

# The effect of an increase of protein intake on whole-body protein turnover in elderly women is tracer dependent.

Citation for published version (APA):

Pannemans, D. L. E., Wagenmakers, A. J. M., Westerterp, K. R., Schaafsma, G., & Halliday, D. (1997). The effect of an increase of protein intake on whole-body protein turnover in elderly women is tracer dependent. *Journal of Nutrition*, 127(9), 1788-1794. <https://doi.org/10.1093/jn/127.9.1788>

## Document status and date:

Published: 01/01/1997

## DOI:

[10.1093/jn/127.9.1788](https://doi.org/10.1093/jn/127.9.1788)

## Document Version:

Publisher's PDF, also known as Version of record

## Document license:

Taverne

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

## The Effect of an Increase of Protein Intake on Whole-Body Protein Turnover in Elderly Women Is Tracer Dependent<sup>1</sup>

Daphne L. E. Pannemans, Anton J. M. Wagenmakers,<sup>2</sup> Klaas R. Westerterp, Gertjan Schaafsma\* and Dave Halliday

Department of Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands and

\*Department of Human Nutrition, TNO Toxicology and Nutrition Institute, 3700 MD Zeist, The Netherlands

**ABSTRACT** To compare the response of whole-body protein turnover with variations in dietary protein level, whole-body protein turnover was measured by different stable isotope methods in six elderly women ( $69 \pm 5$  y) consuming two levels of protein (10 and 20% of total energy, diets A and B, respectively). Protein turnover was measured during 12 h of overnight fasting with  $^{15}\text{N}$ -glycine with urea and ammonia as end products. During the last 4 h of the interval, protein turnover was also estimated by L-[1- $^{13}\text{C}$ ]-leucine infusion. Nitrogen balance [diet A,  $-0.040 \pm 0.015$  g/(kg·d); diet B,  $0.002 \pm 0.053$  g/(kg·d); mean  $\pm$  SD] did not differ significantly between the diet periods, although all subjects were in negative nitrogen balance at the end of diet A. Protein breakdown, as measured with  $^{15}\text{N}$ -glycine, did not differ from results obtained using L-[1- $^{13}\text{C}$ ]-leucine, whereas protein synthesis was found to be significantly lower using the former isotope. The  $^{15}\text{N}$ -glycine method indicated that protein turnover (both synthesis and breakdown) was higher in fasting elderly women when they consumed a 20% rather than a 10% protein diet, whereas the L-[1- $^{13}\text{C}$ ]-leucine method did not show significant differences between the diet periods in the last 4 h of the overnight fasting period. However, the relative increase in net protein breakdown when comparing diet B with diet A, was comparable for both tracers. These data indicate that care is needed with the choice of the tracer used in measuring the components of protein turnover in elderly women with the aim of understanding the physiological basis behind the adequacy of the level of protein intake. *J. Nutr.* 127: 1788–1794, 1997.

**KEY WORDS:** • elderly women • protein intake • whole-body protein turnover • nitrogen balance

Stable isotopes have been used extensively for measuring whole-body protein turnover in children (Van Goudoever et al. 1995), young adults (Conway et al. 1980) and elderly subjects (Golden and Waterlow 1977). Methods with  $^{15}\text{N}$ -labeled tracers, e.g.,  $^{15}\text{N}$ -glycine, have been favored for many years because of their noninvasive character. Protein turnover is calculated by measurement of isotope enrichment in end products of protein breakdown in urine. This method assumes that the whole-body free amino acids are present in one or two metabolically active pools from which urea and ammonia are derived. The  $^{15}\text{N}$  label enters the free amino acid pool and will exchange with other amino acids such that the  $^{15}\text{N}$  label can be regarded as a label for the total free amino acid pool. Urea and ammonia, the urinary end products of N metabolism, are derived solely from the free amino acid pool. Therefore their labeling can be taken as representative of that pool. In the case of  $^{15}\text{N}$ -glycine, the dose is usually given by constant nasogastric infusion, repeated oral doses or in one bolus dose.

Recently, precursor methods have been applied to a wide range of patient groups to derive quantitative information re-

garding protein metabolism. In this case, the label is infused intravenously until the plasma amino acid enrichment reaches a constant value. By giving a priming dose, the plateau can be achieved more rapidly. The protein turnover rate is calculated from the tracer dilution in the plasma and the known infusion rate. However, in the case of leucine, the tracer is infused in the plasma and samples are taken from the plasma, whereas leucine is metabolized within the cells. Because the [1- $^{13}\text{C}$ ]-leucine plasma enrichment is not equal to the enrichment within the cells, plasma  $\alpha$ -ketoisocaproate (KIC)<sup>3</sup> is usually considered to reflect more closely the enrichment of the precursor pool (Bruce et al. 1990, Matthews et al. 1982). KIC is formed intracellularly from leucine by transamination and is released to the plasma. In many studies, it has been shown that plasma KIC enrichment is a good reflection of the intracellular enrichment of leucine (e.g., Matthews et al. 1982).

Protein turnover measurement techniques are based on several assumptions (Halliday and Rennie 1982, Millward et al. 1991, Waterlow et al. 1978); each technique has its own limitations as discussed elsewhere (e.g., Garlick and Fern 1984, Halliday and Rennie 1982, Waterlow et al. 1978). Therefore absolute values for protein turnover rates tend to depend on

<sup>1</sup> The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

<sup>2</sup> To whom correspondence should be addressed.

