the AA pattern is less distorted (MacRae et al. 1997; Berthiaume et al. 2001) with respect to net tissue deposition (Fig. 4).

Non-essential AA Metabolism

There is an obligate need by the mammalian GIT for specific non-essential AA (notably glutamate and glutamine) in both ruminants (Heitmann and Bergman 1978, 1981) and non-ruminants. Indeed oxidation of these two AAs provides 25–47% of the energy requirements of the digestive tract in young pigs (Reeds et al. 1996; Reeds and Burrin 2001) and may help spare glucose oxidation to ensure sufficient remains for absorption and use by glucose-dependent tissues such as brain and erythrocytes (Stoll et al. 1999; van der Schoor et al. 2001) . Metabolism of glutamine is also necessary to provide N to support nucleic acid biosynthesis for cell proliferation and replacement, with 6% directed towards this fate in fasted lambs (Gate et al. 1999; Lobley et al. 2001b). Overall, the metabolic demands of the gastrointestinal tract often result in zero or negative absorption. The latter involves catabolism of all dietary digested glutamine plus oxidation of some released or synthesized by other body tissues (Lobley et al. 2001b; Reeds and Burrin 2001).

Essential Amino Acid Oxidation

While such catabolism of non-essential AA by the GIT will necessitate synthesis elsewhere in the body, with a potential drain on N reserves, nonetheless the most important losses would occur if essential AA were oxidized. A number of studies, based on leucine kinetics, have reported AA oxidation across the GIT, a process sensitive to a range of factors. Thus, in sheep, leucine catabolism decreased both with inclusion of concentrate in the diet (Lobley et al.1996b) and when anti-microbial agents against the GIT microflora were administered (MacRae et al. 1999). In contrast, the presence of intestinal roundworms increased both GIT protein synthesis (by 24%) and leucine oxidation, by 22–41% (Yu et al. 2000). Elevated leucine supply in cattle can also result in increased catabolism across the GIT, even when tissue protein metabolism is unaltered (Lapierre et al. 2002). Conversely, low dietary protein supply reduces leucine oxidation in pigs (van der Schoor et al. 2001).

In such studies, leucine has been used as the tracer AA for a number of reasons, including relative cheapness and the involvement of a simple two-step mechanism to the decarboxylation stage. Unfortunately, leucine, and other branch-chain AA (BCAA), are unique among the essential AA in that the enzymes responsible for their catabolism are distributed across a wide range of tissues e.g., liver, mammary gland, muscle and fat in addition to the GIT (Goodwin et al. 1987; DeSantiago et al. 1998). Such an unusual, widespread distribution then raises the question: does leucine act as a good indicator of GIT oxidation for other essential AA? Recent data from pigs have indicated that lysine can also be oxidized across the GIT (van Goudoever et al. 2000) but threonine cannot (Burrin et al. 2001). As with leucine, catabolism of lysine was sensitive to dietary supply, with near complete suppression at low protein intakes (van Goudoever et al. 2000). Why there is a need to catabolize lysine is unclear. One possibility is that the observed oxidation of enteral tracer might reflect microbial activity in the lumen, as catabolism was observed when labeled lysine was

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Table 1. Oxidation of essential AA across the ovine GIT[z]

	Leucine	Lysine	Methionine	Phenylalanine
GIT oxidation (mmol $^{13}CO_2$/h)	57.2*a*[z]	0.0*c*	–7.6*b*[z]	–2.8*c*
% whole body oxidation	26.5*a*[z]	–1.0*c*	9.9*b*[z]	–4.3*c*
%apparent absorption	36.0*a*[z]	–	15.3*b*[z]	–

[z]Values significantly different from zero.
Values in rows with unlike letters are significantly different ($P < 0.01$).
Data from Lobley et al. (2003).

infused at the duodenum but not when infused into the systemic blood supply (van Goudever et al. 2000). Alternatively, as lysine is a precursor for glutamate, increased oxidation may help meet the extensive needs of the GIT for this AA.

