Variation in the apparent sensitivity of the insulin-mediated inhibition of proteolysis to amino acid supply determines the efficiency of protein utilization

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ABSTRACT

1. The variability between normal individuals in the efficiency of postprandial protein utilization (PPU), a determinant of the apparent protein requirement, was examined in relation to the relative responses of protein synthesis and proteolysis to protein feeding by means of [1–13C]leucine turnover and balance studies.

2. Twenty-five healthy adults were infused intravenously with L-[1-13C]leucine continuously for 9 h. This was started in the postabsorptive state (PA, 3 h) and followed by low-protein feeding (LP, 3 h), and then by isoenergetic high-protein feeding (HP, 3 h). This allowed protein intake to be varied against a constant postprandial insulin level so that the extent of any amino-acid-mediated responses which were additional to those exerted by insulin could be investigated. Leucine oxidation, O, and balance (intake—oxidation), protein synthesis, S, and degradation, D, were calculated from plasma [1-13C]-α-ketoisocaproic acid enrichment and 13CO2 excretion.

3. PPUprotein, calculated as change in leucine balance/change in intake (HP — LP), varied from 0.58 to 0.99 (mean = 0.81 ± 0.10), independently of age or sex. PPUprotein varied directly with the inhibition of D and inversely with the increase in leucine concentration and stimulation of O and S.

4. Efficient PPU, as demonstrated by the top quintile of individuals categorized in terms of PPUprotein, involves maximal inhibition of D by protein feeding with minimal increases in free amino acid concentrations, O and S. Lesser inhibition of D and greater stimulation of S and O characterized the lower, less efficient quintile. This indicates that the efficiency of protein utilization in individuals, and a component of their apparent protein requirement, is determined by the sensitivity of the insulin-mediated inhibition of proteolysis to amino acid supply.

INTRODUCTION

Protein deposition in individuals requires amino acid substrates, energy and an appropriate regulatory response to feeding by pathways of amino acid metabolism which mediate the deposition. This regulatory response, mainly to the protein content of the meal, has been defined as the anabolic drive [1] and involves both neuroendocrine and substrate mediators, of which insulin and amino acids are the best understood. We have recently reported on the

Key words: insulin, leucine, protein degradation, protein requirement, protein synthesis, protein utilization, stable isotopes.
Abbreviations: HP, high protein; LP, low protein; KIC, α-ketoisocaproic acid; PPU, postprandial protein utilization.
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influences of insulin and amino acids as mediators of feeding responses in terms of the changes in leucine kinetics with feeding [2,3]. By employing a three-phase [1-13C]leucine infusion, in the fasted state and during the sequential feeding of low protein and then high protein small frequent meals, we maintained a constant physiological insulin level in the feeding phases which allowed the effects of energy and protein feeding to be evaluated separately [3]. The energy effect was insulin-mediated and was protein-conserving through an inhibition of proteolysis (D), a lowering of amino acid levels and reduced amino acid oxidation (O). The protein effect involved an amino-acid-mediated augmentation of the inhibition of D, additive to the insulin-mediated inhibition, together with an amino-acid-mediated stimulation of protein synthesis (S). These results were consistent with our previous feeding studies in which the amino acid intakes from meals were varied [2]. These and several other previous studies indicate that inhibition of D is a major effect of both insulin [4–8] and amino acids [4,5,7–11] and that amino acid supply appears to be the main determinant of S, an effect which appears to be independent of insulin action [9,11–13].

The practical importance of these studies is in their application as a standardized protocol for assessing apparent protein requirements [14]. We assess metabolic demand for protein from the fasting leucine oxidation rate and the efficiency of postprandial protein utilization (PPU). This is indicated by the slope of the leucine balance–leucine intake relationship measured during the three phases of the infusion. These studies indicated considerable individual variation in the efficiency of protein utilization which, together with some variability in metabolic demand, contributed to variation in apparent protein requirements. We are concerned here with identifying the nature of the differences between individuals in PPU and consequent apparent protein requirement. We have examined therefore the interrelationships between feeding responses of O, D and S, and how these processes vary with PPU, and with the changes in amino acid and insulin concentrations which mediate a large part of these feeding responses. In this way we can identify the most efficient mechanism for protein utilization and the nature of any defective postprandial responses which may increase the apparent protein requirement.

