Nutritional Supplementation Acutely Increases Albumin Fractional Synthetic Rate in Chronic Hemodialysis Patients

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Abstract. Uremic malnutrition is associated with increased risk of hospitalization and death in chronic hemodialysis (CHD) patients. Most nutritional intervention studies in CHD patients traditionally have used concentrations of serum albumin as the primary outcome measure and showed slight or no significant improvements. A recent study showed that intradialytic parenteral nutrition (IDPN) improves whole-body protein synthesis in CHD patients. On the basis of this observation, it was hypothesized that the anabolic effects of IDPN are associated with increases in the fractional synthetic rate of albumin, a direct estimate of acute changes in hepatic albumin synthesis. Seven CHD patients were studied during two hemodialysis (HD) sessions, with and without IDPN, using primed-constant infusion of (^{13}C) leucine 2 h before, during, and 2 h after HD. Plasma enrichments of (^{13}C) leucine and (^{13}C) ketoisocaproate were examined to determine the fractional synthetic rate of albumin. The results indicate that administration of IDPN significantly improves the fractional synthetic rate of albumin during HD (16.2 ± 1.5%/d *versus* 12.8 ± 1.7%/d; $P < 0.05$) in CHD patients in parallel with significant improvements in whole-body protein synthesis (5.05 ± 1.3 mg/kg fat-free mass/min *versus* 3.22 ± 0.3 mg/kg fat-free mass/min; $P < 0.05$). IDPN is protein anabolic in the acute setting in CHD patients, as evidenced by significant concomitant increases in the fractional synthetic rate of albumin and whole-body protein synthesis.

Uremic malnutrition is associated with increased risk of hospitalization and death in chronic hemodialysis (CHD) patients (1,2). Most nutritional intervention studies in CHD patients traditionally have used concentrations of serum albumin as a clinical evaluation of overall protein homeostasis because of its established and significant association with outcomes (3). However, serum albumin concentrations can be difficult to interpret in certain instances, such as presence of inflammatory response and altered fluid state as observed in CHD patients. Furthermore, serum albumin has a relatively long half-life. Not surprising, most studies that have evaluated the effects of nutritional intervention on serum albumin in CHD patients have shown slight or no significant improvements in the serum concentrations of this protein.

We recently showed that intradialytic parenteral nutrition (IDPN) improves whole-body protein synthesis in CHD patients. Because albumin is a prominent protein circulating throughout the body, it is likely that the improvements in whole-body protein as a result of nutritional supplementation are linked with increases in visceral synthesis of albumin. The fractional synthetic rate (FSR) of albumin provides a direct estimate of acute changes in hepatic albumin synthesis. To our knowledge, no study to date has examined the effects of nutritional supplementation on the FSR of albumin in CHD patients. In this study, we hypothesized that the anabolic effects of IDPN may be associated with increases in albumin FSR beyond what could be observed with the hemodialysis (HD) procedure alone. To test this hypothesis, we studied seven CHD patients during two HD sessions, with and without IDPN, using stable isotope infusion techniques for protein turnover assessment. Our results indicate that administration of IDPN significantly improves the FSR of albumin in CHD patients in parallel with marked improvement in whole-body protein synthesis.

Materials and Methods

Patients

Patients were recruited from the Vanderbilt University Outpatient Dialysis Unit. Inclusion criteria for the study were patients who had been on a thrice-weekly CHD program for >6 mo, using a biocompatible HD membrane (Fresenius F80) and an adequate dose of dialysis (single pool Kt/V ≥1.4). Patients who had active infectious or inflammatory disease, had liver failure of any cause, were hospitalized within 3 mo before the study, had recirculation in the vascular access and/or vascular access blood flow <750 ml/min detected on the arteriovenous (AV) shunt, and were receiving steroids and/or immunosuppressive agents were excluded. The whole-body protein synthesis data in this article is derived from seven patients, six of whom were included in our previous publication (4). The Institutional Review Board of Vanderbilt University approved the study protocol, and written informed consent was obtained from all study patients. Patient characteristics are shown in Table 1.

