https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press

British Journal of Nutrition (2005), **94**, 141–153
DOI: 10.1079/BJN20051460
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Review article

The end-product method of measuring whole-body protein turnover: a review of published results and a comparison with those obtained by leucine infusion

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(Received 20 September 2004 – Revised 3 February 2005 – Accepted 16 February 2005)

The present review summarizes the results of all published papers on whole-body protein turnover in man measured by [^{15}N]glycine and the end-product method using both urea and ammonia. It begins with a short account of the underlying assumptions and the justification for the use of [^{15}N]glycine. The results are then compared with those of a large sample of measurements by the 'gold standard' precursor method with continuous infusion of [^{13}C]leucine. The pros and cons of the two methods are compared and it is suggested that there is a place for further work by the less invasive end-product method, particularly for population studies of the genetic, environmental and functional determinants of whole-body rates of protein synthesis.

Protein turnover: [^{15}N]Glycine: End product: Leucine infusion

Protein turnover describes the continual synthesis and breakdown of protein in the body. It is a fundamental biological process in all living organisms, and over the last 50 years workers have attempted to quantify rates of turnover. At the present time the 'gold standard' method of measuring protein turnover in the whole body in man is the 'precursor' method, in which labelled leucine is given by intravenous (IV) infusion for two or more hours until a steady state of enrichment has been achieved in plasma leucine. The purposes of the present review are: to assess what has been achieved over the years by an alternative and historically earlier method, the end-product approach with [^{15}N]glycine; to examine as far as possible the validity of the method by comparing its results with those of the precursor method; to look at the pros and cons of the two methods. The end-product approach would be particularly useful for population studies, which could help to unravel the determinants of individual variation. It is also more easily applied in difficult situations outside a hospital or metabolic ward, as in the studies of Stein *et al.* (1996) on weightless subjects during a space flight or those of Stroud *et al.* (1996) during a crossing of Antarctica. We believe that a wider use of the end-product method would help to extend our knowledge of the extremely important biological function, protein turnover.

The first paper in which the end-product method was used to measure whole-body protein turnover appeared more than 50 years ago (Sprinson & Rittenberg, 1949). These authors used a single oral dose of [^{15}N]glycine and urinary urea as end product. For various reasons (Waterlow *et al.* 1978*a*) the method fell into disuse for many years. It was taken up again and modified by Picou & Taylor-Roberts (1969), who gave the tracer as a continuous infusion by IV and intragastric routes. A further modification was the introduction of a second end product, ammonia, instead of or combined with urea for the calculation of flux, since ammonia has a small pool that turns over very rapidly (Golden & Waterlow, 1977; Waterlow *et al.* 1978*b*). All the results presented in the present review were obtained with [^{15}N]glycine as tracer and both end products combined.

Theory and practice

The simple two-pool model on which the method was originally based is shown in Fig. 1. The end-product method derives from the axiom that two products of the same precursor have the same activity (radioactivity or enrichment) provided that the precursor pool is the only source of the products (Zilversmit, 1960). The assumption then is that the activity of the end product reflects that of the amino-N mixture that is taken up into protein. This mixture does not, of course, have the same amino acid composition as the total free amino-N pool or the plasma pool. An alternative formulation of this assumption is that the proportions of the flux, Q, that go to synthesis and excretion are the same as the proportions of the tracer dose that go to synthesis and

Abbreviation: IV, intravenous.
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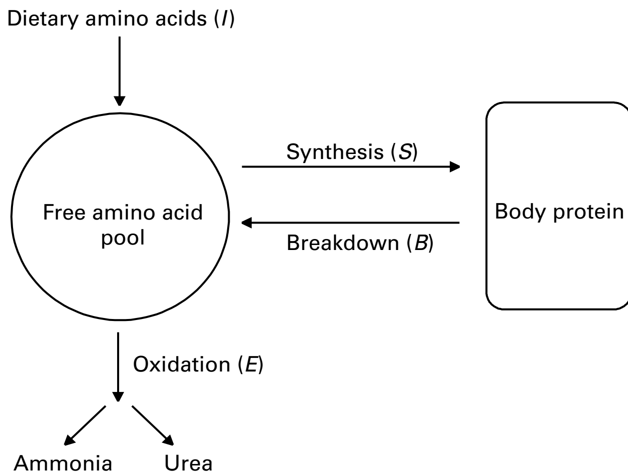

Fig. 1. Simple two-pool model of protein turnover. (From Garlick & Fern, 1985, Courtesy of John Libbey & Company.)

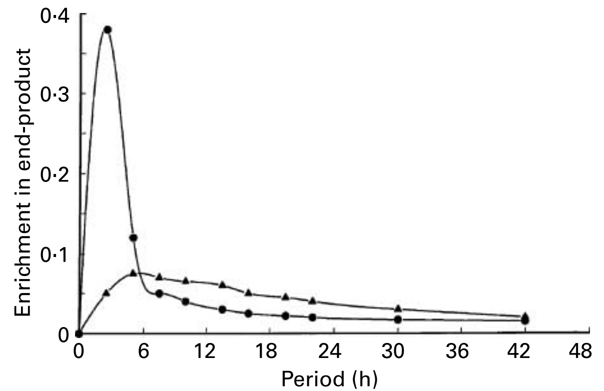

Fig. 2. Time course of excretion of ^{15}N in urinary ammonia (●) and urea (▲) after a single dose of [^{15}N]glycine. (Reproduced with permission from Grove & Jackson, 1995.)

excretion. The crucial measurement is therefore that of the flux: once that is determined, synthesis can be derived from the relation $S = Q - E_T$, where S is synthesis and E_T is total N excretion. It is important to distinguish between the total N loss, E_T, and the loss in the end product, urea or ammonia. In practice the excretion of urea-N + ammonia-N is usually taken as an approximation to E_T, since other sources of urinary N loss and faecal loss can be neglected, being small in comparison with the flux.

Single dose method

Following on from the above, if the tracer is given as a single dose, either by oral or IV route, the flux is defined by the relation:

$$E_{ep(t)}/Q_{(t)} = e_{ep(t)}/d,$$

where d is the dose of tracer, E_{ep} is the amount of end product excreted, e is the amount of tracer excreted in the end product and Q is the flux. t refers to the time period over which the excreted end product is collected. Since e_{ep}/E_{ep} is the ^{15}N enrichment, ε in the total collection, the equation can be rewritten:

$$Q_{(t)} = d/\varepsilon_t.$$

Fig. 2 shows the time course of the excretion of ^{15}N in urinary ammonia and urea after a single dose of [^{15}N]glycine. By 9–12 h the excretion of ^{15}N–labelled ammonia is virtually complete, but that is not the case with urea. To compensate for this, a blood sample is taken after 9–12 h and the amount of [^{15}N]urea retained is determined as the product of plasma [^{15}N]urea concentration and an estimate of total body water (Watson *et al.* 1980), it being assumed that urea is uniformly distributed in the water of the body. The retention of [^{15}N]urea after 9–12 h is usually found to be about 25 % of the dose. Grove & Jackson (1995) suggested that the blood sample taken at 9 h could be replaced by measurement of the amount of labelled urea excreted between 9 and 24 h after the dose of tracer. With ammonia as end product, which turns over rapidly, this extra time of collection could introduce a small but appreciable error from recycling into the ammonia pool of tracer derived from the breakdown of labelled protein; with urea, which turns over more slowly, this source of error can be neglected. It is obviously essential that the collection of urine after the single dose of tracer should be complete, particularly in the first hours. It is possible to divide the 9 h collection into 3 h periods, and to measure the creatinine content of each sample as a check on the completeness of collection.

Multiple oral or continuous infusion

An alternative to the single dose method is to give the tracer by either continuous IV or oral intragastric infusion. More often oral doses are given every hour or so, with food, so that measurements by this multiple or intermittent dose method are almost invariably in the fed state. When the enrichment of the end product has reached a steady state (plateau) ε, $\dot{Q} = \dot{d}/\varepsilon$, where the dots represent rates (amounts/time). This method does not require complete urine collections; its disadvantage is the time needed to reach plateau enrichment in urea −24 to 30 h in children, about 60 h in adults – although this time can be reduced by giving a priming dose of [^{15}N]glycine at the beginning of the study (Dietz *et al.* 1982; Jeevanandam *et al.* 1985; Grove & Jackson, 1995). Results of comparing single and multiple dose methods are discussed later.

By either method, if a steady state is assumed, synthesis and breakdown (B) can be obtained from the flux by the relation:

$$Q = S + E_T = B + I + N,$$

where E_T is total urinary N excretion, not excretion only of end product, I is intake and N is *de novo* synthesis.

It should be noted that the end-product method measures fluxes as uptake into synthesis and excretion, so that $Q = S + E$, and N does not enter into it. The precursor method measures Q from the dilution of tracer by amino acid entering from all sources, so that if the tracer is a non-essential amino acid N has to be taken into account.

