Endogenous glycine and tyrosine production is maintained in adults consuming a marginal-protein diet[1–3]

Neil R Gibson, Farook Jahoor, Lisa Ware, and Alan A Jackson

ABSTRACT

Background: The adequacy of indispensable amino acid supplies has received much attention in studies of protein requirements, but the availability of nitrogen for synthesis and maintenance of the supply of dispensable amino acids has been overlooked.

Objective: We aimed to determine whether nitrogen balance and the endogenous supply of the dispensable amino acids glycine and tyrosine can be maintained with a marginal protein intake.

Design: Phenylalanine, glycine, and tyrosine kinetics were measured in young adults (6 men, 6 women) on 4 occasions during a reduction in habitual protein intake (1.13 $g \cdot kg^{-1} \cdot d^{-1}$) to a marginal intake (0.75 $g \cdot kg^{-1} \cdot d^{-1}$) by using a multiple stable-isotope-infusion protocol.

Results: During the 10-d period of marginal protein intake, nitrogen excretion fell initially, then remained constant such that nitrogen balance was negative for the first 2 d and then positive or zero thereafter. Whole-body protein degradation and synthesis predicted from phenylalanine kinetics declined significantly ($P < 0.05$) over the period of marginal protein intake. Despite the reduction in the amount of glycine and tyrosine derived from whole-body proteolysis, the fluxes of glycine and tyrosine were maintained.

Conclusions: The results show that adaptation to a marginal intake of dietary protein consisted of an overall reduction in whole-body protein turnover, net protein catabolism, and the rate of nitrogen excretion. The conserved nitrogen was sufficient to maintain the endogenous synthesis and hence the supply of glycine and tyrosine. *Am J Clin Nutr* 2002;75:511–8.

KEY WORDS Phenylalanine, glycine, tyrosine kinetics, marginal protein intake, nitrogen balance, protein requirements, dispensable amino acids

INTRODUCTION

There is still controversy over the amino acid and protein requirements of adult humans (1, 2). Although a protein intake of 0.75 $g \cdot kg^{-1} \cdot d^{-1}$ is considered adequate for maintenance of nitrogen balance, it has been argued, on the basis of measurements of oxidative loss, that this marginal protein intake may not be enough to maintain adequate supplies of certain indispensable amino acids, such as lysine (2), if dietary protein is limited in lysine, as in totally cereal-based diets. Although the adequacy of indispensable amino acid supplies has received much attention in most studies of protein requirements, the availability of nitrogen for synthesis and maintenance of the supply of the dispensable amino acids is often overlooked because these amino acids have been categorized as nonessential from a dietary perspective. This has led to the inappropriate perception that dispensable amino acids do not play an important metabolic role. In reality these amino acids are critical for maintenance of physiologic and metabolic homeostasis and are absolutely essential in relatively large amounts for survival. Hence, there is the need for protection and conservation of their pathways for de novo synthesis (3).

The de novo synthesis of the so-called dispensable amino acids is important for survival because they are precursors for the synthesis of numerous metabolites and peptides that are necessary for the maintenance of physiologic homeostasis. One example is the aromatic amino acid tyrosine, which is endogenously synthesized from the essential amino acid phenylalanine. Tyrosine has been shown to be a significant component of the positive acute phase proteins synthesized by the liver. These proteins participate in host-defense mechanisms, and their rates of synthesis increase severalfold under stress (4). Tyrosine is also a precursor for the synthesis of dopamine, norepinephrine, and epinephrine. Glycine is another good example of an amino acid in high demand because it is a precursor for the formation of numerous essential biological compounds such as purines, porphyrins, creatine, glutathione, and, through its interconversion to serine, phospholipids, and cysteine (5). Because glycine is unavailable for reuse after its incorporation into most of these compounds, under certain circumstances—such as severe

[1] From the Institute of Human Nutrition, the University of Southampton, the Clinical Nutrition and Metabolism Unit, Southampton General Hospital, Southampton, United Kingdom (NRG, LW, and AAJ), and the US Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, the Department of Pediatrics, the Baylor College of Medicine, Houston (NRG and FJ).

[2] Supported by a grant from the Biotechnology and Biological Scientific Research Council and by the USDA, Agricultural Research Service (Cooperative Agreement no. 58-6250-1-003).

[3] Address reprint requests to AA Jackson, Institute of Human Nutrition, University of Southampton, Clinical Nutrition and Metabolism Unit, Level C (113) West Wing, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom. E-mail: a.a.jackson@soton.ac.uk.

Received October 20, 2000.

Accepted for publication April 19, 2001.

TABLE 1
Physical characteristics of the subjects

Characteristics	Subjects (n = 6 M, 6 F)
Age (y)	25.6 ± 1.0
Height (m)	1.72 ± 0.02
Weight (kg)	70.3 ± 3.6
Lean body mass (kg)	56.0 ± 3.7
Fat mass (kg)	14.2 ± 1.6

stress—the higher demand for this amino acid may exceed its rate of synthesis. This is especially true during periods of low dietary protein intake, when the availability of labile nitrogen for dispensable amino acid synthesis may be severely reduced. Hence, although studies have shown that nitrogen balance can be maintained with a protein intake of 0.75 $g \cdot kg^{-1} \cdot d^{-1}$ (6), it is not known whether de novo synthesis will allow important dispensable amino acids such as glycine and tyrosine to be maintained in sufficient metabolic supply, rather than being channeled preferentially to one metabolic pathway at the expense of another, such as glutathione synthesis.