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Table 1. Demographic and nutritional characteristics of the study patients ($N = 7$)[a]

Demographics		
Gender (M/F)	5 (71%)/2 (29%)	
Race (black)	7 (100%)	
Age (y ± SD, range)	47 ± 19, 28–76	
Cause of ESRD (%)	5 (71.5%) hypertension	
	1 (14.25%) ADPKD	
	1 (14.25%) FSGS	
Body composition		
body weight (kg)	77.3 ± 17.6	
body mass index (kg/cm^2)	26.4 ± 4.8	
fat mass by DEXA (%)	25.2 ± 12.1	
Baseline biochemistries	Control	IDPN
serum albumin (g/dl)	4.3 ± 0.3	4.1 ± 0.3
serum prealbumin (mg/dl)	45.8 ± 6.2	42.3 ± 6.1
serum transferrin (mg/dl)	194 ± 37	195 ± 31
serum cholesterol (mg/dl)	192 ± 25	163 ± 21
total CO_2 (mg/dl)	22.7 ± 1.8	23.2 ± 2.3
CRP (mg/dl)	0.7 ± 0.4	0.9 ± 0.7

[a] Values are mean ± SD or count and percentage within each count. ADPKD, autossomic dominant polycystic kidney disease; FSGS, focal segmental glomerulosclerosis; DEXA, dual-energy x-ray absorptiometry; IDPN, intradialytic parenteral nutrition; CO_2, total bicarbonate; CRP, C-reactive protein.

Design

This was a randomized crossover study. After review of the inclusion and exclusion criteria, eligible patients were randomly assigned to treatment (IDPN) or control (CTL) periods as a first intervention, using simple randomization. All patients who participated in this study were crossed over and participated in both interventions, with at least 4 wk between each period to allow complete clearance of the labeled products. Within 1 wk before each study, dual-energy x-ray absorptiometry was performed to estimate lean and fat body masses.

The patients were admitted to the General Clinical Research Center the day before the study at approximately 7 p.m., received a meal from bionutrition services, and fasted after that. The last meal was given at least 10 h before the initiation of the study for all patients and consisted of 18% protein and 30% lipids. Energy intake was kept at maintenance levels based on the Harris-Benedict equation.

A schematic diagram of the metabolic study day protocol is depicted in Figure 1. Each metabolic study consisted of a pre-HD phase (a 2-h equilibration phase followed by a 0.5-h basal sampling phase), a 4-h dialysis phase, and a 2-h post-HD phase. In the treatment studies, IDPN was administered starting 30 min after dialysis initiation and continued through the end of dialysis. Constant infusion of isotopes continued throughout the study. Simultaneous blood and breath samples were collected once before the start of the study, three times during the basal sampling phase, six times during IDPN and dialysis, and three times during the post-HD phase.

A dialysis catheter was placed at the venous site of the AV shunt of the forearm at 6 a.m. to collect a baseline blood sample (to assess baseline biochemical nutritional markers and isotopic backgrounds) and then to initiate the isotope infusion. In the present study, two patients had a native fistula and five patients had an artificial graft.

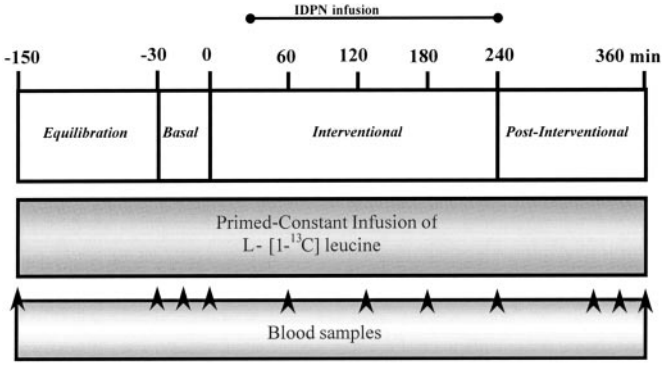

Figure 1. Experimental design. Each study consisted of a 120-min equilibration period (−150 to −30 min), a 30-min basal period, a 240-min hemodialysis (HD) period (0 to 240 min), and a 120-min post-HD period. The arrows indicate blood sampling time points.

