WHOLE-BODY PROTEIN TURNOVER AND ENERGY EXPENDITURE IN POST-VIRAL HEPATOCIRRHOTIC PATIENTS. A STUDY USING MULTIPLE STABLE ISOTOPE TRACERS TO ESTIMATE PROTEIN AND ENERGY REQUIREMENTS AND THE EFFICACY OF A NEW DIET THERAPY BASED ON CHINESE FOOD

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Abstract

L-[1-^{13}C]-leucine and ^{15}N-glycine doubly-labelled tracer experiments revealed accelerated kinetics of leucine, glycine and whole-body protein in post-viral hepatocirrhotic patients. Together with the results of nitrogen balance measurement, the daily protein requirement of these patients was estimated to be higher than 1.2 g/kg/d. Doubly labelled water experiments and $NaH^{13}CO_3$ experiments revealed that the freely living and basal energy expenditure of post-viral hepatocirrhotic patients was not different from that in normal subjects with comparable physical and mental activities. For those freely living in hospital, the energy requirement is estimated to be 150-160 kJ/kg/d. According to the above results, a therapeutic diet formulation based on Chinese food was designed for the patients which contained 1.5 g/kg/d of protein and 150-160 kJ/kg/d. 60-70% of the dietary protein was of vegetable origin, with a branched chain amino acid/aromatic amino acid ratio slightly but significantly higher than the common hospital diet. Patients with compensated post-viral hepatocirrhosis adapted to the diet rapidly. After two months' therapy, the negative nitrogen balance turned positive along with an increase of body weight and urinary creatinine, indicating an improvement of general nutritional status, probably with accumulation of muscle protein. The protein metabolic function of liver itself was also improved as evidenced by a marked increase of plasma albumin and transferrin as well as a correction of the reverted A/G ratio. The decrease of urinary urea excretion and plasma glycine flux imply that the diet therapy suppressed the accelerated metabolism of protein and amino acids in cirrhotic patients. The diet is relatively cheap, can be easily handled by the patients themselves, and hence is also applicable to outpatients.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1. Scientific background

Post-viral hepatocirrhosis is a common disease in China and is the cause of substantial morbidity and mortality, especially in people aged 21-60 years. The disease accounts for an estimated 0.4 to 0.6 percent of the total inpatient hospitalization, a rate much higher than that due to alcoholic cirrhosis in China. Since there is no effective etiological treatment for hepatocirrhotic patients and since most of the severe complications (e.g., encephalopathy, ascites, susceptibility to severe infections) are closely related to protein-energy malnutrition which frequently accompanies the disease, study of the nutritional status of cirrhotic patients and their nutritional therapy has attracted much attention in recent years [1-3]. However, as pointed out by McCullough and colleagues in a recent review [1], the large majority of data has been derived from studies on alcoholic liver disease, and even for this type of cirrhosis, the design of diet therapy (e.g., the quality and quantity of protein, the amount of calorie intake, etc.) and it's clinical evaluation are still important problems which have not yet been studied thoroughly.

Although cirrhosis is generally considered a catabolic disease associated with negative nitrogen balance [1,4], the results of recent studies are contradictory. Urinary 3-methyl-histidine excretion has been reported to be increased in cirrhotic patients [5,6]. Using stable isotope tracer methods, O'Keefe and colleagues [7] reported that plasma tyrosine turnover and whole-body protein synthesis and degradation were accelerated in cirrhotic patients. Among their 18 patients, only one was post-viral hepatocirrhotic. Mullen and colleagues [8] found decreased leucine oxidation but no alteration in rates of whole-body protein turnover or of leucine turnover in their stable alcoholic cirrhotic patients. Millikan et al [9] also found equivalent rates of whole-body protein turnover in normal subjects and alcoholic cirrhotic patients. But Swart and colleagues [10] reported that in their cirrhotic patients (none was definitely post-viral hepatocirrhotic) the nitrogen flux and whole-body protein synthesis were increased either in fed or fasting state. On the contrary, a recent report [11] stated that in alcoholic cirrhosis, muscle protein synthesis was decreased accompanied by a decrease in whole-body protein turnover. Using nitrogen balance measurements to define efficacy, some studies have shown that the protein requirement in cirrhotic patients was about 0.8-1.1 g/kg/d [12,13]. But it has been argued that these results may reflect metabolic adaptation rather than true nutritional requirement [1,10]. Therefore, whether the protein malnutrition in cirrhotic patients, especially in post-viral hepatocirrhotic patients, is due accelerated kinetics of protein metabolism or is secondary to starvation remains uncertain.

Other nutritional requirements have also attracted the attention of many investigators. The basal energy requirement in alcoholic cirrhotic patients has been reported to be similar to normal subjects, within the range of 105 to 125 kJ/kg/d (25 to 30 kcal/kg/d) [1,14,15]. However, no report has been found about the energy expenditure of freely living post-viral hepatocirrhotic patients. Furthermore, although it is generally agreed that high fat diets are harmful to hepatic patients, there appears to be no reason to routinely restrict lipid intake to a very low level [1].

As regards the quality of food protein, the significance of imbalance of branched chain amino acids (BCAA) and aromatic amino acids (AAA) in the development of hepatic encephalopathy has been studied extensively and many people believe that supplements

of BCAA are of value in treating or preventing encephalopathy. Someone reported that BCAA together with adequate protein and calorie supplement improve the nutritional state of cirrhotic patients without the risk of precipitating encephalopathy [16,17]. Vegetable protein has been reported to be better than meat protein for patients with hepatic cirrhosis possibly because of low AAA content and high fibre content [18,19], but it has also been reported that no difference could be seen between vegetable and meat protein [20].

In summary, there is no doubt that protein-energy malnutrition is important in the pathogenesis and treatment of cirrhotic patients, but there are still a series of theoretical and practical problems which need investigation, especially for post-viral hepatocirrhotic patients. Among the goals are the optimal formulation of nutritional therapy and the evaluation of its clinical effect.

As pointed out by Halliday in a recent review [21], the stochastic model system, either the end-product method or the precursor method, both utilizing stable isotope labelled amino acids, has been increasingly employed to quantitate whole body protein turnover in man. The end-product method requires a ^{15}N labelled amino acid as the tracer and measures the production rate of certain end products in urine by tracing the ^{15}N label. The precursor method measures the plasma abundance of ^{13}C or ^{15}N labelled amino acid to calculate the flux of the precursor and, in addition, often needs to measure the rate of transformation of the precursor to its end-product. Thus, for amino acids labelled with ^{13}C at the carboxyl group, the excretion rate of $^{13}CO_2$ is measured [7,8,22] and for ^{15}N labelled amino acids, usually the rate of appearance of ^{15}N in urinary urea or total nitrogen is measured [23,24]. Since urea is formed in liver and the urea production rate is often decreased in cirrhotic patients [25,26], it is likely that methods requiring the measurement of ^{15}N end product production rates might not be suitable or need modifications for such patients. The decarboxylation of most amino acids also partly or entirely occurs in the liver and it has already been reported that in cirrhotic patients, the oxidation of leucine and tyrosine is retarded [7,8]. Which tracer(s) and which method(s) are suitable for measuring whole-body protein turnover in hepatic patients is thus an area for further investigation.