METHODS

Subjects and experimental design
The efficiency and mechanisms of PPU were investigated by means of leucine turnover and balance studies measured during a three-phase (fasting, low and high protein) 9-h constant intravenous infusion of [1-13C]-leucine in 25 adult subjects. The experimental details relating to the infusion protocol have previously been described in full [2], as has its application to studies in 20 of the subjects (10 young and 10 elderly) aimed at evaluating changes with age in metabolic demand, efficiency of PPU and consequent apparent protein requirement [14]. The 25 subjects studied included 10 females (21–81 years) and 15 males (19–91 years). Mean weight, height and age were 66.1 ± 9.6 kg, 1.70 ± 0.08 m and 48.2 ± 23.8 years respectively. All were in good general health, with normal renal and hepatic function, and the elderly subjects were all mobile. The study was approved by the Local Ethics Committee and all subjects gave informed consent after the nature of the protocol had been fully explained to them.

All details of subject recruitment, body composition, assessment of habitual protein intakes and meal composition have been described previously [14] and will only briefly be described here. The measurements in the additional group of five middle-aged men involved an identical protocol.

Subjects were infused initially in the postabsorptive state (3 h) and then sequentially fed low protein (LP) meals (2% protein energy) every 30 min for 3 h, and then isoenergetic high protein (HP) meals (14% protein energy) for 3 h. Meals provided carbohydrate at 60% of energy with dietary protein and fat exchanged isocalorically in the LP and HP meals and with actual protein intakes in the HP diets set to match habitual protein intakes, determined from three 24-h urinary nitrogen measurements [14]. The LP diet was not intended to mimic any real diet but to induce hyperinsulinaemia without supplying substantial amounts of amino acids. Actual energy intakes were 68 ± 6 kJ/kg per 6 h for the group as a whole; the leucine content of the diet was 4.66 mu mol/mg of food N. The variability of the protein intake is indicated by the variability of the leucine intake, taking into account tracer infusion rates of 7 mu mol·h⁻¹·kg⁻¹ in all subjects.

This protocol allows leucine balance to be measured at three levels of leucine intake so that the efficiency of PPU can be calculated from the leucine balance curve [14,15], with whole-body rates of protein synthesis and proteolysis indicated by the leucine turnover data.

Leucine oxidation, balance, the efficiency of PPU and protein turnover
Leucine oxidation (O) and balance (B) were calculated from the excretion rate of 13CO2 and 13C enrichment of plasma 2-ketoisocaproic acid (KIC), and the intake from food and infusion as previously described [2,3,14,16]. The efficiency of PPU is calculated from the change in leucine balance on feeding in relation to change in intake [14,15]. In this protocol there are three balance points
[postabsorptive (PA), low protein (LP) and high protein (HP)], representing separate responses to energy (PA to LP) and protein (LP to HP). This allows calculation of PPU as mediated by both energy and protein from the slope of all three balance points (i.e. equivalent to an entire meal, PPU$_{meal}$), or PPU solely in terms of energy intake (PA and LP balance points) or protein intake (LP and HP balance points). In the present context of exploring the mechanisms of the anabolic drive in terms of protein-mediated postprandial responses, the analysis will be restricted to the LP to HP responses, i.e. PPU$_{protein}$. In practice, with the main part (> 80%) of the change in leucine balance from PA to HP occurring during the LP to HP transition, PPU$_{meal}$ does not differ markedly from PPU$_{protein}$. Leucine flux (Q) was calculated from the $^{13}$C enrichments of infused tracer and plasma $^{13}$C-KIC at plateau by tracer dilution, with endogenous appearance, assumed to reflect the rate of protein degradation through proteolysis, $D$, and leucine non-oxidative disappearance, assumed to reflect the rate of protein synthesis, $S$, calculated from Q, O and leucine intake from food and tracer [17]. $^{13}$CO$_2$ recovery was assumed to be 0.76 in the fasted state and 0.91 in the fed state, as determined previously [18].

Statistical analysis
The Statistica package was used for the data analysis. Values are expressed as means ± S.D. (n = 25). The influence of the dietary treatments was determined by a one-way analysis of variance method with dietary period as classification factor. To analyse differences in mechanisms of PPU, after testing for normality of the distribution of PPU$_{protein}$ between subjects by the Shapiro–Wilk’s W test, subjects were ranked in quintiles in terms of PPU$_{protein}$ and the quintiles comprising the most efficient (group A, n = 5) and least efficient (group B, n = 5) subjects were identified. Differences in leucine kinetics between these two groups were analysed by one-way analysis of variance. Pearson correlation coefficients were calculated to identify any linear relationships between insulin, amino acids and leucine turnover. Significance was set at 5% but for those values close to 5% the actual P values are also quoted.

