The effects of nutrition and trauma on whole-body protein metabolism in man

M. B. CLAGUE, M. J. KEIR, P. D. WRIGHT AND I. D. A. JOHNSTON

Department of Surgery, University of Newcastle upon Tyne, and Department of Medical Physics, Royal Victoria Infirmary, Newcastle upon Tyne, U.K.

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Summary

1. Whole-body protein metabolism was determined by a primed constant-rate infusion of L-[1-14C]leucine in patients before and after elective surgery, the nutritional intake being carefully controlled and the surgical stress in individuals being assessed.

2. Pre-operatively, whole-body protein flux ($P<0.05$) and synthesis ($P<0.05$), along with amino acid oxidation ($P<0.01$), increased with nutritional intake whereas protein breakdown remained unaltered. Whole-body protein balance also correlated with intake ($P=0.001$).

3. Postoperatively, whole-body protein metabolism was determined with patients either fasted (group 1) or fed (group 2) and the change in metabolism in each individual from a pre-operative study, carried out in the fed state, was calculated. Whole-body protein breakdown increased in both groups (group 1, $+0.91 \pm 0.74$ g day$^{-1}$ kg$^{-1}$; mean $\pm$ SD, $n=7$; group 2, $+0.47$, $+0.63$ and $+1.01$ g day$^{-1}$ kg$^{-1}$, $n=3$), the change being significant in those fasted after surgery ($P<0.05$). However, the pattern of change in whole-body protein synthesis was entirely different in each group, rising in those fed throughout ($+0.32$, $+0.41$ and $+0.66$ g day$^{-1}$ kg$^{-1}$, $n=3$) but falling in those fasted after surgery ($-0.38$, $-0.80$ and $-1.33$ g day$^{-1}$ kg$^{-1}$, $n=3$). The changes in metabolism appeared more marked in those undergoing greatest surgical stress.

4. Some of the factors involved in the calculations are discussed and their effects on the overall conclusions are considered.

5. A concept of whole-body protein metabolism in the metabolic response to trauma is advocated whereby protein breakdown is largely obligatory to the response, whereas synthesis responds to substrate availability.

Key words: nutrition, nitrogen balance, protein, trauma.

Introduction

Weight loss and a negative nitrogen balance, with consequent delay in resolution of the clinical state, can arise out of two inter-related aspects of any clinical state: the metabolic response of the body to the disease or its treatment, and any reduction in nutritional intake associated with the condition or its management.

The typical 'dose-response relationship as measured by nitrogen-balance studies to short-term changes in protein intake consists of a negative nitrogen balance, with contraction of the body protein pool, at zero intake rising in a rectilinear or curvilinear fashion to become positive, with expansion of the pool, as protein intake increases [1]. Nitrogen retention within the body appears to reach a plateau at high levels of protein intake. Varying the energy intake also modifies this whole relationship, with increase in energy intake not only providing more fuel for protein deposition but also exerting a 'nitrogen-sparing' effect by reducing gluconeogenesis [2]. A similar effect is also demonstrable with the ketosis associated with prolonged starvation [3].

Changes in the body protein pool associated with the metabolic response to trauma produce a negative nitrogen balance related to the severity of injury [4], the age of the patient [4], the pre-injury nutritional status of the individual [5] and
the nutritional intake after injury [6]. Other factors, such as hormones [7-9] and the administration of synthetic anabolic agents [10], also modify the nitrogen loss which was thought to arise from increased muscle protein catabolism [11].

Proteins are not inert substances, but are continually being synthesized and degraded. With such turnover of protein a negative nitrogen balance can arise from either an increase in body protein breakdown or a reduction in body protein synthesis or a combination of both. Furthermore, restoration of nitrogen equilibrium need not necessarily be achieved simply by reversal of the initiating mechanism (i.e. reducing an elevated breakdown rate), but can also arise through alteration in the other modality (i.e. increasing synthesis up to an elevated breakdown rate).