1.2. Scope of the project

The purposes of this project were to study the relation between protein and energy metabolism in patients with a diagnosis of post-viral hepatocirrhosis and to apply the findings to design a rational formulation of diet therapy during post-viral hepatocirrhosis based on Chinese food, which might also be suitable for other developing countries. It is expected that the results of this work will be of theoretical value in illustrating the significance of protein-energy metabolism in the pathogenesis and prognosis of post-viral hepatocirrhosis as well as of practical value in exploring an economic approach for improving the clinical state and prolonging the life span of patients. The scope of this study involved the following aims:

The first aim was to set up the necessary stable isotope techniques, i.e., the measurement of plasma leucine (Leu) and glycine (Gly) turnover rates with constant infusion of L-[1-^{13}C]-Leu and ^{15}N-Gly; the measurement of protein oxidation rates from breath collections and carbon analysis following continuous infusions of L-[1-^{13}C]-Leu; comparison of methods for estimating whole-body protein synthesis and breakdown rates from the data of L-[1-^{13}C]-Leu and ^{15}N-Gly experiments; measurement of energy expenditure by the ^{2}H and ^{18}O doubly labelled water method, with an attempt of lowering the dose of ^{18}O and

hence the cost; measurement of the CO_2 recovery and the basal energy expenditure of post-viral hepatocirrhotic patients with the $NaH^{13}CO_3$ infusion method.

The second aim was to measure rates of protein and amino acid turnover as well as the energy expenditure of post-viral hepatocirrhotic patients before treatment. It was anticipated that the results would give information about whether the overall protein and amino acid metabolism are in a hyperactive or hypoactive state, and, combined with the nitrogen balance data, would give information for estimating the protein requirement of these patients. It was also expected that the results would give information for estimating the energy requirements of freely living patients with post-viral hepatocirrhotic patients, especially those in the compensated stage.

The third aim was to design the therapeutic diet formulation and to observe its clinical effect. With regard to the formulation of the diet, the following was foreseen: 1) the protein content would be determined according to the pretreatment experimental results on protein and amino acid metabolism; 2) the energy content would be settled according to the energy expenditure determined in the pretreatment experiment; 3) relatively large amounts of vegetable food would be incorporated into the diet so that vegetable protein would account for about 60-70% of the total protein intake and the cost would be lower than in a meat-based diet in developing countries; 4) final conclusions would be drawn from paired comparisons of the data obtained in each individual before and after treatment. It was further expected that the therapeutic diet should improve the clinical state of the patients and be suitable for inpatients as well as for outpatients living at home.

2. EXPERIMENTAL METHODS

2.1. Materials and Equipment

^{15}N-Gly (93.8% abundance and 96% chemical purity) was synthesized by Shanghai Institute of Chemical Engineering. L-[1-^{13}C]-Leu (99% abundance and 99% chemical purity) and $NaH^{13}CO_3$ (87.35% abundance) was purchased from Tracer Technologies. $H_2{}^{18}O$ was supplied by Monsanto Res. Corp., U.S.A., in two batches, the abundances reported by the supplier being 15.0% and 10.2%. When carefully checked by Professor Jin Deqiu of Beijing University they were found to be 14.45% and 9.61% respectively. The latter two values were subsequently used for calculating the total body water and energy expenditure. ^2H_2O, with a measured abundance of 99.9%, was supplied by Guangming Chem. Corp., China. All labelled compounds were processed for intravenous injection and tested to be free from pyrogen, bacteria and toxicity by the Dispensary of Rui Jin Hospital.

Zinc granules (8-30 mesh) were purchased from BDH and purified with nitric acid before use. Trifluoroacetic anhydride was from Merck-Schuchardt. Dowex 1x50 ion exchange resin was from BioRad. All other reagents were local products of analytical grade.

Isotopic determinations of [^{13}C]-leucine and ^{15}N-glycine in plasma were made by electron impact (EI) gas chromatography/mass spectrometry (GCMS) (Finnigan-MAT 4510 GC-MS) in the early stage of the project and later by negative chemical ionization (CI)

GCMS (HP 5989 GCMS system). The analysis of $^{13}CO_2$ abundance in exhaled air was done with a gas isotope ratio mass spectrometer (IRMS) (MAT 251). Gas IRMS (VG-SIRA 24 at Beijing University) was also used for isotopic determinations of ^{18}O and ^{2}H in water from which energy expenditure was calculated by the doubly labelled water method.

2.2. Determination of plasma ^{15}N-Gly and ^{13}C-Leu abundances

1 milliliter (mL)) of heparinized plasma was deproteinized with sulfosalicylic acid and the amino acids were separated out with a small Dowex 1x50 column. The dried amino acids were then turned to their trifluoroacetic butyric derivatives and subjected to selected ion monitoring (SIM) by GCMS analysis. When EI-GCMS was used, ^{15}N-Gly abundance was calculated from the ion strengths of m/z 126, 127, 128, 129 peaks and ^{13}C-Leu abundance was calculated from m/z 227/228 peaks [27]. When CI-GCMS was used, the ion strengths of m/z 172/173 and m/z 284/285 peaks were used for calculating the abundances of Gly and Leu respectively [28].

2.3. Determination of the abundance of $^{13}CO_2$ in expired air and of ^{18}O and ^{2}H in urine

Expired air was blown into a plastic bag, processed on a vacuum line, and the CO_2 was purified and collected in a small glass tube with a liquid nitrogen trap. The tube was sealed, transferred to the inlet of MAT 251, broken in situ, and measured for its $^{13}CO_2$ delta value against a reference CO_2 which has been normalized against PDB. The delta value in each sample was then converted to its respective isotopic abundance.

Water in urine samples was completely vacuum-transferred and sealed in a glass ampoule. For ^{18}O determination, 1 mL of water was transferred into a long-neck flask and frozen with liquid nitrogen. The flask was then evacuated, filled with 13-14 kPa of highly purified CO_2, flame-sealed, and shaken in a 25°C water bath for 12 h to achieve equilibrium of ^{18}O between water and CO_2. The CO_2 was then purified with a vacuum line and subjected to IRMS analysis to obtain the delta value of ^{18}O [30]. For ^{2}H determination, a small glass tube containing 0.2 g purified zinc granules was attached to a vacuum line. After evacuation, 10 μL of water was introduced into the tube, cooled with liquid nitrogen, and the tube was flame-sealed. The sealed tube was heated to 450°C for 2 h and then attached to the inlet of VG-SIRA 24 for analysis of the delta value of ^{2}H [31]. Some of the ^{18}O and ^{2}H delta values of vacuum transferred urine water samples were checked with the values obtained for the same urine samples which were not vacuum-transferred and the results match very well, indicating that isotope fractionation during the vacuum-transfer was negligible.