RESULTS

Leucine oxidation, balance and PPU
Table 1 shows the leucine intake (tracer at 7 μmol h$^{-1}$ kg$^{-1}$ plus food), oxidation, balance and PPU values for all subjects and for the two quintiles representing the highest and lowest values for PPU. Leucine oxidation was unchanged with the LP feeding but increased with HP feeding, so that balance improved with LP meals and became markedly positive with the HP meals. The mean value for PPU$_{protein}$ was 0.81 ± 0.10, individual values varying from 0.58 to 0.99. The variation was independent of sex or age and was normally distributed between subjects (Shapiro–Wilk’s W test: W = 0.98094, P = 0.8969). There were no differences in either mean weight, height or age between the two quintiles identified in terms of the value of PPU$_{protein}$ (group A (3 males, 2 females): weight = 67.0 ± 8.7 kg, height = 1.67 ± 0.10 m, age = 55.8 ± 25.6 years; group B (3 females, 2 males): weight = 61.6 ± 5.9 kg, height = 1.64 ± 0.09 m, age = 62.0 ± 21.0 years). There were no differences between group A and group B in terms of leucine intake in either LP or HP phases. The pattern of responses of leucine oxidation for group A and group B was similar to that for the entire group, although as expected the increase in oxidation with HP meals was less in group A than in group B. Thus for group A, oxidation was lower in the HP phase (P < 0.01) and therefore leucine balance was more positive in the HP phase compared with group B (P < 0.001). The differences in PPU$_{protein}$ between group A and group B were significant (P < 0.001).

Responses of insulin and leucine concentrations
Plasma insulin increased markedly with feeding to a level that was maintained during the feeding of the two diets, and values measured after 120 and 180 min of each phase were not different. Thus for the whole group, the insulin levels were 5.6 ± 3.9 units/ml (postabsorptive) and 44.4 ± 25.2 units/ml (postprandial); for group A, 4.6 ± 1.5 units/ml (postabsorptive) and 44.5 ± 15.7 units/ml (postprandial); and for group B, 6.8 ± 3.0 units/ml (postabsorptive) and 52.4 ± 20.8 units/ml (postprandial), with no differences between group A and group B.

For the entire group, plasma leucine concentrations (Table 2) fell from PA with LP feeding and increased with HP diet to values that were on average higher than PA although not significantly so. For groups A and B, while the patterns of the response were similar, the fall on feeding the LP diet was more marked in group A than in group B (P < 0.05) and the increase on feeding the HP diet was less marked (P < 0.001) so that actual concentrations achieved with the HP diet were lower in group A compared with group B (P < 0.001). This pattern of responses was also observed with KIC.

Leucine turnover
An isotopic steady-state was achieved during each phase as indicated by achievement of plateau enrichment of plasma [1-$^{13}$C]leucine, [1-$^{13}$C]KIC and $^{13}$CO$_2$ during each of the three phases of the study. Leucine was consistently more highly enriched than KIC (KIC/
leucine ratio was 0.77±0.08, but the magnitude of this difference did not change during the infusions.

Table 3 shows leucine flux (Q), and protein turnover as proteolysis, D, and protein synthesis, S. For the group as a whole during the LP diet, Q fell by 10% with significant reductions in D and S. After HP feeding compared with LP, Q increased by 30% with a further inhibition of D and a significant stimulation of S (LP to HP). For groups A and B, values for Q, S and D for PA and LP periods did not differ from each other or from the overall group. However, after HP feeding compared with LP, there was a smaller increase in Q for group A than for group B so that D fell more and S increased less. Thus after the HP meal, values for D were 43±12 and 83±9 μmol·h⁻¹·kg⁻¹ for groups A and B respectively (P < 0.001), while corresponding values for S were 96±14 and 118±12 μmol·h⁻¹·kg⁻¹ (P < 0.05). These responses of leucine turnover and oxidation to protein intake (LP to HP) are shown in Figure 1 as the changes in the respective rates, where significantly different mechanisms are indicated according to the efficiency of PPU. Thus for group A, the most efficient group, protein utilization was achieved by marked inhibition of D (53±7%), with a small increase in S (14±7%). For the least efficient quintile, group B, protein utilization occurred by an increase in S (34±9%) with a small inhibition of D (10±4%).