The concept of metabolic channelling

The formulation set out earlier assumes a single homogeneous precursor pool. As soon as ammonia began to be used as an end product it was realized that it seldom produces the same estimates of flux as urea, and the difference varied with the conditions of measurement, whether in the fasted or fed state and whether the tracer was given by oral or IV route. The estimate of flux based on ammonia is designated Q_A, that on urea Q_U. It is important to recognize that these are not separate fluxes,

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but different estimates of a single flux: that of whole-body amino-N. It was therefore necessary to abandon the original concept of a single homogeneous 'metabolic' pool, and to postulate two pools, spatially and metabolically separate, at least to some extent. The model proposed by Fern *et al*. (1985*a*) is shown in Fig. 3. It is suggested that Q_A is 'biased' towards the peripheral tissues, since urinary ammonia is derived mainly from glutamine (Pitts & Pilkington, 1966), which is synthesized mainly in muscle; and Q_U is 'biased' towards the viscera, since urea is synthesized exclusively in the liver; hence the concept of spatial separation of the two estimates of flux.

Fern *et al.* (1981) also pointed out that Q_A and Q_U tended to vary inversely, which suggested that the best estimate of the 'true' flux is the average of the two different estimates. The flux is the inverse of the end products' activity. There are therefore two possible averages: the arithmetic average $(Q_A + Q_U)/2$, which implies equal amounts of tracer going through the two pathways the harmonic average $2/[(1/Q_A) + (1/Q_U)]$, which implies equal activities in the two pathways. In practice these two averages seldom differ significantly; the arithmetic average is more convenient and will be used throughout the rest of the present review, abbreviated Q_{av} and S_{av}.

[^{15}N]glycine as tracer

The early work on protein turnover was done almost exclusively with [^{15}N]glycine because it was easily available and cheap.

Fern investigated the appropriateness of glycine as a tracer in a remarkable experiment. He compared nine different ^{15}N-labelled amino acids or proteins, and also single oral *v*. IV dosage, all in one subject – himself (Fern *et al.* 1985*b*). The results are shown in Table 1. Taruvinga *et al.* (1979) obtained similar results in rats infused with four different amino acids and measurements of enrichment of urea and ammonia in liver and kidney. Fig. 3 is a possible scheme for interpreting these findings. Since the basic assumption of the end-product method is that the enrichment of N in the end products' precursor pools is the same as in the precursor pool for synthesis, if tracer is diverted towards the end products, so that ε becomes ε^+ where ϵ stands for enrichment, the estimates of flux and synthesis will be falsely low. This is what seems to be happening with alanine and glutamine, the main N transporters, to produce very low estimates of flux and synthesis by both routes of dosage. On the other hand, Jackson & Golden (1980) found no labelling of plasma alanine and glutamate after giving [^{15}N]glycine, a discrepancy that we cannot explain. Aspartate and glutamate are pre-eminent as transaminators. The low flux ratio, Q_A/Q_U, when these amino acids are given orally implies a greater flux through the visceral tissues, consistent with their known role in urea formation. The low flux ratio with leucine, by both rates of dosage, is consistent with the leucine–ketoacid transaminase being located mainly in muscle. Finally, with lysine estimates of flux are uniformly high, indicating very little labelling of the end products' precursor pools. This might be expected, since lysine is not transaminated; it was shown by Äqvist (1951) that of all the amino acids lysine has the least exchange of N with other amino acids and by Read *et al.* (1971, 1972) that when [^{15}N]lysine was given very little ^{15}N appeared in the urine.

Finally, Fern *et al.* (1985*b*) related the fluxes with yeast and wheat to their amino acid composition and obtained excellent agreement between the observed fluxes and a composite of the fluxes obtained with each amino acid (Table 1) multiplied by the proportion of each amino acid in yeast or wheat protein.

This interpretation of Fern's data is inevitably speculative, but it may be reasonable to suppose that an amino acid is more likely to give a 'correct' estimate of whole-body flux the more closely two conditions are fulfilled:

(1) If similar estimates are given by the two end products, i.e. if the flux ratio does not deviate too far from unity, so that the common precursor pool can be regarded as more or less homogeneous;
(2) If the same value for Q_{av} is obtained by the two routes of tracer entry, oral and IV.

These two points minimize the metabolic and the spatial separation postulated in the model of Fig. 3.

On the basis of these criteria glycine is clearly the best: Q_{av} is the same by oral and IV routes and Q_A/Q_U shows the smallest deviation from unity.

Support for the use of glycine as a tracer comes from a little-quoted paper by Matthews *et al.* (1981). After oral dosage of [^{15}N]glycine for 60 h, they measured the quantitative pattern (enrichment × amino acid concentration) of the tracer in plasma amino acids. From this they calculated the expected enrichment in urea. The calculated enrichment would be identical with the

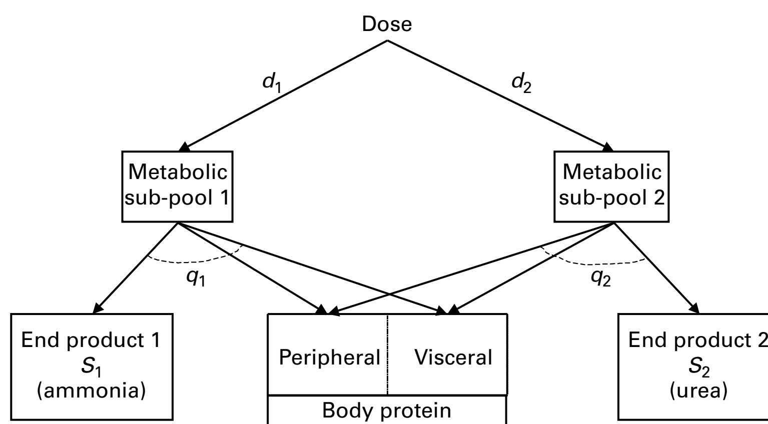

Fig. 3. Fern's hypothesis of metabolic channelling. d_1, d_2, partition of dose; q_1, q_2, fluxes through the two channels; S_1, S_2, synthesis rates of the two end products. (From Fern *et al.* 1985*a*.)

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Table 1. Estimates of nitrogen flux, flux ratio (Q_A/Q_U) and protein synthesis with different ^{15}N-labelled amino acids, using a single dose of tracer in the fed state (Fern *et al.* 1985*b*)

		N flux* (g N/12 h)	Q_A/Q_U	Protein synthesis* (mg protein/kg per h)
Glycine	Oral	27·1	1·08	140
	Intravenous	28·0	0·73	146
Alanine	Oral	16·5	0·94	70
	Intravenous	18·7	0·55	83
Glutamine	Oral	16·8	1·20	60
	Intravenous	17·2	0·50	70
Aspartate	Oral	31·6	2·71	180
	Intravenous	19·9	0·79	94
Glutamate	Oral	33·7	2·29	189
	Intravenous	25·8	0·68	133
Leucine	Oral	42·2	0·49	243
	Intravenous	37·0	0·57	218
Lysine	Oral	126·8	3·06	847
	Intravenous	152·7	2·30	1029
Wheat	Oral	27·3	1·68	135
Yeast	Oral	44·0	1·97	251

* Arithmetic average.

observed enrichment if the pattern of ^{15}N in the precursors of urea was exactly the same as the pattern in plasma amino acids. There was, in fact, quite good agreement: calculated enrichment 0·38, observed 0·44 atom% excess. Further support from the protein side came from the demonstration by Bier & Matthews (1982) that with glycine the distribution of ^{15}N in the amino acids isolated from plasma albumin was similar to that of free amino acids in plasma. It is regrettable that there have not been more such studies.

Analysis of measurements of protein turnover by the end-product method with [^{15}N]glycine

We attempted to find all studies of protein metabolism in man that had been carried out using the end-product method and [^{15}N]glycine. Many papers were already known to us. We searched Medline, Embase and the ISI Science Citation Index databases. We excluded abstracts, letters, PhD theses, book chapters and review articles where data had previously or subsequently been published, to avoid repetition. We found 212 papers that used [^{15}N]glycine and the end-product method. Of these, seventy-seven were included in the tables that follow. The criteria for inclusion were that both end products, ammonia and urea, had been used to calculate results, the number of subjects was reported and that, in studies in the fed state, the protein intake was within a reasonably normal range of 0·5–1·5 g/kg body mass per d.

The analysis included normal adults, children, pregnant women, the elderly and patients in various pathological states. In many of the papers more than one group of subjects was studied. We have reported both synthesis rates and flux ratios.

Comparison of different protocols in the same individuals

Initially we shall consider studies in which the comparisons were made in the same individuals.

Oral v. intravenous dosage of tracer. Fern *et al.* (1981) compared the results with a single dose of [^{15}N]glycine in the same four subjects. There was no difference between oral and IV dosage in either the fed or the fasted state, but the fasted level was only 60 % that in the fed state. In another study (Fern *et al.* 1984), when measurements were repeated on the same individual at different times, there was again no difference between oral and IV routes of dosage.

Single v. multiple dosage or constant infusion. The best comparison of the two protocols is one by Grove & Jackson (1995) in which both protocols were carried out in the same person in eleven out of thirteen subjects. In the single dose method urine was collected for 9 h, with corrections for ^{15}N retained in the urea pool; in the multiple dose method tracer was given orally every 3 h for 15 h. With both methods the subjects were fed every 3 h. The multiple dose method gave values for flux that on average were 25 % lower than those with the single dose method. Two factors may have contributed to this discrepancy: with the multiple dose method a rather large priming dose of [^{15}N]glycine was given 6 h before the multiple doses began, in order that plateau labelling should be achieved in 24 h. This dose may not all have been disposed of by the end of the study; at the same time the 15 h period of dosage may have allowed some re-entry of tracer from protein breakdown into the ammonia pool.