<sup>3</sup> Abbreviations used: en%, energy percentage; FFM, fat-free mass; KIC,  $\alpha$ -ketoisocaproate.

the technique employed. However, it has been shown that there is broad agreement in general among values obtained by different approaches. Reported rates of protein synthesis and protein degradation in young adults obtained with different techniques range from 2 to 5 g/(kg·d) (Conway et al. 1980, Hoffer et al. 1985, Waterlow and Jackson 1981). Acceptable methods to measure protein turnover should not only give similar absolute values but should also reveal similar physiological directional changes in protein turnover whenever they occur (e.g., changes induced by trauma, feeding/fasting or diet of various composition). Garlick et al. (1991) and Young et al. (1987) reviewed the response in protein turnover to differences in protein intake. They concluded that dietary protein affects protein turnover at two levels, i.e., an immediate response to the intake of protein in meals (studies done during the fed state, e.g., Bruce et al. 1990, Garlick et al. 1987, Melville et al. 1989, Young et al. 1987) and a longer-term adaptation after a change in protein intake (studies done in the fed or fasted state after subjects had adapted to a certain level of protein intake, e.g., Conway et al. 1988, Pannemans et al. 1995a and 1995b). Only a few studies have reported on the effect of different levels of protein intake on whole-body protein turnover as measured with different tracers (Motil et al. 1981, Pacy et al. 1994, Quevedo et al. 1994, Yang et al. 1986, Zello et al. 1992), and no such studies have been undertaken in elderly subjects. Previous work of our group revealed a lower protein turnover in elderly women compared with elderly men and young women (Pannemans et al. 1995a and 1995b) at the same protein intake. Therefore we decided to investigate whole-body protein turnover in elderly women in more detail with different tracers. Thus it was of interest to compare the response of whole-body protein turnover with variations in dietary protein level as simultaneously measured with two commonly used tracers, <sup>15</sup>N-glycine and [1-<sup>13</sup>C]-leucine, in the same elderly subjects.

## SUBJECTS AND METHODS

**Subjects.** Six free-living elderly women participated in the study (age, 69 ± 5 y; height, 1.58 ± 0.07 m; weight, 65.9 ± 5.2 kg; fat-free mass, 40.8 ± 1.0 kg; % fat mass, 37.8 ± 4.2). Subjects were recruited with advertisements in the local media and through contacts with alliances for the elderly. All subjects were certified to be in good health by a staff physician. Subjects gave informed consent to participate in the study after the procedures were explained to them. The protocol was approved by the University Ethics Committee.

**Diet.** All subjects consumed two different diets for 2 wk with a wash-out period of at least 4 wk. In diet A, 10.3% of total energy was delivered by protein, 35.8% by fat and 53.9% by carbohydrate. In diet B, 19.6% of total energy was delivered by protein, 28.2% by fat and 52.1% by carbohydrate. Diet components are given in Table 1. To make results of the present study comparable with previously obtained results (Pannemans et al. 1995a and 1995b), we used an isocaloric exchange between protein and mainly fat because this was the easiest way to increase protein intake with commercially available dietary products. For the purpose of the study, three energy levels (7.50, 8.25 and 9.00 MJ/d) were produced. Subjects were placed in the best-fitting energy group according to their estimated energy intake (Pannemans and Westerterp 1993). During the intervention, all meals were provided daily at home. The subjects were not allowed to eat or drink anything else except for water, tea and coffee.

**Body weight and body composition.** Body weight and body composition were measured at the start and at the end of each dietary period. Body weight decreased during the dietary intervention period ( $\Delta$  body weight diet A, -0.5 ± 0.4 kg; diet B, -0.6 ± 0.5 kg in 2 wk). Although subjects lost weight, no effect of this slight negative energy balance was found on protein metabolism. This is in agreement with data from Garlick et al. (1991) who described protein turnover as affected more by protein intake than by energy intake. From this

TABLE 1

### Diet components

| Diet A <sup>1</sup>                  | Diet B                                    |
|--------------------------------------|---|
| Whole-meal bread <sup>2,3,4</sup>    | Whole-meal bread <sup>1,2,3</sup>         |
| Margarine <sup>2,3,4</sup>           | Low fat margarine <sup>1,2,3</sup>        |
| Jam <sup>2,3,4</sup>                 | Jam <sup>2,3</sup>                        |
| Fruit sprinkles <sup>2,3,4</sup>     | Cheese spread 20+ <sup>2,3</sup>          |
| Luncheon meat <sup>2,3,4</sup>       | Luncheon meat <sup>4</sup>                |
| Chocolate sprinkles <sup>2,3,4</sup> | Chocolate sprinkles <sup>2</sup>          |
|                                      | Cheese <sup>1,2,3</sup>                   |
|                                      | Cooked ham <sup>3,4</sup>                 |
|                                      | Chicken breast <sup>3,4</sup>             |
| Orange juice <sup>2,3,4</sup>        | Orange juice <sup>1,2,3</sup>             |
| Soft drink <sup>2,3,4</sup>          | Soft drink <sup>1,2,3</sup>               |
| Powdered milk <sup>2,3,4</sup>       | Evaporated milk <sup>1,2,3</sup>          |
|                                      | Low fat milk <sup>1,2,3</sup>             |
| Biscuit <sup>2,3,4</sup>             | Biscuit <sup>3,4</sup>                    |
| Milk chocolate <sup>3,4</sup>        | Milk chocolate <sup>3,4</sup>             |
| Toast <sup>4</sup>                   | Toast <sup>3</sup>                        |
| Cake <sup>4</sup>                    | Candy bar <sup>1,2,3</sup>                |
| Potato crisps <sup>2</sup>           |   |
| Cookies <sup>2,3,4</sup>             |   |
| Freezer meal <sup>2-5</sup>          | Instant soup based on meat <sup>2,3</sup> |
| Low fat fruit yogurt <sup>4</sup>    | Freezer meal <sup>2-5</sup>               |
|                                      | Low fat fruit yogurt <sup>1,2,3</sup>     |

<sup>1</sup> Diet A contained 10.3% of total energy from protein; diet B contained 19.6% of total energy from protein.

<sup>2</sup> Dietary products given in the energy group of 7.50 MJ.

<sup>3</sup> Dietary products given in the energy group of 8.25 MJ.

<sup>4</sup> Dietary products given in the energy group of 9.00 MJ.

<sup>5</sup> The freezer meal consisted of potatoes, vegetables and meat.