Correlation analysis showed no significant relation-

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Q</th>
<th>D</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(μmol·h⁻¹·kg⁻¹)</td>
<td>(μmol·h⁻¹·kg⁻¹)</td>
<td>(μmol·h⁻¹·kg⁻¹)</td>
</tr>
<tr>
<td>All (n = 25)</td>
<td>PA</td>
<td>125 ± 18^1</td>
<td>110 ± 18^1</td>
<td>99 ± 15^1</td>
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<tr>
<td></td>
<td>LP</td>
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<td>92 ± 14^1</td>
<td>86 ± 12^1</td>
</tr>
<tr>
<td></td>
<td>HP</td>
<td>147 ± 21^1</td>
<td>64 ± 21^1</td>
<td>110 ± 16^1</td>
</tr>
<tr>
<td>Group A (n = 5)</td>
<td>PA</td>
<td>124 ± 15^1</td>
<td>117 ± 15^1</td>
<td>98 ± 17^1</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>111 ± 12^1</td>
<td>91 ± 12^1</td>
<td>84 ± 14^1</td>
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<tr>
<td></td>
<td>HP</td>
<td>126 ± 13^1</td>
<td>43 ± 12^1a</td>
<td>96 ± 14^1a</td>
</tr>
<tr>
<td>Group B (n = 5)</td>
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<td>116 ± 19^1</td>
<td>98 ± 14^1</td>
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<td>HP</td>
<td>160 ± 14^1</td>
<td>83 ± 9^1b</td>
<td>118 ± 12^1b</td>
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</tbody>
</table>
were correlated directly with the increases in KIC and leucine concentrations with protein feeding and KIC (Table 4) did vary significantly with responses of OPU and minimal increase in D resulted in the most efficient protein utilization (highest maximal inhibition of D). A lesser inhibition of D and a greater stimulation of S result in less efficient protein utilization (a lower PPU and a greater increase in S and a greater stimulation of D) result in less efficient protein utilization (a lower PPU and a greater increase in S and a greater stimulation of D). As expected, changes in concentrations of both leucine and KIC (Table 4) did vary significantly with responses of O, S or D to protein feeding. In contrast, changes in concentrations of both leucine and KIC (Table 4) did vary significantly with responses of O, S or D to protein feeding. Thus the increases in KIC and leucine concentrations with protein feeding were correlated directly with the increases in O and S, and inversely with the fall in D. As expected, S, O and D were significantly inter-correlated and each was correlated with PPU\textsubscript{protein}. These interrelationships are shown in Figure 2 where the responses of O, S and D to protein feeding are plotted against PPU\textsubscript{protein}. It is clear that maximal inhibition of D and minimal stimulation of S result in the most efficient protein utilization (highest PPU and minimal increase in O). A lesser inhibition of D and a greater stimulation of S result in less efficient protein utilization (a lower PPU and a greater increase in O). The reason that an increase in S is less effective than a decrease in D can be explained by a slope of only 0.5 for the regression of stimulation of S on inhibition of D (r = 0.84, P < 0.001). This in turn reflects the parallel changes in S and O.

**DISCUSSION**

We have not been able to identify any physiological basis to the variation in PPU between our subjects reported here. All subjects, men and women, young and elderly, were healthy, health conscious and mobile and there were no identifiable age- or sex-related influences on PPU. Body composition cannot influence calculation of PPU as a ratio of balance and intake, and although body composition could reflect lifestyles and physical activity, there was no relationship between body mass index and PPU. Finally, although there was some variation in protein intakes in the HP phase, there was no significant difference between the intakes of groups A and B. On the basis of these considerations the variation in PPU examined here represents normal physiological variation unrelated to any identifiable physical characteristic.

We need to consider whether the results are a methodological artefact. The technical issues relating to true precursor enrichment, variation in the recovery of \(^{13}\text{CO}_2\), the possibility of tracer recycling and the splanchnic sequestration of dietary protein (the first-pass effect), and the influence of the sequence of meals in the current protocol, have been extensively discussed previously in relation to the calculation of leucine kinetics [2,3] and balance [14,16]. However, two issues need to be revisited here.