The delta values of ^{18}O and ^{2}H were converted to their respective abundances with $^{17}R_{V\text{-}SMOW}$ and $^{18}R_{V\text{-}SMOW}$ for ^{17}O and ^{18}O taken as 0.000373 and 0.0020052 and with $R_{V\text{-}SMOW}$ for deuterium taken as 0.00015595 [32].

2.4. Tracer Experiments

2.4.1. Tracer experiment with ^{15}N-Gly and ^{13}C-Leu

Patients with immunologically and pathologically proven post-viral hepatocirrhosis and normal adult volunteers were hospitalized and given a balance diet for

5 days containing 1.2 g/kg/d of protein and 160-167 kJ/kg/d (38-40 kcal/kg/d) of food energy. Urine and feces of the last 48 h were collected for micro-Kjeldahl analysis of nitrogen. In the morning of the 6th day, 100 mg of ^{15}N-Gly and 200 mg L-[1-^{13}C]-Leu dissolved in 500 mL of 5% glucose were infused at a constant rate for 6 h. Blood samples were taken just before and at 4,5,6 h after the start of infusion for analyzing ^{15}N-Gly and ^{13}C-Leu abundances. Samples of expired air were collected in plastic bags at 0, 4, 5, and 6 hours for analyzing $^{13}CO_2$ abundance and partial pressure of CO_2. During the infusion, another sample of expired air was collected in a Douglas bag for measuring the rate of air expiration. The atmospheric pressure and ambient temperature were recorded for converting the measured air volume to standard condition.

The plateau values of plasma ^{15}N-Gly and ^{13}C-Leu enrichment were used to calculate the amino acid fluxes Q_{Gly} and Q_{Leu} (μmol/kg/h) by the following equation:

$$Q = i \times (Ei/Ep - 1) \quad (1)$$

where i is the infusion rate of ^{15}N-Gly or ^{13}C-Leu in μmol/kg/h, Ei is the enrichment of infused ^{15}N-Gly or ^{13}C-Leu, Ep is the plateau ^{15}N-Gly or ^{13}C-Leu enrichment in plasma.

The rate of expiration of $^{13}CO_2$ (μmol/kg/h) was obtained from the product of the rate of CO_2 expiration (μmol/kg/h) and the plateau enrichment of $^{13}CO_2$. Then the rate of whole-body leucine oxidation O_{Leu} was calculated by the following equation [22]:

$$O_{Leu} = \text{Rate of } ^{13}CO_2 \text{ expiration} \times (1/Ep - 1/Ei) \times 100 \div F \quad (2)$$

where F is the fraction of CO_2 produced in the body which is recovered in expired air and is taken as 0.81 for normal subjects and 0.78 for cirrhotic patients (see Section 3.1. of this report).

Leu flux Q_{Leu} was converted to protein flux (Q) by assuming the average Leu content in whole-body protein is 7.9%. The whole-body protein synthesis rate (S) and degradation rate (C) were then calculated according to the two-pool model of Picou-Taylor Roberts [33]:

$$Q = S + E = C + I \quad (3)$$

where I and E are the total nitrogen input rate and output rate. The five parameters are all expressed in g protein/kg/d. The values of E and S were obtained by two different approaches for comparison. One was from O_{Leu} [22] and designated as E_O and S_O, the other was from the total nitrogen excretion rate [34] and designated as E_N and S_N. The conversion factor for nitrogen to protein was taken as 1:6.25 and that for leucine to protein was 7.9:100.

2.4.2. Measurement of energy expenditure

After an overnight fast, the patient or the normal volunteer drank a cup of tracer water (1 g/kg of ^{18}O-water and 0.06 g/kg of ^{2}H-water) at 08.00h. Urine samples were collected before and 4 h after the dose and then at 08.00h of the 5th or 6th, 10th or 11th, 14th or 15th day (in the later stage of this work these were changed to before and 24 h after the dose and then at 08.00h on the 4th or 5th, 7th or 8th and 10th or 11th

day). The samples were kept in airtight plastic bottles at -20°C until processed and measured.

Total body water was calculated by the following equation:

$$TBW(kg) = (d \times APE_d/MW_d)(18.02/APE_{BW}) \times f/1000 \quad (4)$$

where d is the dose of tracer water in g, MW_d and 18.02 are the molecular weights of dose water and distilled water, APE_d is the enrichment of tracer water and APE_{BW} is the enrichment of body water at 0 h obtained by linear regression of the natural logarithms of APEs of urine ^{18}O or ^{2}H against time, and f is the factor for converting the dilution space to total body water and is assumed to be 1/1.01 for ^{18}O and 1/1.04 for ^{2}H [35].

Energy expenditure was obtained by first calculating the CO_2 production rate using the following equation [36]:

$$rCO_2 = (N/2.08)(K_{O^*} - K_D) - 0.015K_DN \quad (5)$$

where N is the total body water in moles obtained from ^{18}O data, K_{O^*} and K_D are the elimination rate constants of ^{18}O and ^{2}H obtained by linear regression. The coefficient for K_DN was estimated by assuming the rate of insensible water loss being 50% of water turnover rate, and the fractionation factors f_1, f_2, f_3 being 0.93, 0.99 and 1.04 [35,36]. The value of RQ was estimated to be 0.88 in this study according to actual calculation of the food quotient from the diet consumed by the patients and normal subjects. The energy expenditure was then calculated from the rate of CO_2 production and RQ.

For comparison, the revised equation of Schoeller [35] was also used to calculate the rCO_2 value which can be rewritten as:

$$rCO_2 = (N/2.08)(1.01K_{O^*} - 1.04K_D) - 0.03N(1.01K_{O^*} - 1.04K_D) \quad (6)$$

2.4.3. Tracer experiment with $NaH^{13}CO_3$

After an overnight fast and bedrest, the patient or normal volunteer was injected intravenously with a priming dose of 28 mg $NaH^{13}CO_3$ followed by a 2-hour continuous infusion of 37 mg $NaH^{13}CO_3$ in normal saline for 2 h. Samples of expired air were collected intermittently for analysis of $^{13}CO_2$ abundance as described in section 2.3. Rates of air expiration and partial pressure of CO_2 were obtained by the method described in section *2.4.1.* The rate of expiration of $^{13}CO_2$ and the rate of infusion of $NaH^{13}CO_3$ (both in μmol/kg/h) were then used to calculate the recovery % of CO_2 produced in the body (Eq.7 and Eq.8).

$$^{13}CO_2 \text{ expiration rate} = CO_2 \text{ expiration rate} \times {}^{13}CO_2 \text{ plateau enrichment} \quad (7)$$

$$\text{Recovery \% of } CO_2 = {}^{13}CO_2 \text{ expiration rate} \div \text{infusion rate of } {}^{13}C \quad (8)$$

The production rate of CO_2, obtained by correcting the CO_2 expiration rate for its recovery %, was also used for the calculation of basal energy expenditure in fasting and resting state, taking the RQ value as 0.88.

