Effects of Low Energy Diets on Protein Metabolism Studies with [¹⁵N]Glycine in Obese Patients

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Summary The effects of low energy diets on protein metabolism in terms of the metabolic pool, active protein pool, and active and inactive protein synthesis rates were studied using [¹⁵N]glycine in five obese patients (percentage of ideal body weight, 120–190%). For 10 days, the patients were given a control diet containing 2,000 kcal of energy and 80 g of protein. For the next 2 weeks, they were given Diet A with 1,100 kcal of energy and 70 g of protein, and for the last 2 weeks given Diet B with 1,100 kcal of energy and 50 g of protein. During the Diet A period, the active protein pool and the active and inactive protein synthesis rates were about the same as during the control diet period, although the metabolic pool tended to be slightly smaller than during the control diet period. During the Diet B period, the metabolic pool, active protein pool, and active protein synthesis rate were all significantly different from the values during the control diet period. The results suggest that protein metabolism in obese patients is not maintained with less than 70 g of protein daily when energy intake was restricted to 1,100 kcal/day.

Key Words obese patients, protein metabolism, energy restriction, low energy diet, metabolic pool, active protein pool, inactive protein pool, active protein synthesis rate, inactive protein synthesis rate, [¹⁵N]glycine

When energy is restricted, the nitrogen balance becomes lower; an increase in the protein intake under energy reductions improves the nitrogen balance (1–3). A detailed picture of this phenomenon has been made possible by using tracer amino acids, which allows estimation of the rate of body protein turnover (4–9). Garlick *et al.* (6) studied the nitrogen flux and protein synthesis and breakdown using

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[^{15}N]glycine in an energy restricted diet at various protein levels. They suggested that restriction of energy intake in obesity does not interfere with whole-body protein turnover, provided that the subject has an adequate intake of protein. A protein-sparing modified fast (4–12) wherein the nitrogen balance is maintained has been used in adults as a method for weight reduction. Thus, the amount of protein intake is an important factor in diets designed for the treatment of obesity.

We have studied the effect of the protein level during energy restriction on the nitrogen balance of obese patients (13). Here we examined the effect of protein levels during energy restriction using [^{15}N]glycine to study whole-body protein metabolism in obese patients; the metabolic pool, active protein pool, and active and inactive protein synthesis rates were estimated from the ^{15}N -enrichment of urinary nitrogen. Sprinson and Rittenberg (14) evaluated their hypothesis on nitrogen metabolism of the metabolic pool and protein synthesis rate. Many investigators (15–17, 20–24, 26–28) have used their theories and techniques. [^{15}N]Glycine is often used as a tracer in studies of protein metabolism on the assumption (15–17) that the labeled glycine participates freely in protein turnover. It is further assumed (15) that the metabolic state does not alter during the course of measurement. We have made such assumptions while using ^{15}N excretion data to estimate protein metabolism.

METHODS

Subjects. Five obese (percentage of ideal body weight: 120–190%) patients, three men and two women, were studied. Their characteristics are summarized in Table 1. All patients were hospitalized during the study and were put on the hospital diet. One patient (Case 4) decided to leave the hospital after the first two parts of the experiment. The full nature and purpose of the study were explained to the subjects and their consent was obtained before starting the study.

Table 1. Characteristics of subjects.

Case no.	Sex	Age	Height (cm)	B.W. (kg)	Ideal B.W. ^a (%)	B.W. loss (kg/2 wk)	
						Diet A	Diet B
1	M	59	161.5	66.5	120.0	–3.0	–1.2
2	M	59	156.0	64.0	127.0	–2.0	–1.2
3	M	51	166.0	75.0	126.3	–2.5	–1.7
4	F	59	149.5	55.5	124.6	–2.5	—
5	F	51	150.8	87.0	190.4	–1.5	–1.7
Mean $\pm$ SD						–2.3 $\pm$ 0.6	–1.5 $\pm$ 0.3

^a Ideal B.W. (kg): (height – 100) $\times$ 0.9.

Experimental diet. The composition of the diet was the same as in a previous report (13). The study period was divided into three parts, with the control diet (energy, 2,000 kcal with 80 g of protein) lasting 10 days, and both Diet A (energy, 1,100 kcal and 70 g of protein) and Diet B (energy, 1,100 kcal and 50 g of protein) lasting two weeks each.

Experiment. Measurement of protein metabolism was started on day 6 of the control diet and on day 10 in Diets A and B. The patients were orally given 7 mg of [^{15}N]glycine (98 atom%) per kg of body weight on the first day of each measurement period. Urine was collected at 0, 1, 2, 3, 5, 7, 12, 24, 36, 48, 60, 72, 96, and 120 h after the administration of [^{15}N]glycine. The samples were assayed for ^{15}N . Urea nitrogen and creatinine in 24-h urine were analyzed with an autoanalyzer (Toshiba TBA 80-S, Toshiba Medical).