Arterial vascular access obtained though the arterial side of the AV shunt was used to perform HD and to sample arterial blood. The venous site of the AV shunt was used to infuse the tracers. Another catheter was placed in a deep vein (on a retrograde insertion) of the contralateral forearm to sample blood draining the forearm muscle bed. At the start of the infusion, patients received a bolus injection of $NaH^{13}CO_3$ (0.12 mg/kg), and L-(1-^{13}C) leucine (7.2 μmol/kg) to prime the CO_2 and leucine pools, respectively. A continuous infusion of leucine (0.12 μmol/kg per min) isotope was then started and continued throughout the remainder of the study.

Patients underwent dialysis for 4 h with blood flow of 400 ml/min and dialysate flow of 500 ml/min. Ultrafiltration rates were determined by the patients' needs and "estimated dry weight" and were similar during both studies. The composition of the dialysate used during the study was identical for all treatments and consisted of 139 mEq/L sodium, 2 mEq/L potassium, 2.5 mEq/L calcium, 200 mg/dl glucose, and 39 mEq/L bicarbonate.

The IDPN infusion was started 30 min after initiation of HD in the treatment period and continued throughout the entire HD procedure (total of 3.5 h of IDPN infusion). The IDPN treatment was based on existing recommendations (5). The solution was given at a rate of 150 ml/h and consisted of 300 ml of amino acids at a concentration of 15%, 150 ml of dextrose at a concentration of 50%, and 150 ml of lipids at a concentration of 20%. Amino acids solution (15% Clinisol; Baxter Healthcare Corp., Deerfield, IL) consisted of nine essential amino acids (1.18 g of lysine, 1.04 g of leucine,1.04 g of phenylalanine, 960 mg of valine, 894 mg of histidine, 749 mg of isoleucine, 749 mg of methionine, 749 mg of threonine, and 250 mg of tryptophan) and eight nonessential amino acids (2.17 g of alanine, 1.47 g of arginine, 1.04 g of glycine, 894 mg of proline, 749 mg of glutamate, 592 mg of serine, 434 mg of aspartate, and 39 mg of tyrosine). This solution provided 188 kcal/h or 3.5 kcal/kg fat-free mass per h. The extra volume, as well as electrolytes that IDPN provided to the patients, were accounted for and removed during HD. Once HD was finished, dialysis lines were disconnected and the 2-h post-HD phase ensued. After the post-HD phase, all catheters were removed, and the patients were given a meal and observed at the General Clinical Research Center until stable, upon which they were discharged.

Simultaneous with each blood sampling, breath samples were collected from the patients via a Douglas bag with duplicate 20-ml samples placed into nonsiliconized glass Vacutainer tubes for measurement of breath $^{13}CO_2$ enrichment. Subjects were asked to breathe through a mask for 1 min each time blood was collected. In addition,

forearm blood flow was estimated using capacitance plethysmography (Model 2560 with URI/CP software v 3.0; Moro Bay, CA). Simultaneous energy expenditure and respiratory quotient were determined by indirect calorimetry using a metabolic cart (Sensormedics 2900, Palo Alto, CA) to measure ventilation rates, CO_2 production, and O_2 consumption.

Analytical Procedures

Blood samples were collected into Venoject tubes that contained 15 mg of Na_2EDTA (Terumo Medical Corp, Elkton, MD). The blood was spun in a refrigerated (4°C) centrifuge (Beckman Instruments, Fullerton, CA) at 3000 rpm for 10 min, and plasma was extracted and stored at −80°C for later analysis.

Nutritional biochemical parameters were done at a specialized ESRD clinical and special chemistry laboratory (Spectra Laboratories, San Juan, CA). Serum albumin was analyzed using bromcresol green technique. Serum prealbumin was analyzed by an antigen-antibody complex assay, and serum transferrin was analyzed by turbidimetric reading (Hitachi 717). C-reactive protein was measured using nephelometric analysis at the Vanderbilt University Medical Center clinical chemistry laboratory. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) in our laboratory. Immunoreactive insulin was determined in plasma with a double-antibody system (Linco Research, St. Louis, MO). Plasma amino acid concentrations were determined by reversed-phase HPLC after derivatization with phenylisothiocyanate (6).