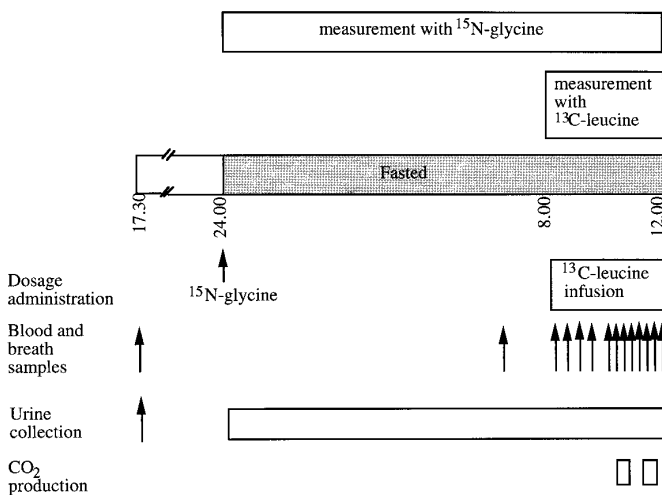
point of view, it is unlikely that the negative energy balance had an effect on the protein turnover data. Body weight, measured simultaneously with protein turnover, was used to express data per kilogram body weight.

Fat-free mass (FFM) was also measured at the start and at the end of each dietary period with deuterium dilution (Van Marken Lichtenbelt et al. 1994) to determine whether muscle mass, e.g., FFM, was lost during these periods. No significant changes in FFM were observed during the dietary intervention period ( $\Delta$  FFM diet A, +0.3 ± 0.9 kg; diet B, +0.1 ± 1.0 kg in 2 wk). Fat-free mass, measured simultaneously with protein turnover, was used to express data per kilogram FFM.

**Protein turnover.** To determine nitrogen balance, the subjects collected 24-h urine for 2 d and total feces for 3 d (during the last week of each diet period). Nitrogen balance was calculated as nitrogen intake minus nitrogen in feces and urine. Each 24-h urine collection started with the second urine in the morning and included the first voiding of the next day. Each daily feces collection started at 0700 h in the morning and continued through 0700 h the next day. The nitrogen content of urine and feces was measured with a Heraeus analyzer (type CHN-O-rapid, Hanau, Germany) and converted to total nitrogen excretion by multiplying with urine volume and fecal weight. Corrections were made for other obligatory losses [8 mg N/kg body weight (FAO/WHO/UNU 1985)].

Whole-body protein turnover was measured with <sup>15</sup>N-glycine given orally (200 mg) and with [1-<sup>13</sup>C]-leucine (99 atom %; Cambridge Isotope Laboratories, Woburn, MA) administered by continuous infusion (see below) on the last day of each diet period.

Rates of protein breakdown and synthesis with the <sup>15</sup>N-glycine method were estimated from the urinary excretion of <sup>15</sup>N in ammonia and urea during the 12 h after tracer administration (Pannemans et al. 1995a). Subjects were not allowed to eat or drink (except water) for at least 6 h before administration of the <sup>15</sup>N-glycine (~1200 h) and during the following 12 h of the study period. Determination of urinary ammonia and urea concentrations, of <sup>15</sup>N enrichment of urinary ammonia and urea and of plasma urea were performed as described earlier (Pannemans et al. 1995a, Read et al. 1982) Calcula-



**FIGURE 1** Experimental design of the protein turnover study in six elderly women. Protein turnover was measured during 12 h of overnight fasting with  $^{15}\text{N}$ -glycine with urea and ammonia as end products. During the last 4 h of the study, protein turnover was also estimated with the use of a  $[1\text{-}^{13}\text{C}]$ -leucine infusion. Blood and expired air were obtained just before the administration of the isotope and at 30-min intervals thereafter during the first 2 h. During the 3rd and the 4th h, blood and expired air samples were collected at 15-min intervals.

tions were done as described by Fern et al. (1981). Rates of whole body protein synthesis and breakdown were derived from the expression  $Q = E + S = I + B$ , where  $E$  is the rate of excretion of total nitrogen in urine,  $S$  is the rate of whole-body protein synthesis,  $I$  is the rate of intake of nitrogen from the diet and  $B$  is the rate of whole-body protein breakdown. Because these studies were conducted in the fasting state, protein breakdown is equal to the calculated flux. A factor of 6.25 was used to convert grams of nitrogen into grams of protein. Whole-body protein turnover was calculated as the harmonic average of the estimates of flux based on urea and ammonia (Fern et al. 1984b).

Whole-body protein turnover was also measured employing a primed continuous infusion of  $[1\text{-}^{13}\text{C}]$ -leucine during the last 4 h of the glycine study (Fig. 1). Subjects reported to the human physiology laboratory at 0700 h, and two venous catheters were inserted with aseptic technique into an antecubital vein of each arm. One catheter was used for withdrawing blood samples, the other for the isotope infusion. A priming dose of  $\text{NaH}^{13}\text{CO}_3$  (0.085 mg/kg) and  $[1\text{-}^{13}\text{C}]$ -leucine (3.8  $\mu\text{mol}/\text{kg}$ ) was administered into the other catheter at 0800 h and immediately followed by the infusion of  $[1\text{-}^{13}\text{C}]$ -leucine [3.6  $\mu\text{mol}/(\text{kg}\cdot\text{h})$ ] at a constant rate (50 mL/h) for 4 h using a calibrated pump (model 561, IVAC, San Diego, CA). Blood and expired air were obtained just before the administration of the isotope and at 30-min intervals thereafter during the first 2 h. During the 3rd and the 4th h, blood and expired air samples were collected at 15-min intervals. Each blood sample was collected with a heparinized syringe and transferred immediately to a tube that was kept on ice. Blood samples were centrifuged at 600 g for 10 min and plasma was stored at  $-20^\circ\text{C}$  until analysis. After derivatization (Wolfe 1992), plasma  $^{13}\text{C}$ -KIC enrichment was measured on a gas chromatography mass spectrometer (Finnigan Incos XL MS with a Varian 3400 GC with 1075 split/splitless injector, Bremen, Germany). Expired air samples were obtained by having the subject breathe normally for 3 min into a 6.75-L mixing chamber. After 3 min, a 20-mL vacutainer was filled with a sample of the mixed expired air.  $^{13}\text{CO}_2$  enrichment in the expired air was measured with a GC continuous flow-IRMS (Finnigan MAT-252). At 3 and 4 h after the start of the infusion, the subject's  $\text{CO}_2$  production rate was determined by means of a computerized open-circuit ventilated hood system.

