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Whole Body Protein Synthesis and Turnover in Normal Man and Malnourished Patients with and without Known Cancer

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Plateau enrichment of ^{15}N-ammonia following 24 hour continuous intravenous infusion of ^{15}N-glycine was used to measure total body protein turnover and synthesis in normal volunteers and malnourished patients, with and without cancer. The mean postabsorptive total body protein synthesis rate in three normal controls was 2.5 g protein/kg/day. Protein synthesis and turnover decreased by a mean of 23% following one week of fasting, and returned to baseline levels following one week of refeeding. In three malnourished patients without known tumor, whole body protein synthesis and turnover was similar to controls; following seven to ten days of total parenteral nutrition (TPN) in the nontumor-bearing patients whole body protein synthesis and turnover decreased by 18%. Three of seven malnourished patients with known tumors had marked elevations in total body protein synthesis and turnover; TPN increased protein turnover in five of seven patients with known cancer. This study suggests that some malignant tumors can increase whole body protein synthesis and turnover in both the malnourished and fed state. This increase in protein turnover may represent a direct effect of the tumor, or reflect concomitant illness.

THE STABLE ISOTOPE of nitrogen(^{15}N) can be used to label amino acid nitrogen and measure protein synthesis rates in man. It is routinely administered by constant intravenous infusion or orally until plateau levels are obtained, and the whole body protein synthesis rate determined from the ^{15}N enrichment of the end product (urea or ammonia). Much of the previous work has been directed at simplifying the theory and improving the methods of analysis.[3,4] Normal values have been obtained,[13] and whole body protein synthesis rates calculated from measured nitrogen intake and output in intravenously fed normal man,[13] malnourished children,[8,11,20] septic man,[6] burned man,[5] and at different ages in orally fed normal man.[23]

Increased demands for protein and calories in the cancer patient have been reported without clear explanation. In an attempt to further understand protein metabolism in cancer patients, we performed the following study. We initially defined a method using ^{15}N-glycine to measure total body protein turnover in normal volunteers in different nutritional states. We then compared whole body protein turnover determinations in these normal volunteers with determinations in malnourished patients. The patients studied were all depleted nutritionally, and were with and without known malignant tumors. All were studied prior to and during total parenteral nutrition.

Materials and Methods

All volunteers and patients were studied according to a protocol approved by the Clinical Research Committee of the Clinical Center, National Institutes of Health. Informed consent was obtained from all patients prior to their acceptance into the study.

^{15}N-glycine was administered to a normal volunteer at a dose of 4 mg/kg/day by constant intravenous infusion for a total infusion time of 50 hours. The oral administration of water was permitted. Urine was col-

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TABLE 1. *Age, Sex, and Body Weight of Normal Volunteers During Study*

Volunteer Number	Sex	Age	Weight Pre-baseline Study (kg)	Weight Post-fasting Study (kg)	Weight Pre-refeeding
1	M	28	71.0		
2	M	31	81.9	77.9	79.4
3	M	27	77.3	73.0	75.0

lected in three-hour aliquots and immediately refrigerated. Twenty-four hour urinary total nitrogen and urea nitrogen excretion were measured. Five milliliter samples from each urinary aliquot were placed on a permutit resin column (ANGC-101, J. T. Baker Co.) to separate urea nitrogen from ammonia nitrogen. Enrichment of urinary urea nitrogen and urinary ammonia nitrogen by ^{15}N was measured using optical emission spectroscopy by methods described previously.[15,17,18]

All subsequent studies were performed with a constant 24-hour intravenous infusion of 2 mg/kg/day of ^{15}N-glycine, and urine was collected in three-hour aliquots, and analyzed for ^{15}N-ammonia. The subjects were allowed only water by mouth during the ^{15}N-glycine studies; if they were receiving total parenteral nutrition solution it was continued at the same rate. Plateau enrichment of urinary ammonia was defined as the mean ^{15}N-ammonia enrichment at 21 and 24 hours. Urinary nitrogen balance studies were performed measuring total urinary nitrogen excretion; nitrogen intake was either zero or the amount of nitrogen in the total parenteral nutrition solution. Plateau urinary enrichment of ammonia was used to measure total body protein flux (Q), which was combined with urinary nitrogen excretion to calculate whole body protein synthesis (S).[10,11,14,19,21]

Two normal volunteers were studied in greater detail. Both volunteers were without illness, obesity, or diabetes. Both had a normal fasting blood cell count and chemistry profile. A 24-hour baseline ^{15}N-glycine infusion was done. Both underwent a complete seven-day fast, in which no calorie or nitrogen intake was allowed. A second ^{15}N-glycine study was performed following the fast period. Finally both ate food *ad lib* for another seven-day period and a third ^{15}N-glycine infusion was performed. Age, sex and body weights for the normal volunteers are given in Table 1.

