Effects of dietary protein restriction on albumin synthesis, albumin catabolism, and the plasma aminogram[1,2]

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The McFarlane technique for the direct measurement of albumin synthesis has recently been applied to man (1–5). However, little attention has been directed: *1*) to protein intake in relation to synthesis, and *2*) to synthesis and catabolism in the same patient on different dietary intakes.

Using indirect methods of measurement, it has been concluded that albumin synthesis is decreased in man by protein restriction (6, 7). These studies are supported by the work of Kirsch et al. (8) who, using the ^{14}C-carbonate technique in the rat, demonstrated a rapid decrease in albumin synthesis following protein restriction.

In protein–calorie malnutrition (PCM) in children, low plasma albumin and characteristic plasma amino acid changes are found (9, 10). Few studies of the plasma aminogram on controlled dietary protein restriction have been reported. No studies relating albumin synthesis to the plasma aminogram have been published.

In view of the above considerations, we felt that a study of albumin synthesis, albumin catabolism, and the plasma aminogram in the same subject before and during protein restriction would be important: *1*) to determine the magnitude of change, measuring albumin synthesis by a direct technique; *2*) to interpret similar studies in patients with cirrhosis, malabsorption, and uremia and in whom protein depletion is frequently present; and *3*) to determine whether any relationship exists between plasma amino acid levels and albumin metabolism.

Materials and methods

Patients

Eight adult male subjects were hospitalized in the metabolic ward of Groote Schuur Hospital. Informed consent was given by each patient. All subjects had normal liver, renal, cardiac, and gastrointestinal function and no evidence of infection.

Protocol

After a 2-week screening period in the hospital on a 70-g protein diet, albumin synthesis and catabolism were measured. The subjects were then placed on a 10-g isocaloric protein diet, supplemented with adequate minerals and vitamins. The full diet was consumed by all subjects. After 4 to 6 weeks of this diet, the synthesis and catabolism studies were repeated. Three days before each study, iodine uptake by the thyroid gland was blocked by the administration of Lugol's iodine (10 minims three times daily) and the bowel was sterilized using neomycin sulfate (1 g four times daily). The Lugol's iodine was maintained for 14 days and the neomycin sulfate for 4 days. On the 1st day of the synthesis rate study, plasma was taken from each subject after an overnight fast for amino acid analysis. The subjects voided urine, and then 80 μCi ^{125}I-labeled human albumin was administered intravenously together with 200 μCi ^{14}C-sodium carbonate (Phillips, Duphar; specific activity 55 mCi/mmole). Blood samples were withdrawn after 10 min, 3 hr, and 6 hr, and then daily for 10 days. Each day, 24-hr urine volumes were collected. The following day, after another overnight fast, 5 μCi ^{14}C-urea were administered and blood samples taken at 3, 3.5, 4, 4.5, and 5 hr. On both days of synthesis rate measurement, a light, nonprotein snack was provided with a liberal fluid intake.

Catabolic rates were derived from urinary ex-

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[2] Supported by the Wellcome Trust, the South African Medical Research Council, the Atomic Energy Board of South Africa, and the Staff Research Fund of the University of Cape Town.

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The American Journal of Clinical Nutrition 25: NOVEMBER 1972, pp. 1174–1178. Printed in U.S.A.

cretion of radioactive iodine as a function of plasma specific activity. Iodination of human albumin was achieved by the McFarlane iodide monochloride technique to give 0.5 to 1.0 g atoms of iodine per molecule of albumin. Free iodine was removed by dialysis against distilled water. Trichloroacetic acid precipitation showed less than 1% free iodine. Carrier albumin was added to reduce radiation damage, and the preparation was sterilized by Seitz filtration. The labeled albumin was used within 48 hr of preparation. The radioactivity of the serum and urine samples was measured in a Packard Autogamma Spectrometer. Plasma volume was obtained by isotope dilution at 10 min. Albumin was measured in duplicate by the method of Fernandez et al. (11). The intravascular albumin pool was estimated by multiplying the plasma albumin concentration by the degradation rate constant (mean of day 3 to day 14).

Albumin synthesis was measured by the technique developed by McFarlane (12) and Reeve et al. (13). The formula used was that proposed by Koj and McFarlane (14). The specific activity of urea at t_0 was derived from the 6-hr sample on the day of the experiment and extrapolated to zero time by applying the slope of exogenously administered ^{14}C-urea injected on day 2.

The hypothetical specific radioactivity of albumin was obtained by extrapolation to zero time, correcting the 6-hr value by a factor derived from the distribution and catabolism of the injected ^{125}I-albumin. When repeated studies were made in the same subject 4 to 6 weeks later, appropriate corrections were made for the residual radioactivity in the albumin. The urea synthesis rate was obtained from the slope of the semilog plot of ^{14}C-urea specific activity versus time.

