EDITORIAL REVIEW

The metabolism and clinical relevance of the keto acid analogues of essential amino acids

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Background

Essential amino acids are not themselves indispensable dietary requirements for the rat but their carbon skeletons are (Rose, 1938). With two exceptions, α-keto acid (Table 1) or α-hydroxy acid analogues maintain the growth of rats almost as well as the corresponding essential amino acids (Richards, 1972). Lysine and threonine are the exceptions: both are irreversibly deaminated (Neuberger & Sanger, 1944; Elliott & Neuberger, 1950) and although the final test of feeding their keto acid analogues has not been undertaken, the α-hydroxy acid analogue of lysine failed to maintain growth (McGinty, Lewis & Marvel, 1924). Recently the clinical tail has wagged the biochemical dog, provoking new interest in the extent to which man can synthesize essential amino acids from their analogues and in the possible therapeutic applications of essential amino acid analogues.

Interest in the metabolism of essential amino acid analogues in man originally arose from the observations that about one-fifth of urea synthesized is hydrolysed to ammonia in the gut (Walser & Bodenlos, 1959) and that both healthy and uraemic men can use ammonia nitrogen for protein synthesis (Richards, Metcalfe-Gibson, Ward, Wrong & Houghton, 1967). In addition it was postulated that urea hydrolysis might substantially increase with the enlarged urea pool in renal failure. It seemed probable that more ammonia nitrogen would be used if it could be channeled into the synthesis of both non-essential and essential amino acids. In fact urea hydrolysis hardly increases at all in renal failure (Walser, 1974; Varcoe, Halliday, Carson, Richards & Tavill, 1975) and no more than about 5% of the urea nitrogen released is re-used for albumin synthesis, either during protein restriction (Varcoe et al., 1975) or when a low-protein diet is supplemented with the keto acid analogues of five essential amino acids (Ell, Richards & Halliday, 1978). If a substantial quantity of urea nitrogen were normally released as ammonia and reincorporated into protein, urea would accumulate (or its excretion would increase) when bacterial hydrolysis of urea was suppressed by antibiotics. In fact, neither the urea pool nor the urea excretion of patients with chronic renal failure increased under these circumstances (Mitch, Lietman & Walser, 1977). Currently, therefore, the interest in these compounds has shifted from a theoretical role in increasing nitrogen reutilization to their unexplained ability to reduce urea synthesis.

Synthesis of essential amino acids in man

Maintenance of nitrogen balance when the appropriate analogues were added to a diet lacking one or more essential amino acids has provided circumstantial evidence of transamination in man of the α-keto acid analogues of phenylalanine, valine, leucine, isoleucine, tryptophan, methionine and histidine in health and uraemia (Gallina, Dominguez, Hoschoian & Barrio, 1971; Richards, Brown, Houghton & Thompson, 1971; Rudman, 1971; Richards, Brown & Lowe, 1972; Walser, Coulter, Dighe & Crantz, 1973). Hydroxy acids of
methionine (Mitch & Walser, 1977a) and phenylalanine (Mitch & Walser, 1977b) have also more or less maintained nitrogen balance. Further indirect evidence has been derived from the incorporation of ammonia $^{15}$N into phenylalanine and valine during administration of their keto acids (Giordano, Phillips, De Pascale, De Santo, Fürst, Brown, Houghton & Richards, 1972), and from the increase in plasma essential amino acid concentrations after feeding their analogues (Rudman, 1971; Sapir, Owen, Pozefsky & Walser, 1974). Finally, transamination of an essential amino acid analogue has been directly proved by the appearance of $^{13}$C-valine in response to an intravenous infusion of $^{13}$C-labelled $\alpha$-ketoisovalerate (Richards, Ell & Halliday, 1977).

**Efficiency of transamination of essential amino acid analogues**

The efficiency of conversion of $\alpha$-keto acid analogues into essential amino acids depends upon the competitive balance between reversible transamination and oxidative decarboxylation (Fig. 1). The outcome depends partly upon substrate concentrations and partly upon relative enzyme activities. The reversible transamination and deamination (reactions 1 and 2, Fig. 1) proceed without preference to one product over the other, the outcome depending upon the relative concentrations of these substrates.

Muscle has the highest capacity (although not the highest activity per unit of tissue) for transamination of branched-chain amino acids in the rat, and liver has the greatest capacity for oxidation of $\alpha$-keto acids (Harper, 1978). Species differences are considerable: the relative branched-chain amino acid dehydrogenase capacity values of liver, kidney and skeletal muscle were 70, 12 and 10% in the rat, 50, 13 and 20% in the monkey, and 30, 2 and 60% in skeletal muscle in man; the relative increase in human muscle was an expression of the greatly reduced specific activities of the enzyme in liver and kidney, the absolute activity in muscle being similar to the rat (Khatra, Chawla, Sewell & Rudman, 1977). The branched-chain amino acid transaminase in human muscle has not yet been measured but if similar to the rat the enzyme balance in man would favour transamination of branched-chain keto acids in preference to oxidation, especially if they were given by intravenous infusion.