Until now, the adequacy of a marginal-protein diet for the maintenance of dispensable amino acid synthesis has not been directly tested. In the present study we tested the hypothesis that the nitrogen-sparing mechanisms associated with a protein intake of 0.75 $g \cdot kg^{-1} \cdot d^{-1}$ are sufficient to maintain adequate rates of synthesis and hence adequate supplies of glycine and tyrosine. Stable-isotope-tracer methods were used to measure whole-body protein, tyrosine, and glycine kinetics in young adults over a 10-d adaptation from their habitual dietary protein intake of ≈1.13 $g \cdot kg^{-1} \cdot d^{-1}$ to a lower marginal protein intake of 0.75 $g \cdot kg^{-1} \cdot d^{-1}$, the current recommended dietary allowance (7).

SUBJECTS AND METHODS

Subjects

Ethical approval for the conduct of all studies was obtained from the Southampton Joint Ethical Committee of the Southampton and South West Hampshire Health Commission, and all subjects gave informed consent for participation after the nature of the protocol had been fully explained to them. Twelve young adults (6 men, 6 women) in good general health and with normal renal and hepatic function were studied. Before the dietary intervention, the height, weight and body composition (by bioelectrical impedance) of each subject were measured. The subjects' physical characteristics are shown in **Table 1**.

Study design and dietary intervention

Each subject underwent a 7-h stable-isotope infusion while in the fasted state (**Figure 1**) to measure phenylalanine, tyrosine, and glycine kinetics on 4 occasions: at baseline and on days 3, 6, and 10 of the marginal-protein diet. The habitual protein intake of each subject was determined from 5-d weighed dietary records. On the 2 d preceding the first isotope infusion, the subjects consumed a diet based on their habitual intakes of protein. This was followed by 10 d of a diet that provided the same amount of energy but a lower (marginal) protein content of 0.75 $g \cdot kg^{-1} \cdot d^{-1}$. Throughout the intervention, diets were supplied as ready-made meals and food portions (Marks and Spencer, Southampton, United Kingdom). The main sources of protein in both the habitual and marginal-protein diets were derived from vegetable and meat. The energy content of both diets was the same and was assessed from resting energy expenditure by indirect calorimetry (Gas Exchange Measurement system; Europa Scientific Ltd, Crewe, United Kingdom) multiplied by a factor of 1.5 to account for physical activity. Dietary energy, protein, and amino acid intakes are summarized in **Table 2**. Amino acid intakes were estimated from food-composition tables of the US Department of Agriculture nutrient database for standard reference (8). Twenty-four–hour urine samples were collected over the 12 d of the study for the estimation of daily nitrogen balance.

Isotope-infusion protocol

[$^{13}C_2$]Glycine (98% ^{13}C), $NaH^{13}CO_2$ (99% ^{13}C), [$^2H_5$]phenylalanine (98% ^2H), [$^2H_2$]tyrosine (98% ^2H), and [$^2H_4$]tyrosine (98% ^2H) were purchased from Mass Trace (Woburn, MA). Solutions were prepared in 0.9% saline by the pharmacy at Southampton General Hospital, and before use they were confirmed to be sterile and pyrogen free.

After a 12-h overnight fast, the subjects arrived at the metabolic unit of the Southampton General Hospital, where they underwent a 7-h isotope-infusion protocol as shown in Figure 1. Intravenous catheters were inserted into superficial veins of both arms, one for continuous infusion of the tracer solutions and the other for repeated blood sampling. After collection of baseline blood (10 mL) and duplicate expired breath samples, priming doses of [$^{13}C_2$]glycine (20 μmol/kg) and $NaH^{13}CO_2$ (5.8 μmol/kg) were given. These were followed immediately by a continuous infusion of [$^{13}C_2$]glycine (15 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) for 7 h. Three hours after the start of the [$^{13}C_2$]glycine infusion, prime doses of [$^2H_5$]phenylalanine (1.6 μmol/kg), [$^2H_2$]tyrosine (0.5 μmol/kg), and [$^2H_4$]tyrosine (0.5 μmol/kg) were administered, immediately followed by continuous infusions of [$^2H_5$]phenylalanine (1.6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) and [$^2H_2$]tyrosine (0.5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Blood samples (5 mL) were taken hourly from hours 3 to 5 and then every 0.5 h until the end of the infusion. Breath samples were taken at 0.5-h intervals from hours 5 to 7. The production rate of carbon dioxide ($\dot{V}CO_2$) was measured by indirect calorimetry (Gas Exchange Measurement system; Europa Scientific Ltd) at three 30-min periods during the study as shown in the protocol diagram (Figure 1).

Sample analyses

Blood was drawn into prechilled tubes containing Na_2EDTA and a cocktail of sodium azide, merthiolate, and soybean trypsin

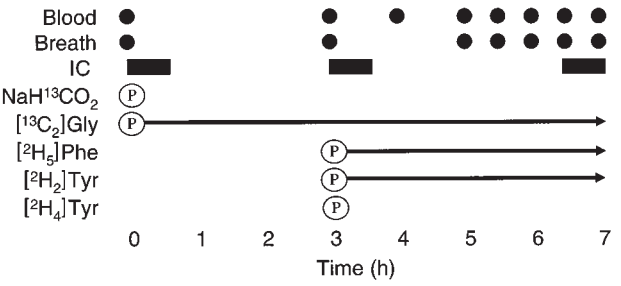

FIGURE 1. Diagram of the isotope-infusion protocol. IC, indirect calorimetry; P, prime.