Urea specific activity was measured on deproteinized plasma samples, incubated with urease to produce $^{14}C\text{-}CO_2$, which was then released by the addition of acid. The volume of gas produced was measured manometrically on a high vacuum gas train, collected in phenylethylamine methanol and counted in a toluene liquid scintillator (2,5-diphenyloxazole and 1,4-Bis-(2,4-methyl-5-phenyloxazolyl) benzene) in a Beckman Automatic Scintillation Spectrometer. Samples were counted to an error of less than 3%. The specific activity of the guanidine carbon of arginine in albumin was obtained by extraction of albumin from the plasma by the acid ethanol method (15). After acid hydrolysis at 110 C, the sample was passed through a bicarbonate resin column to separate arginine that was then incubated with arginase to produce urea. The sample was then treated as for urea. Urea was measured using an AutoAnalyzer. Tryptophan was measured by the method of Hess and Udenfriend (16), whereas the other amino acids were analyzed on a Technicon Amino Acid Analyzer.

Results

Alanine was markedly increased on the restricted protein diet. The other amino acids were unchanged.

The low rate of urea synthesis during protein depletion negates the use of endogenously synthesized ^{14}C-urea. Therefore, ^{14}C-urea was administered the day following the administration of ^{14}C-carbonate similar to the technique employed by Rothschild et al. (4) in cirrhotics. Although it is acknowledged that urea synthesis rates can fluctuate markedly, the conditions of study were identical on both days with stable plasma urea concentrations. Furthermore, the fractional rates of urea synthesis determined by both methods were practically identical in a patient on a normal diet not reported in this study.

The 0 to 6-hr ratios were constant under both experimental conditions, the urinary free iodide excretion in the first 48 hr was not excessive, whereas the final albumin catabolic rates obtained were similar to those reported by other workers.

The lack of correlation between synthetic and catabolic rates on a normal diet, because of the above considerations, are felt by us not to be technical in origin. Jeejeebhoy et al. (3) obtained similar results in their series of patients on high protein diets.

Table 1 shows the absolute albumin synthesis and catabolic rates on the 70-g and 10-g isocaloric protein diet. Patient 2 was reported to be disobeying dietary instructions intermittently. Table 2 summarizes the changes in absolute and fractional synthesis and catabolic rates, plasma urea, plasma albumin, and the plasma volume. Although the plasma volume was unchanged, the plasma urea and the plasma albumin were reduced. Both the fractional and the absolute albumin synthesis and catabolic rates were markedly reduced following protein restriction.

Discussion

Until recently, albumin synthesis measurements were technically inaccurate. There are few studies of albumin synthesis during protein depletion. Using the "synthesis and trans-

fer rate" index described by Matthews (17), Freeman and Gordon (18) and Hoffenberg et al. (6) found that albumin synthesis was reduced in rats, rabbits, and human subjects on a low protein diet. James and Hay (7) studied the fate of injected iodinated albumin using a method of computer analysis in children on varying protein intakes. Their results suggest that the rate of synthesis is more dependent on the immediate dietary protein intake than on the serum albumin concentration or the intravascular albumin mass, and that the reduced rate of synthesis occurs earlier than the compensatory adjustment in catabolism. Kirsch et al. (8), using the direct method of McFarlane (12), drew similar conclusions in rats. Recently, Coles et al. (5) measured albumin synthesis by the method of McFarlane in two patients with severely compromised renal function on 20-g protein diets. The present study represents the first comprehensive attempt to measure albumin synthesis and catabolism independently on different dietary protein intakes in the same subjects free from complicating disease states.

The McFarlane technique has been validated by the agreement obtained between synthesis and catabolic rates when these are estimated independently under steady-state conditions, in which the two are presumed to be equal. Tavill et al. (2), Jeejeebhoy et al. (3), Rosenoer (19), McFarlane (12), and Kirsch et al. (8) obtained fairly good agreement between the albumin synthesis rate measured by the ^{14}C-carbonate technique and the catabolic rate measured with iodinated albumin in a steady state. It is relevant that after 2 weeks on a 70-g protein diet, there is poor correlation between synthesis and catabolic rates measured independently in the same subject in this study. It is possible that at this stage some subjects were still adapting to a protein load greater than that to which they were accustomed.

It is interesting to compare the results of this study with those of Hoffenberg et al. (6) who measured albumin catabolism and "synthesis and transfer" in subjects under the same conditions as reported here (Table 3).