3. RESULTS OBTAINED

3.1. CO_2 recovery and resting energy expenditure obtained from $Na^{13}HCO_3$ experiment

With a priming dose of 4-6 μmol/kg and an infusion rate of about 3 μmol/kg/h, the abundance of $^{13}CO_2$ in expired air reached its plateau at 45 to 60 min after the start of infusion. The $^{13}CO_2$ expiration rate was obtained by multiplying the average plateau enrichment with CO_2 expiration rate (Eq.7) and then the $^{13}CO_2$ expiration rate was divided by the infusion rate of $NaH^{13}CO_3$ to give the recovery % (Eq.8). As shown in Table I, the recovery in normal adults was 80.7 ± 0.3 % (mean ± SEM), very close to the value 81% used by Matthews et al [22] and Wolfe et al [37] in their [1-^{13}C]-Leu tracer experiments. On the other hand, the CO_2 recovery of cirrhotic patients was 78.3 ± 0.8 %, slightly lower than normal. Since the exact mechanism of the partial retention of CO_2 in body is not known, the reason of such a slight decrease of CO_2 recovery in cirrhotic patients remains to be studied. However, the decrease is statistically significant. We therefore used different CO_2 recovery correction factors in our [1-^{13}C]-Leu experiment, i.e., 81% for normal adults and 78% for cirrhotic patients.

In order to obtain the resting energy expenditure, the CO_2 expiration rate was divided by the CO_2 recovery % to give the CO_2 production rate and the RQ value was estimated from the composition of food (see Table VI). As can be seen in Table I, the resting energy expenditure of normal adults is 7206 ± 920 kJ/d (1722 ± 220 kcal/d) or 129 ± 10 kJ/kg/d (30.7 ± 2.4 kcal/kg/d), and that of the patients was 6935 ± 389 kJ/d (1657 ± 93 kcal/d) or 119 ± 6 kJ/kg/d (28.4 ± 1.5 kcal/kg/d), not statistically different from each other. Both values were within the range reported by most authors [1] and the statistical results are in agreement with Jhangiani et. al. [14] and Muller et. al. [15], who found no difference of basal energy expenditures between alcoholic cirrhotics and normal adults with indirect calorimetry.

3.2. Change of leucine turnover parameters in cirrhotic patients

In the [1-^{13}C]-Leu constant infusion experiment, it was found that the plasma ^{13}C-Leu enrichment reached a plateau value at about 4 h after the start of infusion. The $^{13}CO_2$ enrichment in expired air reached its plateau value almost at the same time. As shown in Table II, the plateau enrichments of both plasma ^{13}C-Leu and $^{13}CO_2$ were significantly lower in cirrhotic patients than in normal controls, and decompensated cirrhotic patients had their enrichments lowered more severely than compensated patients.

When leucine turnover rate (Q_{Leu}) was calculated from the plasma plateau enrichment, the tracer infusion rate and the enrichment of labelled tracer according to equation 1, it was found that the turnover rate in cirrhotic patients was significantly faster than in controls. Since the plasma leucine content is usually lower in cirrhotic patients and the plasma leucine pool had been found to be smaller than normal [27], the accelerated leucine flux of patients should imply a real shortening of the turnover time of leucine molecules.

When leucine oxidation rate (O_{Leu}) was calculated from the $^{13}CO_2$ expiration rate and the enrichments of plasma ^{13}C-leucine (Eq.2), the value of normal controls was 31.3 ± 1.2 μmol/kg/h. In contrast to the acceleration of plasma Leu flux, the Leu oxidation rate as well as the fraction of leucine turnover that was oxidized (O_{Leu}/Q_{Leu}) were markedly decreased in cirrhotic patients. This is consistent with the results of Mullen and colleagues obtained in alcoholic cirrhosis [8] but not that of Millikan and colleagues [9].

Since the fraction each amino acid contributes to urea nitrogen production is likely to be equal to each amino acid's fractional contribution to body protein [38], we calculated the leucine catabolic rate E_{Leu} by converting the nitrogen excretion rate to its protein equivalent value (1:6.25) and then the leucine equivalent value (1:0.079). In normal adults, the leucine oxidation rate O_{Leu} matches E_{Leu} very well, giving a ratio of 0.957 for O_{Leu}/E_{leu}. This agrees with the results of De Benoist and colleagues who reported that in preterm infants, the nitrogen retention measured by nitrogen balance was similar to that calculated from leucine retention [34]. On the other hand, in our cirrhotic patients, the O_{Leu}/E_{leu} ratio dropped markedly to 0.594 and 0.486 in the two groups. It is unlikely that this is due to an overestimation of E_{Leu} because there is no reason to believe that leucine deamination is decelerated in cirrhotic patients. Therefore the lowered O_{Leu}/E_{leu} ratio very probably indicates an incomplete oxidation of leucine after its deamination.

Mullen and colleagues suggested that the decrease of leucine oxidation rate is a consequence of decreased plasma leucine level [8]. Another possible mechanism is the impaired activity of leucine decarboxylase in the cirrhotic liver. But it has been claimed that a significant portion of leucine in muscle may be oxidized *in situ* [39,40] but whether there is a change of muscle leucine oxidation rate in hepatic cirrhosis is not yet clear. The exact mechanism remains to be studied.

3.3. Change of whole-body protein turnover in cirrhotic patients studied with [1-^{13}C]-Leu

For the estimation of whole-body protein turnover parameters, Eq.3 was followed. Since the leucine oxidation rate and the O_{Leu}/E_{Leu} ratio were both decreased in cirrhotic patients, we calculated the whole-body protein synthetic rate (S) by estimating the nitrogen excretion rate (E) either from leucine oxidation rate [22] or from the nitrogen excretion rate obtained in nitrogen balance studies [34]. As shown in the lower part of Table II, when E and S were estimated from the oxidation rate of leucine (E_O and S_O), there was an obvious underestimation of E and consequently an overestimation of S leading to an apparent positive nitrogen balance in the patients. Since this is not the actual clinical condition of the patients, we concluded that in the 1-[^{13}C]-Leu experiment, the leucine oxidation rate should not be used to estimate the leucine catabolic rate and nitrogen excretion rate. Instead, E and S may be calculated from the total nitrogen excretion rate obtained from nitrogen balance study (E_N and S_N).

With the synthesis rate calculated from nitrogen excretion rate, it can be seen that the synthesis and the degradation of whole-body protein were both significantly increased in post-viral hepatocirrhotic patients and the change in decompensated was more profound than in compensated patients. Therefore, the negative nitrogen balance of these patients was <u>not</u> due to a suppression of protein synthesis. Instead, the patients were in a state of accelerated protein metabolism and the negative nitrogen balance was due to a

disproportionate increase in the rate of protein breakdown (C) relative to the rate of protein synthesis (S).