The first-pass effect is of particular importance in feeding studies. Splanchnic sequestration of leucine from food is suggested to require a correction of leucine intake in calculating D, and one report identified an apparent age-related increase in splanchnic extraction of leucine [19]. However, the main correlate of splanchnic extraction in that study was body mass index, suggesting to us that as the relative splanchnic mass increases with

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**Table 4 Correlations between responses of KIC and leucine concentrations to protein feeding and responses of leucine turnover and utilization**

<table>
<thead>
<tr>
<th>Variable (n = 25)</th>
<th>[KIC\textsubscript{LP-HP}]</th>
<th>[Leucine\textsubscript{LP-HP}]</th>
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</thead>
<tbody>
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<td>O\textsubscript{LP-HP}</td>
<td>0.50; P &lt; 0.05</td>
<td>0.67; P &lt; 0.01</td>
</tr>
<tr>
<td>S\textsubscript{LP-HP}</td>
<td>0.54; P &lt; 0.05</td>
<td>0.60; P &lt; 0.01</td>
</tr>
<tr>
<td>D\textsubscript{LP-HP}</td>
<td>-0.52; P &lt; 0.05</td>
<td>-0.67; P &lt; 0.01</td>
</tr>
<tr>
<td>PPU\textsubscript{protein}</td>
<td>-0.49; P &lt; 0.05</td>
<td>-0.66; P &lt; 0.01</td>
</tr>
</tbody>
</table>

**Figure 1 Responses of leucine turnover and oxidation to protein intake (LP to HP)**

Mean values are shown for the entire group and for the most (Group A) and least (Group B) efficient quintiles. *P < 0.05 compared with group A.

**Figure 2 Relationships between the efficiency of protein utilization (PPU\textsubscript{protein}) and responses of leucine turnover and oxidation to protein feeding**

The linear fits shown are described by the equations:

- O (HP – LP) = 55.5 – 53.9 \* PPU\textsubscript{protein}; r = -0.96, P < 0.001
- S (HP – LP) = 71.1 – 53.4 \* PPU\textsubscript{protein}; r = -0.43, P = 0.03
- D (HP – LP) = 91.6 – 152 \* PPU\textsubscript{protein}; r = -0.78, P < 0.001
body mass index, so does apparent splanchnic extraction. Since in our study body mass index was not different between groups A and B, it is unlikely that a variation in splanchnic extraction due to body composition could account for our results. This is a complicated issue that has yet to be adequately resolved either experimentally or theoretically [3]. It remains our view that an intravenous route of tracer administration and sampling is most likely to allow measurement of the true weighted mean response of all compartments in the body. In contrast, after intragastric infusion in the fed state, isotope is sampled after passage with amino acids in food through organs which exhibit marked protein accretion, considerable amino acid oxidation and preferential uptake of tracer [20]. As a result, to the extent that amino acids in food act as preferential substrates for these processes, displacing endogenous amino acids, intragastric tracer administration is likely to be associated with both lower sampled enrichment levels of leucine and higher apparent oxidation rates. Because of this we have assumed that there is no need to make adjustments to our data to take account of any so-called first-pass effect and do not believe it likely to be an important influence on our results.

However, the precursor problem does need to be considered as one explanation of our results. The correlated postprandial increases in S and O, an inversely correlated fall in D and in the magnitude of PPUprotein reported here would all occur to a large extent in proportion to the postprandial increase in Q as indicated by a decrease in KIC enrichment. Thus as indicated by the $r^2$ values from the correlations, changes in the KIC enrichment (LP–HP) accounted for 65%, 78% and 74% of changes in O, S and D (LP–HP) respectively, and for 52% of the changes in PPUprotein. The increase in Q (LP–HP), mean 24% and range 2–36%, reflected reductions in KIC enrichment, mean ± S.D. 19 ± 6.5%, range 2–27%. This means that a variation of 13% (i.e., 2 S.D. covering 95% of the subjects) between individuals, in the error resulting from an assumption that the KIC enrichment reflected the true precursor value, would account for over 50% of the variation in the efficiency of PPU and over 70% of the correlated variation in the mechanism by which the variation in PPU occurred. Assumptions about the true precursor enrichment are exemplified by a recent study by Tessari et al. [21]. They showed that in leucine and phenylalanine studies, whether sampling of amino acid for measurement of isotopic enrichment occurred from plasma or whole blood influenced both the extent and mechanism of apparent protein accretion in human skeletal muscle. Thus a cautious approach to the present analysis must be taken. The best evidence in support of the findings being real rather than an artefact of variability in precursor compartmentation is the data in Table 4 showing that much of the variation in O and PPU reflected variability in leucine and KIC concentrations with protein feeding. These are kinetic-model-independent relationships as well as physiologically expected responses.