Ten patients all with protein calorie malnutrition were studied with ^{15}N-glycine prior to and during total parenteral nutrition (TPN). Any patient that the nutritional support service planned to start on TPN was given the opportunity to participate in this study. Baseline 24-hour ^{15}N-glycine infusions were performed as before, and urinary enrichment of ^{15}N-ammonia was measured. During baseline infusions, patients consumed only water by mouth. Following the baseline study the patients were started on TPN (20–25% dextrose, 4.25% Freamine II with added trace elements, vitamins and Intralipid® (Cutter Medical, Berkeley, Calif.) 500 ml twice weekly. After 10 to 14 days, the patients were given a second ^{15}N-glycine study. Again urinary enrichment of ^{15}N-ammonia was measured and protein turnover and synthesis rates calculated and compared with baseline rates.

The patient population was diverse, ranging from no tumor, to large tumor burdens. Table 2 gives data on the patients studied: age, sex, diagnosis, disease extent and body weight compared with ideal weight is listed. Patients #1, 2, and 3 were considered to be without tumor during the study. Patient #1 initially presented with a pleural effusion with elevated amylase, weight loss and did not have cancer. Patient #2 had undergone an 18-month course of treatment for a large retroperitoneal sarcoma and was failing nutritionally. He had undergone surgery, irradiation, and chemotherapy. He subsequently died of hepatic failure, and no tumor was found at necropsy. Patient #3 was recovering from removal of an islet cell tumor, and was believed to be free of macroscopic disease by surgery.

TABLE 2. *Age, Sex, Diagnosis, Tumor Burden, and Weight at the Time of Study*

Patient	Age	Sex	Diagnosis	Tumor Burden	Ideal Weight (kg)	Actual Weight (kg)
1	17	F	Duodenal duplication	None	54	37
2	53	M	Sarcoma	None	64	46
3	70	M	Islet cell tumor	None	68	56
4	64	M	Adenocarcinoma rectum	Local recurrence	65	47
5	24	M	Seminoma	Lung metastases	74	51
6	22	M	Choriocarcinoma	Lung metastases	88	89
7	21	M	Teratocarcinoma	Retroperitoneal lymph node metastases	74	53
8	23	M	Teratocarcinoma	Retroperitoneal lymph node metastases	84	70
9	43	M	Nonfunctional islet cell tumor	Liver metastases	66	46
10	65	F	Sarcoma	Large abdominal mass tumor	56	92

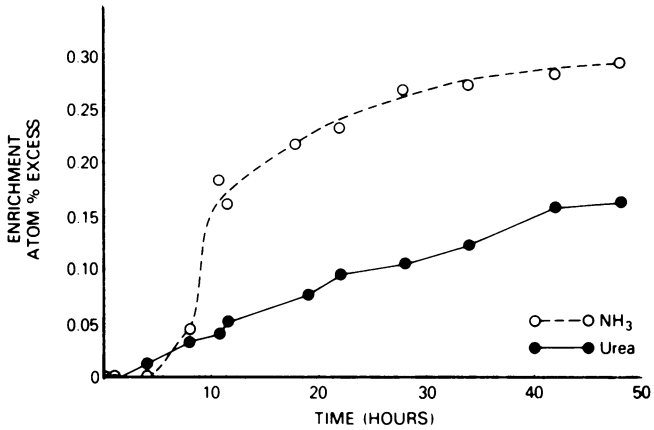

FIG. 1. Comparison of end products, ^{15}N-ammonia (NH_3) versus ^{15}N-urea during a constant intravenous infusion of ^{15}N-glycine in a normal volunteer. The ^{15}N enrichment of ammonia peaks earlier and plateaus earlier; ^{15}N enrichment of urea does not appear to plateau at 50 hours. ^{15}N-ammonia enrichment appears to plateau at 20 hours.