Assay of ^{15}N levels. Urine was assayed for nitrogen by the semimicro Kjeldahl method. Nitrogen in the sample was converted to ammonium sulfate and discharge tubes were prepared by the method of Yamamuro (18) for ^{15}N assay on a ^{15}N analyzer (NIA-1 emission spectrometer, Japan Spectropic Co.).

Theoretical considerations and calculation. Tanaka and Tsuchida (19, 20) reported a method for calculations of metabolic pool size and protein synthesis rate based on a modification of the metabolic model of Olesen *et al.* (21) as shown in Fig. 1. The metabolic pool (Pm), active protein pool (Pa), and active (Sa) and inactive (Si) protein synthesis rates were calculated from the cumulative curve of urinary ^{15}N excretion after [^{15}N]glycine was given. One typical cumulative curve of urinary ^{15}N excretion (Case 4) is shown in Fig. 2. Here, the metabolic calculations were made by the method of Tanaka and Tsuchida (19, 20), which was explained in further detail by Ishii *et al.* (22). The first four equations (Eqs. 1–4) consists of hourly changes in the metabolic model as shown in Fig. 1 when the compound of labeled ^{15}N (^{15}N of a mg) was administered to the subjects. The ^{15}N level in the different pools was expressed as Pm^* , Pa^* , Pi^* , and E^* , and the protein turnover rate as k_1 , k_2 , k_3 , k_5 , and k_6 . When the experimental period is short, k_6 can be ignored.

Fig. 1. Model of pools (mg N/kg) in protein metabolism with designations of each component. (a), amount of administered; k_1 – k_6 , rates (mg N/kg/day).

Fig. 2. Cumulative curve of urinary ^{15}N excretion after administration of $[\text{}^{15}\text{N}]$ glycine and analysis of exponential functions of excretion of the nitrogen component (Case 4, Diet A). $E(t)$, cumulative curve of urinary ^{15}N excretion (%); $E(st)$, saturated value of urinary ^{15}N excretion (%); Y_1 , regression line of $E(st) - E(t)$ in later period; Y_2 , regression line of $E(st) - E(t)$ in earlier period.

Pm^* in metabolic pool (Pm):

$$\frac{d \text{Pm}^*}{dt} = -(k_1 + k_2 + k_5) \text{Pm}^* + k_3 \text{Pa}^* . \quad (1)$$

Pa^* in active protein pool (Pa):

$$\frac{d \text{Pa}^*}{dt} = k_2 \text{Pm}^* - k_3 \text{Pa}^* . \quad (2)$$

Pi^* in inactive protein pool (Pi):

$$\frac{d \text{Pi}^*}{dt} = k_5 \text{Pm}^* . \quad (3)$$

E^* in excreted total nitrogen (E):

$$\frac{dE^*}{dt} = k_1 Pm^* . \quad (4)$$

From the above equations, the solutions obtained are as follows:

$$\begin{aligned} Pm &= \frac{E}{k_1} ; \\ Pa &= \frac{Ak_2}{k_3} ; \\ Sa &= Pmk_2 \quad Si = Pmk_5 ; \\ S &= Sa + Si = Pmk_2 + Pmk_5 . \end{aligned}$$

Hence, the metabolic pool, protein pool, and protein synthesis rates can be calculated using these equations. Each value of k_{1-5} can be calculated from the two regression lines ($Y_1 = R_1^{-\lambda t}$ and $Y_2 = R_2^{-(\lambda+r)t}$) in Fig. 2. The half-life period $T_{1/2}$ is calculated from the slope of Y_1 and Y_2 , and then λ and $\lambda+r$ are obtained from the relationship of $0.693/T_{1/2}$. Accordingly, k_{1-5} can be calculated from following equation:

$$\begin{aligned} k_3 &= \frac{\lambda(\lambda+r) \left(\frac{R_1}{R_2} + 1 \right)}{(\lambda+r) + \lambda \frac{R_1}{R_2}} \\ k_1 &= \frac{R_1 \cdot \lambda r}{(k_3 - \lambda)} \\ k_5 &= \frac{\lambda(\lambda+r)}{k_3} \\ k_2 &= \lambda + (\lambda+r) - k_1 - k_3 - k_5 . \end{aligned}$$

Each pool includes a variety of body tissues. The metabolic pool includes, among other substances, free amino acids in the body; the active protein pool includes serum protein, liver, kidney, and other internal organs with rapid protein turnover; the inactive protein pool includes muscle and connective tissue, the protein turnover of which is slow. The metabolic pool plus the active protein pool is regarded as reserve protein.