The relative efficiencies of enzymes affecting the metabolism of essential amino acid analogues are subject to several influences. Both alanine transaminase and branched-chain amino acid transaminase activity in rats vary with diet and renal function. Interpretation of the effects of diet is complicated by the fact that total starvation, a protein-free diet, and a low-protein diet each exert different effects. Feeding keto acid analogues of branched-chain amino acids creates conditions favouring their transamination because the specific activity of branched-chain amino acid transaminase increases, probably by direct induction of the enzyme (Chan & Walser, 1978).

The efficiency with which keto acids replace essential amino acids also depends upon other factors such as the diet and the route of administration of keto acids. The amount of tyrosine in the diet, for example, determines the efficiency with which phenylpyruvate replaces phenylalanine, and the energy intake partly determines the result of both growth and nitrogen-balance experiments. The relative rates and completeness of absorption of analogues and possible competition between them may also influence their efficiency. The keto analogues of valine and leucine were absorbed from dog small intestine at only 60% of the rate of the amino acids (Weber, Maddrey & Walser, 1977), which are actively absorbed. Phenylpyruvate is absorbed passively along a concentration gradient in everted sacs of hamster small intestine and it seems probable that the amino group is necessary for recognition by the carrier involved in active absorption (Lin, Hagihiara & Wilson, 1962). Urinary wastage does not appear to be a source of inefficiency: less than 1% of the injected radioactivity of $^{14}$C-labelled $\alpha$-ketoisovalerate was excreted in the urine of rats (Chawla & Rudman, 1974).

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Metabolic inter-relationships between $\alpha$-ketoisovaleric acid, valine and isobutyric acid. Reactions 1 and 2 are catalysed by branched-chain amino acid transaminases and 3 by branched-chain amino acid dehydrogenase/decarboxylase.
Gut bacteria might either decrease the effective dose of keto acids by catabolizing them or enhance their efficiency by transaminating and releasing them for absorption as amino acids. Bacterial activity in fact seems relatively unimportant in the metabolism of amino acid analogues in the rat: less than 10% of radioactivity given as 14C-labelled α-ketoisovalerate was recovered in the faeces with or without antibiotic treatment (Chawla & Rudman, 1974); neomycin did not significantly alter the efficiency of the keto analogues of phenylalanine, leucine and valine (Chow & Walser, 1974) but a more effective cocktail of neomycin, polymyxin and bacitracin reduced the efficiency of α-ketoisovalerate in comparison with valine by 25–50%, depending on the concentration of amino acid or analogue in the feed, figures which took into account the fact that antibiotic treatment itself reduced the growth-stunting effect of a valine-deficient diet (Chawla & Rudman, 1974). The unlikely possibility remains that the antibiotic 'cocktail' reduced absorption of α-ketoisovalerate, but not of valine.

Both rat and human tissues have the capacity to transaminate keto analogues of essential amino acids. Perfusion of both rat liver and muscle with keto analogues of valine, leucine, isoleucine, methionine and phenylalanine individually or together produced essential amino acids, more when keto acids were given individually than when given together, probably because of competition for transamination (Walser, Lund, Ruderman & Coulter, 1973). Output of essential amino acids was not the result of their intracellular displacement by analogues, because the tissue-to-medium concentration of essential amino acids was unchanged. Glutamine was the major nitrogen donor in liver; the principal nitrogen donor in muscle was not identified (Walser et al., 1973). Leucine was also synthesized from its keto analogue in human forearm muscle: 52% of infused α-ketoisocaproyltae was extracted in a single passage and about one-third of the extracted analogue appeared as leucine in deep venous blood (Pozefsky & Walser, 1977).

Comparison of the efficiency with which an analogue replaces its amino acid requires careful definition of the term efficiency. Percentage efficiency is best defined (Gaby & Chawler, 1976) as:

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\frac{\text{mol of essential amino acid for a specified physiological response}}{\text{mol of analogue for the same physiological response}} \times 100
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Comparisons of efficiency must be made at several different doses and the essential amino acid under study must be a rate-limiting factor. Gaby & Chawler (1976) correctly observed that if a less than 30% reduction of essential amino acid intake does not reduce growth rate an analogue which was 70% efficient would still maintain growth and appear to be 100% efficient. The alternative definition of efficiency in terms of feed efficiency, defined as weight gain/food intake, may underestimate efficiency of substitution: if, for example, absence of an amino acid causes weight loss the starting point is not zero efficiency but a negative figure; thus if an analogue maintains weight but supports no growth its efficiency would be zero by definition in spite of the fact that it had some effect.

Efficiency of substitution may vary substantially at different intakes: for example, from 80% when the relative effects of one-quarter of the daily requirement of valine for optimal growth was compared with the molar equivalent of α-ketoisovalerate to 37% when the diet contained twice the requirement of valine or its analogue (Chawla & Rudman, 1974). Others, such as α-ketoisocaproate, show little variation, its efficiency of replacement being between 20 and 27% over a wide range of intakes (Chawla, Stackhouse & Wadsworth, 1975); the efficiency of phenylpyruvic acid in place of phenylalanine was 50–70% at different intakes (Chawla et al., 1975). Efficiency of replacement of valine was greater when dietary nitrogen was restricted (Chow & Walser, 1975a).