In sheep, there is also no oxidation of systemic lysine by the GIT (Table 1). Similarly, there is no detectable catabolism of systemic phenylalanine, regardless of whether assessed by release of labeled CO_2 or synthesis of tyrosine (the first product of phenylalanine catabolism). In contrast, under the same conditions, there was oxidation of leucine by the GIT; this decreased net absorption of leucine by 26% and represented 26% of whole body leucine catabolism. Interestingly, methionine was also oxidized, although as a lower proportion (10%) of both total catabolism and absorption (Table 1). Again this raises the question: why does the GIT need to oxidize methionine? One possibility relates to trans-methylation reactions, where the methionine:homocysteine substrate cycle provides one-carbon units, via S-adenosylmethionine, for more than 100 metabolic reactions including membrane lipid biosynthesis and DNA base methylation (a vital process to control gene expression in proliferative cells). The methyl groups used for remethylation originate from folate and betaine, but if supply of these becomes limiting then the toxic intermediate homocysteine (Medina et al. 2001) is catabolized to cysteine, taurine and sulfate with loss of the original methionine carbon skeleton. This is an active process in the GIT (Lobley et al. 1996a) and the pancreas (Xue and Snoswell. 1986). Demands for synthesis of cysteine also increase oxidation of methionine (McNabb et al. 1993).

Endogenous Protein Losses

As already mentioned, AA losses across the GIT can also result from incomplete re-absorption of endogenous secretions. The effect of the absolute losses can be magnified if selective individual AA also become non-available. For example, the high content of threonine in mucins (Mukkur et al. 1985) may account for the low absorption of this AA in young pigs (van Goudoever et al. 2000). Indeed, when these pigs were offered a low-protein milk replacer diet there was no net absorption of threonine and the apparent needs of the GIT were much higher than for lysine (405 vs. 174 μmol kg^{-1} h^{-1}), even though body proteins have, on average, a greater molar content (+33%) for lysine (Campbell and Taverner 1988). Net losses of threonine across the GIT were also increased when the dietary protein content was raised and, in the absence of threonine oxidation (Burrin et al. 2001), this must be due to increased endogenous losses. The ruminant data available show considerable variability in net threonine absorption, again suggestive of differences in endogenous protein losses (see Lapierre and Lobley 2001). Demands for other AA may increase during challenges. For example, when the GIT is challenged with pathogens (an almost continuous process), then digestion-resistant defensins, rich in arginine and lysine, are utilized (Yomogida et al. 1996).

It is difficult to assess the overall impact of endogenous secretions and oxidative losses for individual AA, but some indication can be obtained by examination of AA flows in the vasculature of different parts of the GIT. Most absorption occurs from the small intestine into the vessels of the mesenteric-drained viscera (MDV). In contrast, secretions into the forestomachs, hind-gut and from the pancreas are extracted from the non-MDV part of the total portal-drained viscera (PDV) flow. Differences in the net absorption pattern of AA across the MDV and PDV, therefore, will reflect impacts of endogenous secretions and oxidative losses. Earlier data (MacRae et al.1997) suggested little difference in the relative composition of essential AA absorbed across the MDV and PDV, i.e., the AA pattern of endogenous secretions and oxidative losses was similar to the composition of microbial crude protein. This would appear to contradict both the empirical differences in AA losses (van Goudoever et al. 2000; Fig. 4) and data on poor absorption of mucins (Lien et al. 1997) reported for non-ruminants. More recent evidence in dairy cows (Berthiaume et al. 2001) and sheep (Fig. 5), involving the introduction of more precise analysis (Calder et al. 1999), has revealed marked differences in PDV:MDV AA appearance, however. The ratio values for lysine, phenylalanine, methionine and leucine were inversely related to their extent of oxidation in sheep. A low fraction was observed also for threonine, in agreement with probable endogenous losses discussed above. The lowest ratio (and thus the largest "loss") was found for tryptophan and clearly metabolic data on the fate of this AA are urgently needed as this may impact on whether appropriate AA compositions are available to post-GIT tissues.

Overall, the current data would suggest that oxidative losses of essential AA across the GIT are restricted to the branch-chain AA, methionine and enteral lysine. These losses can be influenced by protein intake, AA supply plus both microbial and parasite demand. Greater losses are probably associated with endogenous secretions and this can lead to marked imbalances in the net absorption of individual AAs. The clearest example of this is threonine but a similar situation may also exist for tryptophan. Again, factors that increase endogenous secretions, especially the mucins, will reduce both the pattern and absolute amounts of AA available for tissue anabolism and secretion.