TABLE 2
Daily energy, protein, and selected amino acid intakes during the habitual- and marginal-protein diets[1]

Intake	Habitual-protein diet	Marginal-protein diet
Energy (kJ/d)	154.7 ± 4.7	154.7 ± 4.7
Protein (g/d)	1.13 ± 0.04	0.75 ± 0.00[2]
Glycine (mg·kg^{-1}·d^{-1})	38.4 ± 5.9	22.6 ± 2.1[2]
Serine (mg·kg^{-1}·d^{-1})	35.3 ± 4.6	22.8 ± 2.1[2]
Threonine (mg·kg^{-1}·d^{-1})	35.5 ± 5.1	18.7 ± 1.8[2]
Phenylalanine (mg·kg^{-1}·d^{-1})	40.3 ± 4.9	28.9 ± 2.5[2]
Tyrosine (mg·kg^{-1}·d^{-1})	37.7 ± 4.9	22.7 ± 2.1[2]

[1] $\bar{x}$ ± SEM.
[2] Significantly different from the habitual-protein diet, $P < 0.05$ (paired *t* test).

inhibitor and immediately centrifuged at 4°C; the plasma was then removed and stored at −70°C for later analysis.

Plasma amino acids were isolated by ion exchange (AG50W-X8 resin, 100–200 mesh, hydrogen form; Bio-Rad Laboratories, Hercules, CA) chromatography and then converted to the *n*-propyl ester heptafluorobutyramide derivative. The tracer-tracee ratios of the amino acids were determined by negative chemical ionization gas chromatography–mass spectrometry on an HP5890 gas chromatograph coupled to an HP5989 quadrapole mass spectrometer (Hewlett-Packard, Palo Alto, CA). The analysis selectively monitored ions at mass-to-charge ratios 595–599, 383–388, and 293–295 for tyrosine, phenylalanine, and glycine, respectively, in 3 separate runs.

Breath samples for measurement of $^{13}CO_2$ were collected in a breath bag with a one-way valve and immediately transferred to a 10-mL evacuated glass tube and stored at room temperature until analyzed by isotope ratio mass spectrometry (ANCA system; Europa Scientific Ltd) monitoring ions at mass-to-charge ratios 44 and 45.

During the day, individual urine samples were collected in containers and temporarily stored at 4°C. Twenty-four–hour specimens were pooled and thoroughly mixed, and the total volume was recorded. A 20-mL sample of each 24-h sample was then stored at −70°C until analyzed for total nitrogen by the Kjeldahl method.

Calculations and statistics

Phenylalanine, tyrosine, and glycine kinetics

Whole-body amino acid flux was calculated by using the 2-pool steady state approach (9), whereby the flux (Q) of amino acids is determined from tracer dilution:

$$Q = i[(\mathrm{Ei/Ep}) - 1] \quad (1)$$

where i is the tracer infusion rate (μmol·kg^{-1}·h^{-1}) and Ei and Ep are the tracer-tracee ratios of the infusate and of either plasma glycine, phenylalanine, or tyrosine at plateau, respectively. The term −1 corrects for the contribution of the tracer infusion to the flux.

Phenylalanine hydroxylation, ie, the conversion rate of phenylalanine to tyrosine (Q_{PT}), was calculated with the following equation:

$$Q_{PT} = Q_{Tyr} \times (E_{Tyr}/E_{Phe}) \times Q_{Phe}/(i_{Phe} + Q_{Phe}) \quad (2)$$

where Q_{Tyr} and Q_{Phe} are the fluxes for tyrosine and phenylalanine (μmol·kg^{-1}·h^{-1}) estimated from infusions of [$^2H_2$]tyrosine and [$^2H_5$]phenylalanine, respectively. E_{Tyr} and E_{Phe} are the plateau tracer-tracee ratios of [$^2H_4$]tyrosine and [$^2H_5$]phenylalanine in plasma, respectively, and i_{Phe} is the rate of infusion of [$^2H_5$]phenylalanine (μmol·kg^{-1}·h^{-1}). The expression $Q_{Phe}/(i_{Phe} + Q_{Phe})$ corrects for the contribution made by the tracer infusion.

Glycine oxidation (Ox) was calculated from $\dot{V}CO_2$ in breath (mL/min) and the enrichment of plasma glycine as shown:

$$\mathrm{Ox} = (\dot{V}CO_2 \times E_{CO_2} \times 44.6 \times 60)/(\mathrm{Bwt} \times \mathrm{RF} \times E_{Gly} \times 2) \quad (3)$$

where E_{CO_2} is the isotopic enrichment of carbon dioxide (atom percent excess), Bwt is body weight (kg), and RF adjusts for the fraction of carbon dioxide recovered in the breath during the fasted state (0.76) (10). E_{Gly} is the tracer-tracee ratio of glycine. The terms 44.6 and 60 convert mL/min to μmol/h and the term 2 compensates for the formation of two $^{13}CO_2$ molecules from the oxidation of one [$^{13}C_2$]glycine molecule. Nonoxidative disposal of glycine was calculated by subtracting the rate of glycine oxidation from glycine flux. Furthermore, the synthesis rate of endogenous glycine was estimated from the difference between total glycine flux and glycine derived from whole-body protein breakdown, assuming the noncollagen protein composition of amino acids to be 4.9% for glycine (11).