The result on the protein requirement of post-viral hepatocirrhotic patients is somewhat different from the results of other authors on their alcoholic cirrhotic patients. The patients of our group showed a negative nitrogen balance when their diet contained 1.2 g/kg/d of protein while Swart et. al. [13] and Freund et. al. [41] found in their alcoholic cirrhotic patients that 0.8-1.0 g/kg/d are adequate. One probable reason for such a discrepancy is that the diet formulations of different authors contain different ratios of animal versus vegetable proteins. But it is also probable that this discrepancy is due to a difference between the pathological basis of the two types of cirrhosis.

3.4. Change of glycine turnover parameters in cirrhotic patients

As listed in Table III, the glycine metabolism was also in a accelerated in cirrhotic patients. The glycine flux (Q_{Gly}) was significantly elevated. Since plasma glycine may come from other sources in addition to food intake and protein degradation, we calculated the parameters of glycine turnover in a way similar to that described by Robert and colleagues [42]. The rates of glycine incorporation into protein via protein synthesis (S_{Gly}) and the rate of glycine release from protein degradation (C_{Gly}) were calculated from the protein synthesis and breakdown rates obtained with [1-^{13}C]-Leu in the doubly labelled experiment. The molar ratio (7.9:8.3) of leucine and glycine for the above calculation and the content of glycine in whole body protein (4.9 g/100 g) were adopted from Stegink and colleagues [43]. It can be estimated from the data of Table III that in normal adults under the balance diet used in this study, about 47% of the plasma glycine flux was for protein synthesis, about 38% was for conversion to other compounds in the body, and about 15% was disposed as nitrogen excretion. In cirrhotic patients, all the three components were elevated in a more or less parallel manner. The proportions of glycine flux utilized for protein synthesis, for conversion to other compounds, and disposed as nitrogen excretion were 53:36:11 in compensated cirrhotics and 51:39:10 in decompensated cirrhotics. Therefore, glycine metabolism, involving its interconversion with other amino acids and its catabolism, is also in a hyperactive state in post-viral hepatocirrhotic patients.

3.5. Energy expenditure of cirrhotic patients studied with doubly labelled water

The dose of ^{2}H-water used in this study was similar to literature values but the dose of ^{18}O-water was lower than those used in most reported studies mainly due to economical consideration. Preliminary experiments revealed that at the dose used in this study and within the observation period, the delta values of most urine samples fell in the range of 100-600 for ^{2}H and 20-150 for ^{18}O. In these ranges, the average precisions (expressed as coefficients of variation) of single determinations were 0.032% (0.011 to 0.138%) for ^{18}O and 0.192% (0.062 to 0.493%) for ^{2}H. The average precisions of duplicate urine samples were 0.492% (0.016 to 1.943%) for ^{18}O and 0.685% (0.002 to 2.417%) for ^{2}H. Good linear regression curves were obtained in most cases, with an average correlation coefficient of 0.998 for ^{18}O and 0.997 for ^{2}H.

Energy expenditure was measured in 23 individuals, 13 of whom were normal volunteers including 10 laboratory workers and 3 hospitalized convalescent adults. The other 10 were hospitalized compensated cirrhotic patients. During the experiment, the

laboratory workers were working and living as usual, and the patients or convalescent subjects were staying in hospital without special restriction of activity.

As can be seen in Table IV, the values of total body water of both normal subjects and compensated cirrhotic patients were within the range reported in literature. Similar to the result of Schoeller [35], the energy expenditure calculated by Eq.6 is consistently slightly lower than calculated by Eq.5. The energy values listed in Table III were expressed in kJ. When converted to kcal, the values from Eq.6 became 48.43 ± 2.02 and 44.12 ± 2.18 kcal/kg/d for normal male and female laboratory workers, 36.50 ± 0.73 and 33.02 kcal/kg/d for the two hospitalized healthy males and one female, 37.54 ± 1.88 and 34.46 ± 2.12 kcal/kg/d for male and female cirrhotic patients. The values of hospitalized healthy adults are slightly lower than the literature values of adults freely living at home but not doing work (e.g., 39.2 ± 5.6 kcal/kg/d for females reported by Stein and colleagues [44], 44.6 ± 8.1 and 39.0 ± 2.8 kcal/kg/d for males and females respectively reported by Schoeller and Webb [45]). But the values of laboratory workers are slightly higher than literature values of normal subjects doing similar professional work (e.g., 43.6 ± 0.7 kcal/kg/d for 3 males and 38.0 for one female reported by Schoeller and van Santen [46]). This discrepancy is probably due to the longer working time (6 days a week) and heavier additional physical activities (e.g., traffic and housekeeping) of Chinese laboratory workers which are in addition to their professional work.

Similar to the basal energy expenditures measured in the $NaH^{13}CO_3$ experiment, no difference was found between the freely living energy expenditures of cirrhotic patients and the hospitalized adults having similar activities. When compared with the basal energy expenditure, it can be estimated that about 20-30% of the total energy expenditure of hospitalized subjects was consumed for additional activities, and, for the laboratory workers, it was about 35-40%.

3.6. Design of the therapeutic diet and its clinical effect

3.6.1. Formula for the therapeutic diet

Based on the results of above experiments, the therapeutic diet formulation was designed to have the following features:

a) the protein content was settled to be 1.5 g/kg/d because both the whole-body protein turnover and the turnover of leucine and glycine were found to be at a hyperactive state, and there was a negative nitrogen balance when the balance diet contained 1.2 g/kg/d of protein;

b) the energy intake was settled to be 150-166 kJ/kg/d (36-40 kcal/kg/d) according to the measured energy expenditure of patients which averaged 163 kJ/kg/d for males and 150 kJ/kg/d for females;

c) vegetable protein accounted for 60-70% of the total protein intake instead of 30-40% in common hospital diet for cirrhotic patients;

d) the BCAA/AAA ratio appeared to be slightly but significantly higher than the common diet. An example of the daily menu of the therapeutic diet is listed in Table V.

The average nutrient intake of the patients in the two months' therapy calculated from the daily records of the actually ingested foods [47,48] are shown in Table VI. Also shown in the table are the average actual nutrient intakes of the control patients in the two months' period of observation. The protein intake of the latter group averaged 0.92 g/kg/d and the energy intake averaged 106 kJ/kg/d which was in fact just at the level required for maintaining basal energy expenditure. Evidently, such a lowered energy intake might lead to an exhaustion of energy source stored in the body, which would in turn aggravate the protein malnutrition.

From the average actual food intake of each individual, their respiratory quotients were estimated. Because of the low fat content in Chinese diet, the RQ values of both groups were about 0.88. The value 0.885 ± 0.004 of the control patients is probably overestimated, since these patients were not in an energy balance state, and the mobilization of body fat might make the actual respiratory quotient lower than that estimated from food.