Patients 4–10 all had known neoplastic tissue present. All were at least one week postoperative prior to inclusion in this study, and none received chemotherapy or radiation therapy during this study. Patient #4 had known locally recurrent adenocarcinoma of the rectum following abdominoperineal resection. He had a draining open wound that appeared to be necrotic. Patient #5 had seminoma with known lung metastases, and was scheduled for radiation therapy. Patient #6 had a choriocarcinoma with massive mediastinal and pulmonary metastases. He also had a large amount of necrotic bulk tumor in the right upper part of the abdomen. Patients #7 and 8 both had teratocarcinoma of the testis with extensive disease and were treated following retroperitoneal lymph node dissection to remove bulk tumor. Patient #9 had a malignant nonfunctioning islet cell tumor of the pancreas with liver metastases confirmed by arteriographic examination, and was a candidate for hepatic artery infusion of chemotherapy. Patient #10 had a huge retroperitoneal soft tissue sarcoma and was studied prior to surgical resection of this 12 kg tumor.

Results

Comparison of End-products

Figure 1 shows the result of a 50-hour intravenous infusion of ^{15}N-glycine (4 mg/kg/day) in a normal volunteer. The ^{15}N enrichment of ammonia was compared with the enrichment of urea on each aliquot of urine during the study. The ^{15}N enrichment of ammonia becomes greater than urea enrichment at ten hours of ^{15}N-glycine infusion, and it remains greater than urea throughout the entire infusion. In previous studies, ammonia has been the preferred end-product, because of greater enrichment, and earlier plateau than urea.[17,21,22] However, in our volunteer, enrichment of ammonia did not appear to plateau as early as expected, but it did plateau at 20 hours of infusion. Enrichment of urea did not appear to plateau during the entire 50-hour infusion. For our clinical studies with multiple infusions in the same volunteer or patient, we chose to measure enrichment of ammonia at 20–24 hours following ^{15}N-glycine infusion. We measured ^{15}N-ammonia enrichment at these two time points in all patients, and it did not differ by greater than 10%. We took the mean of the enrichment at 21 and 24 hours, and used it to calculate protein turnover.

Control Subjects

The mean baseline whole body protein turnover in the three normal volunteers was 2.9 g protein/kg/day, and the mean baseline whole body protein synthesis rate was 2.4 g protein/kg/day. These levels agreed well with data reported by others using the same method when corrected for age.[21,22] In the two controls who fasted for seven days, total body protein synthesis and turnover decreased in both cases. In one, synthesis decreased 14%, and the other 30%; both had similar 4 kg weight losses during the week fast. Following the week of refeeding, both increased their whole body protein turnover and synthesis levels back to baseline. One regained 1.5 kg, and the other regained 2 kg. All the values for total body protein turnover and synthesis in the volunteers under different nutritional manipulations are given in Table 3.

Malnourished Patients

In the three protein–calorie malnourished patients without demonstratable tumor present (patients number 1–3), whole body protein turnover and synthesis rates were in the same range as normal volunteers (Figs. 2 and 3). Following TPN in these patients, whole body protein turnover and synthesis decreased

TABLE 3. *Flux and Synthesis During Normal Volunteer Studies*

Volunteer Number	Baseline		Fast		Refeed	
	Q	S	Q	S	Q	S
1	2.2	2.0	—	—	—	—
2	3.4	2.9	2.7	2.5	3.2	2.7
3	3.1	3.1	2.3	1.6	3.2	2.6
$\bar{x}$	2.9	2.4	2.5	2.05	3.2	2.65

Where Q is whole body protein flux in grams protein/kg/day.
Where S is whole body protein synthesis in grams/protein kg/day.
Baseline is the initial ^{15}N-glycine study during the fed state. Fast is the second study following a week of fasting, and Refeed is the third study following a week of eating *ad lib*.

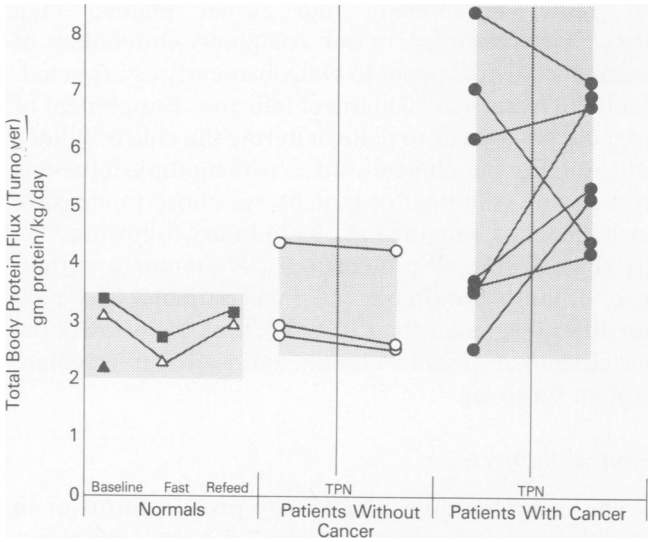

FIG. 2. Total body protein turnover in normal volunteers and patients with and without known cancer. Protein turnover in normals appears to decrease with fasting and increase back to baseline levels with refeeding following a fast. Protein turnover in malnourished patients without known cancer is in same range as normals and appears to decrease slightly following total parenteral nutrition (TPN). Protein turnover in malnourished patients with known cancer is more variable than other groups, but appears to be greater than other groups in some patients before and after TPN.

