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E & PMG Workshop

Peptide utilization by tissues: current status and applications of stable isotope procedures

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Classical concepts of protein metabolism in mammalian species consider only the utilization of free amino acids (AA) in blood or plasma as substrates for tissue protein synthesis; the AA being derived from gastrointestinal tract (GIT) absorption and from protein degradation. However, peptides are important products of protein degradation in the intestinal lumen and there have been reports which indicate that some dietary peptides, resistant to lumen digestion and enterocyte hydrolysis, appear intact in plasma (Adibi, 1971; Gardner *et al.* 1991). This has raised questions relating to their metabolism and the potential role of circulating peptides in protein metabolism. Over the last few years considerable evidence that peptides can be used as a source of AA for protein synthesis in humans and other single-stomached animals (Adibi, 1987; Lochs *et al.* 1988; Furst *et al.* 1990) has resulted in the selective clinical use of di- and tripeptides in parenteral nutrition as a means of increasing the solubility and stability of certain AA (Grimble & Silk, 1989).

In ruminants the suggestion that peptides may be involved in protein metabolism has come from a number of observations. For example, only 30–80% of the essential AA disappearing from the intestine of sheep can be accounted for by the net flux of free AA in the portal-drained viscera (PDV; Tagari & Bergman, 1977; MacRae *et al.* 1993). This difference between their disappearance from the small intestine and their appearance in portal blood may reflect gut metabolism of AA. Alternatively, it may indicate a net transfer of AA as protein and/or peptides, a finding which would be consistent with the apparent net flux of peptide-AA (PAA) across the GIT of sheep and steers (Seal & Parker, 1991; Webb *et al.* 1992). Peptide utilization has been demonstrated across the mammary gland of lactating dairy goats (Backwell *et al.* 1994*b*, see p. 458) and across the hindlimb of growing calves (McCormick & Webb, 1982), where there appears to be a net uptake of PAA in the fed state and a net output during fasting, suggesting that the availability and utilization of PAA may change with the nutritional or physiological status of the animal.

While such evidence indicates that peptides may have a role in protein metabolism *in vivo*, a better understanding of this requires consideration of three interrelated questions: first, can tissues utilize peptides as a source of AA for protein synthesis?; second, does this occur *in vivo*?; third, if so, then what are the source(s) of these peptides? The present paper shows how the use of selective ion monitoring of stable isotopically labelled AA is being applied to help answer some of these questions.

CURRENT UNDERSTANDING OF PEPTIDE METABOLISM

Can tissues use peptides as a source of AA for tissue protein synthesis?

In the last 10–20 years there has been a surge of interest in the use of peptides in parenteral nutrition. Tyrosine, cysteine and glutamine, which are relatively insoluble or unstable in free form, are of particular use in peptide form in clinical conditions where these AA are considered to become 'indispensable' for a variety of metabolic reasons (for review, see Furst, 1985; Furst *et al.* 1990). Studies in single-stomached animals have shown that peptides resistant to plasma hydrolysis do not appear in the urine, but disappear rapidly from the circulation after infusion or injection. These studies suggest that they are utilized by tissues (Adibi *et al.* 1977) and that the rate of clearance is affected by molecular structure (Hubl *et al.* 1989). The peptides alanyl-tyrosine and glycyl-tyrosine have been used to supply tyrosine in chronic renal failure (Druml *et al.* 1989), while glutamine, tyrosine and branched-chain AA have been administered in dipeptide form to supply these AA in acute pancreatitis (Steininger *et al.* 1989). Liver (Lochs *et al.* 1986, 1988), kidney (Lochs *et al.* 1988) and skeletal muscle (Roth *et al.* 1988) can all hydrolyse small peptides and, indeed, positive N balance was maintained in baboons (*Papio cynocephalus/anubis*) receiving total parenteral nutrition of a mixture of small peptides only (Steinhardt *et al.* 1984).