3.6.2. The clinical effect of the therapeutic diet

As shown in Table VII, the effect of therapeutic diet after two months' application is significant. Although most of our patients suffered with different degrees of anorexia, some accompanied by nausea and vomiting, they adapted rapidly, usually within one week, to the high protein, high calorie diet. When they were re-examined two months later, the negative nitrogen balance turned positive along with an increase of body weight and an increase of creatinine in urine, indicating that there was an improvement of general nutritional status probably with an accumulation of muscle protein. In the same time, the protein metabolic function of liver itself was also improved as evidenced by a marked increase of plasma albumin, a correction of the reverted A/G ratio and an elevation of serum transferrin. On the contrary, in the control patients receiving identical medical treatment but having their average daily intake containing only 0.92 g/kg/d of protein and 106 kJ/kg/d of energy supply, no improvement of the above parameters was found in the two months' period.

Along with the improvement of the above parameters, the elevated glycine flux and urea excretion rate in urine was reduced in the treated group while in the control group these rates remained at the original high level. In a recent group of five patients, it has been found that the high leucine turnover rate and protein turnover rate, as well as the high whole-body protein synthesis and breakdown rates were also reduced by 2 months' application of the therapeutic diet (Table VIII). The leucine oxidation rate was slightly elevated, but was still slower than the leucine catabolic rate calculated from nitrogen excretion. These results indicate that the therapeutic diet is able to suppress the hypermetabolic state of protein in the patients. It seems unlikely that such an effect is simply due to a supplement of energy, because in the balance diet used in the [1-^{13}C]-Leu and ^{15}N-Gly experiment containing 1.2 g/kg/d of protein, the energy supply is almost the same as the therapeutic diet and yet the patients were in negative nitrogen balance and had very high turnover rates of whole-body protein and amino acids. More probably, the effect is due to the combined supplements of protein and energy so that the excessive degradation of body protein for supplying energy is no more needed and accumulation of body protein and regeneration of hepatic function becomes possible [1]. In addition, the

slightly higher proportion of BCAA/AAA in the diet might also play a role in providing more adequate raw materials (various amino acids) for the synthesis of body protein.

Therefore, our results on post-viral hepatocirrhotic patients before and after diet therapy support the idea that cirrhosis is a catabolic disease, partly due to undernutrition and partly due to disturbance of protein and amino acid metabolism [1,19]. The results also agree with the idea that there is an elevated protein requirement in cirrhotic patients [7,10] and vegetable protein is beneficial to these patients [19].

4. CONCLUSION

1. In this work, several stable isotope techniques have been used for the study of the dynamic aspect of protein-energy malnutrition in post-viral hepatocirrhotic patients. The established techniques involve: the L-[1-^{13}C]-Leu and ^{15}N-Gly doubly labelled tracer experiment for studying the overall metabolic status of leucine, glycine, and whole-body protein; the ^{18}O and ^{2}H doubly labelled water method for studying the energy expenditure of freely living adults; the NaH$^{13}CO_3$ infusion experiment for studying the CO_2 recovery rate and basal energy expenditure.

2. By the L-[1-^{13}C]-Leu and ^{15}N-Gly doubly labelled tracer experiment, it was found that in post-viral hepatocirrhotic patients, the overall metabolism of leucine, glycine and whole-body protein were in a hyperactive state. Together with the results of nitrogen balance measurement, it was concluded that high protein intake was necessary for these patients and the daily requirement was estimated to be higher than 1.2 g/kg/d.

3. By the doubly labelled water experiment and the NaH$^{13}CO_3$ experiment, it was found that the energy requirement of post-viral hepatocirrhotic patients was not different from normal subjects having similar physical and mental activities. The actual daily requirement of calorie intake would depend on the actual activities of each patient. For those freely living in hospital (probably also for those freely living and resting at home), it was estimated that 150-160 kJ/kg/d would be necessary.

4. According to the above results, a high protein, high calorie therapeutic diet formulation based on Chinese food was designed for post-viral hepatocirrhotic patients which has the following features: the protein content is 1.5 g/kg/d, the energy supply is 150-160 kJ/kg/d, 60-70% of the protein is of vegetable origin, and the BCAA/AAA ratio is slightly but significantly higher than common hospital diet for cirrhotic patients.

5. The effect of the above therapeutic diet is significant. Patients with post-viral hepatocirrhosis adapted to the diet rapidly. After two months' diet therapy, the general negative nitrogen balance turned positive along with an increase of body weight and urinary creatinine, indicating an improvement of general nutritional status probably with an accumulation of muscle protein. The protein metabolic function of liver itself was also improved as evidenced by the increase of plasma albumin, A/G ratio and transferrin. The decrease of urinary urea excretion and the slow down of plasma glycine and leucine turnover as well the synthesis and degradation of whole-body protein implicate that the diet therapy is able to suppress the hypermetabolic state of protein-amino acids in cirrhotic patients. No similar improvement was seen in the control patients receiving identical medical treatment but freely taking common hospital diet.

6. The leucine oxidation rate of post-viral hepatocirrhotic patients was significantly decreased while the total nitrogen excretion rate was increased, suggesting that the classical method of L-[1-^{13}C]-Leu tracer experiment for measuring whole-body protein turnover should be modified for these patients. In order to calculate the whole-body protein synthesis rate, it would be better to estimate the nitrogen excretion rate by the data of nitrogen balance measurement instead of by the leucine oxidation rate. Further inves-tigation would be needed to search for better method of studying whole-body protein turnover in these patients.

5. PAPERS PUBLISHED ON WORK UNDER THE CONTRACT

Six papers are in preparation on work done under this contract [49-54].

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TABLE I. CO_2 RECOVERY AND RESTING ENERGY EXPENDITURE FROM $NaH^{13}CO_3$ EXPERIMENT

	Normal Adults (n = 6)	Cirrhotic Patients (n = 9)
Plateau Enrichment of $^{13}CO_2$ (%)	0.03088 ± 0.00263 [f]	0.03163 ± 0.00115 [n]
CO_2 Expiration Rate (mmol/kg/h)	83.0 ± 6.8	74.0 ± 3.4 [n]
$^{13}CO_2$ Expiration Rate (μmol/kg/h)	2.48 ± 0.12	2.31 ± 0.06 [n]
$NaH^{13}CO_3$ Infusion Rate (μmol/kg/h)	3.07 ± 0.15	2.96 ± 0.12 [n]
CO_2 Recovery (%)	80.7 ± 0.3	78.3 ± 0.8 [a]
CO_2 Production Rate (mmol/kg/h)	102.7 ± 8.1	94.7 ± 5.0 [n]
RQ (Calculated from food)	0.884 ± 0.024	0.886 ± 0.007 [n]
Resting Energy Expenditure (kJ/d)	7206 ± 920	6935 ± 389 [n]
(kcal/d)	1722 ± 220	1657 ± 93 [n]
Resting Energy Expenditure (kJ/kg/d)	129 ± 10	119 ± 6 [n]
(kcal/kg/d)	30.7 ± 2.4	28.4 ± 1.5 [n]

f: All experimental data are expressed in mean ± SE in the text and tables of this report except stated elsewhere, the letter 'n' in parenthesis indicates the number of individuals.

a: t-test revealed a p value < 0.05 between the two groups;

n: t-test revealed no significant difference between the two groups.