Whole-body protein kinetics

Whole-body protein kinetics was estimated from phenylalanine and tyrosine kinetics (9). Because phenylalanine is not synthesized endogenously, its flux in the fasted state is an index of whole-body protein breakdown because it represents phenylalanine derived entirely from whole-body proteolysis. Similarly, the rate of hydroxylation of phenylalanine to tyrosine can be considered an index of phenylalanine oxidation and also an index of net protein oxidation. Finally, assuming that most phenylalanine not oxidized is used for protein synthesis, then the disposal rate of nonoxidative phenylalanine, derived by subtracting phenylalanine hydroxylation from phenylalanine flux, can be considered an index of whole-body protein synthesis rate.

Nitrogen balance

Total daily nitrogen excretion was determined from the 24-h urinary nitrogren measurements with additional adjustments for fecal nitrogen losses of 12 mg·kg^{-1}·d^{-1} and miscellaneous nitrogen losses of 8 mg·kg^{-1}·d^{-1} (12). The nitrogen content of the diet was estimated with the use of a factor of 6.25 g N/g protein. Daily nitrogen balance was determined by subtracting the total nitrogen loss from the daily nitrogen intake.

Statistical analysis

The data were analyzed by repeated-measures analysis of variance (ANOVA) with the use of the STATVIEW II statistical package, version 4.5 (Abacus Concepts, Inc, Berkeley, CA). When there were significant differences over time, individual time points were compared with univariate post-ANOVA contrasts. The significance of differences was assumed at $P < 0.05$, and numerical data are expressed as means ± SEMs.

RESULTS

The age and physical characteristics of the subjects are shown in Table 1. The subjects were aged 25.6 ± 1.0 y and were within the normal range of ideal body weight and body mass index. The men and women were matched for age and body mass index.

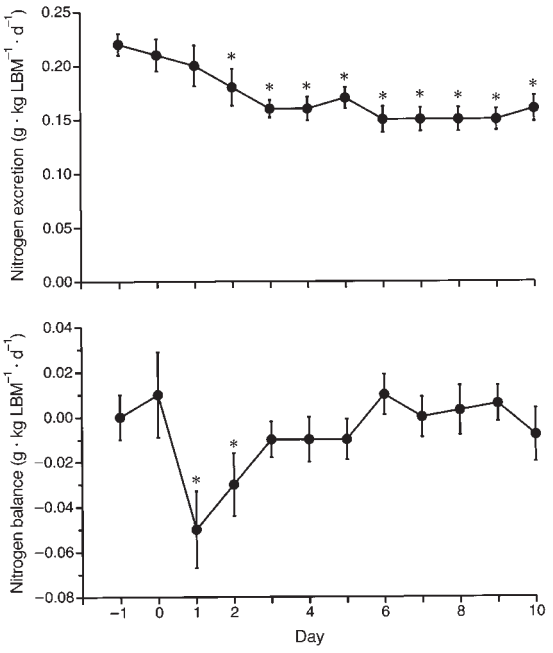

FIGURE 2. Mean (±SEM) daily nitrogen excretion and balance for all subjects. *Significantly different from baseline, $P < 0.05$ (ANOVA and Tukey's test). LBM, lean body mass.

However, the men were significantly taller and heavier and had less body fat mass than the women. All kinetic data are expressed in terms of lean body mass (LBM) for the whole group unless otherwise stated.

Daily nitrogen excretion decreased gradually from baseline (days –1 and 0), reaching a significantly lower rate after 2 d of the marginal protein intake (**Figure 2**). Thereafter, from days 3 to 10, the rate of nitrogen excretion plateaued and remained significantly slower than the mean baseline rate. The average rate of nitrogen excretion at plateau (days 3–10) was 28% slower than the mean baseline rate (156 compared with 216 mg·kg LBM^{-1}·d^{-1}).

Also shown in Figure 2 are the nitrogen balance data. After 1 d of the marginal-protein diet, nitrogen balance decreased significantly to a negative value and remained lower after 2 d. However, after 3 d, as nitrogen excretion decreased to a lower steady state value, nitrogen balance returned to a value that was not significantly different from baseline.

Phenylalanine kinetics data at baseline and on days 3, 6, and 10 of the marginal-protein diet are shown in **Figure 3**. After 3, 6, and 10 d of the marginal-protein diet, there were significant reductions from baseline in phenylalanine flux and nonoxidative disposal. The oxidation rate of the phenylalanine appeared slower after 3 d of dietary treatment, but this value was not significantly different from baseline.

Glycine and tyrosine kinetics data are shown in **Figure 4**. Glycine flux, oxidation, and nonoxidative disposal rates did not change significantly from baseline during the marginal-protein diet, nor did tyrosine flux. Endogenous glycine synthesis rates increased significantly from baseline on days 3, 6, and 10. Although all of the other glycine kinetic variables showed an upward trend on day 6 of the diet, these increases were not significantly different from baseline. On average, tyrosine flux was 1.3 times the phenylalanine flux, and glycine flux was 3.5 times phenylalanine flux on a molar basis. In 3 subjects, tyrosine flux was consistently twice as high as phenylalanine flux.