Calculations of the rate of protein turnover were performed as described by Matthews et al. (1980). In short, leucine turnover is measured from the dilution of  $[1\text{-}^{13}\text{C}]$ -leucine infusion in plasma KIC at isotopic steady state  $Q = i[(E_i/E_p) - 1]$  where  $i$  is the  $[1\text{-}^{13}\text{C}]$ -

leucine infusion rate [ $\mu\text{mol}/(\text{kg}\cdot\text{h})$ ];  $E_i$  is the enrichment of the  $[1\text{-}^{13}\text{C}]$ -leucine infused (MPE);  $E_p$  is the  $[1\text{-}^{13}\text{C}]$ -KIC enrichment in the plasma at steady state (MPE). The rate of leucine oxidation is  $O = F^{13}\text{CO}_2 [(1/E_p) - (1/E_i)] \times 100$ , where  $F^{13}\text{CO}_2$  is the rate of  $^{13}\text{CO}_2$  released by leucine tracer oxidation [ $\mu\text{mol } ^{13}\text{C}/(\text{kg}\cdot\text{h})$ ]. From these calculations, the rate of leucine incorporation into protein (nonoxidative leucine disappearance) is calculated, as described above, where  $Q = S + O = B + I$ . The leucine parameters were converted to corresponding estimates of whole-body protein turnover by multiplying the leucine values by the constant (24 h/d)/(590  $\mu\text{mol}$  leucine/g protein) to give values of g protein/d (Matthews et al. 1980).

**Statistical analyses.** When comparisons were made between diet A and diet B and between the tracers, a Wilcoxon nonparametric test was used. Significance was set at  $P < 0.05$ . Data are given as means  $\pm$  SD. The software program Statview 512+ (Abacus Concepts, Berkeley, CA) was used for the analyses.

## RESULTS

**Nitrogen balance.** Despite the significant differences in the protein content of diets A and B, the nitrogen excretion in urine and feces was not significantly different when subjects ate diet A or diet B nor were there any differences in nitrogen balance between the two diet periods (Table 2). However, during diet A, mean nitrogen balance was negative ( $P < 0.05$ ), whereas mean nitrogen balance did not differ significantly from zero during diet B.

**Protein turnover.** Protein turnover was measured in the postabsorptive state, and, as expected, protein breakdown was significantly higher than protein synthesis independent of the way in which data were presented (g/d, g/kg body weight, g/kg FFM) (Table 3).

As measured with  $^{15}\text{N}$ -glycine, protein turnover (breakdown and synthesis) was significantly greater when subjects consumed 20% of energy (en%) as protein in the diet rather than 10 en% ( $P < 0.05$ ), whereas no differences in protein turnover between the diets were measured with  $[1\text{-}^{13}\text{C}]$ -leucine. Protein oxidation, measured with  $[1\text{-}^{13}\text{C}]$ -leucine, was significantly higher when subjects consumed diet B compared with diet A.

Protein breakdown values measured with  $^{15}\text{N}$ -glycine were not different than those obtained with  $[1\text{-}^{13}\text{C}]$ -leucine when subjects consumed both diets. However, protein synthesis rates were significantly lower when measured with  $^{15}\text{N}$ -glycine than with  $[1\text{-}^{13}\text{C}]$ -leucine (diets A and B). Table 4 shows the protein balance data as derived by both tracers during both diet periods, as calculated from protein synthesis minus protein

**TABLE 2**

*Nitrogen balance data in six elderly women after consuming diets differing in protein content<sup>1</sup>*

|                      | Diet A <sup>2</sup> | Diet B           |
|----------------------|---------------------|------------------|
|                      | g/kg·d              |                  |
| Intake               | 0.13 $\pm$ 0.01*    | 0.20 $\pm$ 0.02  |
| Urine                | 0.13 $\pm$ 0.01     | 0.15 $\pm$ 0.05  |
| Feces                | 0.03 $\pm$ 0.02     | 0.03 $\pm$ 0.02  |
| Balance <sup>3</sup> | -0.04 $\pm$ 0.02    | 0.002 $\pm$ 0.05 |

<sup>1</sup> Values are means  $\pm$  SD.

<sup>2</sup> Diet A contained 10.3% of total energy from protein; diet B contained 19.6% of total energy from protein.

<sup>3</sup> Corrections [8 mg N/(kg·d)] were made for obligatory losses (FAO/WHO/UNU 1985). \*Significantly different than diet B, Wilcoxon test,  $P < 0.05$ .

TABLE 3

Protein turnover as measured with  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine in six elderly fasting women<sup>1</sup>

|   | Diet A          |              | Diet B        |              |
|---|-----------------|--------------|---------------|--------------|
|   | Glycine         | Leucine      | Glycine       | Leucine      |
| <i>g/protein/d</i>                        |                 |              |               |              |
| Breakdown                                 | 175.1 ± 11.4**  | 225.3 ± 40.4 | 242.9 ± 34.8  | 237.7 ± 17.8 |
| Synthesis                                 | 116.6 ± 17.0*** | 201.8 ± 36.2 | 171.2 ± 28.2* | 205.7 ± 14.3 |
| Oxidation                                 | —               | 23.5 ± 5.3** | —             | 32.0 ± 7.8   |
| <i>g protein/(kg · d)</i>                 |                 |              |               |              |
| Breakdown                                 | 2.7 ± 0.4**     | 3.4 ± 0.6    | 3.7 ± 0.7     | 3.6 ± 0.3    |
| Synthesis                                 | 1.8 ± 0.4***    | 3.1 ± 0.5    | 2.6 ± 0.6*    | 3.1 ± 0.2    |
| Oxidation                                 | —               | 0.4 ± 0.1**  | —             | 0.5 ± 0.1    |
| <i>g protein/(kg FFM<sup>2</sup> · d)</i> |                 |              |               |              |
| Breakdown                                 | 4.3 ± 0.4**     | 5.5 ± 0.9    | 6.0 ± 1.0     | 5.8 ± 0.3    |
| Synthesis                                 | 2.9 ± 0.5***    | 4.9 ± 0.8    | 4.2 ± 0.8*    | 5.0 ± 0.2    |
| Oxidation                                 | —               | 0.6 ± 0.1**  | —             | 0.8 ± 0.2    |

<sup>1</sup> Values are means ± SD; diet A contained 10.3% of total energy from protein; diet B contained 19.6% of total energy from protein. \* $P < 0.05$  differences between  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine within each diet (Wilcoxon test); \*\* $P < 0.05$  differences between the diets as measured with the same tracer as tested with a Wilcoxon nonparametric test.