The method of choice for measuring albumin FSR in this study was through plasma enrichments of (^{13}C) leucine and (^{13}C)ketoisocaproate (KIC), as explained in our previous publications (4,7). Albumin was precipitated from plasma by adding 2 ml of 10% TCA to defibrinogenized plasma. The pellet was solubilized in 0.5 ml of water before adding 2.5 ml of 96% ethanol containing 1% TCA. After mixing and centrifugation, the supernatant was collected. Although the recovered sample contained >90% of the albumin, SDS-PAGE showed some contaminating bands. Removal of these bands was accomplished via the addition of 2 ml of 26.8% ammonium sulfate. The ammonium sulfate precipitated the albumin but not the contaminating bands. The precipitate was washed twice with 2 ml of 0.2 M PCA, and the resulting pellet was analyzed using combustion isotope ratio mass spectrometry. Enrichment measurements were made in duplicate, and duplicates had a coefficient of variation <1%. The ^{13}C-combustion analyses were performed via Dumas combustion in a Europa ANCA-NT system and subsequent isotope analysis in a Europa 20/20 Stable Isotope Analyzer. This is a continuous flow system (Helium of 99.999% as carrier gas) with combustion of samples to CO_2 at 1000°C in a quartz column with O_2 (99.994% purity) introduction for 40 s. The dried, isolated protein samples were transferred to tin capsules and introduced into the system via an autosampler. The CO_2 from the combustion process then passed through a water trap and was separated from all other gases produced by a gas chromatograph set at 55°C. The gas peaks subsequently flowed into a magnetic sector mass spectrometer for isotope analysis via computer control. The mass spectrometer's precision was 0.2 per mil for ^{13}C ratio analysis as stated by the manufacturer and verified by multiple analyses of laboratory standards.

Calculations

Details on the calculations of leucine kinetics can be found in our previous publications (4,8). Albumin FSR was calculated using the mean plasma KIC enrichment (KIC^{me}) during each period as the tracer precursor enrichment and the changes in enrichment of leucine bound to albumin during each period, as shown in the formula FSR (%/d) = $[(P^{fe} - P^{se})/t)/(KIC^{me})] \times 100\%$.

KIC^{me} was calculated by determining the average KIC enrichment for the time points of each period. The change in albumin enrichment was calculated by subtracting the isotopic enrichment of leucine bound to albumin at the start of each period (P^{se}) from the enrichment of leucine bound to albumin at the end of each period (P^{fe}). This change in product enrichment was divided by time (t) using the units of day so that albumin FSR could be expressed as percentage synthesized per day.

Statistical Analyses

For each protocol, mean variables for each phase (before, during, and after HD) were calculated as the average of the time points for each phase. Values presented in the text and figures are means ± SEM unless otherwise noted. For comparisons between study periods (IDPN *versus* CTL), a paired *t* test was used for parametric distribution and Wilcoxon signed-rank test for nonparametric distribution. Differences between the mean values within phases (before, during, and after HD) were assessed using a repeated-measures ANOVA. Analyses were completed with the SPSS statistical software package (SPSS, version 11.5, Chicago, IL). $P < 0.05$ was required to reject the null hypothesis of no difference between the means.

Results

Blood Chemistries and Patient Characteristics

Table 1 depicts baseline biochemical nutritional markers, including serum albumin, serum prealbumin, serum transferrin, and serum cholesterol, as well as CO_2 and C-reactive protein for the two study protocols: control and IDPN. These measurements were similar between CTL and IDPN protocols, and there were no statistical differences. As suggested by the above-mentioned biochemical markers, the population studied was in an overall adequately nourished status and not inflamed. Measurement of pre- and post-HD blood chemistries, including blood urea nitrogen, showed expected changes after HD treatment without any significant difference between the two separate HD sessions within patients (data not shown).

Albumin FSR

Table 2 shows components of FSR of albumin for each study period and protocols. As can be seen, at baseline, there was no significant difference in the albumin FSR between the two occasions that patients were studied (8.3 ± 1.5 and 8.8 ± 0.7%/d, CTL and IDPN; NS). During HD, albumin FSR increased in both protocols. However, the increase in albumin FSR was significantly higher when IDPN was infused (84% with IDPN *versus* 54% with control; $P < 0.05$). Figure 2 shows these results for each study patient. In the post-HD phase, albumin synthesis did not change in the CTL protocol, remaining elevated compared with baseline. In the protocol with nutritional supplementation, albumin synthesis decreased significantly (73%; $P < 0.05$) post-HD compared with during HD but still remained numerically higher than baseline values. There were no significant differences in albumin FSR in the post-HD phase between the two protocols.