Hepatic Catabolism

The liver is considered a major site of AA catabolism, with the necessary oxidative enzymes present for all essential and non-essential AA. Under steady state conditions, supply beyond the liver will reflect peripheral tissue needs, plus any

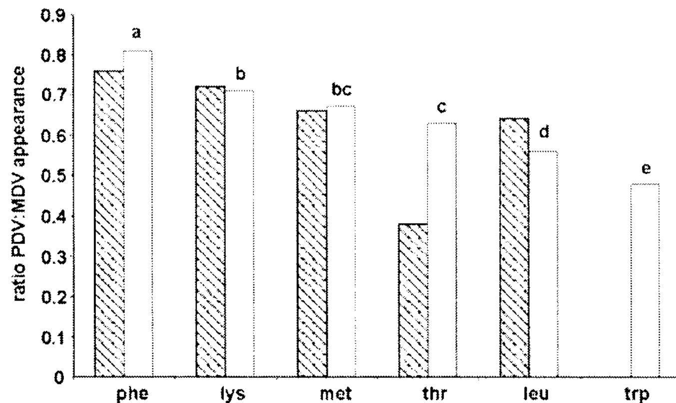

Fig. 5. Ratio of AA appearances across the portal drained viscera (PDV) to that net absorbed across the mesenteric drained viscera (MDV). Data are for dairy cows (▧) [from Berthiaume et al. (2001)] and sheep (□) (Lobley et al. 2003). For the sheep data, columns with unlike letters are significantly different ($P < 0.01$).

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post-hepatic oxidation. For some AA (e.g., phenylalanine, histidine) the liver is either the exclusive (Raiha et al. 1973) or predominant (Hryb and Feigelson 1983; Lichter-Konecki et al. 1999) site and, therefore, post-hepatic supply will approximate to whole body gain, under normal dietary conditions. Many factors can regulate oxidation of individual, or all, AA and any stimulus that improves (reduces) productive N gain or output, but without changing AA absorption, will result de facto in decreased (increased) AA catabolism by the liver. Unfortunately, such observations do not answer the key question: does the liver regulate supply to the peripheral tissues? Is suppression of AA oxidation and ureagenesis an "active" event in control of anabolism or is hepatic oxidation used merely as a "passive" mechanism to remove material either unwanted or unused by other tissues? If oxidation is "actively" controlled then specific signals are required that allow tight integration between peripheral and splanchnic tissues. Furthermore, such signals have to encompass all the main AA, so either separate signals must exist for each AA or there is a common (but not yet identified) process that senses excesses or deficiencies in general. On the other hand "passive" regulation must also have a mechanism by which the liver can recognize and respond to material surplus to anabolic needs. What would be the time-scale of such a mechanism—minutes, hours or days?

These questions may be answered if we reconsider how liver dynamics are usually presented. Traditionally, data for hepatic removal of AA are expressed as a fraction of the amount absorbed and, indeed, such extractions can be high. For example, absorbed histidine and phenylalanine can be totally removed across the liver in non-growing sheep (Lobley and Milano 1997). In practice, however, the liver is presented not with just absorbed AA but a mixture that also includes re-circulated AA (derived from recently absorbed material that bypassed the liver, plus that released by breakdown of tissue proteins). Re-circulation accounts for the largest proportion of hepatic supply, ranging from 72 to 98% in sheep, pigs and cattle (Reynolds et al. 1994; Hanigan et al. 1998; Le Floc'h et al. 1999; Lobley et al. 2001a). Thus, of the total AA inflow to the liver, only 1–20% is extracted per pass (e.g., Reynolds et al. 1994; Wray-Cahen et al. 1997; Lobley et al. 2001a). Furthermore, over a wide range of total inflows, the fractional extraction for most individual AA appears to be constant (Wray-Cahen et al. 1997; Hanigan et al. 1998; Lobley et al. 2001a).

A few AA do not fit the constant extraction pattern but these can be explained by known metabolic phenomena. For example, hepatic detoxification of benzoic acid formed from plant organic acids is vital but requires glycine. This function imposes an initial demand that can result initially in removal of not only the equivalent of the glycine absorbed but also deplete of systemic glycine i.e., there is a negative, post-hepatic glycine supply. Once these detoxification needs are met, however, extraction constancy is re-established (Lobley et al. 2001a). In contrast, the ability of the liver to remove the BCAA is limited and, once exceeded, all additional inputs effectively bypass the liver i.e., the extraction ratio falls to zero (Wray-Cahen et al. 1997; Lobley et al. 2001a).