Variability. A study by Fern *et al.* (1984) allows an estimate of the intrinsic variability of the method when measurements were repeated on the same two subjects over a period of 3–4 years. The results are shown in Table 2. The CV were of the order of 5–6 %, with, as mentioned earlier, no difference between oral and IV dosage. In five other subjects measured on two occasions at intervals of 25–66 weeks, the mean difference between the two measurements was 10 %. Of course, there may have been real temporal changes. Glynn *et al.* (1988) made two studies, at an interval of 1 month, on a patient with Guillain-Barré syndrome. The tracer was given IV over 1 h and the patient was fed by nasogastric tube. Synthesis rates differed by only 4 %. This patient is of interest because her muscle mass was only 17 % of body weight yet her whole-body synthesis rate was very high – at 310 mg protein/kg per h almost twice that in normal subjects, presumably because a larger proportion of her lean body mass consisted of visceral protein with a high rate of turnover.

Comparison of different protocols in different individuals

Whole-body protein synthesis in normal adults. There are four different variants of the method. The results are shown in Table 3. With single oral dosage in the fasted state, synthesis is almost 20 % lower than in the fed state. The difference between

Table 2. Variability of protein synthesis rates measured on five occasions (oral) or three occasions (intravenous, IV) over a period of 3·5 to 4 years, using a single dose of [^{15}N]glycine and urea and ammonia as end products in the fed state (Fern *et al.* 1984)

			Protein synthesis* (g protein/9 h)		
Subject		Number of studies	Mean	SD	CV (%)
1	Oral	5	100	5·9	5·2
	Intravenous	3	105	4·7	4·5
2	Oral	5	96	6·6	6·8

* Arithmetic average.

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these methods is 34 mg protein/kg per h (95 % CI 4, 64; $P=0\cdot03$). As in the study of Grove & Jackson (1995) multiple dosage gives rates 25 % lower than oral dosage, the difference being 42 mg protein/kg per h (95 % CI −1, 85; $P=0\cdot05$). The IV single dose, usually administered over 1 h, gives rates similar to oral dosage. With constant IV infusion the results are much lower, probably because these infusions were continued for 2–3 d, which would allow a substantial amount of recycling, thus increasing the labelling and decreasing the estimate of flux. The variability between groups is high, suggesting that there were undetected differences in technique. In the largest group (A) the inter-individual CV is 21 %. The contrast between using the between-group SD and the within-group SD is seen most clearly with constant IV infusion fed (G), where protein synthesis in the three groups is similar (actual data 90, 98 and 104 mg protein/kg per h), but variation between individuals within each group was high (SD of actual data 28, 19 and 13 mg protein/kg per h, respectively).

Whole-body protein synthesis in various physiological states. The end-product method has been quite widely applied to subjects in different physiological states. Synthesis rates in this group are shown in Table 4. In premature babies the average rate of synthesis is more than twice as high as in adults. One feature of studies in premature infants is that glycine may not be available for urea synthesis, so that urea gets zero or very low labelling (Jackson *et al.* 1981). Authors have therefore reported data based on labelling of ammonia only. We have not included these data in Table 4. However, an analysis of these studies (using the same method, multiple oral dose) shows a mean rate of protein synthesis of 404 mg protein/kg per h, not dissimilar from the mean of 444 mg protein/kg per h shown in Table 4 (JC Waterlow, unpublished results). These babies weighed approximately 2 kg and on average were growing at a rate of approximately 15 g/kg per d. If the new tissue contains 12 % protein they would be depositing about 75 mg protein/kg per h. Thus, synthesis far exceeds deposition; the same situation has been found in growing animals and in children recovering from malnutrition (Reeds & Harris, 1981). Synthesis rates fall progressively as children become older and are growing less. In pregnancy the rate is similar to that in normal adults and does not appear to change as pregnancy progresses. Pregnancy, however, poses particular problems in respect of how best to express results. The interpretation can vary considerably depending on whether rates of synthesis are expressed as absolute values or relative to body weight or lean body mass (Duggleby & Jackson, 2002). The average rate in the elderly is a little lower than in younger people; in one study in which the results in the two sexes were reported separately (Pannemans *et al.* 1995), synthesis was substantially lower in women than in men even when the rates were based on lean body mass rather than on body weight.

Whole-body protein synthesis in pathological states. The results of thirty-nine studies are summarized in Table 5. As with normal subjects, the values with the IV tracer tend to be lower than when the tracer is given orally. The synthesis rate in malnourished infants, at 132 mg/kg per h, is only about half that of recovered children (Table 4). In an interesting study by Golden & Golden (1992), children were given varying amounts of Zn during the recovery period. The first measurements were made in the early stage of recovery, and so are not included in Table 5. Their results suggest that Zn promotes protein synthesis. The undernourished adults do not show the same depression of synthesis as the infants, but the extent of their malnutrition was much less severe. Moreover, the Indian labourers in the study had less body fat and a lower proportion of muscle to non-muscle tissue than controls, which would tend to raise their synthesis rates. The low synthesis rates per kg in obesity perhaps reflect the dilution of lean body mass by fat. Otherwise it is difficult to discern any pattern. The high synthesis rate in sickle cell disease may result from the constant replacement of erythrocytes, while the low rate in AIDS is presumably the consequence of an inactive immune system. Tomkins *et al.* (1983) showed that protein turnover rates were very high in children with severe infections, but this study was not included here because only ammonia was used as an end product.

Comparison of whole-body protein synthesis measured by the end-product and precursor methods. Use of the end-product method with glycine as a tracer is best supported by comparison with a method that rests on completely different assumptions, such at the precursor method with leucine–α-ketoisocaproic acid. Very briefly, in this method [^{13}C]leucine is given by constant IV infusion, usually with a priming dose so that plateau

Table 3. Whole-body protein synthesis rates by [^{15}N]glycine and the end-product method in normal healthy adults

	Method		n*	Protein synthesis (mg protein/kg per h)				Reference¶
				N†	Mean‡	Between-group SD‡	Within-group SD‡ (n, N)§	
A	Oral, single dose	Fed	22	146	179	48·0	37·3 (17, 114)	1–9, 10§, 11§, 12§, 13–16, 17§
B	Oral, single dose	Fasted	13	130	145	30·0	33·0 (11, 117)	8, 11§, 12§, 15, 18–21
C	Oral, multiple dose	Fed	6	48	137	27·4	17·6	4, 6, 22–25
D	Oral, multiple dose	Fasted	No studies	–	–	–	–	
E	Intravenous, single dose	Fed	5	16	183	19·8	56·5 (2, 10)	9§, 11§, 17§, 26
F	Intravenous, single dose	Fasted	5	36	167	53·0	49·4 (4, 32)	11§, 27–29
G	Constant infusion	Fed	3	9	96	6·0	20·1	30, 31
H	Constant infusion	Fasted	3	42	63	11·9	12·5	32–34

* n = number of groups included.
† N = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ Papers that did not report an SD could not be included in calculation of within-group SD; n = number of groups included, N = number of individuals/results included.
¶ References: 1, Stein *et al.* (1996); 2, Marchini *et al.* (1996); 3, Soares *et al.* (1991); 4, Jackson *et al.* (2000); 5, Gausseres *et al.* (1997); 6, Grove & Jackson (1995);7, Willommet *et al.* (1992); 8, McNurlan *et al.* (1987); 9, Fern *et al.* (1984); 10, Fern & Garlick (1983); 11, Fern *et al.* (1981); 12, Swart *et al.* (1988); 13, Soares *et al.* (1994); 14, Acheson *et al.* (1995); 15, Robinson *et al.* (1990); 16, Stein *et al.* (1990); 17, Fern *et al.* (1985*b*); 18, Conway *et al.* (1995); 19, Pannemans *et al.* (1995); 20, Zillikens *et al.* (1993); 21, Bos *et al.* (2000); 22, Badaloo *et al.* (1989); 23, de Benoist *et al.* (1985); 24, Jackson *et al.* (1997); 25, Jackson *et al.* (1987); 26, Pacy *et al.* (1994); 27, Bettany *et al.* (1996); 28, Ang *et al.* (1995); 29, Nissim *et al.* (1983); 30, Jeevanandam *et al.* (1986); 31, Ma & Jiang (1990); 32, Richards *et al.* (1993); 33, Tracey *et al.* (1988); 34, Jeevanandam *et al.* (1987).