TABLE II. PARAMETERS OF LEU AND WHOLE-BODY PROTEIN TURNOVER OBTAINED IN L-[1-^{13}C]-LEU EXPERIMENT

	Normal (n = 11)	Compensated Cirrhosis (n = 12)	Decompensated Cirrhosis (n = 7)
Plasma Leu Enrichment (%)	3.21 ± 0.21	1.80 ± 0.11 [a]	1.45 ± 0.06 [a,c]
$^{13}CO_2$ Enrichment (%)	0.00953 ± 0.00095	0.00442 ± 0.00015 [a]	0.00313 ± 0.00038 [a,b]
Leu Turnover (μmol/kg/h)			
Q_{Leu} - Plasma Leu Flux	135.6 ± 7.8	221.5 ± 7.6 [a]	262.0 ± 12.3 [a,b]
E_{Leu} - Leu in nitrogen excretion	32.8 ± 0.8	36.9 ± 0.5 [a]	40.9 ± 1.3 [a,b]
O_{Leu} - Leu oxidation rate	31.3 ± 1.2	21.9 ± 1.1 [a]	19.7 ± 2.2 [a,m]
O_{Leu}/Q_{Leu}	23.8 ± 1.5	9.9 ± 0.4 [a]	7.5 ± 0.8 [a,c]
O_{Leu}/E_{Leu}	95.7 ± 3.4	59.4 ± 3.2 [a]	48.6 ± 5.7 [a,m]
Whole-body Protein Turnover (g/kg/d)			
Q	5.39 ± 0.31	8.82 ± 0.30 [a]	10.43 ± 0.49 [a,b]
E_N (calculated from nitrogen excretion)	1.30 ± 0.03	1.47 ± 0.02 [a]	1.63 ± 0.05 [a,b]
E_O (calculated from O_{Leu})	1.24 ± 0.04	0.88 ± 0.05 [a]	0.78 ± 0.09 [a,m]
I	1.35 ± 0.03	1.27 ± 0.02 [n]	1.27 ± 0.02 [n,m]
S_N (Q-E from nitrogen excretion)	4.09 ± 0.31	7.35 ± 0.31 [a]	8.80 ± 0.48 [a,c]
S_O (Q-E from Leu oxidation)	4.15 ± 0.31	7.94 ± 0.27 [a]	9.64 ± 0.45 [a,b]
C	4.05 ± 0.32	7.54 ± 0.30 [a]	9.16 ± 0.50 [a,b]
Nitrogen Balance (g prot/kg/d)			
Calculated from nitrogen excretion	+0.05 ± 0.03	−0.20 ± 0.03 [a]	−0.36 ± 0.04 [a,b]
Calculated from O_{Leu}	+0.10 ± 0.05	+0.40 ± 0.04 [a]	+0.49 ± 0.10 [a,m]

a: t-test revealed a p value <0.001 between cirrhotics and normal subjects;
b: t-test revealed a p value <0.01 between decompensated and compensated cirrhotics;
c: t-test revealed a p value <0.05 between decompensated and compensated cirrhotics;
n: t-test revealed no significant difference between cirrhotics and normal sujcets;
m: t-test revealed no significant difference between decompensated and compensated cirrhotics.

TABLE III. GLYCINE TURNOVER PARAMETERS FROM DOUBLE LABELLED TRACER EXPERIMENT

Parameters (in μmol/kg/d)	Normal (n = 11)	Compensated cirrhosis (n = 12)	Decompensated cirrhosis (n = 7)
Q_{Gly} - Gly Flux	229.3 ± 6.2	364.0 ± 9.7 [a]	466.8 ± 17.9 [a,c]
E_{Gly} - Gly disposal in nitrogen excretion	35.5 ± 0.9	40.1 ± 0.6 [a]	44.3 ± 1.4 [a,c]
I_{Gly} - Gly input from food	36.7 ± 0.9	34.6 ± 0.5 [n]	34.5 ± 0.6 [n,m]
S_{Gly} - Gly used in protein synthesis	111.4 ± 8.4	200.0 ± 8.4 [a]	239.5 ± 13.0 [a,d]
C_{GLy} - Gly from protein degradation	110.1 ± 8.6	205.4 ± 8.0 [a]	249.3 ± 13.7 [a,c]
X_{Gly} - Gly/other amino acids interconversion	82.4 ± 10.5	124.0 ± 13.5 [b]	183.0 ± 16.8 [a,d]

a: t-test revealed a p value < 0.001 between cirrhotics and normal subjects;

b: t-test revealed a p value < 0.01 between cirrhotics and normal subjects;

c: t-test revealed a p value < 0.01 between the two groups of cirrhotics;

d: t-test revealed a p value < 0.05 between the two groups of cirrhotics;

n: t-test revealed no significant difference between cirrhotics and normal subjects;

m: t-test revealed no significant difference between the two groups of cirrhotics.

TABLE IV. ENERGY EXPENDITURE OF CIRRHOTIC PATIENTS AND HEALTHY ADULTS MEASURED WITH DOUBLY LABELLED WATER METHOD

Subject (n)	Age (y)	BW (kg)	k_O (d^{-1})	k_H (d^{-1})	TBW (%BW)	Energy Expenditure (Equation (5)) (kJ/d)	(kJ/kg/d)	Energy Expenditure (Equation (6)) (kJ/d)	(kJ/kg/d)
Healthy Laboratory Workers									
male (4)	26 ±4	58 ±1	0.0957 ±0.0052	0.0682 ±0.0053	59.5 ±1.8	12103 ±285	209 ±7	11616 ±270	203 ±8
female (6)	30 ±9	49 ±4	0.1163 ±0.0088	0.0868 ±0.0084	52.0 ±1.9	9488 ±690	192 ±10	9169 ±657	185 ±9
Hospitalized Healthy Adults									
male (2)	49 ±4	63 ±4	0.0910 ±0.0048	0.0684 ±0.0068	56.5 ±7.1	9931 ±207	159 ±3	9542 ±190	153 ±3
female (1)	57	57	0.1197	0.0971	52.7	8184	144	7878	138
Cirrhotic Patients, Compensated									
male (7)	52 ±9	63 ±5	0.1083 ±0.0095	0.0866 ±0.0083	59.3 ±2.1	10355 ±488	163 ±8	9964 ±468	157 ±8
female (3)	64 ±5	55 ±8	0.0986 ±0.0021	0.0774 ±0.0025	57.4 ±1.3	8011 ±1047	150 ±9	7706 ±1003	144 ±9