Body protein synthesis has been shown to increase after food ingestion [12] and with the supply of intravenous nutrients to normal volunteers [13] and patients postoperatively [14]. Conflict exists, however, over the mechanism which gives rise to the negative nitrogen balance in the stressed individual. Whereas increased body protein breakdown has been demonstrated in septic man [15], after inoculation [16] and after burn injury [17] or major skeletal trauma [18, 19], results after elective surgery suggest that the protein loss arises from reduced synthesis [20-22]. This apparent disparity could be attributed to the specificity of the response to different injury states, or to the varying nutritional status and degree of trauma in each group of individuals studied, such that the two sets of results represent opposite ends of the same spectrum.

To try to resolve such conflict and define the role of synthesis and breakdown in protein accretion associated with varying nutritional intake, whole-body protein metabolism was determined in pre-operative and postoperative patients, the latter undergoing varying but measurable surgical stress, with controlled levels of dietary intake.

Materials and methods

Kinetic model and analytical technique

Whole-body protein metabolism can be determined by measuring turnover of some component of protein, an amino acid or nitrogen, tagged with an appropriate label and extrapolating the results to body protein [23]. Similar results have been obtained by employing a variety of labels, amino acids, infusion and sampling techniques [23]. The lack of a facility for mass spectrometry obviated the use of an amino acid tagged with a stable isotope for this study, whereas the availability of liquid-scintillation counting in the clinical setting deemed the use of 3H or 14C label appropriate. Simplification of the kinetic model can be achieved by employing an amino acid with no known metabolic pathways other than incorporation or release from proteins, and oxidation with irreversible loss of the label at an early step to avoid recycling through other metabolites. The isotopically labelled essential amino acid L-[1-14C]leucine met such requirements, only possessing the metabolic pathways outlined above, with the 14C label being released irreversibly at the step after transamination [24]. Although studies with L-[1-14C]leucine [20] had previously been undertaken on a small number of surgical patients, the duration of infusion (8-10 h) and requirement for some method of collecting expired air (mouthpiece or hood) reduced patient compliance in the postoperative period. The technique was therefore modified to incorporate a priming dose and reduce the length of infusion, and a method was devised to measure the oxidation rate of the amino acid without the need to collect expired air. A tracer dose of L-[1-14C]leucine (code CFA 273, The Radiochemical Centre, Amersham, U.K.; specific radioactivity 0.1 mCi/mmol, 97-99% pure) was infused as a priming dose [25], approximately 3 x 10^8 d.p.m. pre-operatively and 5 x 10^8 d.p.m. postoperatively, followed by a constant-rate infusion for 2.5 h (i.d.p.m./h: about 9 x 10^8 d.p.m./h), with a preceding 1.5 h infusion of sodium [14C]bicarbonate (code CFA 431; The Radiochemical Centre, Amersham; specific radioactivity 59 mCi/mmol) to calibrate the body’s bicarbonate pool [26].

A sample of venous blood was drawn before and on three occasions during the latter part of each infusion (at 0, 60, 75 and 90 min into the [14C]bicarbonate infusion, and 90, 120 and 150 min into the L-[1-14C]leucine infusion) with chemical separation of the various 14C-labelled components [27] in plasma, as summarized in Fig. 1, to determine and confirm the attainment of plateau values. Plasma proteins were precipitated with perchloric acid [28]; the amount of labelled leucine incorporated was ascertained by the difference in radioactivity (d.p.m.) between the sample of untreated plasma and that in which the proteins had been removed, providing an index of the degree of metabolic stress imposed on the patient [29]. Plasma [14C]bicarbonate was measured with the use of lactic acid [30] and the value obtained was applied to that during the sodium [14C]bicarbonate infusion to deduce the rate of oxidation of the amino acid [26]. Finally,
Protein flux, nutrition and injury