More recently, in a series of studies designed to examine the role of peptides in the supply of AA to the mammary gland for milk-protein synthesis, a dual-labelled tracer technique has been developed. This has shown that there is direct incorporation of infused PAA into milk protein by the mammary gland of lactating goats (Backwell *et al.* 1994*b*). Briefly, the technique involved infusion of a dipeptide XY°, where Y° is a ^{13}C-labelled AA, into the external pudic artery (EPA) supplying half the mammary gland, coupled with a simultaneous (jugular) infusion of the same AA, Y*, but labelled with ^{2}H (Fig. 1). Two peptides were used, glycyl-L-[^{13}C]phenylalanine and glycyl-L-[^{13}C]leucine, coupled with simultaneous jugular infusions of L-[ring-^{2}H]phenylalanine and L-[5,5,5-^{2}H]leucine respectively; these latter infusions were to allow correction for systemic peptide hydrolysis and subsequent mammary use of the free AA released. Direct incorporation of ^{13}C-labelled AA derived from the infused peptide resulted in a higher ^{13}C-labelled AA enrichment in casein prepared from the peptide-infused half of the gland (Fig. 2). The ^{13}C:^{2}H values for leucine and phenylalanine in casein secreted from the half of the gland receiving the close-arterial peptide infusion were 10·4 (SE 1·5) and 11·9 (SE 2·1) % higher ($P<0·01$) than those for the non-infused side (Fig. 3). This demonstrates that the higher enrichments were not simply due to a difference in fractional extraction rate of AA between the gland halves and that the mammary gland has the inherent ability to utilize peptide AA for milk-protein synthesis.

While it is obvious that tissues do have the ability to assimilate peptide AA, how this occurs is unclear. Possible mechanisms include uptake of intact peptides by specific transporter systems, followed by intracellular hydrolysis by cytoplasmic peptidases; or hydrolysis by plasma membrane peptidases followed by uptake of AA residues by AA transport systems. Both of these mechanisms appear to operate in the gut (Adibi, 1971; Matthews, 1975) and kidney (Ganapathy & Leibach, 1983), whilst in the liver there appears to be cell surface hydrolysis by membrane peptidases and AA uptake (Lochs *et al.* 1986).

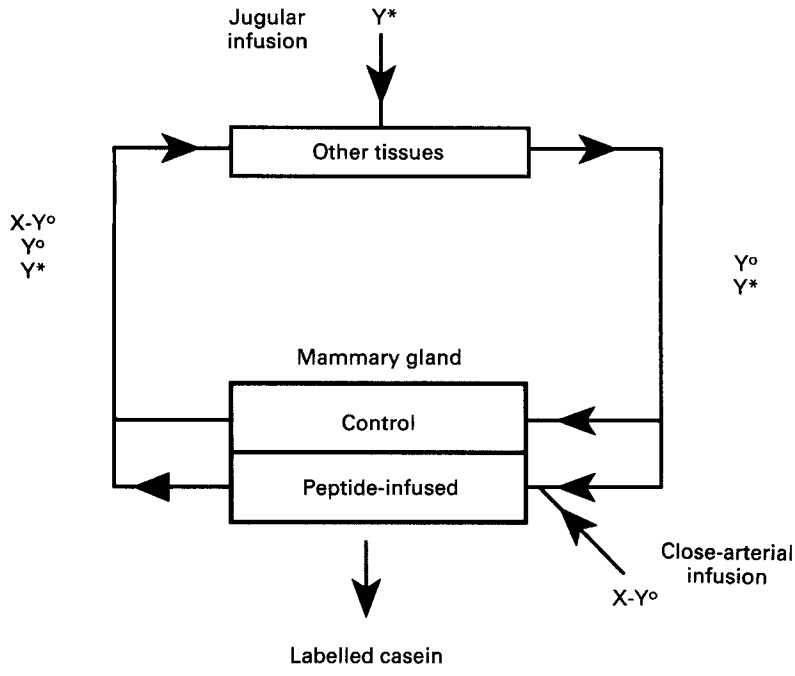

Fig. 1. Diagrammatic representation of a kinetic isotope approach to demonstrate the utilization of peptides by the mammary gland of lactating goats for milk-protein synthesis. The technique involves infusion of peptides, X-Y°, where Y° is a ^{13}C-labelled amino acid (AA), into the external pudic artery supplying half the mammary gland and a simultaneous systemic infusion of AA, Y*, where Y* is a ^{2}H-labelled AA, to allow for whole-body recycling of Y° released on hydrolysis of X-Y°.