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Table 4. Whole-body protein synthesis rates by [^{15}N]glycine and the end-product method in various physiological states

	Method		n*	Protein synthesis (mg protein/kg per h) N†	Mean‡	Between-group SD‡	Reference§
Premature infants	Oral multiple dose	Fed	5	52	441	65	1–3
Children‖	Oral, single or multiple dose¶	Fed					
Mid recovery			2	20	254	9	4, 5
Recovered			4	43	230	37	4–6
Pregnant women	Oral, single or multiple dose	Fed					
Early gestation			2	15	181	19	7, 8
Mid gestation			4	46	162	41	7–10
Late gestation			4	46	163	39	7–10
Elderly	Oral, single dose or constant infusion	Fed	3	20	106	9	11–12
		Fasted**	6	64	144	48	13–14

* *n* = number of groups included.
† *N* = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ References: 1, Pencharz *et al.* (1989); 2, Catzeflis *et al.* (1985); 3, Van Goudoever *et al.* (1995); 4, Golden & Golden (1992); 5, Waterlow et al. (1978*b*); 6, Jackson *et al.* (1983); 7, de Benoist *et al.* (1985); 8, Willommet *et al.* (1992); 9, Jackson et al. (2000); 10, Duggleby & Jackson (2001); 11, Arnal *et al.* (1999); 12, Golden & Waterlow (1977); 13, Pannemans *et al.* (1995); 14, Pannemans *et al.* (1997).
‖ Children were recovering or had recovered from malnutrition.
¶ No difference in rates of protein synthesis using a single or multiple dosage, in separate subjects.
** In one study (Pannemans *et al.* 1995) results were given separately for both sexes: protein synthesis was 183 and 114 mg protein/kg per h for men and women, respectively.

enrichment is achieved in 1–2 h. Flux is calculated from the enrichment in plasma of α-ketoisocaproic acid, a transamination product of leucine that gives a better estimate than plasma leucine of the precursor activity at the site of synthesis. Oxidation, the equivalent of N excretion in the end-product method, is calculated by measuring the output of $^{13}CO_2$, allowing for some of it being retained in the body. Finally, as with the end-product method, synthesis is obtained by subtracting oxidation from flux.

Few comparisons of the two methods have been made in the same patients at the same time. Golden & Waterlow (1977) fed six elderly patients by nasogastric tube for 24 h, with [^{15}N]glycine added to the feed. In three of the subjects [^{14}C]leucine was given by IV infusion; in the other three it was added to the feed. If the results with leucine had been corrected by using α-ketoisocaproic acid, the two methods would have agreed to within about 10 %. Pacy *et al.* (1994) compared synthesis rates (Table 6) and found good agreement at the two higher levels of protein intake but not at the lowest. Pannemans *et al.* (1997) made a similar comparison in elderly women (Table 6). Interpretation of these results is complicated by differences in the route of dosage. Of interest is a study by Nissim *et al.* (1983), who gave a pulse dose of [^{15}N]glycine and from compartmental analysis of the plasma decay curve calculated a synthesis rate of 147·5 mg protein/kg per h, compared with a rate of 121·9 from the end-product average. Although this study involved only a single tracer, it is a true comparison between precursor and end-product methods.

The other comparison that can be made is between the mean rates of protein synthesis obtained by the end-product method with those obtained by the 'gold standard' precursor method. The difficulty in comparing the two approaches is that there are so many variants of both methods. From a practical point of view, the most useful comparison is between the most used variants. For the end-product approach the method that has been preferred by most workers is the single oral dose of tracer in the fed state; for the precursor approach it is the single IV infusion. The data we have used for the precursor method are derived from an analysis of forty-three papers studying some 356 subjects. For the purposes of this comparison the fasting–feeding protocol (where the measurement is carried out over a period of fasting followed by a period of food intake) is regarded a single study. The results are shown in Table 7.

The difference in the fed state is less than 10 %: 179 mg protein/kg per h by the end-product method *v.* 164 mg protein/kg per h by the precursor method. It is greater in the fasted state (difference 33 mg protein/kg per h; 95 % CI 13, 52; $P=0{\cdot}0013$). Thus there is a discrepancy in the effect of fasting that we cannot explain – no difference from feeding with the precursor method, quite a large difference by the end-product method. This is clearly a finding that needs further investigation.

An important finding is that the variability both between and within groups is lower with the precursor method, probably because the protocol is more rigidly standardized.

The flux ratio

The end-product method, however, does allow us to estimate the flux ratio. On Fern's hypothesis, the flux ratio is of interest because it could give information about the relative activity in two pathways that are metabolically and spatially separate. Table 8 shows the data in normal adults in fed and fasted states, with the four different variants of the end-product method and [^{15}N]glycine. In some of the studies synthesis rates were reported rather than fluxes. In such cases the flux ratio was calculated as S_A/S_U. If the subjects were in N balance, and excretion was equal to intake, adding it to the values for synthesis would lead to a negligible difference between S_A/S_U and Q_A/Q_U. In addition, in some studies, mean Q_A and mean Q_U have been reported but not mean (Q_A/Q_U). In forty-four of the studies, data were given for individuals. In thirty-seven of these (mean Q_A)/(mean Q_U) differed from mean (Q_A/Q_U) by less than 5 %. We have therefore accepted (mean Q_A)/(mean Q_U) as a representative summary index of mean (Q_A/Q_U) when that was all that was reported. The within-group SD could not be calculated as many papers did not report an SD for the flux ratio.

The flux ratios are all lower with IV than with oral dose, although the differences are not statistically significant except

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Table 5. Whole-body protein synthesis rates by [^{15}N]glycine and the end-product method in various pathological states (fed or fasting)

	*n**	*N*†	Protein synthesis (mg protein/kg per h) Mean‡	Between-group SD‡	Reference§
Oral – single or multiple dose					
Children					
Malnourished	1	10	132	‖	1
Obese on habitual diet¶	1	16	121	‖	2
Obese after 2 weeks on weight-reducing diet¶	1	16	178	‖	2
With cancer	3	20	262	23	3, 4
Adults					
Undernourished, males	4	21	205	28	5–7
Undernourished, females	2	14	146	0	7
Undernourished, elderly	2	17	134	9	8
Obese, on habitual diet	1	8	79	6**	9
Obese, after 4 weeks on weight-reducing diet	1	8	70	23**	9
With hypopituitarism	1	8	165	‖	10
After surgery	1	11	183	46**	11
With cancer	1	11	181	35**	11
With cirrhosis of the liver	5	49	174	32	12, 13
With sickle cell disease	1	6	250	21**	14
With AIDS	1	9	132	36**	15
Intravenous – single dose or constant infusion					
Adults					
After surgery	12	82	125	55	16–21
After multiple trauma	15	181	92	10	22–28
With gastrointestinal disease on TPN	10	68	140	111††	16, 19, 20, 29–32
With gastrointestinal disease	5	49	105	28	31–34
With cirrhosis of the liver	4	22	122	26	35
With cancer	16	172	121	47	17, 31–34, 36–38
With AIDS	4	16	102	7	39

TPN, total parenteral nutrition.
* *n* = number of groups included.
† *N* = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ References: 1, Waterlow *et al.* (1978*b*); 2, Pencharz *et al.* (1988); 3. Vaisman *et al.* (1993); 4, Kien & Camitta (1983); 5, Soares *et al.* (1991); 6, Soares *et al.* (1994); 7, Vaisman *et al.* (1992); 8, Bos *et al.* (2000); 9, Stein *et al.* (1991); 10, Binnerts *et al.* (1992); 11, Glass *et al.* (1983); 12, Swart *et al.* (1988); 13, Zillikens *et al.* (1993); 14, Badaloo *et al.* (1989); 15, Stein *et al.* (1990); 16. Lowry *et al.* (1986); 17, Yoshida *et al.* (1996); 18, Taggart *et al.* (1991); 19, Powell-Tuck *et al.* (1984); 20, Ma & Jiang (1990); 21, Bonau *et al.* (1984); 22. Jeevanandam *et al.* (1991*a*); 23, Petersen *et al.* (1993); 24. Jeevanandam *et al.* (1993); 25, Jeevanandam *et al.* (1992); 26, Petersen *et al.* (1994); 27, Jeevanandam *et al.* (1991*b*); 28, Jeevanandam *et al.* (1989); 29, Glynn *et al.* (1987); 30. Powell-Tuck & Glynn (1985); 31, Jeevanandam *et al.* (1988); 32, Jeevanandam *et al.* (1987); 33, Jeevanandam *et al.* (1984); 34, Fearon *et al.* (1988); 35, Kondrup *et al.* (1997); 36, Richards *et al.* (1993); 37, Mihranian *et al.* (1983); 38, Dresler *et al.* (1987); 39, Lieberman *et al.* (1994).
‖ Not possible to calculate an SD or SD not reported in paper.
¶ In this study protein intake was 1.8 and 2.3 g protein/kg per d for habitual and weight-reducing diets, respectively.
** SD reported in paper, therefore within-group SD.
†† In two studies, rates of protein synthesis were unusually high at 370 mg/kg per h.

Table 6. Comparison of whole-body protein synthesis rates by precursor and end-product methods in the same subjects

A

Previous protein intake (g protein/kg per d)	Leucine Mean	SD	*n*	Glycine Mean	SD	*n*
0·36	170	26	5	128	31	5
0·77	174	17	5	176	33	5
1·59	193	7	6	209	78	5

B

Protein:energy ratio of previous diet	Leucine Mean	SD	*n*	Glycine Mean	SD	*n*
10·6	129	21	6	75	17	6
19·6	129	8	6	108	25	6

Protein synthesis in mg protein/kg per h.

A, Pacy *et al.* (1994), young adults. Leucine given by continuous intravenous infusion. Data based on enrichment in plasma of α-ketoisocaproic acid. Glycine by intravenous dosage over 1 h. Both in fed state. B, Pannemans *et al.* (1997), elderly women. Leucine by continuous intravenous infusion. Data based on enrichment in plasma of α-ketoisocaproic acid. Glycine by single oral dose. Both in fasted state.