Table 2. Components of albumin fractional synthetic rate during study periods[a]

	Pre-HD	During HD	Post-HD
KIC MPE (%)			
CTL	7.8 ± 0.8	7.1 ± 0.7	7.3 ± 0.7
IDPN	7.6 ± 0.5	4.6 ± 0.2[b,d]	6.9 ± 0.6[c]
Albumin MPE (final − initial)			
CTL	0.0009 ± 0.00015	0.0021 ± 0.0003[d]	0.0010 ± 0.0002[c]
IDPN	0.0009 ± 0.00001	0.0017 ± 0.0001[d]	0.0009 ± 0.00012[c]
Albumin FSR (% per day)			
CTL	8.3 ± 1.5	12.8 ± 1.7[c]	12.5 ± 2.4
IDPN	8.8 ± 0.7	16.2 ± 1.5[b,d]	11.8 ± 1.7[c]

[a] CTL, control; HD, hemodialysis; MPE, molar percent excess.
[b] $P < 0.05$ for CTL *versus* IDPN.
[c] $P < 0.05$ for post-HD *versus* during HD.
[d] $P < 0.05$ for during HD *versus* pre-HD.

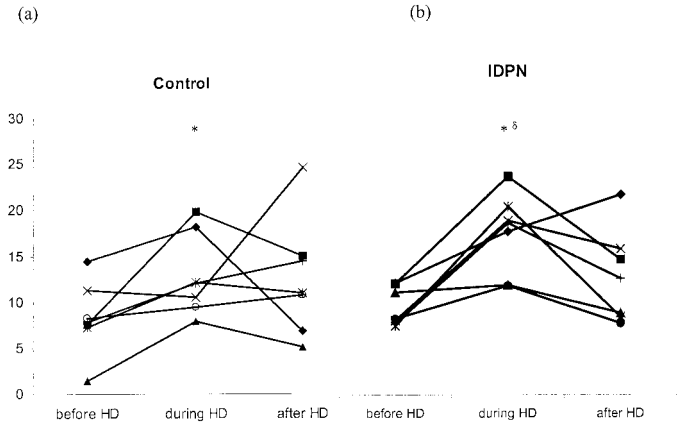

Figure 2. The increment in albumin fractional synthetic rate (FSR) during each study period for each individual patient. (a) Results for control protocol. (b) Results for intradialytic parenteral nutrition (IDPN) protocol. *Significant difference from the baseline period for the mean; δSignificant difference between protocols.

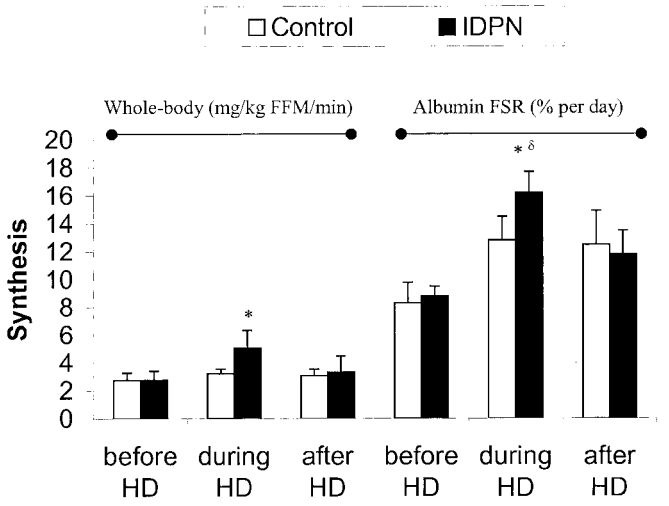

Figure 3. Whole-body protein synthesis and FSR of albumin during each study period for both protocols (control and IDPN). *Significant difference from the baseline period; δSignificant difference in albumin FSR between protocols during HD.