<sup>2</sup> FFM, fat free mass.

breakdown. Because subjects were studied in the postabsorptive state, breakdown was higher than synthesis, resulting in negative net protein balances. Both tracers showed that the absolute difference between breakdown and synthesis was higher during consumption of diet B ( $P < 0.05$ ) compared with diet A, and the absolute difference between breakdown and synthesis was also higher when measured with  $^{15}\text{N}$ -glycine than with  $[1-^{13}\text{C}]$ -leucine. However the relative increase in net balance was similar for both tracers ( $^{15}\text{N}$ -glycine,  $25 \pm 27\%$ ;  $[1-^{13}\text{C}]$ -leucine,  $36 \pm 4\%$ ).

## DISCUSSION

Previous work of our group had revealed a lower protein turnover in elderly women compared with elderly men and young women (Pannemans et al. 1995a and 1995b) when they consumed the same level of protein. We decided therefore to study the effect of the level of protein intake on whole-body protein turnover in elderly women in more detail, with two commonly used labeled amino acids,  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine, to investigate two questions. First, are whole-body protein turnover data as measured with  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine comparable? Second, do both tracer techniques detect comparable changes in protein turnover as induced by a change in dietary protein intake? Before discussing these points, it is necessary to consider the changes in nitrogen balance as a result of changing the protein intake.

**Nitrogen balance.** At the end of each dietary period, the nitrogen balance was determined over 3 d. There were no significant differences in nitrogen excretion via feces and urine nor were differences found in the resulting nitrogen balance. Nevertheless, all subjects were in negative nitrogen balance when they consumed diet A, which provided the recommended intake of 0.8 g protein/kg body weight (FAO/WHO/UNU 1985). These results do not agree with previously obtained data in elderly women (Pannemans et al. 1995a) who were in nitrogen balance when they ate a diet providing the same amount of protein. There were no differences in protein intake ( $0.127 \pm 0.020$  g N/kg body weight in the previous study vs.  $0.125 \pm 0.011$  g N/kg body weight in this study). Because energy intake was slightly lower in the previous study, the energy percentage given as protein was somewhat higher (12 vs. 10 en% in the present study). There were no differences in fecal nitrogen excretion ( $0.021 \pm 0.007$  vs.  $0.033 \pm 0.021$  g N/kg body weight); however, urinary nitrogen excretion was higher in the present study ( $0.098 \pm 0.028$  vs.  $0.125 \pm 0.013$  g N/kg body weight). The difference in nitrogen balance between the studies probably indicates that a protein intake of 0.8 g/kg body weight is near the daily requirement, leading to a net balance in some subjects and a small net loss of nitrogen in others. Urine collections were complete in both studies as indicated by 24-h urinary creatinine excretion values.

In the past, protein requirements of the elderly have been studied extensively. Cheng et al. (1978) determined protein

TABLE 4

Protein balance data as measured with  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine in six fasting elderly women<sup>1</sup>

|                                      | Diet A          |               | Diet B        |             |
|--------------------------------------|-----------------|---------------|---------------|-------------|
|                                      | Glycine         | Leucine       | Glycine       | Leucine     |
| Balance, g/d                         | -58.4 ± 11.7*** | -23.5 ± 5.3** | -71.7 ± 14.3* | -32.0 ± 7.8 |
| Balance, g/(kg · d)                  | -0.9 ± 0.2***   | -0.4 ± 0.1**  | -1.1 ± 0.2*   | -0.5 ± 0.1  |
| Balance, g/(kg FFM <sup>2</sup> · d) | -1.4 ± 0.3***   | -0.6 ± 0.1**  | -1.8 ± 0.4*   | -0.8 ± 0.2  |

<sup>1</sup> Values are means ± SD; protein balance was calculated as synthesis minus breakdown. \* $P < 0.05$  differences between  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine within each diet as (Wilcoxon test); \*\* $P < 0.05$  differences between the diets as measured with the same tracer (Wilcoxon test).

<sup>2</sup> FFM, fat-free mass.

requirements in 15 elderly subjects and recommended a protein intake level of 0.8 g/(kg·d). Uauy et al. (1978) reported the same recommendation for elderly women [0.83 g/(kg·d)], whereas the protein intake for elderly men was established at 0.70–0.85 g/(kg·d). In another study, it was estimated that the protein requirements for elderly men were 0.72 g/(kg·d) (Zanni et al. 1979). Gersovitz et al. (1982) studied the long-term effects of a protein intake of 0.8 g/(kg·d) (as recommended by the FAO) on nitrogen balance in elderly men and women. Because most of the subjects were in negative nitrogen balance, it was concluded that the current recommended protein intake was too low for the majority of subjects  $\geq 65$  y. In the present study, a protein intake of 1.3 g/kg (diet B) resulted in a mean nitrogen balance that did not differ significantly from zero; at this protein intake, the elderly women were in nitrogen balance. It is therefore concluded on the basis of the current study and on retrospective nitrogen balance data that a safe protein intake for elderly adults would probably be higher than 0.8 g/(kg·d) because this amount may not always be sufficient to reach nitrogen balance, especially not in the frail elderly who have a low energy intake, low energy expenditure, low body weight and low physical activity levels.

**Are whole-body protein turnover data as measured with both tracers comparable?** Using  $^{15}\text{N}$ -glycine as a tracer, protein synthesis and degradation can be calculated by measurement of isotope enrichment in the urinary end products urea and ammonia. As described previously (Fern and Garlick 1983, Waterlow et al. 1978), both end products gave different results, with urea always giving higher values than ammonia (not shown). The difference between the end products implies anatomical compartmentation, and it was suggested that the average of the two flux rates given by ammonia and urea would give the best estimate for whole-body protein turnover. Therefore the harmonic average of both end products was taken as the basis for this calculation. When comparing this average with the  $[1-^{13}\text{C}]$ -leucine data, it is seen that both methods gave similar values for protein breakdown, whereas leucine gave higher results for protein synthesis than did  $^{15}\text{N}$ -glycine. In the past, only a few studies have been published comparing these two methods. In line with our findings in elderly women, Van Goudoever et al. (1995) observed higher rates of protein turnover in preterm infants with the  $[1-^{13}\text{C}]$ -leucine method than with  $^{15}\text{N}$ -glycine. In another study, Golden and Waterlow (1977) demonstrated similar rates of protein synthesis in elderly people when employing  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine tracers simultaneously.