Most previous studies of feeding influences on leucine kinetics have addressed the limited question of whether an inhibition of proteolysis or a stimulation of protein synthesis occurs as alternative strategies of assumed equal value for mediating protein deposition. However, in practical terms the important question relates to how much protein deposition occurs, i.e., whether inhibition of proteolysis or stimulation of protein synthesis has any advantage as a feeding strategy for protein utilization.

The most advantageous mechanism is one which can minimize the postprandial increase in amino acid concentrations. Thus for leucine (and many other amino acids), minimal feeding increases in leucine concentration would be expected to minimize the postprandial increase in O, channel amino acids into protein deposition as opposed to oxidative catabolism and increase PPU. In theory, either a stimulation of S or inhibition of D should be equally effective at this. Certainly insulin has been shown on various occasions to lower amino acid concentrations by either stimulating protein synthesis [22] or inhibiting proteolysis [23]. However, the problem becomes more complicated when changes in amino acid concentrations are considered not only in terms of an outcome of changes in S or D but also as regulators of such changes. If amino acid concentrations have to increase to achieve these protein anabolic responses then it may be difficult to avoid increases in amino acid oxidation. The key question then becomes the shape and relative slopes of the concentration–response curves. In practice, S, D and O are not processes which occur within a common amino acid pool because of intracellular compartmentation (e.g., leucine oxidation is mitochondrial). Furthermore, an extracellular increase in amino acid concentrations can signal changes in intracellular S and D and presumably intracellular amino acid concentrations in different compartments. Examples are the inhibition of intracellular proteolysis by increases in extracellular amino acid concentrations through a receptor-mediated mechanism in the perfused liver [24] and the effect of amino acids on cell swelling and consequent stimulation of S and inhibition of D in hepatocytes and in other cells [25]. Since our understanding of the mechanism by which amino acids influence S and D at the cellular and molecular level is poor, it is not possible to predict from first principles the most effective postprandial response of S and D to minimize O and maximize protein deposition. However, in the present studies we have been able to evaluate this in a limited way, relating changes in plasma leucine to changes in whole-body rates of S, D and O and consequent protein utilization. The results show that maximal inhibition of D and minimal stimulation of S result in the most efficient protein utilization (minimal increase in O and highest PPU). A lesser
inhibition of $D$ and a greater stimulation of $S$ result in less efficient protein utilization (a greater increase in $O$ and a lower PPU).

We are interested in this because of the importance of PPU as a determinant of the protein requirement of individuals. The protein requirement reflects metabolic demand for and efficiency of utilization of dietary amino acids, measurable, at least as standardized quantities, from postabsorptive leucine oxidation and PPU in the protocol described here [14]. The lower value for PPU for group B reported here means an apparent protein requirement which was 35% higher compared with group A.

The first important factor that could potentially explain the physiological variation in PPU is responsiveness to insulin. The studies were designed to maintain a relatively constant insulin level during the feeding phase at the characteristic postprandial level for the individual, and this varied markedly between subjects. We assume this reflects the wide range of insulin sensitivity of glucose disposal present in this population. The increase in insulin in response to LP involved reductions in $D$, $S$ and in some cases in $O$, accompanied by reductions in concentrations of leucine and KIC. The apparent insulin sensitivity of $D$ ($D_{PA-LP}/\text{insulin}_{PA-LP}$) varied markedly over a 60-fold range. However, there was no identifiable relationship between this variability and the variability in PPU. This means in effect that, notwithstanding the large variation in insulin responses to feeding, no functional impairment of insulin action on protein and amino acid metabolism was identified.