21 ml of heparinized venous blood
Store at 4°C for up to 4½ h

Centrifuge at 2 x 10³ rev./min for 10 min
9 ml of plasma
Count 2 x 1 ml samples for radioactivity

Add 4 ml of plasma to
0.5 ml of perchloric acid and
centrifuge at 3.5 x 10³ rev./min for
10 min

3 ml of deproteinized plasma.
Bubble with CO₂ for 3 min. Add
2 x 1.125 ml samples to outer compartment of ¹⁴CO₂-trapping vial.
Add 0.5 ml of ceric sulphate in H₂SO₄ (1 mol/l). Incubate at 37°C for
30 min. Add 100 μl of satd. soln. of sodium citrate and leave for a further
30 min. Remove inner tube and count
Hyamine for radioactivity and residual material in vial

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The intake of leucine (I mmol/h) was controlled and known and the rate of oxidation (C mmol/h) can be measured. The flux of the pool (Q mmol/h) can be obtained from the equation:

\[ Q = \frac{i(d.p.m./h)}{SA \text{ plateau}} \times \frac{1}{(d.p.m./mmol)} \]  

The rate of entry for leucine into the free pool from protein (B mmol/h) and its exit from that pool into protein (S mmol/h) can then be calculated from eqn. (1). Values for body protein metabolism (g day⁻¹ kg⁻¹) can then be derived, assuming that body protein contains on average 8% leucine [32].

**Patient studies**

Two series of studies were carried out. In the first set a single study lasting 6 h was undertaken in 22 pre-operative patients over the age of 45 years admitted for elective surgery. The majority of these patients were admitted for surgery for 'mechanical defects' (i.e. inguinal hernia, varicose veins, peripheral vascular disease and osteoarthritis). All patients were apyrexial and free of any metabolic disease or endocrine disorders. Burn injury [17], trauma [18, 19], surgery [20–22], neoplasia [33, 34] and infection [15, 16] have all been shown to alter body protein metabolism to a varying extent, and it would have been difficult to distinguish the effect of nutrition from that of the disease process in such individuals. The
results of five of the patients studied were subsequently excluded, these patients having evidence of being metabolically stressed by their disease (i.e. peripheral vascular disease or cholelithiasis) at the time of their study as assessed both by their symptomatology (i.e. ischaemic rest pain or jaundice) and increase in the percentage of the infused labelled amino acid incorporated into plasma proteins (normal value of $6.7 \pm 0.5\%$; mean $\pm$ SD; $n=16$; value in symptomatic patients $8.3-20.6\%$ [29]).

Patients were allocated at random to receive one of four levels of an enteral diet, Clinifeed 400 Chocolate (30 g of actual protein and $3350 \text{kJ/l}$ of feed; Roussel Laboratories Ltd, U.K.; as the model in reality monitors leucine kinetics, the protein intake derived for the study is calculated from the leucine content of 2.40 mmol/100 mmol in the diet and the leucine content of 8% of whole-body protein, i.e. 39.3 g of derived protein/l of feed) in hourly batches (0, 0.8, 1.25 and 1.8 ml h$^{-1}$ kg$^{-1}$), commencing 2 h before starting the infusions and preceded by an overnight fast. No attempt was made to control or monitor dietary intake before the study other than to ensure that the patients had not undergone any recent weight change. Sampling for determination of whole-body protein metabolism was made with patients sitting at rest 5-6 h after starting the feeds, at which time an equilibrium should have been achieved [12], and this was subsequently confirmed by attainment of plateau values in plasma (three samples each with a value of $< 5\%$ of the mean and not obviously rising or falling).

In the second series of studies whole-body protein metabolism was determined in ten patients pre-operatively and again 1-3 days after surgery. All patients received the enteral feed during the pre-operative study, but were either fasted ($n=7$) or received the same nutritional intake (0.8 ml h$^{-1}$ kg$^{-1}$) during the postoperative study ($n=3$).