DISCUSSION

Although endogenously synthesized amino acids such as glycine and tyrosine are conventionally regarded to be nonessential in the diet, they are essential for metabolic homeostasis because they serve a critical role as precursors for the formation of numerous peptides and metabolites that are essential for survival. Much of the debate over protein requirements has focused on the adequacy of indispensable rather than of dispensable amino acids (1, 2). However, at the moderate protein intake of 0.75 g·kg^{-1}·d^{-1} recommended by the FAO/WHO/UNU (7), the supply of dispensable amino acids may also be limited. The importance of nitrogen conservation for synthesis and supply of dispensable amino acids such as glycine and tyrosine has been largely overlooked and inadequately tested. In the present study, we determined nitrogen excretion and balance and whole-body protein kinetics as well as the kinetics of glycine and tyrosine in healthy young adults during adaptation from their habitual protein intake to the intake recommended by the FAO/WHO/UNU. The results showed that adaptation to this lower intake of dietary protein consisted of an overall reduction in whole-body protein turnover and a decrease in net protein catabolism and in the excretion rate of nitrogen. This conservation of nitrogen was associated with maintenance of both nitrogen balance and the endogenous supply of glycine and tyrosine.

Although nitrogen excretion started to decrease immediately after the subjects began consuming the marginal-protein diet, the initial reductions were smaller than the 26% reduction in dietary nitrogen intake. As a consequence, the subjects were in negative nitrogen balance during the first 2 d of the marginal-protein diet.

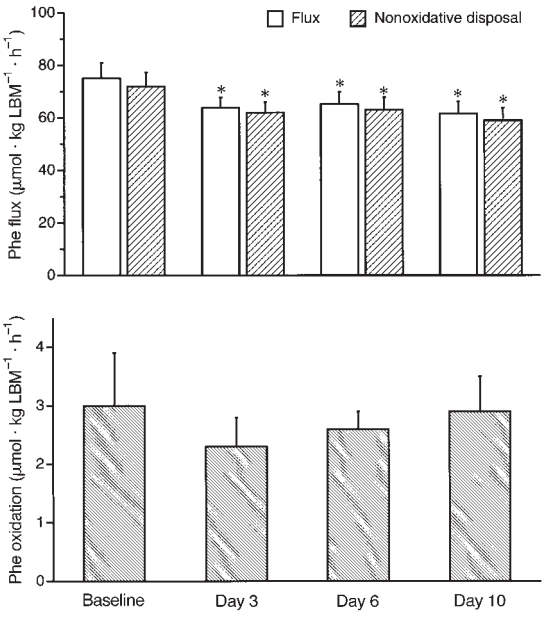

FIGURE 3. Mean (±SEM) phenylalanine flux and nonoxidative disposal and phenylalanine oxidation at baseline and on days 3, 6, and 10 of the marginal-protein diet. *Significantly different from baseline, $P < 0.05$ (ANOVA and Tukey's test). LBM, lean body mass.

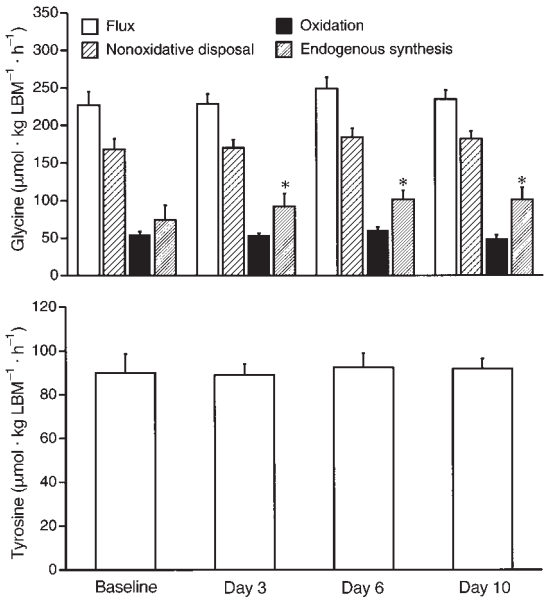

FIGURE 4. Mean (±SEM) glycine flux, nonoxidative disposal, oxidation, and endogenous synthesis and tyrosine flux at baseline and on days 3, 6, and 10 of the marginal-protein diet. *Significantly different from baseline, $P < 0.05$ (ANOVA and Tukey's test). LBM, lean body mass.

After 2 d, although nitrogen excretion was significantly slower, nitrogen conservation was not complete because the rate of excretion decreased further to a lower plateau value on day 3. From days 3 to 10, the mean reduction in nitrogen excretion from baseline (61 ± 3 mg·kg LBM^{-1}·d^{-1}) was almost identical to the mean reduction in dietary nitrogen intake (58 ± 3 mg·kg LBM^{-1}·d^{-1}), indicating that nitrogen conservation was achieved. Concomitantly, nitrogen balance improved, returning to a value similar to that at baseline on day 3 of the diet.