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Table 7. Comparison of whole-body protein synthesis rates in normal healthy adults by end-product and precursor methods. End-product data by oral, single dose, synthesis is arithmetic average of estimates from ammonia and urea; precursor data by constant intravenous infusion

Method		n*	N†	Protein synthesis (mg protein/kg per h)		
				Mean‡	Between-group SD‡	Within-group SD‡§
End product‖	Fed	22	146	179	48	37
Precursor¶	Fed	21	168	164	19	13
End product	Fasted before feeding	–	–	–	–	–
Precursor	Fasted before feeding	21	168	160	13	12
End product	Fasted	13	130	145	30	33
Precursor	Fasted	22	188	178	25	23

* n = number of groups included.
† N = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ Those papers that did not report an SD could not be included in calculation of within-study SD.
‖ For references for results by the end-product method, see Table 3 rows A and B.
¶ References for results by the precursor method will be presented in a forthcoming book (Waterlow, In Press).

for the single dose in the fasted state (B *v*. F): mean difference 50·4 (95 % CI 28, 73; $P=0·0007$). This implies a tendency for the precursors of ammonia to be more highly labelled, and for those of urea to be less highly labelled with IV dosage, which is what would be expected on the basis of the Fern hypothesis. There is also a statistically significant difference between method A (carried out in the fed state) and method B (carried out during fasting). The mean difference is 38·5 (95 % CI 19, 58; $P=0·0004$). This again suggests that in fasting the pattern of flux is biased towards peripheral tissues.

In Table 9 are also collated data on the flux ratio in subjects in various physiological and pathological states. We took the same approach to the data as for Table 8, described earlier. There is very little variation in the normal subjects, from premature infants to the elderly, which implies a remarkable degree of stability throughout life. In malnourished infants the ratio is low, suggesting a relatively high flux through the visceral pathway, in keeping with their reduced muscle mass. The same effect was found in undernourished male adults in whom, as mentioned earlier, muscle mass was greatly reduced (Soares *et al.* 1991, 1994).

An outstanding change is a high flux ratio in two groups, trauma and obesity, implying on our hypothesis a relatively greater flux through peripheral than visceral tissues. In trauma this is the opposite of what one would expect, since these subjects should be producing in the liver large amounts of acute-phase proteins (e.g. Preston *et al.* 1995). Perhaps this result indicates enhanced activity of the immune system throughout the body. There are no studies on subjects with infection rather than trauma.

The finding of a high flux ratio in obese patients is surprising. The difference from the mean in normal subjects with single or multiple oral dosage in the fed state is highly significant ($t = 3·2$). We have no explanation for this finding, which suggests a previously unrecognized difference in metabolism between normal and obese people, and clearly deserves further study.

The precursor and end-product methods are complementary; the choice between them will depend on the purpose of the exercise and on practical considerations. There are some results that cannot be obtained by the end-product method, such as consecutive fasting/feeding measurements or studies of C balance, both of which are major contributions of Young's group at Massachusetts Institute of Technology. On the other hand, the end-product

Table 8. Flux ratio (Q_A/Q_U) measured by [^{15}N]glycine and the end-product method in normal healthy adults

	Method		n*	N†	$Q_A/Q_U \times 100$		Reference§
					Mean‡	Between-group SD‡	
A	Oral, single dose	Fed	20	122	70·4	20·4	1–16
B	Oral, single dose	Fasted	6	47	108·9	19·8	6, 10, 13, 17, 18
C	Oral, multiple dose	Fed	6	48	84·2	18·6	3, 5, 19–22
D	Oral, multiple dose	Fasted	No studies	–	–	–	
E	Intravenous, single dose	Fed	5	16	64·0	9·9	7, 8, 10, 23
F	Intravenous, single dose	Fasted	5	36	58·5	11·0	10, 24–26
G	Constant infusion	Fed	3	9	70·5	5·0	27, 28
H	Constant infusion	Fasted	No studies	–	–	–	

* n = number of groups included.
† N = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ References: 1 Marchini *et al.* (1996); 2, Soares *et al.* (1991); 3, Jackson *et al.* (2000); 4, Gausseres *et al.* (1997); 5, Grove & Jackson (1995); 6, McNurlan *et al.* (1987); 7, Fern *et al.* (1985b); 8, Fern *et al.* (1984); 9, Fern & Garlick (1983); 10, Fern *et al.* (1981); 11, Soares *et al.* (1994); 12, Acheson *et al.* (1995); 13, Robinson *et al.* (1990); 14, Willommet *et al.* (1992); 15, Stein *et al.* (1996); 16, Stein *et al.* (1990); 17, Conway *et al.* (1995); 18, Bos *et al.* (2000); 19, Badaloo *et al.* (1989); 20, de Benoist *et al.* (1985); 21, Jackson *et al.* (1997); 22, Jackson *et al.* (1987); 23, Pacy *et al.* (1994); 24, Bettany *et al.* (1996); 25, Ang *et al.* (1995); 26, Nissim *et al.* (1983); 27, Jeevanandam *et al.* (1986); 28, Ma & Jiang (1990).

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https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press

Table 9. Flux ratio (Q_A/Q_U) measured by [^{15}N]glycine and the end-product method in various physiological and pathological states

	*n**	*N*†	$Q_A/Q_U \times 100$ Mean‡	Between-group SD‡	Reference§
Oral – single or multiple dose, fed state					
Premature infants	5	52	62·7	14·6	1–3
Children					
Malnourished	2	20	65·5	8·7	4, 5
Recovered from malnutrition‖	4	69	80·8	14·5	4–6
Obese on habitual diet¶	1	16	97·0	**	7
Obese after 2 weeks on weight-reducing diet¶	1	16	177·1	**	7
Adults					
Pregnant women, all stages	8	97	61·1	12·2	8–10
Elderly	2	21	88·9	12·3	11, 12
Undernourished, males	4	21	60·1	0·9	13–15
Undernourished, females	2	14	86·5	6·5	15
Undernourished, elderly	2	14	81·0	5·2	16
Obese on habitual diet	2	14	80·8	37·8	17, 18
After surgery	1	11	121·7	**	19
With cancer	1	11	114·5	**	19
With AIDS	1	9	34·6	**	20
Intravenous – single dose or constant infusion, fasting or TPN					
Adults					
After surgery	7	50	108·0	25·2	21–24
After multiple trauma	2	16	94·0	0	25
With gastrointestinal disease on TPN	4	24	111·7	24·7	26, 27
With cancer	6	75	73·4	12·0	21, 28

TPN, total parenteral nutrition.
* *n* = number of groups included.
† *N* = number of individuals/results included.
‡ Mean and SD are weighted for study size.
§ References: 1, Van Goudoever *et al.* (1995); 2, Pencharz *et al.* (1989); 3, Catzeflis *et al.* (1985); 4, Waterlow *et al.* (1978*b*); 5, Golden & Golden (1992); 6, Jackson *et al.* (1983); 7, Pencharz *et al.* (1988); 8, de Benoist *et al.* (1985); 9, Willommet *et al.* (1992); 10, Duggleby (1999); 11, Arnal *et al.* (1999); 12, Golden & Waterlow (1977); 13, Soares *et al.* (1991); 14, Soares *et al.* (1994); 15, Vaisman *et al.* (1992); 16, Bos *et al.* (2000); 17, Wolman *et al.* (1985); 18, Stein *et al.* (1991); 19, Glass *et al.* (1983); 20, Stein *et al.* (1990); 21, Yoshida *et al.* (1996); 22, Ma & Jiang (1990); 23, Taggart *et al.* (1991); 24, Powell-Tuck *et al.* (1984); 25, Jeevanandam *et al.* (1991*a*); 26, Glynn et al. (1987); 27, Powell-Tuck & Glynn (1985); 28, Fearon *et al.* (1988).
‖ In one study (Jackson *et al.* 1983) flux ratio was 81 in children given 1·7 g protein/kg per d and was 47 in those given 0·7 g protein/kg per d.
¶ In this study protein intake was 1·8 and 2·3 g protein/kg per d for habitual and weight-reducing diets, respectively
** Not possible to calculate an SD or SD not reported in paper.

method may be the one of choice for population studies aimed at determining whole-body rates of protein synthesis and their functional correlates in genetically or environmentally different groups. We hoped also that the flux ratio would give interesting information about the partition of protein metabolism between peripheral and visceral tissues. This hope has been only partially fulfilled, but the results have raised some points for further investigation.

The practical pros and cons of the two methods are summarized in Table 10. There is a powerful argument for using only ammonia as end product, particularly for field studies, since no blood samples are needed. A good example of the information that can be obtained with ammonia alone is a study by Tomkins *et al.* (1983) in Nigeria, which showed well-defined differences in whole-body protein synthesis and breakdown in children with varying degrees of infection and malnutrition. Another, more academic, advantage of ammonia

Table 10. Pros and cons of precursor and end-product methods

	Precursor method	End-product method
Invasiveness		
Blood	Two in-dwelling cannulae, for infusion and blood samples	For SD, two blood samples at beginning and end, unless urine sample collected between 9 and 24 h
	Multiple samples at plateau	For MD, no samples
Urine	No samples	For SD, two samples; collection must be complete
Breath	Continuous or multiple sampling of CO_2 output at plateau	No samples needed
Freedom of movement	Restricted	Unrestricted except for urine collection
Equipment	Complex: GC–MS	Relatively simple: isotope ratio MS
Cost	High	Relatively low
Facilities needed	Metabolic ward	Can be free-living
Optimum groups for study	Small groups: basic research	Larger groups: population studies

SD, single dose method; MD, multiple dose method.