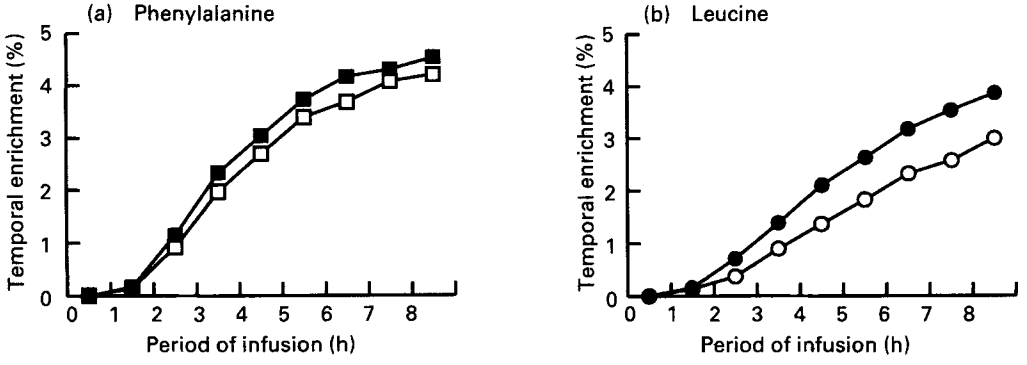

Fig. 2. Temporal enrichments of (a) [1-^{13}C]phenylalanine and (b) [1-^{13}C]leucine from milk casein obtained from the peptide-infused (■ or ●) and control (□ or ○) halves of the gland during an 8 h constant infusion of (a) glycyl-L-[1-^{13}C]phenylalanine and (b) glycyl-L-[1-^{13}C]leucine. Data illustrated are from one animal. Similar temporal labelling patterns were observed in casein from all the animals studied. In all cases enrichments were higher in casein prepared from the peptide-infused half of the gland than in that prepared from the control (non-infused) half.

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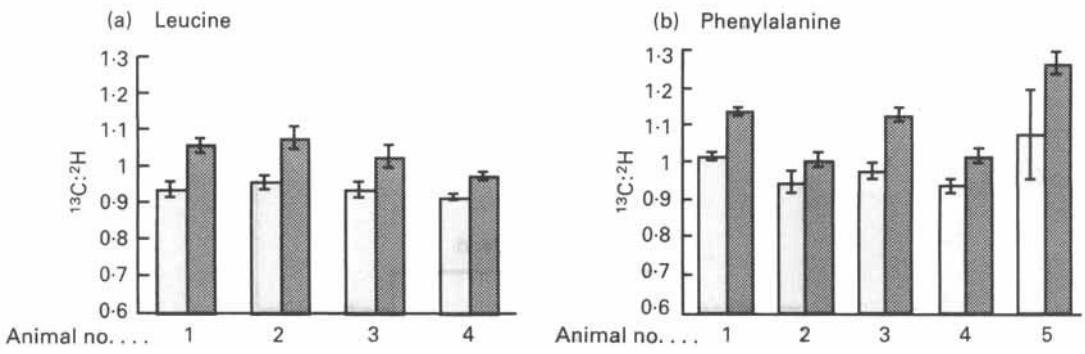

Fig. 3. Isotopic $^{13}C:^{2}H$ ratios in casein prepared from peptide-infused (▩) and control (non-infused, □) halves of the mammary gland during 8 h constant infusions of (a) glycyl-L-[^{13}C]leucine and [^{2}H]leucine or (b) glycyl-L-[^{13}C]phenylalanine and [^{2}H]phenylalanine. Values are the means with their standard errors from the last 3–5 h of the infusion. Statistical significance of the difference between the control and peptide-infused samples was determined by Student's paired *t* test for animals nos. 1–5 respectively with (a) leucine: $P<0{\cdot}001$, n 6; $P=0{\cdot}043$, n 4; $P=0{\cdot}05$, n 5; $P<0{\cdot}001$, n 5; (b) phenylalanine: $P<0{\cdot}001$, n 6; $P=0{\cdot}088$, n 4; $P=0{\cdot}03$, n 4; $P=0{\cdot}036$, n 4; $P=0{\cdot}026$, n 4.