Data analysis. Data were analyzed by Student's *t*-test and *p* values of less than 0.05 were considered to be statistically significant.

RESULTS

Body weight

The mean weight loss was 2.3 ± 0.6 kg in the Diet A period and 1.4 ± 0.3 kg in

Table 2. Urinary nitrogen excretion.

	<i>n</i>	Control ^a (5)	Diet A ^b (5)	Diet B ^c (4)
Total	(g N/day)	9.8±0.9	9.5±0.7	7.4±1.3*
Urea	(g N/day)	8.3±0.5	8.3±0.9	5.8±1.4*
Urea/Total	(%)	85.9±3.7	88.7±3.5	82.4±3.9
Creatinine	(g N/day)	1.3±0.1	1.3±0.3	1.3±0.3

^a Control diet contained 2,000 kcal of energy and 80 g of protein. ^{b,c} Diets A and B both contained 1,100 kcal, with 70 g of protein in A and 50 g of protein in B. Values are means±SD. * Significantly different from control diet ($p < 0.05$).

Fig. 3. Size (mg N/kg) of the metabolic pool (Pm), active protein pool (Pa), and inactive protein pool (Pi), and the rate (mg N/kg/day) of active and inactive protein synthesis in Case 4.

the Diet B period, or a loss of 3.6 ± 0.5 kg for the 4 weeks (Table 1).

Urinary nitrogen excretion

Excretion of nitrogen in the urine on the three diets is shown in Table 2. Total nitrogen excretion while on the control diet and Diet A was about the same; that on

Table 3. Protein pool and protein synthesis rate.

Diet period	n	Protein pool ^a (mg N/kg)			Protein synthesis rate ^b (mg N/kg/day)		
		Pm	Pa	Pm + Pa	Sa	Si	Sa + Si
Control diet	5	245 ± 88	175 ± 114	420 ± 59	397 ± 341	246 ± 84	641 ± 296
Diet A	5	179 ± 86	171 ± 107	350 ± 79	357 ± 345	158 ± 86	515 ± 401
Diet B	4	484 ± 112*	75 ± 78	583 ± 69*	93 ± 84	254 ± 64	385 ± 90
Difference ratio							
Diet A/Control		0.74 ± 0.22	1.02 ± 0.14	0.84 ± 0.20	0.87 ± 0.26	0.67 ± 0.27	0.75 ± 0.29
Diet B/Control		2.42 ± 1.02*	0.41 ± 0.23*	1.47 ± 0.23*	0.23 ± 0.08*	1.19 ± 0.38	0.67 ± 0.41

^a Pm, Pa, and Pm + Pa represent the metabolic pool, active protein pool, and the sum of values of Pm and Pa, respectively. ^b Sa, Si, and Sa + Si represent the active protein synthesis rate, inactive protein synthesis rate, and the sum of Sa and Si, respectively. Control diet contained 2,000 kcal and 80 g of protein. Diets A and B contained 1,100 kcal of energy and 70 g and 50 g of protein, respectively. Values are means ± SD. * Significantly different among the same column at $p < 0.05$.

Diet B was significantly lower. The pattern for urinary urea nitrogen was the same. The ratio of urea to total nitrogen excretion was about the same on the different diets. There were no changes in creatinine excretion during each diet period.

Pool size and protein synthesis rate

Pool size and protein synthesis rate in each diet period are shown in Table 3. Changes for one patient (Case 4) are shown in Fig. 3. In the Diet A period, the active protein pool (Pa) and active (Sa) and inactive (Si) protein synthesis rates were similar to those during the control diet period, whereas the metabolic pool (Pm) tended to be lower. In the Diet B period, protein metabolism was different from that during the control diet period; Pm was significantly larger, Pa tended to be smaller, Pm+Pa were significantly larger, and Sa was lower than during the control diet period, although the Si value was similar on the control diet. Thus, protein metabolism during the Diet A period was maintained at the same level during the control diet, but during the Diet B period it was not. Differences from the control diet (difference ratio) were usually greater during the Diet B period: Pm, Pa, Pm+Pa, and Sa had difference ratios (Table 3) to the control diet that were significantly different from those during the Diet A period.

DISCUSSION

The method of Picou and Taylor-Roberts (23) for the study of whole-body nitrogen turnover in different physiological and pathological states has been widely used; this field has been extensively reviewed by Waterlow *et al.* (24). Reviews on the measurement of protein turnover with ^{15}N concluded that the single-dose approach is useful for measurements of protein synthesis and breakdown, and that it can be used in the same way as the constant infusion or repeated dose approach. We chose the single dose approach because it is less of a burden on the patient (the process is simple and convenient) and can be repeated many times.