Whole-Body Protein Homeostasis

The components of whole-body protein homeostasis (synthesis, breakdown, and net balance) have been previously published with a similar patient population (4). Nonetheless, we elected to include whole-body protein synthesis in this article to emphasize the unidirectional increases in both whole-body protein and albumin synthesis with nutritional supplementation. Whole-body protein synthesis (Figure 3) at baseline was similar between protocols. During dialysis without nutritional supplementation, whole-body protein synthesis had a slight nonsignificant increase of 17%. However, with the administration of IDPN, whole-body protein synthesis increased 83% ($P < 0.05$ compared with pre-HD), consistent with our previously published data (4). Of note, this increase was in the same extent and direction of the increase in albumin FSR (83 *versus* 84%; Figure 3). After cessation of HD and thus the infusion of nutrients, we observed an abrupt decrease in whole-body protein synthesis, which was very similar to the decrease in albumin FSR (66 and 73%, respectively; Figure 3).

Plasma Amino Acids and Metabolic Hormones

We also measured the concentrations of individual plasma amino acids and a panel of metabolic hormones, including insulin, cortisol, glucagon, growth hormone, insulin growth factor I, epinephrine, and norepinephrine. Our results are consistent with our previous findings (4), in that we observed a fourfold increase in plasma arterial insulin levels with IDPN ($P < 0.05$ *versus* CTL; Figure 4) along with significant increases in plasma levels of essential amino acids and nonessential amino acids (data not shown).

Discussion

The results of this study indicate that nutritional supplementation, in the form of IDPN, improves the hepatic synthesis of albumin as a part of improvements in the whole-body protein homeostasis. This is evidenced by significant increases in the FSR of albumin above and beyond what is observed as a result

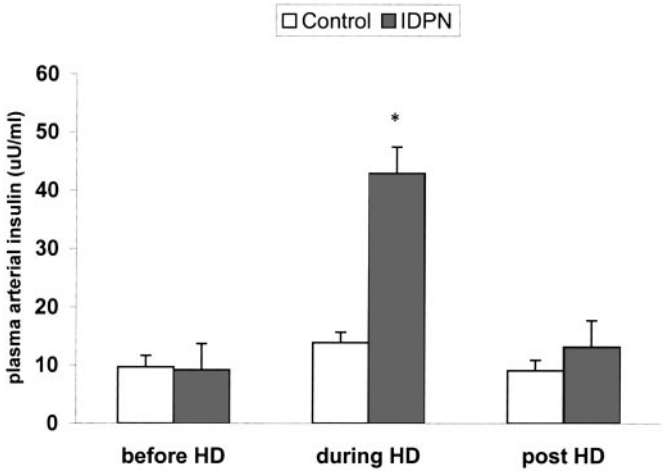

Figure 4. Plasma arterial insulin levels during each study period for both protocols (control and IDPN). *Significant difference between protocols during HD.

of HD alone. To our knowledge, this is the first study to show this concomitant unidirectional change in whole-body protein and albumin synthesis in response to acute administration of IDPN in CHD patients.

Given the significant association with increased hospitalization and death risk, treatment of uremic malnutrition has been an area of great interest. Unfortunately, the limited number of nutritional intervention studies in CHD patients have produced mixed results (9–12). Potential explanations for the lack of agreement among studies seem to be based in different study designs and the inherent methodologic problems of the outcomes measured. Indeed, by using more precise methods, namely primed-constant stable isotope infusions, we have shown remarkable acute improvements in net protein and energy balances with administration of IDPN (4). However, a question remained on whether the improvements in protein synthesis would translate into improvements in serum albumin concentrations, the most commonly used outcome measure and most significant predictor of clinical outcome in CHD patients (13).

Serum albumin is part of the complex metabolism of visceral albumin, and the concentration of serum albumin is the net result of its synthesis, breakdown, volume of distribution, and exchange between intra- and extravascular spaces, as well as losses (14,15). Albumin is synthesized in the liver and secreted into the bloodstream, where it is rapidly equilibrated and has a half-life of ~20 d. Equilibration between intra- and extravascular albumin pools is slower than within the intravascular space, varying from 7 to 10 d, depending on the tissue (15,16). Supplementation with a mixture of amino acids and glucose has been shown to optimize anabolic activity of the liver to synthesize albumin in healthy individuals (17). Availability of essential amino acids is limited by normal events associated with HD (7,18), and treatment with IDPN increased amino acid availability in the present study. Furthermore, it is possible that a portion of the response to IDPN is due to increases in circulating insulin, which has been shown to positively modulate albumin FSR (17). Further research will be required to differentiate the effects caused by increased availability of amino acids *versus* insulin.