Do tissues use peptides in vivo*?*

Information on the extent to which tissues do use peptides *in vivo* is, at present, largely indirect and inconclusive. The inability to account for the rate of disappearance of protein from the small intestine of sheep as net flux of free AA in the portal-drained viscera (Tagari & Bergman, 1977; MacRae *et al.* 1993) was referred to earlier. Arterio–venous (A–V) difference measurements across the mammary gland of dairy cows (Bickerstaffe *et al.* 1974; Metcalf *et al.* 1994) indicate that the uptake of certain AA such as phenylalanine and tyrosine are insufficient to account for their output as milk protein, so alternative substrates such as PAA must be used. Mammary-gland glutathione uptake is more than sufficient to account for the amount of cysteine in secreted milk (Pocius *et al.* 1981). However, glutathione is a special case; most PAA will be present as products of protein degradation (either of dietary or endogenous origin) and can range from dipeptides up to proteins such as albumin. Techniques to prepare peptides of defined size from physiological fluids are currently inadequate to provide answers to the important biological questions involved. Variations associated with these procedures have also led to much confusion and uncertainty in the literature. Thus, both Seal & Parker (1991) and Koeln *et al.* (1993) measured circulating concentrations of AA and PAA in steers and sheep as the difference in AA concentrations of plasma samples, deproteinized with perchloric or sulphosalicylic acid, before and after acid-hydrolysis and reported that 60–80% of total AA in plasma is in the form of peptides. However, acid deproteinization is not completely efficient and AA released on hydrolysis may have been of protein rather than peptide origin. We have modified this approach to include a gel-filtration step which removes residual protein in the column void volume; only material of molecular weight <1500 Da is collected. This method has shown that the

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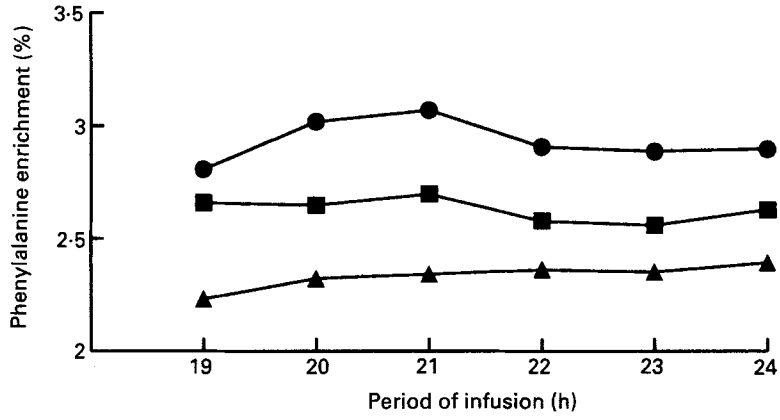

Fig. 4. [^{13}C]Phenylalanine enrichments in casein (▲), arterial blood (■) and arterial plasma (●) during a 24 h constant infusion of [^{13}C]phenylalanine into the jugular vein of lactating goats. Casein-bound phenylalanine enrichments are consistently lower than those of blood or plasma phenylalanine indicating that sources other than free amino acids contribute precursor for milk-protein synthesis.

PAA contribution from small peptides is 10–15% of total AA, although the value may be greater for alanine, phenylalanine and lysine (20–30%). These findings are more consistent, with values of 8·2 and 10·3% reported for PAA in rat plasma from the portal vein and aorta respectively (Gallibois *et al.* 1991); these values were obtained using acid deproteinization followed by ultrafiltration through molecular-weight filters with a nominal 3000 Da cut-off. Preliminary findings from applications of the modified gel-filtration procedure indicate a small uptake of each AA in peptide form by the mammary gland of goats.