Approval was granted by the Isotope Advisory Panel in London, U.K., for two studies in any individual over the age of 45 years with infusion of up to $5 \mu$Ci of sodium $[1^{4}\text{C}]$bicarbonate and $25 \mu$Ci of L-[1-$^{14}\text{C}$]leucine on each occasion (maximum calculated total absorbed dose of about 290 mRads for each individual [35], with less than one-third of this being absorbed by the bone marrow). The protocol as a whole was approved by the Local Ethical Committee and informed consent was obtained from each patient immediately before each study.

Details of the patients allocated to each series and level of intake are shown in Table 1. The lack of uniformity in allocation of numbers arises in part from exclusion of metabolically stressed individuals from the pre-operative series and individuals who declined their second study after surgery. Although protein synthesis has been shown to fall with age in animal studies and children [36-38], no such pattern has been delineated in adult man [38,39], so that the age span (45-77 years) investigated here should have no influence on the result. Examination of the results of the small numbers in each dietary group pre-operatively confirmed this, with no demonstrable fall in synthesis with age. The mean age of each subgroup was comparable. The effect of gender in this age group has not been investigated, but again no pattern was discernible in the small numbers studied here. The weights of patients covered a wide range (47-101 kg), but any variations in response due to weight differences were minimized by providing intake and calculating results in terms of unit body mass (g day$^{-1}$ kg$^{-1}$), although this did not allow for differences in body composition between individuals.

### TABLE 1. Age, weight and sex ratio of patients studied

<table>
<thead>
<tr>
<th>Dietary intake* (g of protein day$^{-1}$ kg$^{-1}$)</th>
<th>Age (years)</th>
<th>Sex ratio (M:F)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-operative studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ($n=2$)</td>
<td>65 (64-65)</td>
<td>1:1</td>
<td>61 (56-66)</td>
</tr>
<tr>
<td>0.75 ($n=6$)</td>
<td>58 (50-65)</td>
<td>5:1</td>
<td>69 (47-101)</td>
</tr>
<tr>
<td>1.18 ($n=4$)</td>
<td>58 (45-57)</td>
<td>2:2</td>
<td>71 (49-90)</td>
</tr>
<tr>
<td>1.67 ($n=5$)</td>
<td>59 (45-77)</td>
<td>4:1</td>
<td>73 (61-86)</td>
</tr>
<tr>
<td><strong>Pre-operative/postoperative studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed/fasted ($n=7$)</td>
<td>59 (45-67)</td>
<td>4:3</td>
<td>76 (60-95)</td>
</tr>
<tr>
<td>Fed/fed ($n=3$)</td>
<td>60 (53-65)</td>
<td>2:1</td>
<td>69 (47-101)</td>
</tr>
</tbody>
</table>
Results at each level of dietary intake in preoperative studies were compared by using analysis of variance. The changes postoperatively in each facet of whole-body protein metabolism in those patients undergoing surgery were evaluated by a paired t-test.

Results

The results of changes in whole-body protein metabolism with short-term variations in nutritional intake in pre-operative patients are shown in Table 2. Protein flux, synthesis and amino acid oxidation increase, and the balance changes from a negative one with fasting to an increasingly positive balance with rising intake. Protein breakdown remains the same at all levels of intake. A levelling off in protein balance and synthesis seems to occur at the higher levels of intake, with a concomitant increase in the rate of amino acid oxidation.

The effect of elective surgery on whole-body protein breakdown and synthesis is shown in Table 3. Only three values for body protein synthesis are shown in the seven patients in group 1, because the value for the rate of oxidation (C) in the initial four patients studied was obtained by subtraction of the activity in untreated plasma from that after addition of lactic acid, the residual radioactivity in the untreated plasma too high to enable small differences on addition of lactic acid to be accurately defined. This led to modification of the method with trapping and counting of the evolved $^{14}$CO$_2$ directly in the three remaining patients, with good duplication in all samples. Protein breakdown increased significantly after surgery in patients fed pre-operatively but fasted postoperatively. The number of patients who received an identical nutritional intake for both studies were too small ($n = 3$) for statistical analysis, but whereas protein breakdown was elevated postoperatively in both groups of patients, the response of protein synthesis was different between the groups, falling in those fasted after surgery but rising in those fed.