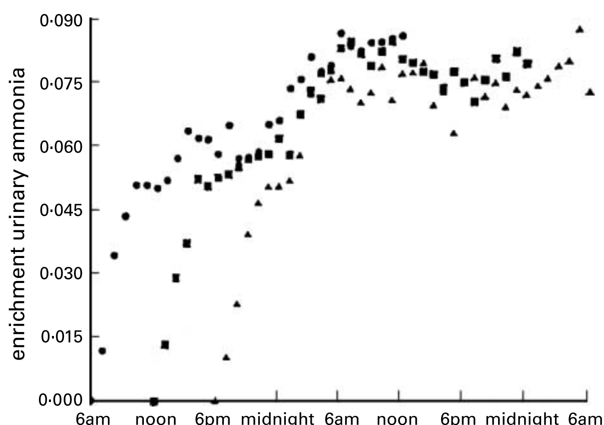

Fig. 4. Oral doses of [^{15}N]glycine were given as a tracer, hourly for 36 h, to normal adults who were consuming hourly feeds, and the enrichment in urinary ammonia was measured. The studies were started at either 06.00 hours in six subjects (●), at 12.00 h in five subjects (■); or at 18.00 h in two subjects (▲). Values are the mean for each time point. (From Jackson *et al.* 1997)

as end product is that its enrichment may undergo rapid and short-term changes of a kind that cannot be observed with urea. In a study in which hourly feeds and [^{15}N]glycine were given for 36 h a step-like pattern was observed in which a plateau of ammonia enrichment occurred about 4 h after the start of feeding, followed by a second plateau during the night, suggesting a circadian process. The second plateau could be caused by increased breakdown of labelled protein during the night. Indications of such a step could be seen in figures reproduced by other authors (Catzeflis *et al.* 1985; Jeevanandam *et al.* 1985).

No combination of pools turning over by first order kinetics could produce such a step. It was therefore suggested that it resulted from a pool of protein that turned over by life-cycle kinetics. Such a system, modelled by computer, produced curves that agreed quite well with those observed experimentally (Slevin *et al.* 1991). It seemed possible that the life-cycle pool might consist of protein of the villous cells of the gastrointestinal mucosa. Its turnover could not be related in any simple way to feeding, since food was infused throughout the experiment. In a further study of this effect, subjects received hourly feeds + tracer for 36 h (Jackson *et al.* 1997). They were divided into three groups, starting the experiment at 06.00 hours, noon or 18.00 hours. Enrichment of ammonia reached a plateau in about 4 h from the start; then at about midnight, regardless of the time at which the experiment started, the enrichment began to rise to a second plateau, which was achieved in about 4 h and maintained until the evening. At the second plateau the calculated flux was 71 % of that at the first plateau (Fig. 4).

This pattern suggests a circadian rhythm in which the first phase lasts from about 06.00 hours to 18.00 hours and the second from 18.00 hours to 06.00 hours. In the second phase there is an increase in the enrichment of ammonia, which could arise in several ways: decreased breakdown of poorly labelled body protein or increased breakdown of highly labelled protein, as observed in the evidence for recycling (q.v.), where very rapid turnover of even a small part of the total protein pool can have a significant effect on labelling of the precurser (K Slevin and JC Waterlow, unpublished results). This breakdown could be occurring in a pool turning over by lifetime kinetics. There is not the evidence to decide between these possibilities. It would be interesting to see if a similar pattern was found with a 24 h infusion of leucine in which feeding was continued throughout.

Circadian rhythms have been described in the secretion of some proteins by the liver (Marckmann *et al.* 1993) and by the exocrine pancreas (Maouyo *et al.* 1993) and in the turnover of bone (Eastell *et al.* 1992). This is a subject that deserves further investigation.

Conclusion

Many problems remain about the metabolic basis of the end-product method. It is difficult to find a consistent explanation for some of the results in Table 1: the differences between the estimates of flux by the oral and IV routes and the differences in flux ratio between different amino acids and routes of tracer dosage. One general point may be relevant: the first five amino acids in Table 1 are non-essential and therefore undergo *de novo* synthesis, which is not the case for the amino acids used as tracers in the precursor method. As explained earlier, *de novo* synthesis of the tracer amino acid will not affect the estimate of protein synthesis, but the location of *de novo* synthesis, central or peripheral, will have a profound effect on the flux ratio. We believe that these unexplained findings, derived from a single unique experiment, should not undermine confidence in the empirical usefulness of the method, but rather should stimulate further research.

At the end of the day the question of the validity of the results for whole-body protein turnover by either method is still an open one. Therefore we have to rely on the reasonable agreement between the two sets of results, based on different assumptions, that they are both measurements of a real biological function – whole-body protein synthesis.

We dissent strongly from the view expressed by Bier (1989) in an important review: '…the approach of "validating" nitrogen flux calculations against related values derived from "primary" and "reciprocal" pool methods is unconvincing, since…protein inflow and outflow rates do not always agree with protein kinetic parameters obtained from rigorous solution of a more comprehensive model'. There can never be a fully comprehensive model of a system as complicated as the whole body; in the same paper Bier refers to '…a structurally and mathematically correct model as a reference…'. Such a 'correct' model is a chimera; one can but proceed by successive approximations, and when two approximations based on different assumptions agree reasonably well, that is one step forward.

Leaving aside the philosophical problem of defining what is true, we argue that the end-product method, applied in a strict and standardized way, provides a reasonable estimate of whole-body protein turnover. We hope to see it measured in the future in as wide a range of subjects as metabolic rate has been: in different ethnic groups, in old and young, in fat and thin people, tall and short, active and inactive. Protein turnover is a basic biological function and we need to know more about it.

References

Acheson KJ, Decombaz J, Piguet-Welsch C, Montigon F, Decarli B, Bartholdi I & Fern EB (1995) Energy, protein and substrate metabolism in simulated microgravity. *Am J Physiol* **269**, R252–R260.

https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press

https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press

Ang B, Halliday D & Powell-Tuck J (1995) Whole-body protein-turnover in response to hyperinsulinemia in humans postabsorptively with [N-15] glycine as tracer. *Am J Clin Nutr* **61**, 1062–1066.

Äqvist SEG (1951) Metabolic interrelationships among amino acids studies with isotopic nitrogen. *Acta Med Scand* **5**, 1046–1064.

Arnal MA, Mosoni L, Boirie Y, *et al.* (1999) Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr* **69**, 1202–1208.

Badaloo A, Jackson AA & Jahoor F (1989) Whole body protein turnover and resting metabolic rate in homozygous sickle cell disease. *Clin Sci (Lond)* **77**, 93–97.

Bettany GEA, Ang BC, Georgiannos SN, Halliday D & Powell-Tuck J (1996) Bed rest decreases whole-body protein turnover in post-absorptive man. *Clin Sci (Lond)* **90**, 73–75.

Bier DM (1989) Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. *Diabetes Metab Rev* **5**, 111–132.

Bier DM & Matthews DE (1982) Stable isotope methods for *in vivo* investigations. *Fed Proc* **41**, 2679–2685.

Binnerts A, Swart GR, Wilson JHP, Hoogerbrugge N, Pols HAP, Birkenhager JC & Lamberts SWJ (1992) The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. *Clin Endocrinol (Oxf)* **37**, 79–87.

Bonau RA, Ang SD, Jeevanandam M & Daly JM (1984) High branched chain amino acid solutions: relationship of composition to efficacy. *JPEN J Parenter Enteral Nutr* **8**, 622–627.

Bos C, Benamouzig R, Bruhat A, Roux C, Mahe S, Valensi P, Gaudichon C, Ferriere F, Rautureau J & Tome D (2000) Short-term protein and energy supplementation activates nitrogen kinetics and accretion in poorly nourished elderly subjects. *Am J Clin Nutr* **71**, 1129–1137.

Catzeflis C, Schütz Y, Micheli JL, Welsch C, Arnaud MJ & Jéquier E (1985) Whole body protein synthesis and energy expenditure in very low birth weight infants. *Pediatr Res* **19**, 679–687.

Conway JM, Thorp JW, Stein TP, Seale JL & Rumpler WV (1995) Decreased protein synthesis during dry saturation diving. *Undersea Hyperb Med* **22**, 219–227.

de Benoist B, Jackson AA, Hill JS & Persaud C (1985) Whole-body protein turnover in Jamaican women during normal pregnancy. *Hum Nutr Clin Nutr* **39**, 167–179.

Dietz WH, Wolfe MH & Wolfe RB (1982) A method for the rapid determination of protein turnover. *Metab Clin Exp* **31**, 749–754.

Dresler CM, Jeevanandam M & Brennan MF (1987) Metabolic efficacy of enteral feeding in cancer and non-cancer patients. *Metabolism* **36**, 82–88.

Duggleby SL (1999) Protein turnover and urea kinetics during pregnancy, maternal body composition and fetal growth. PhD Thesis, University of Southampton.

Duggleby SL & Jackson AA (2001) Relationship of maternal protein turnover and lean body mass during pregnancy and birth length. *Clin Sci (Lond)* **101**, 65–72.