slightly in each patient. Turnover had a mean decrease of 7%, and synthesis had a mean decrease of 30% on TPN. In the seven patients (numbers 4–10) with varying amounts of tumor present and degrees of illness, whole body protein flux and synthesis appeared to be greater than the controls and other patients (Table 4, Figs. 2 and 3). Five of seven patients had an increase in total body protein turnover while on TPN, for a mean increase of 30% with much variability (Fig. 2); two patients showed a definite decrease in protein turnover. Four of seven patients in this more progressive disease group showed a decrease in protein synthesis on TPN, but the net effect on all seven patients was essentially no change in total body protein synthesis on TPN (Fig. 3).

Discussion

The use of stable isotopes of nitrogen for clinical studies of protein metabolism has considerable appeal. Because of the absence of radioactivity, ^{15}N can be used safely in normal controls, in young children, and for repetitive studies in patients. The choice of ^{15}N enrichment of urinary ammonia appears to be the preferred end-product because of the greater enrichment at a given ^{15}N dose and the shorter infusion time to plateau.[17,21,22] While absolute plateau levels are rarely obtained, the present study compares enrichment at two time points 21 and 24 hours of intravenous infusion, where the difference between ^{15}N enrichment of urinary ammonia has reached less than 10% in all cases. The mean baseline total body protein synthesis rate (2.4 g protein/kg/day) of our three normal controls using this method agreed well with levels reported by others when one considers age differences.[22]

Whole body protein synthesis is affected by nutritional state. In the classic original study of Picou and Taylor-Roberts total body protein synthesis was noted to be increased in malnourished infants and to decrease following refeeding.[11] The confusion in this study was clarified by Waterlow in his monograph when he stated that all the children had been partially refed prior to the initial study.[22] Waterlow repeated this study and found that malnourished children have decreased rates of total body protein synthesis prior to recovery from malnutrition.[20] In the two control volunteers who fasted for seven days in this study, whole body protein synthesis and turnover was found to decrease in both cases. These findings probably represent a metabolic adaptation to food deprivation, aimed at deriving energy from adipose tissue and conservation of protein reserves.[2] As expected when both of these subjects were provided with food *ad lib*, protein synthesis rates returned to normal levels.

Protein turnover and synthesis is effected by patho-

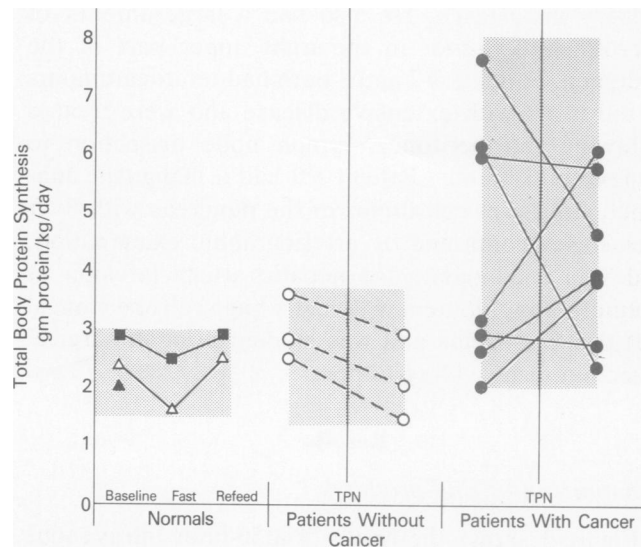

FIG. 3. Total body protein synthesis in normal volunteers and patients with and without known cancer. Protein synthesis in normals appears to decrease with fasting and increase back to baseline levels with refeeding following a fast. Protein synthesis in malnourished patients without known cancer is in the same range as normals, and appears to decrease slightly following total parenteral nutrition (TPN). Protein synthesis in malnourished patients with cancer appears to be greater than other groups in some (3/7) patients prior to TPN and some (3/7) patients following TPN, but the data is more variable in these patients.