A decrease in total nitrogen excretion in response to a reduction in protein intake is well documented (12–14). In a similar study, in which subjects were habituated to a high protein intake of 1.82 g·kg^{-1}·d^{-1} for 2 wk and then placed on a marginal intake of 0.77 g·kg^{-1}·d^{-1} for 9 d, Quevedo et al (13) reported a gradual reduction in nitrogen excretion that was significantly lower from day 4 onward. As expected, they observed a concomitant and significant improvement in nitrogen balance on day 4 onward. However, in contrast with our present findings, they reported that nitrogen balance remained modestly negative throughout the 9 d of marginal protein intake because the mean reduction in nitrogen excretion observed from days 4 to 9 (102 mg·kg^{-1}·d^{-1}) was less than the reduction in dietary nitrogen intake of 168 mg·kg^{-1}·d^{-1}. The persistent negative balance shown in the study by Quevedo et al (13) may have been partly due to the lower energy intake than in the present study (140 compared with 155 kJ·kg^{-1}·d^{-1}). Another explanation is that amino acid–oxidizing enzymes must have been markedly up-regulated to dispose of the excess amino nitrogen supplied during adaptation to the surfeit protein intake and that this up-regulation persisted to some degree during the period of marginal protein intake. As a consequence, nitrogen conservation was not achieved after 9 d of the marginal-protein diet because of the higher activity of amino acid–deaminating and nitrogen excretion pathways. For example, a study by Thorpe et al (15) showed that phenylalanine flux and oxidation were affected by the amount of protein consumed 2 d before the study began, indicating that oxidating enzymes were up-regulated by prior protein intake and that control of protein intake before a study begins is important. The prior intakes of our subjects were remarkably uniform at ≈1.1 g·kg^{-1}·d^{-1}, equivalent to their habitual intakes. This is a modest amount of dietary protein [compared with the quantities of protein consumed by the subjects in the study by Quevedo et al (13)] that is considered to be sufficient for the maintenance of both amino acid and nitrogen balances in healthy adult subjects (2). Hence, it is unlikely that this adequate protein intake would have caused any up-regulation of the amino acid–deaminating pathways of our subjects.

In the present study, to reestablish nitrogen balance, the 25% reduction in nitrogen intake had to be matched by an equivalent reduction in net amino-nitrogen catabolism and hence in nitrogen excretion. The ≈24% reductions observed in nitrogen excretion (Figure 2) suggest that this was accomplished on day 3 of the marginal protein intake. Both the flux and nonoxidative disposal of phenylalanine decreased on day 3, indicating an overall deceleration of protein turnover in response to the lower dietary protein intake. Conversely, in the studies by Quevedo et al (13), daily rates of synthesis and degradation estimated from leucine kinetics remained unchanged and increased respectively. The findings in the present study indicate that whole-body protein turnover decreases significantly in healthy young individuals adapting successfully to a protein intake of 0.75 g·kg^{-1}·d^{-1}. Because of the increased requirement for amino acids to support various aspects of the metabolic-immunologic response to stressful stimuli, Young and Marchini (16) have argued that the reduction in whole-body protein breakdown in response to a protein intake of 0.75 g·kg^{-1}·d^{-1} would probably diminish an individual's capacity to mount an adequate response to a stressful stimulus. That is, though sufficient for maintenance of nitrogen balance in good health, a protein intake of 0.75 g·kg^{-1}·d^{-1} may not permit the protein metabolic responses required to mount a successful host response to stress.

In the present study, plasma phenylalanine flux decreased significantly from baseline with the marginal-protein diet, indicating a suppression of whole-body proteolysis. An inhibition of whole-body protein breakdown can only be beneficial for the conservation of nitrogen if the proportion of amino acid flux that is oxidized is reduced or remains unchanged. In the current model, the main outflows from the amino acid pool are for protein synthesis and for oxidation. On day 3 phenylalanine oxidation decreased by ≈25%. Although not significant, this change was similar to the decrease in flux, indicating no change in the proportion of flux that was oxidized. Similarly, although the percentage decrease in the rate of oxidation was almost identical on days 6 and 10, these values were not significant because of high intersubject variability. The change in nonoxidative phenylalanine disposal, an index of the protein synthesis rate, mirrored the pattern of changes in phenylalanine flux and oxidation over the study period, suggesting that the suppression of proteolysis and subsequent decrease in amino acid availability caused a decrease in protein synthesis. These results indicate that the successful adaptation to this lower intake of dietary protein consisted of an overall reduction in whole-body protein turnover and net protein catabolism.

The trend for a reduction in phenylalanine oxidation observed in this study agrees with the findings of other studies, which

reported similar reductions in fasted leucine and phenylalanine oxidation in response to a reduction in protein intake to ≈0.75 g·kg^{-1}·d^{-1} (13, 17). However, the decrease in phenylalanine oxidation was not significant, and in a similar study, Zello et al (18) failed to show a significant decrease in leucine oxidation in men after their protein intakes were reduced to 0.6 g·kg^{-1}·d^{-1}. In contrast with our present finding that both the phenylalanine flux and the nonoxidative disposal rate (indexes of protein degradation and synthesis) were reduced, Quevedo et al (13) reported no significant changes in leucine flux and nonoxidative disposal rate in subjects whose protein intakes were reduced from 1.82 to 0.77 g·kg^{-1}·d^{-1} for 9 d. On the other hand, Pacy et al (17) reported that fasted adult men with a marginal protein intake of 0.77 g·kg^{-1}·d^{-1} had significantly slower leucine and phenylalanine fluxes than did subjects with a higher protein intake of 1.59 g·kg^{-1}·d^{-1}, indicating slower rates of protein degradation. They failed, however, to detect any influence of diet on fasting protein synthesis calculated from the nonoxidative disposal of both phenylalanine and leucine. Taken together, the findings of Quevedo et al (13) and of Pacy et al (17) suggest that adaptation to a marginal protein intake involves changes in the rates of protein degradation and net catabolism, whereas our present data suggest that adaptation to a marginal protein intake involves changes in the rates of protein degradation, synthesis, and net catabolism.