Indirect evidence for the utilization of peptides by the mammary gland has been obtained by examination of the kinetics of milk-casein labelling during long-term (24 h) infusions of ^{13}C-labelled AA (Backwell *et al.* 1994*a*). These kinetics suggest that free AA are not transferred directly from blood (or plasma) to casein but first appear to mix with intracellular pools which may be derived partly from the degradation of intracellular or constitutive proteins (Bequette *et al.* 1994). The plateaux ratios between blood, plasma and casein differ between AA and this indicates that sources other than the labelled plasma free AA contribute precursors for milk-protein synthesis.

Fig. 4 shows that the enrichment of casein-bound phenylalanine attained only 80–90% ($P<0·05$) of the level of enrichment in blood or plasma, thus implicating peptides or small proteins in the supply of phenylalanine to the mammary gland (Backwell *et al.* 1994*a*). This is consistent with the observation that A–V transfers of phenylalanine alone do not account for phenylalanine output in milk protein (Bickerstaffe *et al.* 1974; Metcalf *et al.* 1994). In contrast, during infusion of [^{13}C]leucine, casein-bound enrichments were similar to those in blood or plasma (values not shown), indicating the potential net specificity of peptide utilization.

Where do circulating peptides come from?

One obvious source of circulating peptides is from absorption of dietary peptides across the GIT. Until the 1950s it was generally accepted that the endproducts of dietary

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protein degradation in the small intestine were free AA, which were then absorbed by specific transport systems. Subsequently, evidence accumulated indicating that distinct GIT peptide transport systems exist (for review, see Grimble, 1994) and, furthermore, that peptide transport required an inwardly directed proton gradient (Ganapathy *et al.* 1987). While it is well established that the enterocyte has sufficient peptidase activity to hydrolyse absorbed peptides to free AA, there are a number of reports of PAA in the circulation of both single-stomached animals (Gardner *et al.* 1983, 1991; Gallibois *et al.* 1991) and ruminants (Seal & Parker, 1991; Webb *et al.* 1992). Other studies also suggest a substantial uptake of peptide material across the GIT of ruminant species (Koeln *et al.* 1993). Such observations, of course, do not preclude the possibility that these peptides could arise as products of serum or intracellular protein turnover across the GIT tissue bed and more work is needed to apply the newer methodologies for peptide estimation to A–V difference studies of this type.

Another potential source of circulating peptides must be the liver. Studies which have examined the metabolism of free AA across the splanchnic tissues have shown that in dogs (Elwyn *et al.* 1968), sheep (Wolff *et al.* 1972) and lactating cows (Baird *et al.* 1980) most AA absorbed across the gut, with the exception of the branched-chain AA, are apparently removed by the liver resulting in no net output of AA to the peripheral circulation. This has led to the suggestion (Elwyn, 1970) that the peripheral metabolism of plasma proteins synthesized by the liver may represent a significant source of AA for peripheral tissues, including muscle (McCormick & Webb, 1987) and gut (Rérat *et al.* 1992).

SUMMARY AND PERSPECTIVES

While there can be no doubt that tissues have the inherent ability to utilize peptides which are supplied to them for protein synthesis, the extent to which this does, in fact, occur *in vivo* has yet to be adequately resolved. Measured trans-organ fluxes across various tissues indicate that for some AA the uptake in free form may not account for total AA requirement. This has been confirmed across the lactating mammary gland where ^{13}C-labelled peptides and AA have been used to establish not only that the mammary gland can use peptides but that the free AA in blood or plasma do not represent the only AA precursor for protein synthesis (i.e. that other AA precursors must be involved).

Confirmation of the involvement of such precursors in protein metabolism will require further development of better analytical methods for the measurement of trans-organ fluxes of PAA and protein-AA. Furthermore, if they do represent a significant source of AA for protein synthesis, then their nature, in terms of molecular size and AA composition, and origin, whether dietary or endogenous, need to be elucidated.

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REFERENCES

Adibi, S. A. (1971). Intestinal transport of dipeptides in man: Relative importance of hydrolysis and intact absorption. *Journal of Clinical Investigation* **50,** 2266–2275.

Adibi, S. A. (1987). Experimental basis for use of peptides as substrates for parenteral nutrition: a review. *Metabolism* **36,** 1001–1011.