Winterer *et al.* used [^{15}N]glycine to study the protein turnover rate and found that protein synthesis and breakdown are maintained at the normal rate in moderately obese but otherwise healthy women after three weeks on a diet consisting only of high-quality animal protein (1.5 g/kg of the ideal body weight), which provided only 440 kcal/day (4). Pencharz *et al.* (8) reported that the effects of an energy restricted (46 kJ/day/kg), adequate protein diet (1.47 g/day/kg) on the nitrogen metabolism of five obese, rapidly growing adolescents (two males and three females) were assessed by means of nitrogen-balance measurements and determination of whole-body nitrogen turnover. Restriction of energy intake in obesity does not interfere with whole-body protein turnover, provided that the subject receives adequate protein in the diet (25). Obese subjects maintain protein metabolism with energy restriction while using endogenous fat as their principal energy source. The level of protein intake is important for the maintenance of protein turnover during energy restriction (1-3). Therefore, the protein level must

be appropriate in diets for the treatment of obesity.

This study examined protein metabolism in two low energy diets with the same energy levels but different amounts of protein. Whole-body protein metabolism was maintained on Diet A but not on Diet B. It is thought that the loss of body protein is due to deficient protein intake in view of the significant changes in the metabolic state during the Diet B period. It seems that Pm is significantly larger because some of the lost body protein flows into the Pm and that Sa is significantly lower because the protein synthesis ability decreases. Our results indicate that the protein level of Diet A was suitable for the maintenance of protein metabolism with energy restricted to 1,100 kcal/day.

We reported elsewhere (13) that the protein level of Diet A is needed to maintain the nitrogen balance, at least when energy intake is restricted to 1,100 kcal/day. The study reported here confirmed the results of our previous report (13) from the aspect of whole-body protein turnover. Our data on the effect of low energy diets on protein metabolism in obese subjects were in agreement with the results of other investigators (4-9). However, our experiment had some points of difference from previous investigations (4-9); the composition of Diets A and B was natural foods, and the energy level was higher (1,100 kcal) to allow maintenance of the basal metabolic rate. The energy intake (15 ± 3 kcal/kg) was about one-third of the maintenance energy intake (45 ± 2 kcal/kg) for young Japanese adults (3). It is not clear whether protein metabolism can be maintained even with the protein level

Table 4. Comparison of pool size and protein synthesis rates in adults of normal weight and in obese patients.

	Adults of normal weight ^a				Obese patients ^b
	Sprinson and Rittenberg (14)	Olesen <i>et al.</i> (21)	Yamanaka (27)	Honda (28)	This study
Pool size (g N)					
Pm ^c	19.7	36.7	5.7	12.2	19.9 ± 5.4^d
Pa ^c	7.1	31.9	16.8	3.6	12.7 ± 9.6
Protein synthesis rate (g N/day)					
Sa ^e	19.2	50.1	27.1	25.4	28.3 ± 27.4
Si ^e	14.1	19.1	19.6	19.9	15.9 ± 4.8
Sa + Si ^e	33.3	69.2	46.9	45.3	44.3 ± 26.7

^a The results for normal-weight adults are cited from Tanaka and Tsuchida (19, 20); they recalculated data from other investigations (14, 21, 27, 28) using their own method. ^b The results for obese patients are data obtained during the control diet for this study. ^c Pm and Pa represent the metabolic pool and active protein pool, respectively. ^d Values are means $\pm$ SD. ^e Sa, Si, and Sa + Si represent the active protein synthesis rate, inactive protein synthesis rate, and the sum of Sa and Si, respectively.

of Diet A when healthy subjects are put under such severe energy restriction. Sim *et al.* (26) reported on the whole-body protein turnover in normal-weight adults receiving amino acids by intravenous infusion under reduced energy intake. They indicated (26) that whole-body protein synthesis and breakdown were reduced to 62% and 75% of the control values, respectively, although the protein intake was sufficient. Tanaka and Tsuchida (20) reported on the metabolic pool, active protein pool, and active and inactive protein synthesis rates in normal-weight adults, calculated from the data of Sprinson and Rittenberg (14), Olesen *et al.* (21), Yamanaka (27), and Honda (28). Our results with the control diet were similar to theirs, as shown in Table 4. The results suggest that there are no essential differences in the protein metabolism of obese and normal-weight subjects.

Our results showed that protein metabolism in obese patients is not maintained on protein levels less than that of Diet A (70 g/day) when energy intake is restricted to 1,100 kcal/day. In general, obese patients need enough protein intake for protein metabolism when energy intake is restricted for the reduction of body weight.

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