Duggleby SL & Jackson AA (2002) Protein, amino acid and nitrogen metabolism during pregnancy: how might the mother meet the needs of her fetus. *Curr Opin Clin Nutr Metab Care* **5**, 503–509.

Eastell R, Simmons PS, Colwell A, Assiri MA, Burritt MF, Russell RGG & Riggs BL (1992) Nyctohemeral changes in bone turnover assessed by serum bone Gla-protein concentration and urinary deoxypyridinoline excretion: effects of growth and ageing. *Clin Sci (Lond)* **83**, 375–382.

Fearon KC, Hansell DT, Preston T, Plumb JA, Davies J, Shapiro D, Shenkin A, Calman KC & Burns HJ (1988) Influence of whole body protein turnover rate on resting energy expenditure in patients with cancer. *Cancer Res* **48**, 2590–2595.

Fern EB & Garlick PJ (1983) The rate of nitrogen metabolism in the whole body of man measured with [^{15}N]-glycine and uniformly labelled [^{15}N]-wheat. *Hum Nutr Clin Nutr* **37**C, 91–107.

Fern EB, Garlick PJ, McNurlan MA & Waterlow JC (1981) The excretion of isotope in urea and ammonia for estimating protein turnover in man with [^{15}N]glycine. *Clin Sci (Lond)* **61**, 217–228.

Fern EB, Garlick PJ, Sheppard HG & Fern M (1984) The precision of measuring the rate of whole body nitrogen flux and protein synthesis in man with a single dose of [^{15}N]-glycine. *Hum Nutr Clin Nutr* **38C**, 63–73.

Fern EB, Garlick PJ & Waterlow JC (1985*a*) The concept of the single body pool of metabolic nitrogen in determining the rate of whole-body nitrogen turnover. *Hum Nutr Clin Nutr* **39**C 85–99.

Fern EB, Garlick PJ & Waterlow JC (1985*b*) Apparent compartmentation of body nitrogen in one human subject: its consequences in measuring the rate of whole-body synthesis with ^{15}N. *Clin Sci (Lond)* **68**, 271–282.

Garlick PJ & Fern EB (1985) Whole body protein turnover: theoretical consideration. In *Substrate and Energy Metabolism.* [JS Garrow and D Halliday, editors]. London: John Libbey.

Gausseres N, Catala I, Mahe S, Luengo C, Bornet F, Guy-Grand B & Tome D (1997) Whole-body protein turnover in humans fed a soy protein-rich vegetable diet. *Eur J Clin Nutr* **51**, 308–311.

Glass RE, Fern EB & Garlick PJ (1983) Whole-body protein turnover before and after resection of colorectal tumours. *Clin Sci (Lond)* **64**, 101–108.

Glynn MJ, Metzner S, Halliday D & Powell-Tuck J (1987) Whole body protein metabolism in parenterally fed patients. Glucose versus fat as the predominant energy source. *Clin Nutr* **6**, 91–96.

Glynn MJ, Powell-Tuck J & Halliday D (1988) Reproducibility of whole body protein turnover measurements in an ‘ideal’ metabolic subject. *Eur J Clin Nutr* **42**, 273–275.

Golden BE & Golden MH (1992) Effect of zinc on lean tissue synthesis during recovery from malnutrition. *Eur J Clin Nutr* **46**, 697–706.

Golden MHN & Waterlow JC (1977) Total protein synthesis in elderly people: a comparison of results with [^{15}N]glycine and [^{14}C]leucine. *Clin Sci Mol Med* **53**, 277–288.

Grove G & Jackson AA (1995) Measurements of protein turnover in normal men using the end-product method with oral [^{15}N]-glycine: comparison of single dose and intermittent dose regimes. *Br J Nutr* **74**, 491–507.

Jackson AA, Duggleby SL & Grove G (2000) Whole body protein turnover can be measured non-invasively in women using the end product method with (^{15}N) glycine to show changes with the menstrual cycle and pregnancy. *Eur J Clin Nutr* **54**, 329–336.

Jackson AA & Golden MHN (1980) [^{15}N]Glycine metabolism in normal man: the metabolic α-amino-nitrogen pool. *Clin Sci (Lond)* **58**, 577–582.

Jackson AA, Golden MH, Byfield R, Jahoor F, Royes J & Soutter L (1983) Whole-body protein turnover and nitrogen balance in young children at intakes of protein and energy in the region of maintenance. *Hum Nutr Clin Nutr* **37**, 433–446.

Jackson AA, Persaud C, Badaloo V & de Benoist B (1987) Whole-body protein turnover in man determined in three hours with oral or intravenous ^{15}N-glycine and enrichment in urinary ammonia. *Hum Nutr Clin Nutr* **41**, 263–276.

Jackson AA, Shaw JC, Barber A & Golden MH (1981) Nitrogen metabolism in preterm infants fed human donor breast milk: the possible essentiality of glycine. *Pediatr Res* **15**, 1454–1461.

Jackson AA, Soares MJ, Grove G & Waterlow JC (1997) Enrichment in urinary ammonia and urea with hourly oral doses of [^{15}N]glycine; evidence for a step function and a circadian rhythm in protein turnover. *Clin Sci (Lond)* **93**, 265–271.

Jeevanandam M, Brennan MF, Horowitz GD, Rose D, Mihranian MF, Daly J & Lowry SF (1985) Tracer priming in human protein studies with [^{15}N]glycine. *Biochem Med* **34**, 214–225.

Jeevanandam M, Horowitz GD, Lowry SF & Brennan MF (1984) Cancer cachexia and protein metabolism. *Lancet* **1**, 1423–1426.

Jeevanandam M, Legaspi A, Lowry SF, Horowitz GD & Brennan MF (1988) Effect of total parenteral nutrition on whole body protein kinetics in cachetic patients with benign or malignant disease. *JPEN J Parenter Enteral Nutr* **12**, 229–236.