Active or Passive Control by Liver of Removal of AA

So how does this information help in understanding the role of the liver in partitioning AA to peripheral tissues? First, the observed constancy of fractional extraction during high rates of AA infusion (Wray-Cahen et al. 1997; Lobley et al. 2001a) demonstrates that hepatic transport and catabolism kinetics are not saturated within normal protein intakes, except for the BCAA. This finding will simplify the construction of models of liver metabolism. Second, the low rates of removal compared with flow (substrate supply) through the tissue account for the rise in arterial AA concentrations observed following absorption and allow the majority of absorbed AA to be made available to peripheral tissues. For example, in pregnant, dry cows, the fractional removal of total methionine inflow to the liver is 0.08 (Hanigan et al. 1998). In consequence, any additional methionine absorbed would require nine hepatic passes before 50% is removed and even after a further 15 passes 10% would still be available to the animal for productive purposes. Thus, the majority of any extra methionine absorbed is initially made available to the peripheral tissues but any not used within a finite time-scale (each pass has a 20–30 s duration) will eventually be extracted by the liver. Third, although as discussed earlier, some of the hepatic AA removal forms part of vital processes (e.g., synthesis of plasma proteins), most appear to be extracted as a function of

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Table 2. Relationships between essential AA (g d^{-1}) absorption, post-hepatic supply, mammary gland uptake and use for milk protein output in lactating dairy cows[x]

AA	Histidine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
Absorbed	37	117	108	32	85	63
Post-liver	27	140	111	22	44	57
Mammary gland uptake	23	101	88	24	43	40
Milk protein output	22	82	68	23	42	37
Milk protein: post-liver[y]	0.82[y]	0.58	0.61	1.01[y]	0.94[y]	0.64

[x]From Lapierre et al. (2000), Lobley and Lapierre (2001) and Blouin et al. (2002); composite data from four separate studies.
[y]Values are not significantly different from unity.

AA supply (i.e., blood flow × concentration). In consequence, because plasma concentrations are maintained within finite limits through release of AA from protein breakdown, then complete suppression of hepatic oxidation is an improbable goal.

The data suggest, therefore, that the liver does not appear be an "active" regulator of AA extraction and catabolism but rather "responds" to inflow (Hanigan et al. 1998; Lobley et al. 2001a). This allows integration with the needs of the peripheral tissues but with the role of the liver being to remove AA in excess of use, rather than regulation of supply per se. The effect of this can be seen with recent data that compare absorption, post-hepatic supply, and milk protein output in dairy cows. For three essential AA, methionine, histidine and phenylalanine (plus tyrosine), the ratio of milk AA output to post-hepatic supply was not different from unity (Lapierre et al. 2000; Lobley and Lapierre 2001; Table 2). This indicates that these AA are catabolized mainly by the liver. In contrast, for other essential AA, more of the BCAA, lysine and threonine can pass beyond the liver than were needed to support net requirements for milk protein output. Instead, post-hepatic supply of these latter AA balanced the amounts extracted by the mammary gland (Table 2). Thus, post-hepatic supply and mammary gland total use were again balanced. Nonetheless, under AA-limiting situations, supply to the mammary gland of leucine (Bequette et al.1996a) or lysine (Guinard and Rulquin 1994; Metcalf et al. 1996; Tagari et al. 2000; Thieverge et al. 2002) can become similar to net milk protein needs. Thus, use of these AA by the mammary gland is variable, even when milk output is unchanged, and yet post-hepatic supply matches these needs. Again, this is most easily explained by the liver removing any non-utilized material, rather than regulating absolute supply to the mammary gland.

Such a mechanism also has the advantage of not requiring separate "control" for each AA, but instead removal of "excess" based on total inflow can encompass one, more or all AA. This system would predict that post-hepatic net appearance could be zero, or even negative, if AA are supplied to the mammary gland before being "sensed" by the liver. This is what happens when AA are infused via the jugular vein on top of a basal ration. This caused an increase in milk protein output but post-splanchnic appearance either equalled (histidine, methionine) or exceeded (threonine, phenylalanine, tryptophan) net absorption across the PDV (Berthiaume et al. 2002 and unpublished data).