TABLE 4. *Flux and Synthesis in Patients Before and During Total Parenteral Nutrition*

Patient Number	Pre-TPN		TPN		Net Change With TPN	
	Q	S	Q	S	Q	S
1	4.4	3.6	4.3	2.9	−2%	−19%
2	2.7	2.5	2.5	1.4	−7%	−44%
3	2.8	2.7	2.5	2.0	−11%	−26%
4	8.6	7.6	7.1	4.7	−17%	−38%
5	2.5	2.0	5.1	3.9	+104%	+95%
6	6.2	5.9	6.7	5.7	+8%	−3%
7	3.7	2.6	5.3	3.9	+43%	+50%
8	3.6	2.8	4.1	2.7	+14%	−4%
9	7.0	6.1	4.3	2.3	−39%	−62%
10	3.5	3.1	6.8	5.9	+94%	+90%
NTB (#1–3) $\bar{x}$ ± SEM	3.3 ± 0.6	2.9 ± 0.3	3.1 ± 0.6	2.1 ± 0.4	−6.7 ± 2.6	−30 ± 7
TB (#4–10) $\bar{x}$ ± SEM	5.0 ± 0.9	4.3 ± 0.8	5.6 ± 0.5	4.2 ± 0.5	+30 ± 20	18 ± 23

Q = whole body protein flux in grams protein/kg/day.
S = whole body protein synthesis in grams protein/kg/day.
NTB = patients without known tumor present.
TB = patients with known tumor present.

logic states. Total body protein synthesis has been shown to be increased in septic patients,[6] and in children recovering from severe burns.[5] From this current study, it appears that whole body protein turnover and synthesis maybe increased in the presence of some advanced malignant tumors. In the various protein–calorie malnourished patients without proven tumor, the whole body protein turnover and synthesis rates were similar to controls. However, prior to TPN, three of seven patients with known progressive malignant tumor had a markedly elevated level for protein turnover and synthesis and, on TPN, five of seven patients had markedly elevated protein turnover (Figs. 2 and 3). These data imply that in some advanced cancer patients, as in septic and burned patients, there is a metabolic demand on the host to increase whole body protein turnover and synthesis. In comparing uncomplicated starvation to cancer cachexia it has been noticed that occasionally large protein and caloric loads are needed to help the patients with tumors achieve a positive nitrogen balance.[1] Protein metabolism is an important part of whole body energy metabolism.[24] This increase in protein turnover in some cancer patients may partly explain the increased energy requirements also reported in these patients. Increases in whole body protein synthesis have been reported in tumor bearing rats, and it was noticed that the rates of protein synthesis by the tumors, themselves, was very rapid in different dietary conditions.[16] Whether the increases in total body protein synthesis noticed in these cancer patients are a direct result of tumor protein synthesis or an indirect effect on the host by the advanced tumor state is speculative.

Total parenteral nutrition did not increase whole body protein turnover or synthesis in the malnourished patients without known tumor who were studied. There is conflicting data in the literature as to what refeeding does to total body protein synthesis,[11,20] but most recent work shows that it increases total body protein synthesis.[22] This study used each volunteer subject and patient as his own control. In the volunteer subjects oral refeeding increased total body protein turnover and synthesis compared with fasting levels in both cases. In the malnourished postabsorptive non-tumor-bearing patient, whole body protein turnover decreased by 7% and synthesis decreased by 30% following TPN. Recently Sim, reported that TPN decreased whole body protein turnover by 21% in normal volunteers, compared with oral feeding levels.[13] We reported similar findings in rats fed intravenously compared with orally.[9] It may be that gastrointestinal processing of food normally accounts for a large part of whole body protein turnover, and the decrease in protein synthesis with TPN reflects the need for less digestive processing of food. Alternatively, it is conceivable that depletion by TPN involves a decrease in protein breakdown.

In the seven patients with known cancer, five increased protein turnover on TPN, and three increased protein synthesis. The failure of some of these patients to decrease protein turnover and synthesis on TPN like normal volunteers and malnourished, nontumor patients may represent an effect of the tumor in these patients. This may partly explain why reversal of protein energy malnutrition in some cancer patients has required greater substrate than expected by normal guidelines for basal energy expenditure.[12]

Diseases like cancer, sepsis, and severe burns may pose a severe nutritional demand on a patient for increased protein synthesis. Whether this increased synthesis is a direct effect of the tumor for actual increased synthesis, or an indirect effect on the host by the tumor will need to be studied subsequently. This increase in protein turnover in the presence of progressive malig-

nant disease may simply represent a breakdown in the normal metabolic integrity of the patient with disease.

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