Discussion

The overall values for whole-body protein metabolism obtained in the pre-operative non-stressed patients are in agreement with those found by others in this age group [40]. The reduction in protein synthesis in those who were

### Table 2. Relationship of whole-body protein metabolism (g day$^{-1}$ kg$^{-1}$) to protein intake (calorie/nitrogen ratio 200:1) in non-stressed pre-operative patients

<table>
<thead>
<tr>
<th>Intake (I)</th>
<th>Flux (Q)</th>
<th>Synthesis (S)</th>
<th>Oxidation (C)</th>
<th>Breakdown (B)</th>
<th>Balance (S-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ($n = 2$)</td>
<td>2.00</td>
<td>1.52</td>
<td>0.48</td>
<td>2.00</td>
<td>-0.48</td>
</tr>
<tr>
<td>Mean</td>
<td>2.25</td>
<td>1.87</td>
<td>0.37</td>
<td>2.25</td>
<td>-0.37</td>
</tr>
<tr>
<td>0.75 ($n = 6$)</td>
<td>2.57</td>
<td>2.34</td>
<td>0.22</td>
<td>1.80</td>
<td>+0.54</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.63 ± 0.47</td>
<td>2.20 ± 0.53</td>
<td>0.53 ± 0.12</td>
<td>1.87 ± 0.47</td>
<td>+0.34 ± 0.14</td>
</tr>
<tr>
<td>1.18 ($n = 4$)</td>
<td>4.26</td>
<td>3.97</td>
<td>0.49</td>
<td>3.08</td>
<td>+0.91</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.57 ± 0.93</td>
<td>3.26 ± 0.97</td>
<td>0.36 ± 0.11</td>
<td>2.39 ± 0.93</td>
<td>+0.87 ± 0.07</td>
</tr>
<tr>
<td>1.67 ($n = 5$)</td>
<td>4.15</td>
<td>3.49</td>
<td>0.66</td>
<td>2.47</td>
<td>+1.02</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.98 ± 0.71</td>
<td>3.23 ± 0.58</td>
<td>0.75 ± 0.20</td>
<td>2.31 ± 0.71</td>
<td>+0.93 ± 0.20</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>N.S.</td>
<td>=0.001</td>
</tr>
</tbody>
</table>
TABLE 3. Effect of surgery on whole-body protein breakdown ($B$, g day$^{-1}$ kg$^{-1}$) and synthesis ($S$, g day$^{-1}$ kg$^{-1}$) in patients fed before surgery and fasted postoperatively (group 1) and those in whom nutritional intake was identical for both studies (group 2)

The 'degree of trauma', calculated from the percentage change in incorporation of label into plasma proteins [29], the percentage and actual change in each facet of metabolism individually and for each group as a whole (mean ± SD) are shown.

<table>
<thead>
<tr>
<th>Degree of trauma</th>
<th>Whole-body protein metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breakdown ($B$)</td>
</tr>
<tr>
<td></td>
<td>Pre-operative</td>
</tr>
<tr>
<td>Group 1 (fasted postoperatively)</td>
<td>+36</td>
</tr>
<tr>
<td></td>
<td>+76</td>
</tr>
<tr>
<td></td>
<td>+102</td>
</tr>
<tr>
<td></td>
<td>+111</td>
</tr>
<tr>
<td></td>
<td>+167</td>
</tr>
<tr>
<td></td>
<td>+194</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2 (fed postoperatively)</td>
<td>+26</td>
</tr>
<tr>
<td></td>
<td>+52</td>
</tr>
<tr>
<td></td>
<td>+100</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P = 0.02$

fasted compared with those fed [12, 41] and the rise in synthesis as intake is provided [13, 14] or increased [36, 42, 43] are also consistent with previous results. The lack of change in protein breakdown with reduced intake has also been demonstrated previously when protein and energy intake were changed simultaneously [12], although protein breakdown has been shown to decline with a reduction in protein intake when the level of energy intake is maintained [43]. The role of energy intake and substrate availability in producing these changes is still uncertain [38, 40] and needs further evaluation.