TABLE V. AN EXAMPLE OF THE DAILY MENU OF THE THERAPEUTIC DIET

Time	Name of food	Gross Weight (g)[a]	Protein (g)	Fat (g)	Carbohydrate(g)
7 am	Staple food	50	3.1	0.6	39.0
	Optional				
	1.Dried meat floss	30	18.0	4.0	0.4
	2.Bean in soy sauce	50	20.0	10.0	10.5
	3.Dried bean curd	100	14.1	0.8	4.2
	Bread or steamed bread	50	3.1	/	24.4
	Egg	50	5.0	4.0	/
9 am	Milk or Soy bean milk	200	6.6	8.4	10.0
12 am	Staple food	100	6.2	1.2	78.0
	Optional				
	1.Lean meat	100	16.7	2.8	1.0
	2.Bream	100	18.5	6.6	0.2
	3.Eel	100	17.2	1.2	0.6
	Vegetable and/or fruits	150	1.2	0.1	2.5
	Egg	50	5.0	4.0	/
6 pm	Staple food	100	6.2	1.2	78.0
	Optional				
	1.Mushroom	50	7.2	0.9	29.0
	2.Fresh bean curd	100	6.3	0.9	25.0
	Optional				
	1.Bean curd sheets	25	8.0	0.7	1.3
	2.Lean meat	50	8.3	1.4	0.5
	Vegetable and/or fruits	150	1.2	0.1	2.5
8 pm	Milk or Soy bean milk	200	6.6	8.4	1.0
Daily	Vegetable oil	15	1.0	15.0	/

a: All amounts are expressed in g except the amount of milk, soy bean milk and vegetable oil which are in mL.

TABLE VI. AVERAGE INTAKES OF PATIENTS TREATED AND UNTREATED WITH THERAPEUTIC DIET

Composition	Treated Group		Control Group	
	d^{-1}	$kg^{-1}d^{-1}$	d^{-1}	$kg^{-1}d^{-1}$
Protein (g)	94.5 ± 2.9	1.57 ± 0.04	55.6 ± 2.5[a]	0.92 ± 0.10[a]
Fat (g)	74.5 ± 4.0	1.24 ± 0.07	46.3 ± 3.2[a]	0.77 ± 0.09[a]
Carbohydrate (g)	317.0 ± 9.0	5.28 ± 0.14	213.0 ± 10.0[a]	3.53 ± 0.38[a]
Energy Supply (kJ)	9956 ± 168	166 ± 3	6418 ± 277[a]	106 ± 11[a]
(kcal)	2378 ± 40	39.7 ± 0.6	1533 ± 66[a]	25.4 ± 2.7[a]
Amino Acids (μmol)				
Valine	55.4 ± 1.0	0.95 ± 0.03	28.5 ± 1.4[a]	0.47 ± 0.04[a]
Leucine	74.2 ± 0.7	1.28 ± 0.04	38.7 ± 2.2[a]	0.64 ± 0.06[a]
Isoleucine	36.9 ± 0.4	0.63 ± 0.02	20.0 ± 1.1[a]	0.33 ± 0.03[a]
Phenylalanine	32.0 ± 0.3	0.55 ± 0.01	16.9 ± 0.9[a]	0.28 ± 0.03[a]
Tyrosine	17.2 ± 0.2	0.29 ± 0.01	10.8 ± 0.5[a]	0.18 ± 0.02[a]
BCAA/AAA	3.39 ± 0.04		3.14 ± 0.05[b]	
RQ (estimated from food)	0.879 ± 0.004		0.885 ± 0.004[n]	

a: t-test revealed a p value <0.001 between treated group and control group;

b: t-test revealed a p value <0.01 between treated group and control group;

n: t-test revealed no significant difference between the two groups.

TABLE VII. EFFECT OF THERAPEUTIC DIET ON PARAMETERS RELATED TO PROTEIN METABOLISM

Parameters	Treated Group		Control Group	
	Before Treatment	After 2 months' Treatment	Before 2 months' Obsevation	After 2 months' Observation
Plasma Albumin (g/dL)	2.86 ± 0.16	3.82 ± 0.18 [b]	3.11 ± 0.15	3.07 ± 0.15 [n]
Plasma A/G ratio	0.68 ± 0.06	1.19 ± 0.10 [a]	0.77 ± 0.05	0.76 ± 0.06 [n]
Serum Transferrin (mg/dL)	106 ± 12	180 ± 9 [a]	123 ± 5	121 ± 4 [n]
Urinary Creatinine (g/d)	0.66 ± 0.07	0.90 ± 0.06 [a]	0.60 ± 0.04	0.61 ± 0.04 [n]
Urinary Urea (g/d)	9.56 ± 0.34	8.15 ± 0.34 [a]	9.55 ± 0.38	9.98 ± 0.21 [n]
Glycine Flux (μmol/kg/h)	448 ± 24	344 ± 13 [a]	365 ± 28	407 ± 33 [n]
Nitrogen Balance (g prot/kg/d)	−0.26 ± 0.05	+0.34 ± 0.05 [a]	−0.22 ± 0.06	−20.35 ± 0.03 [n]
Body Weight (kg)	57.0 ± 1.5	60.2 ± 1.8 [b]	62.3 ± 3.6	62.4 ± 0.35 [n]

a: Paired t-test of the data before and after treatment revealed a p value <0.001;

b: Paired t-test of the data before and after treatment revealed a p value <0.01

n: Paired t-test of the data before and after two months' observation revealed no significant difference.

TABLE VIII. LEU AND WHOLE-BODY PROTEIN TURNOVER BEFORE AND AFTER DIET THERAPY

	Before Treatment (n = 5)	After Treatment (n = 5)
Leu Turnover (μmol/kg/h)		
Q_{Leu} - Plasma Leu Flux	211.2 ± 7.8	152.3 ± 7.6 [a]
E_{Leu} - Leu in nitrogen excretion	35.3 ± 0.8	40.4 ± 2.4 [a]
O_{Leu} - Leu oxidation rate	23.1 ± 0.8	25.3 ± 0.5 [b]
O_{Leu}/Q_{Leu}	11.1 ± 0.7	16.8 ± 0.9 [a]
O_{Leu}/E_{Leu}	65.9 ± 3.4	63.5 ± 3.6
Whole-body Protein Turnover (g/kg/d)		
Q	8.41 ± 0.31	6.06 ± 0.30 [a]
E (calculated from N excretion)	1.41 ± 0.03	1.61 ± 0.10
I	1.17 ± 0.02	1.82 ± 0.02 [a]
S (Q-E from N excretion)	6.98 ± 0.30	4.45 ± 0.24 [a]
C	7.24 ± 0.30	4.24 ± 0.30 [a]
Nitrogen Balance (g prot/kg/d)	−0.26 ± 0.02	0.21 ± 0.08 [a]

a and b: $p<0.01$ and <0.05 (paired t-test) before and after two months' diet therapy.