Adibi, S. A., Krzysik, B. A. & Drash, A. L. (1977). Metabolism of intravenously administered dipeptides in rats: effects on amino acid pools, glucose concentration and insulin and glucagon secretion. *Clinical Science and Molecular Medicine* **52,** 193–204.

Backwell, F. R. C., Bequette, B. J., Metcalf, J. A., Wray-Cahen, D., France, J., Crompton, L., Beever, D. E., Lobley, G. E. & MacRae, J. C. (1994*a*). Peptide utilisation by the mammary gland of lactating dairy goats. *Animal Production* (In the Press).

Backwell, F. R. C., Bequette, B. J., Wilson, D., Calder, A. G., Wray-Cahen, D., Metcalf, J. A., MacRae, J. C., Beever, D. E. & Lobley, G. E. (1994*b*). The utilisation of dipeptides by the caprine mammary gland for milk protein synthesis. *American Journal of Physiology* (In the Press).

Baird, G. D., Lomax, M. A., Symonds, H. W. & Shaw, S. R. (1980). Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows *in vivo* in relation to lactation and nutrient supply. *Biochemical Journal* **186,** 47–57.

Bequette, B. J., Backwell, F. R. C., Wilson, D., Walker, A., Calder, A. G., Wray-Cahen, D., Metcalf, J. A., Lobley, G. E. & MacRae, J. C. (1994). Kinetics of milk casein labelling in dairy goats at different stages of lactation. *British Journal of Nutrition* **72,** 211–220.

Bickerstaffe, R., Annison, E. F. & Linzell, J. F. (1974). The metabolism of glucose, acetate, lipids and amino acids in lactating cows. *Journal of Agricultural Science* **82,** 71–85.

Druml, W., Roth, E., Lenz, K., Lochs, H. & Kopoa, H. (1989). Phenylalanine and tyrosine metabolism in renal failure: dipeptides as tyrosine source. *Kidney International* **36,** Suppl. 27, S-282–S-286.

Elwyn, D. H. (1970). The role of the liver in regulation of amino acid and protein metabolism. In *Mammalian Protein Metabolism*, pp. 523–557 [H. N. Munro, editor]. New York: Academic Press.

Elwyn, D. H., Parikh, H. C. & Shoemaker, W. C. (1968). Amino acid movements between gut, liver and periphery in unanaesthetized dogs. *American Journal of Physiology* **215,** 1260–1275.

Furst, P. (1985). Peptides in parenteral nutrition. *Clinical Nutrition* **4,** 105–115.

Furst, P., Albers, S. & Stehle, P. (1990). Dipeptides in clinical nutrition. *Proceedings of the Nutrition Society* **49,** 343–359.

Gallibois, I., Pitre, F., Parent, G. & Savoie, L. (1991). Analysis of bound amino acids in the plasma of fed rats: a new preparation procedure. *Journal of Nutritional Biochemistry* **2,** 25–30.

Ganapathy, V. & Leibach, F. H. (1983). Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-border membrane vesicles from the rabbit. *Journal of Biological Chemistry* **258,** 14189–14192.

Ganapathy, V., Miyamoto, Y. & Leibach, F. H. (1987). Driving force for peptide transport in mammalian intestine and kidney. *Contributions to Infusion Therapy and Clinical Nutrition*, vol. 17, pp. 54–68 [S. A. Adibi, W. E. Fekl, P. Furst and M. Oehmke, editors]. Basel: Karger.

Gardner, M. L., Illingworth, K. M., Kelleher, J. & Wood, D. (1991). Intestinal absorption of the intact peptide carnosine in man and comparison with intestinal permeability to lactulose. *Journal of Physiology* **439,** 411–422.

Gardner, M. L. G., Lindblad, B. S., Burston, D. & Matthews, D. M. (1983). Transmucosal passage of intact peptides in the guinea-pig small intestine *in vivo*: a reappraisal. *Clinical Science* **64,** 433–439.

Grimble, G. K. (1994). The significance of peptides in clinical nutrition. *Annual Review of Nutrition* **14,** 419–447.

Grimble, G. K. & Silk, D. B. A. (1989). Peptides in human nutrition. *Nutrition Research Reviews* **2,** 87–108.