The second important factor is the response to amino acid supply from the protein feeding. In this case the present data clearly demonstrate the important role of amino acids per se as mediators of PPU that we and others have discussed previously [1,2,3,9–13,23]. However, the new findings arise from analysis of the variability in increases in amino acid levels and kinetic responses. This allows us to discriminate between changes in $S$ and $D$ in terms of both their relative efficiency as mediators of protein deposition and their effectiveness in limiting changes in amino acid levels with feeding. Since we have not directly altered amino acid levels a cautious analysis is required in terms of cause and effect considerations of the interrelationships between leucine concentrations and $D$, $S$ and $O$ described in Table 4. In fact, previous work shows that while the parallel changes in $S$ and $O$ with leucine concentrations observed here can be achieved by directly manipulating amino acid supply, the response of $D$ to either increased protein intakes [2] or increasing amino acid infusion rates and concentrations [26] is increased inhibition, the opposite of the response reported here. This implies that in the present work it is the variable response of $D$ and net deposition in individuals with the same intake that causes the variable increases in leucine concentrations, with responses of $O$ and $S$ following the changes in leucine concentrations. Consequently, individuals with a maximal inhibition of $D$ on feeding exhibited the most efficient protein utilization because of minimal increases in leucine concentrations and $O$. This would suggest that the inhibition of $D$ in response to dietary amino acids is the key response. In contrast, the increase in $S$ due to increased amino acid concentrations and linked to stimulation of $O$ appears to be a consequence of an inadequate response. Clearly if $S$ could be activated by feeding through an effect independent of the need to increase amino acid levels, such as the insulin-mediated stimulation of $S$ in skeletal muscle in the young rat [27], which can actually lower amino acid levels while increasing net protein accretion [22], this would separate any increase in $S$ from amino acid oxidation. However, there is little evidence that this occurs in the human adult [23,25]. Thus the stimulation of $S$ is less effective than inhibition of $D$ as a feeding strategy. This is indicated by the regression of stimulation of $S$ on inhibition of $D$ with protein feeding having a slope of only 0.54. In contrast, inhibition of $D$ in response to amino acid feeding and presumably a small increase in concentrations, can promote amino acid channelling into protein thus minimizing any further increase in concentrations or oxidation. These arguments lead us to conclude that the variation in the effectiveness of this feeding response between subjects explains the inverse relationship between inhibition of $D$, stimulation of $O$ and PPU.

We have quantified the feeding response most conducive to protein utilization, the inhibition of $D$, in terms of its amino acid sensitivity (inhibition of $D$ per unit leucine intake: both as $\mu$mol leucine·h\(^{-1}\)·kg\(^{-1}\)). The relationship between this parameter and PPU\(_{\text{protein}}\) is shown in Figure 3. The magnitude of the amino acid sensitivity parameter varies markedly over a > 20-fold

**Figure 3** Relationship between the efficiency of protein utilization (PPU\(_{\text{protein}}\)) and the amino acid sensitivity of proteolysis

The relationship is described by the equation:

$$\text{PPU}_{\text{protein}} = 0.686 - 0.255 \times (D_{\text{HP-LP}}/D_{\text{LP}}); \ r = -0.76, \ P < 0.001, \text{where } I = \text{intake.}$$
range between our subjects for reasons we have failed to identify. There was no significant relationship between postprandial insulin and the amino acid sensitivity of $D (r = -0.075, P = 0.72)$ in this data set. Furthermore, the present results do not indicate whether the variability shown in Figure 3 would still be apparent in these subjects if examined by varying only amino acid intakes without supplying energy and stimulating insulin levels. In other words, the extent to which the amino-acid-mediated inhibition of $D$ requires a prior insulin-mediated inhibition is not indicated by our results.

The mechanisms by which either insulin or dietary amino acids inhibit proteolysis are poorly understood [28] with most known about hepatic autophagy. Work from Mortimore’s laboratory has explored amino-acid-mediated inhibition [29], and shown a receptor-mediated leucine-activated inhibition [24,30]. Vom Dahl and Haussinger [25] have explored how changes in cell volume act as an important regulator of proteolysis in liver showing that inhibition of proteolysis by both insulin and some amino acids is mediated by increases in cell volume. These two mechanisms are important in that they allow changes in extracellular amino acid concentrations to mediate inhibition of intracellular proteolysis while limiting increases in intracellular amino acids and oxidation rates as observed in group A. However, it remains to be seen which specific mechanisms of amino-acid-mediated proteolysis inhibition are defective and responsible for the less efficient protein deposition in group B.

In summary, the current studies show that efficient protein utilization is achieved by the inhibition of proteolysis rather than an increase in protein synthesis. This is because this mechanism is associated with a minimization of postprandial increases in amino acid concentrations and oxidation. Furthermore, in a healthy population of adults of all ages and both sexes, there appears to be variation in the efficiency and mechanism of protein utilization after protein feeding. This is a result of an apparent marked variation in the sensitivity of insulin-activated proteolysis to inhibition by amino acids, the main determinant of the efficiency of PPU.

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