The pattern depicted by protein balance in response to increasing intake (see Table 2) is similar to that obtained from conventional nitrogen-balance studies. A negative balance occurs with fasting, this becoming positive in a rectilinear or curvilinear fashion as intake increases, tending towards a plateau at higher levels. Zero protein balance in this study was achieved at an intake of only 0.4 g of protein day$^{-1}$ kg$^{-1}$ and an energy intake of about 33.5 kJ day$^{-1}$ kg$^{-1}$. Although these values, taken in combination, are low [44–46], they may be a reflection of the mode of feeding and method of calculation rather than any disagreement in the actual values. Nitrogen-balance studies are usually carried out over 24–96 h in individuals who are meal-fed during the day and fasted at night. The results presented here represent protein metabolism on an hour-to-hour basis, extrapolated for convenience and comparison with other results to 24 h, in patients at rest and receiving continuous feeding. This latter method of feeding is likely to be more efficient, particularly as our results suggest that balance and synthesis reach a plateau at the higher levels of intake, a situation that would produce a surge in oxidation [43] during the period of absorption after a meal.

The results show that, in the short term at any rate, changes in body protein balance in relation to protein intake are brought about by modifying the rate of protein synthesis with no apparent change in protein breakdown. There also appears to be an upper limit to the capacity to synthesise protein above which the excess ingested amino acids are largely oxidized, a phenomenon already described elsewhere [43, 47].

In calculating these results it is necessary to make several assumptions, and their validity and the effect of any error on the results require evaluation.

(a) A 'steady state' existed, in that the free leucine pool did not change in size and the rates at which leucine entered and left the pool were constant.

(b) The L-[1-13C]leucine was metabolized in
the body in a manner identical with its unlabelled counterpart.

(c) There was no significant recycling of labelled leucine, incorporated into proteins during the infusion, back into the free leucine pool.

(d) The average leucine content of body protein is 8%.

(e) The sampled pool is representative of the pool at the site of protein synthesis.

(f) Although the body protein pool cannot truly be called homogeneous, the measured rates of protein metabolism are considered representative of the body as a whole and not biased towards any particular tissue or sub-pool.

A 'steady state' was deemed to exist because of the attainment of plateau values. Similarity of handling of labelled and unlabelled compounds is a prerequisite of all isotope studies, but is difficult to validate in practice. Some recycling of labelled leucine must occur, but should be minimal because of the short duration of the study and the presence of plateau values (i.e. significant recycling would cause the plasma level of L-[1-14C]leucine to rise slowly during the constant rate infusion as more of the label becomes first incorporated and then released from the protein pool). The leucine content of the body cannot be confirmed clinically, but alteration in the value would still produce the same pattern of results, provided that, as seems likely, variation between individuals is minimal.

The true two assumptions are more difficult to justify and probably impose some degree of error on the results, particularly that of the relationship of the plasma leucine pool to the precursor pool for protein synthesis (assumption e). Leucine for this precursor pool must originate either from plasma through the extracellular space, or from the products of protein degradation within the cell. Incorporation of leucine into protein directly and exclusively from the extracellular space would make the specific radioactivity of L-[1-14C]leucine in plasma representative of that at the site of protein synthesis, whereas admixing of the two components in one or more intracellular precursor pools would result in dilution of the plasma value by unlabelled leucine released intracellularly. The remaining alternative, of the leucine being entirely recycled intracellularly, seems to be not only illogical (i.e. would result in no uptake of dietary amino acids or interchange between organs) but can also be discounted by the detection of label in various tissue proteins in animals and in plasma proteins in this instance.