Despite the 16% reduction in the rate of protein degradation and, hence, in protein-derived glycine, there was no significant change in glycine flux. According to the 2-compartment model of protein turnover described by Waterlow et al (19), in the fasted state, the flux of a dispensable amino acid represents the sum of its inflow into the free pool from protein and peptide breakdown and from de novo synthesis. Assuming a metabolic steady state, the rate of inflow into the free pool will then be exactly equal to the rate of outflow toward oxidation and nonoxidative reactions such as protein synthesis. Therefore, the finding of no significant change in glycine flux despite a 16% reduction in the protein breakdown rate, and hence of protein-derived glycine, indicates that glycine flux was maintained by increased de novo synthesis of the amino acid. Furthermore, the observation that glycine oxidation did not decrease in response to the lower protein intake suggests that the outflow of glycine for nonoxidative reactions was maintained because of its increased de novo synthesis. Hence glycine supply for metabolic requirements is not compromised in subjects consuming a marginal intake of protein.

Our present findings do not agree with those of 2 previous studies, which reported that glycine flux decreased in response to a low protein intake or to an intake (0.6 g·kg^{-1}·d^{-1}) of an amino acid mixture devoid of dispensable amino acids (20, 21). On closer examination, however, direct comparisons with these previous investigations may not be appropriate. For example, Yu et al (20) reported significantly slower glycine flux in adults fed an artificial amino acid mixture (equivalent to 0.6 g protein·kg^{-1}·d^{-1}) totally devoid of dispensable amino acids than in adults fed a diet containing both indispensable and dispensable amino acids (equivalent to 1.5 g protein·kg^{-1}·d^{-1}). However, when the artificial amino acid mixture was made complete by the addition of dispensable amino acids, they reported no significant differences in glycine flux, as observed in our present study. On the other hand, glycine kinetics was measured in the study by Gersovitz et al (21) by using intermittent oral administration of [^{15}N]glycine in subjects receiving submaintenance amounts of protein (0.4 g·kg^{-1}·d^{-1}). With this protocol they reported a flux of ≈450 μmol·kg^{-1}·h^{-1}, more than twice the values measured in our study. These higher values may have been due either to the faster turnover of glycine-nitrogen or to splanchnic uptake and metabolism of some of the orally administered tracer.

Glycine is regarded as a dispensable amino acid, meaning that it can be synthesized in sufficient quantities to meet the usual needs of the organism. Many studies have attempted to estimate the de novo synthesis of glycine on the basis of measurements of its flux and oxidation and an estimates of its rate of release from protein breakdown (11, 22). Using the same approach with our data, we found a significant increase from baseline in endogenous glycine synthesis on days 3 and 6. However, because the content of glycine in different body proteins varies and the turnover rates of individual proteins are considerably different, we were reluctant to put too much emphasis on the validity of these absolute values for endogenous glycine synthesis. Nevertheless, to maintain nonoxidative glycine disposal constant in the face of a 16% reduction in protein-derived glycine implies that de novo glycine synthesis was stimulated by ≈16%.

If glycine synthesis were stimulated by ≈16% with a marginal-protein diet, the ability to maintain this rate of formation would require the availability of ≈36 μmol N·kg LBM^{-1}·h^{-1} at the same time that the dietary intake of nitrogen decreased by 179 μmol N·kg^{-1} LBM·h^{-1}. This implies that other mechanisms for conserving nitrogen would have to be in operation. Again, there is some debate over these mechanisms for conserving nitrogen. Young et al (23) showed that urea production, excretion, and hydrolysis increased linearly with nitrogen intake, whereas we previously showed that adaptation to diets low in protein mostly involves changes in the disposal of urea in the body. With low-protein diets there is enhanced hydrolysis of urea in the lower bowel, with the salvaged urea nitrogen being returned to the system for further metabolic uses (3, 24). When the consumption of protein is reduced, nitrogen balance is restored in large part because of a decrease in the urinary excretion of urea (24, 25). However, within the normal range of protein consumption, this decrease in urea excretion is not a simple reflection of an equivalent reduction in the rate of urea production. As the protein intake decreases, there is a shift in the relative disposal of urea, with a decreased proportion being excreted through the kidney and an increased proportion being hydrolyzed in the lower bowel (26, 27). On the basis of the results of our previous studies (3, 24–26), a conservative estimate can be made that at intake of 1.1 g protein·kg^{-1}·d^{-1}, urea production would be ≈230 mg N·kg LBM^{-1}·d^{-1}, with 175 mg N·kg LBM^{-1}·d^{-1} being excreted in urine and 55 mg N·kg LBM^{-1}·d^{-1} undergoing hydrolysis. However, at an intake of 0.75 g protein·kg^{-1}·d^{-1}, urea production would be ≈200 mg N·kg LBM^{-1}·d^{-1}, with 100 mg N·kg LBM^{-1}·d^{-1} being excreted in urine and 100 mg N·kg LBM^{-1}·d^{-1} undergoing hydrolysis (3, 24). Thus, with the lower-protein diet an additional amount of urea nitrogen, ≈45 mg urea N·kg LBM^{-1}·d^{-1} (or 135 μmol N·kg LBM^{-1}·h^{-1}), would be salvaged. Usually only a part of the salvaged urea nitrogen is used for the formation of amino acids, estimated at ≈66% of that potentially available (3, 28). On the basis of these conservative estimates, a diet providing 0.75 g protein·kg^{-1}·d^{-1} would make available for synthesis of amino acids ≈88 μmol extra nonessential N·kg LBM^{-1}·h^{-1}, of which 41% (36 μmol N·kg LBM^{-1}·h^{-1}) could contribute to maintaining the endogenous synthesis of glycine. This is possible because it was