Together, these various observations support the concept that hepatic extraction of AA has two main components. First, needs for vital hepatic processes (export protein synthesis, production of key metabolites) and, second, removal of AA in excess of the needs of peripheral tissues. The latter appears to be a function of supply (via the portal vein and hepatic artery) and allows a short-term control of supply to peripheral tissues. Furthermore, the immediate needs and metabolic activity of peripheral tissues are probably the largest determinant of how much AA is extracted and catabolized by the liver.

Peripheral Tissues

So what factors regulate utilization of AA by the peripheral tissues? If we assume conditions where no individual AA is limiting then there are three possible constraints. These are: supply, uptake and use.

Efforts to relate anabolic response to blood flow or arterial concentration, i.e., AA supply, have proved unsuccessful (Lescoat et al. 1996). Indeed, supply via the blood is greatly in excess of net gain by both the mammary gland (Bequette et al.1996a,b) and muscle (Biolo et al. 1992) (Fig. 6a). Regulation by transport into the tissues is an interesting concept, especially as there is some evidence that inward transport of glucose may limit mammary gland responses (Cant et al. 1999). If similar constraints existed for AA then this would provide a focus for genetic selection based on increased transporter number or activity. Direct measurement of AA transport is difficult to assess in vivo, except for small laboratory species (Banos et al. 1973; Hundal et al. 1989), but indirect measures, based on kinetic transfers, can be applied to larger mammals (Biolo et al. 1992). This approach has shown that retention by ovine hind-quarter tissue (mainly muscle) is only 9–39% of inward transport (Hoskin et al. 2001, 2003; Fig. 6b). Thus, most of the inward transport must be matched by a corresponding efflux (or counter-transport) from the cell. Even when net retention is negative, as under sub-maintenance intakes (Biolo et al. 1992; Hoskin et al. 2001), both inward (and outward) transport flows are large (Fig. 6b).

Rates of protein synthesis and inward transport are closer, however (Fig. 7). While care has to be exercised that this is not a consequence of the dynamic model used, the data suggest that either AA flow through the cell drives protein synthesis or that inward transport is stimulated by the rate of synthesis. In the latter case, protein synthesis would reduce the concentration of cell free AA slightly and thus create a "gradient" stimulating inward flow.

Although, under most circumstances, AA transport does not constrain net anabolism, this is not always the case.

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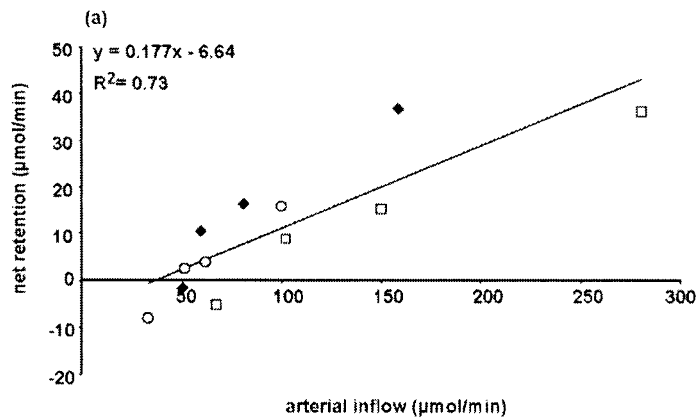