A review of the literature [23] showed that workers at various times have produced results supporting each of the two above feasible proposals. Such results could arise because different amino acids have been examined by using a variety of techniques in vivo and in vitro on various cell types, tissues and species. The picture is no clearer when tissue studies with labelled leucine alone are examined [48-50]. The only work comparable with our study in which this problem was specifically addressed suggested that there was admixing of both the intracellular and extracellular components, with the specific radioactivity at the site of protein synthesis being 80% of the plasma value [39]. Recalculating our results using this or any value down to 65% of the measured value in plasma did not alter the overall pattern or significance of our result, although the actual values for protein metabolism changed. The only situation in which the results could differ from those depicted would be if the relative contribution of each component changed with dietary intake, such that the extracellular fraction increased as flow of substrate from the gastrointestinal tract into plasma rose. The pattern might then change (i.e. synthesis and breakdown would be under-estimated at low intakes, and a pattern could arise whereby synthesis would remain unchanged and breakdown fall with increasing intake). However, no evidence exists at this time to support this concept of a significant change in the contribution of each component to the amino acid pool with varying influx from the bowel.

The lack of homogeneity of the body protein pool is a major problem (assumption f). Although the use of a constant-rate infusion can overcome any heterogeneity within the free leucine pool, provided that all sub-pools have equilibrated, no similar mechanism enables the lack of uniformity within the protein pool to be completely disregarded. Numerous proteins exist with various pool sizes and half-lives. These individual proteins may not respond in a similar manner to a stimulus, such as dietary intake, and the results obtained could be a reflection of a large change in a single protein which may represent only a small part of the total protein mass (e.g. albumin). Such a statement can only be discounted by recourse to studies of individual proteins (e.g. 3-methylhistidine excretion as an index of muscle protein breakdown) in man [51] and carcass analyses in animals [52, 53] under similar circumstances. Results from such studies do not suggest that our results are biased towards a single tissue or protein.

After elective surgery it can be seen that a completely different mechanism exists in producing the negative nitrogen balance. Whole-body protein breakdown rises significantly post-operatively in the group of patients with sufficient
numbers to allow statistical analysis (see Table 3: group 1). It is noteworthy that breakdown fell slightly in the two patients who were least traumatized and rose most in the individual most traumatized in this group. Although the pattern did not hold up to linear regression analysis, a picture of increasing breakdown as the severity of injury rises is suggested in both groups of patients studied here and in the only other study (burn injury) where the trauma could be quantified [17]. Such a lack of correlation could arise from the small numbers studied, an error in deriving the actual value for protein degradation or the absence of any truly linear relationship.

The same assumptions apply in calculating actual values as stated above and similar arguments apply regarding their validity, but two points merit repetition. The increased endogenous release of unlabelled amino acid from the elevated protein breakdown could reduce the specific radioactivity at the site of protein synthesis even more than the estimated 80% of the plasma value proposed above, decreasing further as the degree of trauma and protein breakdown rises. Such a statement is difficult to substantiate, certainly in vivo. Assuming that such a phenomenon exists and applying a sliding scale to the calculation of the actual specific radioactivity will produce significant correlations between body protein synthesis, breakdown and the degree of trauma, just as we have done in a previous publication [33]; but the sliding scale tends to bias the result in favour of significance. Such results therefore lend no further support to the proposal that intracellular specific radioactivity might change, but merely serve to highlight a plausible proposition. Further studies are obviously required to resolve this.

The second point raised concerns the lack of homogeneity of the protein pool once more and whether the results are a reflection of changes in a small but rapidly turning-over pool rather than representative of the body as a whole. Examination of studies of individual proteins show that, from a protein from a relatively small pool with a rapid turnover, such as albumin [54], to one from a large pool with a slower turnover, such as muscle protein [11, 51, 55-58], all behave in a way consistent with the results depicted in the body as a whole. Several of these studies also suggest a relationship between protein catabolism and the extent of injury.