shown previously that [^{15}N]ammonia gets incorporated into glycine (29). Although the exact route is not certain, some possibilities include threonine degradation from threonine synthesized in the colon and de novo glycine formation through the peroxisomal aminotransferase pathway with the formation of serine, glycine, or both. For glycine, the amino donor is thought to be alanine and the carbon donor is glyoxylate, although the possible sources of glyoxylate from within the intermediary metabolism carbon pathways are not known.

Of course there are limitations to the whole-body protein turnover model that was used to derive this increase in de novo glycine synthesis. For example, the model assumes that phenylalanine flux relates to the rate of glycine influx from proteolysis according to a fixed ratio of phenylalanine to glycine in whole-body protein. Invariably, this ratio is not fixed in all body proteins, and different body proteins turn over at different rates. Therefore, the rates of release of these 2 amino acids may not be equal in the true biological system because of the different rates of breakdown of individual proteins. For example, muscle amino acids will turn over at a slower rate than in the splanchnic pool; therefore, the rates at which phenylalanine and glycine enter the plasma pool from these individual protein compartments may vary (4). Thus, a 16% reduction in phenylalanine flux does not necessarily translate into a 16% reduction in the rate of release of glycine from protein breakdown. Hence, it may not be necessary to impute an increase in de novo glycine synthesis to maintain its flux.

A similar adaptation in tyrosine kinetics was observed in response to the marginal protein intake. Again, despite a reduction in tyrosine derived from protein breakdown, tyrosine flux did not change significantly, indicating that the endogenous supply of tyrosine is maintained. Traditionally this amino acid has been considered conditionally indispensable because it is thought to be synthesized exclusively from phenylalanine and because tyrosine inclusion in the diet exerts a sparing effect on the dietary phenylalanine requirement. In classic phenylketonuria there is a definite requirement for tyrosine when phenylalanine 4-monooxygenase is absent or greatly diminished. There is also evidence that tyrosine is indispensable to the diet in other diseases, such as alcoholic cirrhosis in malnourished patients (30). Thus, in the fasted state tyrosine is derived solely from phenylalanine hydroxylation and protein breakdown. Because the typical tyrosine content of protein is slightly less than the phenylalanine content, the tyrosine flux we observed appears to be high. Once again, this may have been due to a limitation in the whole-body protein turnover model as discussed above. Nevertheless, in the present study of young healthy adults, the marginal amount of dietary protein appears to be adequate for the maintenance of tyrosine production.

Finally, because our kinetic measurements were made only in the fasted state, they may not represent the response in the fed state. There is reason to believe that the same trend would not have been observed in the fed state. In agreement with our present findings, a similar study of fasted adult men by Motil et al (31) showed that endogenous lysine flux, an index of protein breakdown, and nonoxidative lysine disposal, an index of protein synthesis, were ≈15% lower when subjects had a marginal protein intake of 0.6 $g \cdot kg^{-1} \cdot d^{-1}$ compared with an adequate intake of 1.5 $g \cdot kg^{-1} \cdot d^{-1}$. In the fed state there was a marked decrease in endogenous lysine flux to almost identical values with both diets, but the increase in nonoxidative lysine disposal elicited by feeding was 24% lower in subjects consuming the marginal-protein diet. Hence, in the fed state, protein breakdown was suppressed to lower but similar rates with both diets. Protein synthesis, on the other hand, was stimulated to a greater extent by the high protein intake. As a consequence, net protein synthesis was 50% less in subjects who consumed the marginal protein intake of 0.6 $g \cdot kg^{-1} \cdot d^{-1}$ than in those who had an adequate intake of 1.5 $g \cdot kg^{-1} \cdot d^{-1}$. From these findings we can extrapolate that in the fed state in the present study, nonoxidative disposal—and hence net protein synthesis—would have been lower with the marginal-protein diet even though endogenous phenylalanine flux would have been suppressed to the same value with both diets. Furthermore, although the same amounts of glycine and tyrosine would have been released from protein breakdown with both diets, the absolute amounts would have been markedly lower in the fed state than in the fasted state.

In summary, we observed a rapid adaptation to a moderate protein intake, equivalent to the amount recommended by the FAO/WHO/UNU expert group in 1985 (7). During adaptation, several countermeasures for the conservation of nitrogen are brought into play, including decreased whole-body protein turnover, net protein catabolism, and nitrogen excretion. Yet glycine and tyrosine fluxes are maintained, suggesting that to a considerable extent the metabolic supply of these dispensable amino acids is maintained.

We are grateful to Professor Y Lu for his assistance with these studies and to Margaret Frazer and Melanie Del Rosario for their invaluable technical help with the specimen analyses.

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