TABLE 1

Rates of absolute albumin synthesis and catabolism[a] in subjects on 70-g and 10-g isocaloric protein diets

Patient	70-g Protein diet		10-g Isocaloric protein diet	
	Rates		Rates	
	Synthesis	Catabolism	Synthesis	Catabolism
1	398	250	101	184
2	225	138	257[b]	82
3	327	163	82	123
4	307	168	79	125
5	161	211	88	133
6	95	171	33	102
7	256	147	83	89
8	193	132	126	53
Mean ± SD	245 ± 98	173 ± 40	85 ± 28	111 ± 39

[a] Expressed in milligrams per kilogram per day. [b] Reported to be eating a 70-g protein diet intermittently.

TABLE 2

Plasma albumin, plasma urea, plasma volume, rates of fractional and absolute albumin synthesis, and rate of catabolism

	70-g Protein diet[a]	10-g Isocaloric protein diet[a]	Differences between two diets
Plasma albumin, g/100 ml	4.11 ± 0.45	3.71 ± 0.35	$t = 1.99$, $0.02 < P < 0.05$
Plasma urea, mg/100 ml	15.5 ± 5.3	7.0 ± 4.2	$t = 3.55$, $0.001 < P < 0.01$
Plasma volume, ml	2,330 ± 515	2,247 ± 428	$t = 0.38$, $0.7 < P < 0.8$
Fractional synthesis rate, %/day	14.9 ± 6.2	5.9 ± 2.1	$t = 3.86$, $0.001 < P < 0.01$
Absolute synthesis rate, mg/kg/day	245 ± 98	85 ± 28	$t = 4.42$, $P < 0.001$
Fractional catabolic rate, %/day	10.9 ± 2.65	6.5 ± 2.7	$t = 3.33$, $0.001 < P < 0.01$
Absolute catabolic rate, mg/kg/day	173 ± 98	111 ± 39	$t = 3.14$, $0.001 < P < 0.01$

[a] Mean ± SD of eight observations.

The results obtained by the different methods are similar. While this work was being completed, Coles et al. (5) reported reduced albumin catabolism and synthesis rates of 120 and 100 mg/kg per day in two uremic patients on 20-g protein diets. They concluded that changes in albumin metabolism in uremia were probably secondary to the dietary changes rather than to the renal disease itself. This study, using a direct technique for measuring albumin synthesis, therefore confirms the work of previous investigators and serves to emphasize the importance of measuring albumin catabolism and particularly albumin synthesis under controlled dietary conditions.

Studies in normal adults on low protein or protein-free diets have shown varying patterns in plasma amino acids. Tuttle et al. (20) and Swendseid et al. (21) showed a reduction in valine and alpha-amino *n*-butyric acid on a low protein diet, but a rise in methionine, glutamine, asparagine, glycine, and alanine. However, Adibi (22) found a marked elevation of plasma alanine concentration and, to a lesser extent, an elevation of the glycine concentration in normal subjects after 2 weeks on an isocaloric protein-free diet. This is similar to the findings of a raised plasma alanine after 4 to 6 weeks on a 10-g isocaloric protein diet reported here. When one considers that alanine, together with glycine, glutamic acid, and aspartic acid constitute 80% of the free amino acids in the body, it is hardly surprising that any changes would be detected first in this group. In fact Felig, Owen, Wahren, and Cahill (23) consider that alanine serves as a vehicle of transport for nitrogen arising from amino acid catabolism in the periphery. However, as plasma amino acid concentrations may have no bearing on hepatic intracellular amino acid levels, the relationship of the low synthesis rates to the elevated plasma alanine levels may even be fortuitous or the result of two processes acting at different sites.

TABLE 3

Comparison of the results of Hoffenberg et al. (6) and those of the present study

	Normal diet		10-g Protein diet	
	Present study	Hoffenberg et al. (6)	Present study	Hoffenberg et al. (6)
Fractional synthesis rate[a]	14.9	8.9[b]	5.9	5.8[b]
Fractional catabolic rate[a]	10.9	8.8	6.5	6.5
Absolute synthesis rate[c]	245	150[b]	85	103[b]
Absolute catabolic rate[c]	173	151	111	103

[a] Percent of intravascular pool/day. [b] "Synthesis and transfer." [c] Expressed in milligrams per kilogram per day.

Summary

Albumin synthesis, albumin catabolism, and the plasma aminogram were studied in eight patients on both normal and low protein diets.

The fractional and absolute synthesis rates of albumin were markedly reduced by protein restriction. Catabolic rates were reduced to a lesser degree.

Plasma alanine was increased by protein restriction. The other amino acids were unaffected.

We are grateful to Dr. R. Kirsch for his suggestions and to the Department of Chemical Pathology for the urea estimations.

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