Comparison of the results with previous studies is complicated by the differences in the experimental protocols, e.g., differences in tracers, subjects and the feeding status of the subjects. In summary, it can be concluded that protein turnover rates measured with leucine and glycine are either comparable or turnover rates measured with leucine are somewhat higher.

**Does the increase in protein intake lead to comparable changes in protein turnover with both tracers?** Protein synthesis and protein breakdown rates, as determined with  $^{15}\text{N}$ -glycine, were higher when subjects consumed the high protein diet. However, when using the  $[1-^{13}\text{C}]$ -leucine method, no such differences were observed, although the oxidation rates, as measured with  $[1-^{13}\text{C}]$ -leucine, were higher for the high protein intake. Net protein breakdown rates increased significantly as measured with both tracers when subjects consumed the high protein diet, and the relative increase in net protein breakdown (breakdown minus synthesis) was also similar in magnitude for both tracers.

In the past, a few other investigators have reported on the

effect of dietary protein levels on protein turnover as measured with stable isotope tracers in humans. The present  $^{15}\text{N}$ -glycine data are in agreement with data previously published from our laboratory concerning the effect of protein intake on protein kinetics in both young and elderly fasting subjects (Pannemans et al. 1995a and 1995b). As in the present study, we found significantly higher protein turnover rates when subjects consumed the high protein diet in both groups. Using the same tracer, Meredith et al. (1989) and Gersovitz et al. (1980) described the effects of a variable protein intake on protein turnover measured during 60 h of feeding and fasting. Both groups found increased overall fluxes with increasing protein intake. Previous studies measuring the effect of protein intake on protein metabolism as measured with  $[1-^{13}\text{C}]$ -leucine are also more or less in agreement with the present data, i.e., increasing the protein intake has no effect on protein turnover of fasting subjects as measured with  $[1-^{13}\text{C}]$ -leucine (Pacy et al. 1994, Yang et al. 1986, Zello et al. 1992). Quevedo et al. (1994) described the effect of a reduced protein intake and concluded that changing the protein intake from high to moderate intake levels had no effect on rates of protein synthesis and breakdown in fasting subjects, whereas oxidation rates decreased. The latter result was also described by Pacy et al. (1994), whereas others found no effect of protein intake on oxidation rates in fasting subjects when changing from marginal to surfeit protein intake (Motil et al. 1981). In contrast with the present study, Motil et al. (1981) reported increased protein breakdown and synthesis rates with increasing protein intake, whereas Yang (1986) described decreased protein fluxes as intake increased from 0 to 1.5 g/(kg·d). Recently, Casteneda et al. (1995) reported increased protein fluxes with increasing protein intake in fed elderly women, whereas no effects were observed on protein synthesis and breakdown. The effects of protein intake on leucine flux probably is less pronounced in fasting than in fed subjects (Motil et al. 1981, Pacy et al. 1994, Quevedo et al. 1994). In summary it can be concluded that the results of the present study are in agreement with data previously obtained under similar experimental conditions for both the  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine tracers.

It remains unclear why protein turnover increased in subjects consuming the high protein diet with  $^{15}\text{N}$ -glycine as tracer, whereas measurements done with  $[1-^{13}\text{C}]$ -leucine did not detect any difference. To detect changes in protein turnover, the reproducibility of the technique used is important. However, the coefficients of variation of the leucine tracer technique and the glycine tracer technique are similar [6.8 and 6.7%, respectively (Bier et al. 1981, Fern et al. 1984a)]. It is not known why these differences were observed. Because both techniques rely on several assumptions, the differences may be attributable to the differences in assumptions made for both techniques. Promotion of the concept that a single given methodology represents the gold-standard approach to the estimation of whole-body protein turnover, applicable in both health and disease and to all age groups, would be difficult to defend. Direct comparison of results in studies in which  $^{15}\text{N}$ -glycine has been employed is often difficult in that so many variations on the central methodological theme have been reported (length of the study, oral or intravenous administration of the label, amount of tracer or choice of the end product). Given that the ammonia pool of the body is small and rapidly turning over, any pathophysiological disturbance of this pool could markedly affect the measured  $^{15}\text{N}$  enrichment in urinary ammonia. It has been reported in some neonatal studies that no  $^{15}\text{N}$  enrichment was measurable in urinary urea following  $^{15}\text{N}$ -glycine administration, thus precluding the harmonic mean calculation (Van Goudoever et al. 1995).

There is only one published study reporting the effect of dietary protein intake on whole-body protein turnover data as obtained with  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine, measured simultaneously in the same subjects, i.e., the study of Pacy et al. (1994) who used both  $^{15}\text{N}$ -glycine and  $[1-^{13}\text{C}]$ -leucine to measure whole-body protein synthesis and degradation in healthy subjects given diets with increasing protein intakes [0.36, 0.77 and 1.59 g/(kg · d)]. They concluded that the  $^{15}\text{N}$ -results generally supported the leucine infusion data. However, measurements were done only in the fed state because the relatively long period required to clear all  $^{15}\text{N}$  from the metabolic pool precluded measurements in both fed and fasted subjects.