Hubl, W., Druml, W., Langer, K. & Lochs, H. (1989). Influence of molecular structure and plasma hydrolysis on the metabolism of glutamine-containing dipeptides in humans. *Metabolism* **38,** Suppl. 1, 59–62.

Koeln, L. L., Schlagheck, T. G. & Webb, K. E. (1993). Amino acid flux across the gastrointestinal tract and liver of calves. *Journal of Dairy Science* **76,** 2275–2285.

Lochs, H., Morse, E. & Adibi, S. A. (1986). Mechanism of hepatic assimilation of peptides. *Journal of Biological Chemistry* **261,** 14976–14981.

Lochs, H., Williams, P. E., Morse, E. L., Abumrad, N. N. & Adibi, S. A. (1988). Metabolism of dipeptides and their constituent amino acids by liver, gut, kidney and muscle. *American Journal of Physiology* **254,** E588–E594.

McCormick, M. E. & Webb, K. E. (1982). Plasma free, erythrocyte free and plasma peptide amino acid exchange of calves in steady state and fasting metabolism. *Journal of Nutrition* **112,** 276–282.

McCormick, M. E. & Webb, K. E. (1987). Serum proteins as carriers of amino acids to and from the hindlimbs of fed and fasted calves. *Journal of Animal Science* **64,** 586–593.

MacRae, J. C., Lobley, G. E., Bruce, L. A., Luo, Q., Calder, A. G. & Farningham, D. A. H. (1993). Leucine kinetics across the portal drained viscera of sheep. *Journal of Animal Science* **71,** Suppl. 1, 755 Abstr.

Matthews, D. M. (1975). Intestinal absorption of peptides. *Physiological Reviews* **55,** 537–608.

Metcalf, J. A., Beever, D. E., Sutton, J. D., Wray-Cahen, D., Evans, R. T., Humphries, D. J., Backwell, F. R. C., Bequette, B. J. & MacRae, J. C. (1994). The effect of supplementary protein on *in vivo* metabolism of the mammary gland in lactating dairy cows. *Journal of Dairy Science* (In the Press).

Pocius, P. A., Clark, J. H. & Baumrucker, C. R. (1981). Glutathione in bovine blood: possible source of amino acids for milk protein synthesis. *Journal of Dairy Science* **64,** 1551–1554.

Rérat, A., Simoes-Nuñes, C., Mendy, F., Vaissade, P. & Vaugelade, P. (1992). Splanchnic fluxes of amino acids after duodenal infusion of carbohydrate solutions containing free amino acids or oligopeptides in the non-anaesthetized pig. *British Journal of Nutrition* **68,** 111–138.

Roth, E., Kerner, J., Ollenschlager, G., Karner, J., Simmel, A., Furst, P. & Funovics, J. (1988). Alanylglutamine reduces muscle loss of alanine and glutamine in post-operative anaesthetised dogs. *Clinical Science* **75,** 641–648.

Seal, C. J. & Parker, D. S. (1991). Isolation and characterisation of circulating low molecular weight peptides in steer, sheep and rat portal and peripheral blood. *Comparative Biochemistry and Physiology* **99B**, 679–685.

Steinhardt, H. J., Paleos, G. A., Brandl, M., Fekl, W. E. & Adibi, S. A. (1984). Efficacy of a synthetic dipeptide mixture as a source of amino acids for total parenteral nutrition in a subhuman primate (baboon). *Gastroenterology* **86,** 1562–1569.

Steininger, R., Karner, J., Roth, E. & Langer, K. (1989). Infusion of dipeptides as nutritional substrates for glutamine, tyrosine and branched-chain amino acids in patients with acute pancreatitis. *Metabolism* **38,** Suppl. 1, 78–81.

Tagari, H. & Bergman, E. N. (1977). Intestinal disappearance and portal blood appearance of amino acids in sheep. *Journal of Nutrition* **108,** 790–803.

Webb, K. E., Matthews, J. C. & DiRienzo, D. B. (1992). Peptide absorption: a review of current concepts and future perspectives. *Journal of Animal Science* **70,** 3248–3257.

Wolff, J. E., Bergman, E. N. & Williams, H. H. (1972). Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. *American Journal of Physiology* **223,** 438–446.

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