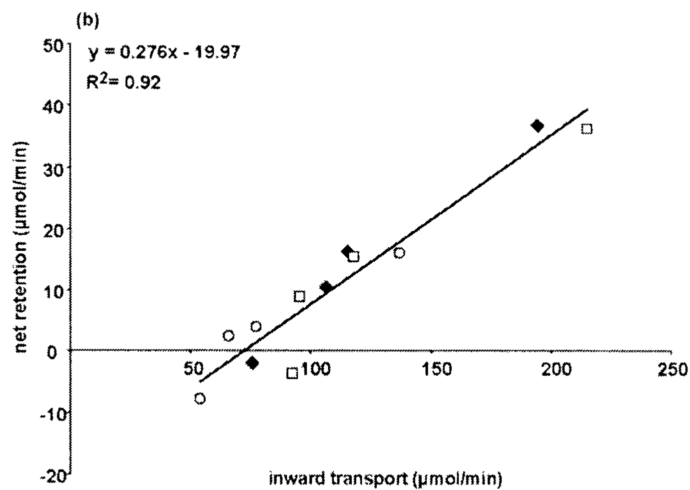

Fig. 6. AA kinetics (◆, leucine; ○ threonine; (□,valine) in response to alteration of intake from 0.5 maintenance through to 2.5 maintenance [from Hoskin et al. (2001, 2003)]. Response in net retention to either (a) altered AA supply via arterial inflow or (b) transport of AA into muscle cells.

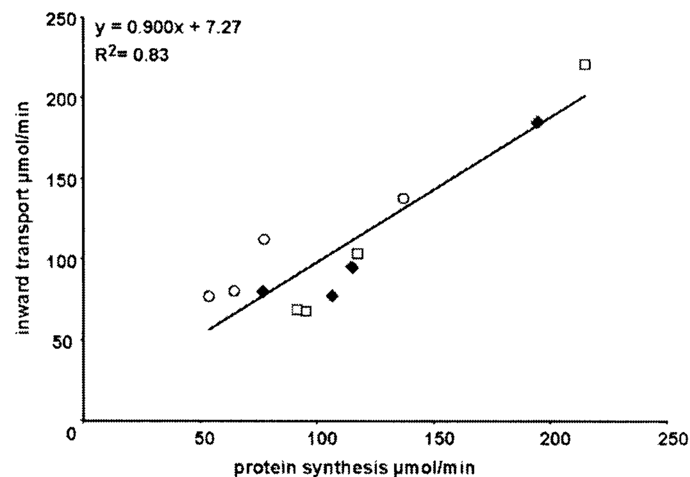

Fig. 7. Relationship between protein synthesis in muscle of the ovine hind-quarters and the rate of transport of AA into the cells. Values from Hoskin et al. (2001, 2003) based on kinetics of (◆) leucine, (○) threonine and (□) valine in response to alteration of intake from 0.5 maintenance through to 2.5 maintenance.

During severe limitation of a single AA, rates of inward transport and retention may be similar. Thus, in lactating goats maintained under leucine- or histidine-deficient conditions most of the arterial supply is extracted (inward transport), with the venous outflow from the udder then nearly devoid of the AA (Bequette et al. 1996a; Bequette et al. 2000). This means also that AA efflux is suppressed and this offers another potential point of control on anabolism. Nonetheless, as neither supply nor inward transport appear to limit anabolism normally, then other regulatory mechanisms must operate. These will certainly include hormones, and both insulin and IGF-1 play important roles

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in regulating lean tissue growth in both ruminants and non-ruminants (Dubreuil et al. 1998; Lobley 1998; Davis et al. 2001). The past few years have seen a marked increase in our understanding of how nutrient-hormone interactions control both synthesis (Yoshizawa et al. 1998; Balage et al. 2001; Kimball and Jefferson 2002) and degradation (Larbaud et al. 2001; Combaret et al. 2001) of proteins. These mechanisms and the implications for tissue anabolism are reviewed elsewhere in this symposium (Davis 2002).

CONCLUSION

The dynamics of protein synthesis and breakdown are vital to maintaining homeothermy, allowing animals to function and respond to normal physiological events and to enable environmental challenges to be met quickly and effectively. As part of normal function, both digestive and defence mechanisms probably incur a penalty and lead to catabolism and loss of protein and AA. These may be reduced, but not eliminated, by good husbandry and the possibility that these processes create demands for specific AA needs to be considered in future ration formulation schemes. Recent evidence would indicate that obligate catabolism of AA by the liver is relatively minor and that hepatic oxidation of AA reflects removal of material surplus to peripheral tissue requirements, rather than regulating supply to productive processes. Research, therefore, should be directed at understanding mechanisms of protein gain at the target tissues. Except under special circumstances, anabolic gain at the peripheral tissues is not constrained by either AA supply or transport into the cells. Instead, responses are regulated by intracellular events that alter the relationship between protein synthesis and degradation. These will probably involve nutrient-hormonal interactions.

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