To make results of the present study directly comparable to previous studies (Pannemans et al. 1995a and 1995b), we choose to use the  $^{15}\text{N}$ -glycine method. Protein turnover was measured in the postabsorptive state with an orally administered dose (200 mg) of  $^{15}\text{N}$ -glycine. Protein turnover was calculated on the basis of excreted end products, urea and ammonia, during 12 h, according to an established procedure (Fern et al. 1981). This method was used because of its simplicity and its convenience for use outside the laboratory, especially when elderly people are involved. To investigate whole-body protein turnover in more detail and to allow a comparison between the two most commonly used stable isotopes, it was decided to employ the  $^{13}\text{C}$ -leucine method as well. Furthermore, the  $^{15}\text{N}$ -glycine method gives no information about the protein oxidation levels; thus the  $^{13}\text{C}$ -leucine method was used to directly measure this variable. However, this method requires a continuous infusion of the tracer, which means that subjects have to be in the laboratory during the 4-h measurement. For practical reasons, this period was chosen at the end of the overnight fasting period and not in the middle of the night. The differences in time needed to collect the necessary data (12 h in the case of  $^{15}\text{N}$ -glycine and 4 h in the case of  $^{13}\text{C}$ -leucine) resulted in the protocol as described. This might be another possible explanation for the differences between the two tracers, i.e., the fact that glycine measurements were done over 12 h after a 6-h fast, whereas leucine measurements were done over 4 h after a 14-h fast.

In summary, protein breakdown as measured with  $^{15}\text{N}$ -glycine is comparable to results from  $[1-^{13}\text{C}]$ -leucine, whereas protein synthesis is significantly lower. Furthermore, measurements with  $^{15}\text{N}$ -glycine as tracer revealed higher protein turnover rates when subjects consumed the high protein diet, whereas no significant differences in protein turnover were found during consumption of the normal and high protein diet with the  $[1-^{13}\text{C}]$ -leucine method. Leucine oxidation rates were higher though when subjects consumed the high protein diet. Both tracers showed that the absolute difference between breakdown and synthesis (net protein breakdown) was higher during consumption of the high protein diet ( $P < 0.05$ ). Despite the fact that the absolute difference was higher as measured with the  $^{15}\text{N}$ -glycine method compared with the  $[1-^{13}\text{C}]$ -leucine method, the relative increase in net protein breakdown during consumption of the high protein diet is comparable for both tracers. These data indicate that care is needed with the choice of the tracer used when the components of protein turnover are measured in elderly women with the aim of understanding the physiological basis behind the adequacy of the level of protein intake.

#### ACKNOWLEDGMENTS

The authors thank Annemie Gijzen and Frank van de Vegt for their excellent technical support and Nicole Duysens and Tanja Hermans for assistance with diet composition during the study protocols.

#### LITERATURE CITED

- Bier, D. M., Motil, K. J. & Matthews, D. E. (1981) Energy intake and whole body protein dynamics in man. In: *Nutrition and Child Health: Perspectives for the 1980s* (Tsang, R. C. & Nichols, B. L., eds.), pp. 161–180. Alan Liss, Inc., New York, NY.
- Bruce, A. C., McNurlan, M. A., McHardy, K. C., Broom, J., Buchanan, K. D., Calder, A. G., Milne, E., McGaw, B. A., Garlick, P. J. & James, W.P.T. (1990) Nutrient oxidation patterns and protein metabolism in lean and obese subjects. *Int. J. Obes.* 14: 631–646.
- Cheng, A.H.R., Gomez, A., Bergan, J. G., Lee, T., Monckenberg, F. & Chichester, C. O. (1978) Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination wheat-soy-milk mixture. *Am. J. Clin. Nutr.* 31: 12–22.
- Conway, J. M., Bier, D. M., Motil, K. J., Burke, J. F. & Young, V. R. (1980) Whole body lysine flux in young adult men: effects of reduced total protein and of lysine intake. *Am. J. Physiol.* 239: E192–E200.
- Conway, J. M., Marable, N. L. & Bodwell, C. E. (1988) Whole body leucine and energy metabolism in adult women. *Eur. J. Clin. Nutr.* 42: 661–669.
- FAO/WHO/UNU, E. C. (1985) Energy and protein requirements. Technical Report Series number 724 World Health Organization, Geneva, Switzerland.
- Fern, E. B. & Garlick, P. J. (1983) The rate of nitrogen metabolism in the whole body of man measured with  $^{15}\text{N}$ -glycine and uniformly labelled  $[^{15}\text{N}]$ -wheat. *Hum. Nutr. Clin. Nutr.* 37C: 91–107.
- Fern, E. B., Garlick, P. J., McNurlan, M. A. & Waterlow, J. C. (1981) The excretion of isotope in urea and ammonia for estimating protein turnover in man with  $[^{15}\text{N}]$ glycine. *Clin. Sci. (Lond.)* 61: 217–228.
- Fern, E. B., Garlick, P. J., Sheppard, H. G. & Fern, M. (1984a) The precision of measuring the rate of whole-body nitrogen flux and protein synthesis in man with a single dose of  $[^{15}\text{N}]$ -glycine. *Hum. Nutr. Clin. Nutr.* 38C: 63–73.
- Fern, E. B., Garlick, P. J. & Waterlow, J. C. (1984b) The concept of the single body pool of metabolic nitrogen in determining the rate of whole-body nitrogen turnover. *Hum. Nutr. Clin. Nutr.* 38C: 85–99.
- Garlick, P. J. & Fern, E. B. (1984) Whole body protein turnover: theoretical considerations. In: *Substrate and Energy Metabolism* (Garrow, J. S. & Halliday, D., eds.), pp. 7–15. J. Libbey, London, UK.
- Garlick, P. J., McNurlan, M. A. & Ballmer, P. E. (1991) Influence of dietary protein intake on whole body protein turnover in humans. *Diabetes Care* 14: 1189–1198.
- Garlick, P. J., McNurlan, M. A., McHardy, K. C., Calder, A. G., Milne, E., Fearn, L. M. & Broom, J. (1987) Rates of nutrient utilization in man measured by combined respiratory gas analysis and stable isotopic labelling: effect of food intake. *Hum. Nutr. Clin. Nutr.* 41C: 177–191.
- Gersovitz, M., Bier, D., Matthews, D., Udall, J., Munro, H. N. & Young, V. R. (1980) Dynamic aspects of whole body glycine metabolism: influence of protein intake in young adults and elderly males. *Metabolism* 29: 1087–1094.
- Gersovitz, M., Motil, K., Munro, H. N., Scrimshaw, N. S. & Young, V. R. (1982) Human protein requirements: assessment of the adequacy of the current Recommended Dietary Allowance for dietary protein in elderly men and women. *Am. J. Clin. Nutr.* 35: 6–14.
- Golden, M.H.N. & Waterlow, J. C. (1977) Total protein synthesis in elderly people: a comparison of results with  $[^{15}\text{N}]$ glycine and  $[^{14}\text{C}]$ leucine. *Clin. Sci. Mol. Med.* 53: 277–288.
- Halliday, D. & Rennie, M. J. (1982) The use of stable isotopes for diagnosis and clinical research. *Clin. Sci. (Lond.)* 63: 485–496.
- Hoffer, L. J., Yang, R. D., Matthews, D. E., Bistrian, B. R., Bier, D. M. & Young, V. R. (1985) Effects of meal consumption on whole body leucine and alanine kinetics in young adult men. *Br. J. Nutr.* 53: 31–38.
- Matthews, D. E., Motil, K. J., Rohrbach, D. K., Burke, J. F., Young, V. R. & Bier, D. M. (1980) Measurement of leucine metabolism in man from a primed, continuous infusion of L-[ $^{13}\text{C}$ ]leucine. *Am. J. Physiol.* 238: E473–E479.
- Matthews, D. E., Schwarz, H. P., Yang, R. D., Motil, K. J., Young, V. R. & Bier, D. M. (1982) Relationship of plasma leucine and  $\alpha$ -ketoisocaproate during a L-[ $^{13}\text{C}$ ]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* 31: 1105–1112.
- Melville, S., McNurlan, M. A., McHardy, K. C., Broom, J., Milne, E., Calder, A. G. & Garlick, P. J. (1989) The role of degradation in the acute control of protein balance in adult man: failure of feeding to stimulate protein synthesis as assessed by L-[ $^{13}\text{C}$ ]leucine infusion. *Metabolism* 38: 248–255.
- Meredith, C. N., Zackin, M. J., Frontera, W. R. & Evans, W. J. (1989) Dietary protein requirements and body protein metabolism in endurance-trained men. *J. Appl. Physiol.* 66: 2850–2856.
- Millward, D. J., Price, G. M., Pacy, P.J.H. & Halliday, D. (1991) Whole-body protein and amino acid turnover in man: what can we measure with confidence? *Proc. Nutr. Soc.* 50: 197–216.
- Motil, K. J., Matthews, D. E., Bier, D. M., Burke, J. F., Munro, H. N. & Young, V. R. (1981) Whole-body leucine and lysine metabolism: response to dietary protein intake in young men. *Am. J. Physiol.* 240: E712–E721.
- Pacy, P. J., Price, G. M., Halliday, D., Quevedo, M. R. & Millward, D. J. (1994) Nitrogen homeostasis 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, D.L.E., Halliday, D. & Westerterp, K. R. (1995a) Whole body protein turnover in elderly men and women: responses to two levels of protein intake. *Am. J. Clin. Nutr.* 61: 33–38.
- Pannemans, D.L.E., Halliday, D., Westerterp, K. R. & Kester, A.D.M. (1995b)