The results described in this paper can be combined with those of others to advance a concept of protein turnover in the metabolic response to trauma under differing circumstances. Such a concept will also explain away the apparent disparity in results obtained previously. This concept is illustrated in Fig. 2.

Fig. 2(b) illustrates the response with the effects of changing nutrition neutralized. This is derived basically from our results in patients in whom nutrition was maintained (see group 2, Table 3) and supported by the results obtained after major skeletal trauma [18, 19]. Both protein synthesis and breakdown rise along with the

![Diagram](image-url)
degree of trauma, breakdown rising more rapidly than synthesis, thus accounting for the increased negative nitrogen balance with increasing severity of injury [4].

Fig. 2(a) illustrates the response when the nutritional intake is reduced postoperatively. This is derived basically from our results in patients fasted postoperatively (see group 1 in Table 3). The additional increment in protein loss over the above situation is brought about by a marked suppression of protein synthesis, although breakdown may fall slightly. A similar picture with a fall in synthesis has been shown when patients given glucose and saline intravenously postoperatively have been compared with those receiving parenteral amino acid solutions [14], and in normal individuals when measurements made in the fasted state are compared with those made when nutrition is provided [28].

Examination of our latter results will enable a point to be delineated where breakdown remains unaltered but synthesis is reduced (i.e. degree of trauma of about +80 in group 1 patients in Table 3). Previous groups of patients studied after elective surgery have either been fasted or received reduced nutritional intake postoperatively, and the mean result of each group presented could represent this point. This is supported by the following evidence. In half of the patients for whom individual results are presented, protein breakdown did rise postoperatively, but this has been overlooked by concentrating on mean results. Although it is not possible in retrospect to assess the degree of trauma in these patients, if all the patients were to be scattered along our 'regression lines' about the above-suggested point then there should be some correlation between changes in breakdown and synthesis postoperatively in individual patients. Patients with a fall in breakdown should have an even greater reduction in synthesis, and synthesis should increase only in those with markedly elevated breakdown. Such a correlation does exist \( B = 0.79S + 13; r = 0.71; P < 0.005; n = 15 \), on pooling results from two of the papers [20, 21] and lends support to the results of previous workers complying with the concept presented. The reduction in postoperative nutritional intake as well as the grouping together of patients undergoing various severity of injury masked the true response to trauma.

At this point speculation can be made regarding the effects of prolonged reduced intake and aging on the response, nitrogen losses being reduced under both circumstances [11, 59]. Reduced synthesis and breakdown have been demonstrated in the elderly [39], in obese individuals on a reduced intake for several weeks [60], in anorexic patients with neoplasia [33] and in malnourished children with infection compared with normally nourished children with the same infection [61]. This would result in a shift of the zero degree of trauma to the left (see Fig. 2a). Injury will then cause a move to the right, depending on the degree of trauma, but as the individual started further to the left than normal he would only reach a position where protein breakdown and synthesis are not as divergent as if he had started in the normal position, and so will excrete less nitrogen than the normally fasted [59] or younger individual [4] undergoing the same stress.

In Fig. 2(c) the expected response when patients are given an increased intake postoperatively is depicted. The nitrogen loss is reduced as synthesis is increased towards the elevated breakdown rate, and a positive balance could even be achieved if sufficient intake is given. This aspect is supported by the response of preoperative patients to nutritional intake (in our study) and that seen after burn injury, when balance was achieved as nutritional intake was increased in line with the burn surface area [17].

In conclusion it may be stated that the body responds in a similar way to many forms of stress, the results of previous workers merely reflecting the large spectrum of response obtainable. The effect of that stress, whether it is accidental injury or elective surgery, on whole-body protein synthesis and breakdown in a patient depends on the age and nutritional status of the individual as well as the degree of trauma. Breakdown appears to be largely related to the severity of injury, whereas synthesis responds to substrate availability.

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