Clinical relevance

In chronic renal failure essential amino acid analogues offer a means of delivering essential amino acids without their nitrogen, thereby reducing nitrogen retention (by re-utilizing retained non-protein nitrogen for essential amino acid synthesis) without compromising nutrition (Schloerb, 1966; Richards et al., 1967). Similarly, nitrogenous intoxication might be minimized in patients with liver failure, and in children with deficiencies of urea-cycle enzymes (Close, 1974; Walser, 1975a). Although potential benefit would be expected to be limited to the effects of withdrawing nitrogen from the non-protein nitrogen pool to convert the analogues into an optimal supply of essential amino acids (without reasonable expectation of further benefit from the generation of an excessive supply of amino acids), the therapeutic effects of essential amino acid analogues are apparently greater than this (Richards, 1975). A substantial improvement in nitrogen balance and reduction in blood urea concentration was
obtained when analogues were added to an already marginally sufficient diet (Walser et al., 1973). The improvement could not be attributed solely to re-utilization of retained nitrogen because the change in nitrogen balance was greater than if the analogues had been transaminated with complete efficiency and was greater than the benefit obtained from essential amino acids themselves (Walser, 1975b). Further, the reduction of urinary urea nitrogen induced by daily infusions of five keto acid analogues with the remaining four essential amino acids continued for several days afterwards (Sapir et al., 1974). Likewise infusions of only the branched-chain analogues during the first week of a total fast reduced urinary urea nitrogen excretion during that week and the next (Sapir & Walser, 1977). Possibly a disproportionately great effect of branched-chain amino acids, especially leucine, upon the regulation of protein synthesis (Kirsch, Frith & Saunders, 1976; Harper, 1978) may explain the wider anabolic effects of a branched-chain keto acid supplement. Whatever the mechanism, several lines of evidence converge upon the conclusion that essential amino acid analogues have an anabolic action which is not simply explained by their transamination to essential amino acids or to their acting as a ‘nitrogen sponge’ (Gaby & Chawla, 1976).

It is difficult to prove that any supplements (essential amino acid or keto acid) confer a long-term nutritional benefit, both because of the difficulty in setting up appropriate nitrogen-balance studies and because of the paucity of other measures of nutrition. Essential amino acid supplements to a 16–20 g of protein daily diet improved the nitrogen balance of uraemic patients (Bergström, Fürst & Norée, 1975) and there are substantial theoretical and now some experimental grounds for believing that anything essential amino acid analogues can do in uraemia their analogues might do better. Clinical benefit has also been described (Walser et al., 1973; Walser, 1975b; Norée & Bergström, 1975), but is difficult to measure.

The usefulness of essential amino acid analogues in the treatment of liver failure has yet to be defined but may be enhanced by the reduced activity of branched-chain keto acid dehydrogenase in cirrhotic liver (Khatra et al., 1977). Infusion of five essential amino acid analogues and four essential amino acids to cirrhotic patients with porto-systemic encephalopathy lowered blood ammonia concentration but clinical benefit was hard to judge (Maddrey, Weber, Coulter, Chura, Chapannis & Walser, 1976). Isolated results of treatment of children with urea-cycle enzyme deficiencies have been impressive and encouraging. Plasma ammonia, glutamine and alanine were reduced in a 13 year-old girl with carbamyl phosphate synthetase deficiency whose health greatly improved (Batshaw, Brusilow & Walser, 1975, 1976). Plasma ammonia was reduced to normal and citrulline by 50% in an infant with neonatal citrullinaemia; it became possible to improve the diet with improved growth and development and diminished symptoms (Thoene, Batshaw, Spector, Kuloovich, Brusilow, Walser & Nyhan, 1977).

Treatment with essential amino acid analogues is feasible. Although the keto acids are unstable their sodium and especially their calcium salts are much more stable; unpublished work suggests that their stability is sufficient for clinical use, especially if kept dry. The hydroxy acid analogues are at least as stable and are easier to prepare than the keto acids; current evidence suggests that they are effective by first being oxidized to the keto acid (Gordon, 1965). The finding of both branched-chain keto acids and hydroxy acids in the urine of patients with maple-syrup urine disease (Scrivner & Rosenberg, 1973) indicates that the enzymatic capacity for the conversion of hydroxy to keto acid almost certainly exists in man. On balance essential amino acid analogues are neither more nor less pleasant to take than the amino acids themselves; both may sometimes induce nausea and vomiting. Hypercalcaemia sufficient to cause symptoms develops in a small proportion of patients given substantial amounts of calcium with the analogues (Mitch, Gelman & Walser, 1978), but no other serious unwanted effects have been described.

Key words: amino acids, keto acid analogues, transamination.

References