- Effect of variable protein intake on whole body protein turnover in young men and women. *Am. J. Clin. Nutr.* 61: 69–74.
- Pannemans, D.L.E. & Westerterp, K. R. (1993) Estimation of energy intake to feed subjects at energy balance as verified with doubly labelled water: a study in the elderly. *Eur. J. Clin. Nutr.* 47: 490–496.
- Quevedo, M. R., Price, G. M., Halliday, D., Pacy, P. J. & Millward, D. J. (1994) Nitrogen homeostasis in man: diurnal changes in nitrogen excretion, leucine oxidation and whole body leucine kinetics during a reduction from a high to a moderate protein intake. *Clin. Sci. (Lond.)* 86: 185–193.
- Read, W.W.C., Harrison, R. A. & Halliday, D. (1982) A resin-based method for the preparation of molecular nitrogen for  $^{15}\text{N}$  analysis from urinary and plasma components. *Anal. Biochem.* 123: 249–254.
- Uauy, R., Scrimshaw, N. S. & Young, V. R. (1978) Human protein requirements: nitrogen balance response to graded levels of egg protein in elderly men and women. *Am. J. Clin. Nutr.* 31: 779–785.
- Van Goudoever, J. B., Sulkers, E. J., Halliday, D., Degenhart, H. J., Carnielli, V. P. & Sauer, P.J.J. (1995) Whole body protein turnover in preterm appropriate for gestational age and small for gestational age infants: comparison of  $^{15}\text{N}$  glycine and  $^{13}\text{C}$  leucine administered simultaneously. *Pediatr. Res.* 37: 381–388.
- Van Marken Lichtenbelt, W. D., Westerterp, K. R. & Wouters, L. (1994) Deuterium dilution as a method for determining total body water: effect of test protocol and sampling time. *Br. J. Nutr.* 72: 491–497.
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) Summary of methods of measuring total protein turnover. In: *Protein Turnover in Mammalian Tissues and in the Whole Body*, pp. 327–338. North Holland Publishing Co., Amsterdam, The Netherlands.
- Waterlow, J. C. & Jackson, A. A. (1981) Nutrition and protein turnover in man. *Br. Med. Bull.* 37: 5–10.
- Wolfe, R. R. (1992) Radioactive and Stable Isotopes Tracers in Biomedicine: Principles and Practice of Kinetic Analysis, p. 427. Wiley-Liss, New York, NY.
- Yang, R. D., Matthews, D. E., Bier, D. M., Wen, Z. M. & Young, V. R. (1986) Response of alanine metabolism in humans to manipulation of dietary protein and energy intakes. *Am. J. Physiol.* 250: E39–E46.
- Young, V. R., Gucalp, C., Rand, W. M., Matthews, D. E. & Bier, D. M. (1987) Leucine kinetics during three weeks at submaintenance to maintenance intakes of leucine in men: adaptation and accommodation. *Hum. Nutr. Clin. Nutr.* 41C: 1–18.
- Zanni, E., Calloway, D. H. & Zezulka, A. Y. (1979) Protein requirements of elderly men. *J. Nutr.* 109: 513–524.
- Zello, G. A., Telch, J., Clarke, R., Ball, R. O. & Pencharz, P. B. (1992) Reexamination of protein requirements in adult male humans by end-product measurements of leucine and lysine metabolism. *J. Nutr.* 122: 1000–1008.