https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press

Jeevanandam M, Leland D, Shamos RF, Casano SF & Schiller WR (1991*a*) Glucose infusion improves endogenous protein synthesis efficiency in multiple trauma victims. *Metabolism* **40**, 1199–1206.
Jeevanandam M, Lowry SF & Brennan MF (1987) Effect of the route of nutrient administration on whole-body protein kinetics in man. *Metabolism* **36**, 968–973.
Jeevanandam M, Lowry SF & Horowitz GD (1986) Influencing dietary intake on whole body protein kinetics in normal man. *Clin Nutr* **5**, 41–48.
Jeevanandam M, Petersen SR & Shamos RF (1993) Protein and glucose fuel kinetics and hormonal changes in elderly trauma patients. *Metabolism* **42**, 1255–1262.
Jeevanandam M, Shamos RF & Petersen SR (1992) Substrate efficacy in early nutrition support of critically ill multiple trauma victims. *JPEN J Parenter Enteral Nutr* **16**, 511–520.
Jeevanandam M, Young DH & Schiller WR (1989) Endogenous protein-synthesis efficiency in trauma victims. *Metabolism* **38**, 967–973.
Jeevanandam M, Young DH & Schiller WR (1991*b*) Obesity and the metabolic response to severe multiple trauma in man. *J Clin Invest* **87**, 262–269.
Kien CL & Camitta BM (1983) Increased whole-body protein turnover in sick children with newly diagnosed leukaemia or lymphoma. *Cancer Res* **43**, 5586–5592.
Kondrup J, Nielsen K & Juul A (1997) Effect of long-term refeeding on protein metabolism in patients with cirrhosis of the liver. *Br J Nutr* **77**, 197–212.
Lieberman SA, Butterfield GE, Harrison D & Hoffman AR (1994) Anabolic effects of recombinant insulin-like growth factor-1 in cachectic patients with the acquired immunodeficiency syndrome. *J Clin Endocrinol Metab* **78**, 404–410.
Lowry SF, Legaspi A, Jeevanandam M, Horowitz GD, Albert JD & Brennan MF (1986) Body protein kinetics during perioperative intravenous nutritional support. *Surg Gynecol Obstet* **163**, 303–309.
Ma EL & Jiang ZM (1990) Determination of protein turnover changes in perioperative patients by the ^{15}N-glycine constant infusion method. *Proc Chin Acad Med Sci Peking Union Med Coll* **5**, 97–101.
McNurlan MA, McHardy KC, Broom J, Milne E, Fearns LM, Reeds PJ & Garlick PJ (1987) The effect of indomethacin on the response of protein synthesis to feeding in rats and man. *Clin Sci (Lond)* **73**, 69–75.
Maouyo D, Sarfati P, Guan D, Morisset J & Adelson JN (1993) Circadian rhythm of exocrine pancreatic secretion in rats: major and minor cycles. *Am J Physiol* **264**, G792–G800.
Marchini JS, Moreira EAM, Moreira MZ, Hiramatsu T, de Oliveira JED & Vannucchi H (1996) Whole-body protein metabolism turnover in men on a high or low calorie rice and bean Brazilian diet. *Nutr Res* **16**, 435–441.
Marckman P, Sanstrom B & Jesperson J (1993) Dietary effects on circulation fluctuations in human blood coagulation factor VII and fibrinolysis. *Atherosclerosis* **101**, 225–234.
Matthews DE, Conway JM, Young VR & Bier DM (1981) Glycine nitrogen metabolism in man. *Metabolism* **30**, 886–893.
Mihranian MH, Daly JM, Ang SD, Jeevanandam M & Brennan MF (1983) Whole-body protein-turnover, synthesis, and catabolism in surgical patients receiving branched-chain amino-acid enriched solutions. *Surg Forum* **34**, 80–84.
Nissim I, Yuskoff M & Segal S (1983) A model for determination of total body protein synthesis based upon compartmental analysis of the plasma [^{15}N]glycine decay curve. *Metabolism* **32**, 646–653.
Pacy PJ, Price GM, Halliday D, Quevedo MR & Millward DJ (1994) Nitrogen homoeostasis in man: the diurnal responses of protein synthesis and degradation and amino acid oxidation to diets with increasing protein intakes. *Clin Sci (Lond)* **86**, 103–118.
Pannemans DLE, Halliday D & Westerterp KR (1995) Whole body protein turnover in elderly men and women: responses to two protein intakes. *Am J Clin Nutr* **61**, 33–38.
Pannemans DLE, Wagenmakers AJM, Westerterp KR, Schaafsma G & Halliday D (1997) The effect of an increase of protein intake on whole-body protein turnover in elderly women is tracer dependent. *J Nutr* **127**, 1788–1794.
Pencharz PB, Clarke R, Archibald EH & Vaisman N (1988) The effect of a weight-reducing diet on the nitrogen metabolism of obese adolescents. *Can J Physiol Pharmacol* **66**, 1469–1474.
Pencharz PB, Clarke R, Papageorgiou A & Farri L (1989) A reappraisal of protein turnover values in neonates fed human milk or formula. *Can J Physiol Pharmacol* **67**, 282–286.
Petersen SR, Holaday NJ & Jeevanandam M (1994) Enhancement of protein synthesis efficiency in parentally fed trauma victims by adjuvant recombinant human growth hormone. *J Trauma* **36**, 726–733.
Petersen SR, Jeevanandam M & Harrington T (1993) Is the metabolic response to injury different with or without severe head injury? Significance of plasma glutamine levels. *J Trauma* **34**, 653–660.
Picou D & Taylor-Roberts T (1969) The measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amounts of dietary protein. *Clin Sci (Lond)* **36**, 283–296.
Pitts RF & Pilkington LA (1966) The relation between plasma concentrations of glutamine and glycine and utilization of their nitrogen as sources of urinary ammonia. *J Clin Invest* **45**, 86–93.
Powell-Tuck J, Fern EB, Garlick PJ & Waterlow JC (1984) The effect of surgical trauma and insulin on whole-body protein turnover in parenterally-fed undernourished patients. *Hum Nutr Clin Nutr* **38**, 11–22.
Powell-Tuck J & Glynn MJ (1985) The effect of insulin infusion on whole-body protein metabolism in patients with gastrointestinal disease fed parenterally. *Hum Nutr Clin Nutr* **39**, 181–191.
Preston T, Fearon KCH, McMillan DC, Winstanley FP, Slater C, Shenkin A & Carter DC (1995) Effect of ibuprofen on the acute-phase response and protein metabolism in patients with cancer and weight loss. *Br J Surg* **82**, 229–234.
Read WWC, McLaren DS & Tchalian M (1971) Urinary excretion of nitrogen from ^{15}N-labelled amino acids in the malnourished and recovered child. 1. Glycine and lysine. *Clin Sci (Lond)* **40**, 375–380.
Read WWC, McLaren DS & Tchalian M (1972) Urinary excretion of nitrogen from (^{15}N) valine, (^{15}N) leucine and (^{15}N) isoleucine in malnourished and recovered children. *Clin Sci (Lond)* **42**, 139–143.
Reeds PJ & Harris CI (1981) Protein turnover in animals: man in his context. In *Nitrogen Metabolism in Man*, pp. 391–408 [JC Waterlow and JML Stephen, editors]. London: Applied Science Publishers.
Richards EW, Long CL, Nelson KM, Tohver OK, Pinkston JA, Navari RM & Blakemore WS (1993) Protein turnover in advanced lung cancer patients. *Metabolism* **42**, 291–296.
Robinson SM, Jaccard C, Persaud C, Jackson AA, Jequier E & Schutz Y (1990) Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men. *Am J Clin Nutr* **52**, 72–80.
Slevin K, Jackson AA & Waterlow JC (1991) A model for the measurement of whole body protein turnover incorporating a protein pool with life-time kinetics. *Proc R Soc Lond* series *B*, **243**, 81–92.
Soares MJ, Piers LS, Shetty PS, Jackson AA & Waterlow JC (1994) Whole body protein turnover in chronically undernourished individuals. *Clin Sci (Lond)* **86**, 441–446.
Soares MJ, Piers LS, Shetty PS, Robinson S, Jackson AA & Waterlow JC (1991) Basal metabolic rate, body composition and whole body protein turnover in Indian men with different nutritional status. *Clin Sci (Lond)* **81**, 419–425.
Sprinson DB & Rittenberg D (1949) The rate of interaction of the amino acids of the diet with the tissue proteins. *J Biol Chem* **180**, 715–726.
Stein TP, Leskiw MJ & Schluter MD (1996) Diet and nitrogen metabolism during spaceflight on the shuttle. *J Appl Physiol* **81**, 82–97.
Stein TP, Nutinsky D, Condoluce D, Schluter MD & Leskiw MJ (1990) Protein and energy substrate metabolism in AIDS patients. *Metabolism* **39**, 876–881.
Stein TP, Pumpler WV, Leskiw MJ, Schluter MD, Staples R & Bodwell CE (1991) Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man. *Metab Clin Exp* **40**, 478–483.

Stroud MA, Jackson AA & Waterlow JC (1996) Protein turnover rates of two human subjects during an unassisted crossing of Antarctica. *Br J Nutr* **76**, 165–174.

Swart GR, Van den Berg JW, Wattimena JL, Rietveld T, van Vuure JK & Frenkel M (1988) Elevated protein requirements in cirrhosis of the liver investigated by whole body protein turnover studies. *Clin Sci (Lond)* **75**, 101–107.

Taggart DP, McMillan DC, Preston T, Shenkin A, Wheatley DJ & Burns HJG (1991) Effect of surgical injury and intraoperative hypothermia on whole-body protein-metabolism. *Am J Physiol* **260**, E118–E125.

Taruvinga M, Jackson AA & Golden MHN (1979) Comparison of ^{15}N-labelled glycine, aspartate, valine and leucine for measurement of whole-body protein turnover. *Clin Sci (Lond)* **57**, 281–283.

Tomkins AM, Garlick PJ, Schofield WN & Waterlow JC (1983) The combined effects of infection and malnutrition in children. *Clin Sci (Lond)* **65**, 313–324.

Tracey KJ, Legaspi A, Albert JD, Jeevanandam M, Matthews DE, Brennan MF & Lowry SF (1988) Protein and substrate metabolism during starvation and parenteral refeeding. *Clin Sci (Lond)* **74**, 123–132.

Vaisman N, Clarke R, Rossi M, Goldberg E, Zello GA & Pencharz PB (1992) Protein turnover and resting energy expenditure in patients with undernutrition and chronic lung disease. *Am J Clin Nutr* **55**, 63–69.

Vaisman N, Stallings VA, Chan H, Weitzman SS, Clarke R & Pencharz PB (1993) Effect of chemotherapy on the energy and protein-metabolism of children near the end of treatment for acute lymphoblastic-leukemia. *Am J Clin Nutr* **57**, 679–684.

Van Goudoever JB, Sulkers EJ, Halliday D, Degenhart HJ, Carnielli VP, Wattimena JL & Sauer PJ (1995) Whole-body protein turnover in preterm appropriate for gestational age and small for gestational age infants: comparison of [^{15}N]glycine and [1-^{13}C]leucine administered simultaneously. *Pediatr Res* **37**, 381–388.

Waterlow JC (In press) *Protein Turnover in Man*. Wallingford: CABI Publishing.

Waterlow JC, Garlick PJ & Millward DJ (1978*a*) *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: North-Holland.

Waterlow JC, Golden MHN & Garlick PS (1978*b*) Protein turnover in man measured with ^{15}N: comparison of end products and dose regimes. *Am J Physiol* **235**, E165–E174.

Watson PE, Watson TD & Ball R (1980) Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr* **33**, 27–39.

Willommet L, Schutz Y, Whitehead R, Jequier E & Fern EB (1992) Whole body protein metabolism and resting energy expenditure in pregnant Gambian women. *Am J Physiol* **263**, E624–E631.

Wolman SI, Sheppard H, Fern M & Waterlow JC (1985) The effect of tri-iodothyronine (T_3) on protein turnover and metabolic rate. *Int J Obes* **8**, 459–463.

Yoshida S, Noake T, Tanaka Y, Ishibashi N, Shirouzu Y, Shirouzu K & Kakegawat Stein TP (1996) Effect of fentanyl citrate anesthesia on protein turnover in patients with esophagectomy. *J Surg Res* **64**, 120–127.

Zillikens MC, Van den Berg JW, Wattimena JL, Rietveld T & Swart GR (1993) Nocturnal oral glucose supplementation. The effects on protein metabolism in cirrhotic patients and in healthy controls. *J Hepatol* **17**, 377–383.

Zilversmit DB (1960) The design and analysis of isotope experiments. *Am J Med* **29**, 832–848.

https://doi.org/10.1079/BJN20051460 Published online by Cambridge University Press