The measurement of albumin FSR with stable isotopic-labeled amino acids is an important and sensitive tool for the study of the visceral protein response to nutrition and other physiologic and/or pathologic stimuli. The technique allows assessment of albumin synthesis in the liver during the infusion of labeled isotopes of essential amino acids (19). In brief, when labeled leucine is infused, the enrichment of its ketoacid, α-ketoisocaproic acid (KIC), is used as an index of the enrichment of the precursor pool used for liver protein synthesis (19). The validity of the method has been supported by studies by Nair *et al.* (20), in which the authors demonstrated that the enrichment of plasma KIC is a reliable surrogate of the intrahepatic leucine-tRNA or hepatic vein leucine enrichments (21). A number of studies, both in animals and in humans with and without chronic disease states, have shown that nutrient intake can modulate albumin synthesis, and this can be precisely monitored by appropriate methods such as the studies with isotopic labeled amino acids (22–24) and studies of mRNA expression in the liver (25,26).

In addition to its robust relationship to clinical outcome as a surrogate nutritional marker, albumin is considered to have important biologic properties. Albumin is the major protein carrier in the plasma and acts as a buffer pool to stabilize plasma concentrations of calcium, tryptophan, and hormones, including cortisol, testosterone, and estrogens. The nutritive function of albumin arises from its availability to cells as a source of amino acids. Finally, albumin is one of the few intracellular proteins that have a free thiol group and therefore has significant antioxidant capacity. It is interesting that Himmelfarb and McMonagle (27) reported that albumin is the major plasma protein target of oxidant stress in CHD patients and that plasma albumin is substantially more oxidized in CHD patients than in healthy volunteers. We have also reported that hypoalbuminemic CHD patients are at substantially increased risk for oxidative stress (28). As oxidative stress has been proposed as the potential link leading to the increased cardiovascular risk in CHD patients, an increase in albumin synthetic rate can potentially prevent the adverse consequences of oxidative stress and improve cardiovascular outcomes in these patients (29).

An interesting observation in our study is that albumin FSR was significantly increased during HD despite that HD is an inflammatory event (30), which should lead to a decrease in albumin synthesis as a result of its negative acute-phase reactant properties. Although this was an unexpected finding at first, it is nevertheless consistent with the published literature. Ruot *et al.* (31) in animal studies, Mansoor (32) *et al.* in head trauma patients, and Fearon *et al.* (33) in cancer patients all have shown increased albumin FSR during inflammatory episodes. Our findings are consistent with our earlier report (30), which was confirmed recently by Raj *et al.* (34). Therefore, although an increase in FSR of albumin during an inflammatory response is counterintuitive, it is nevertheless true and consistent with current literature. This suggests that other crit-

ical mechanisms determine the serum concentration of albumin during episodes of an inflammatory response.

Although the results presented in this study are intriguing, some potential limitations should be considered. Our study population is well nourished and without evidence of inflammation. CHD patients with evidence of uremic malnutrition and/or inflammation may potentially have a different response to the nutritional supplementation. Clearly, the current study should be extended to a larger patient population with more diverse nutritional and inflammatory status. This study also represents only short-term effects of one-time administration of IDPN, and it would only be speculative to project the effect of IDPN over a longer period of time on the basis of this report. Although the techniques used in this study are associated with a margin of error, these are small and the techniques are well accepted and validated. In addition, we have used these techniques in several study populations and have found them to be reliable and reproducible (30,35–37). Finally, further research is needed to determine the mechanisms of the observed effects, such as increased availability of amino acids and/or insulin action.

In conclusion, the results of this study indicate that IDPN is protein anabolic in the acute setting in CHD patients. This is evidenced by significant concomitant increases in the FSR of albumin and whole-body protein synthesis. Further research is needed to establish the effects of IDPN on more diverse patient populations as well as its long-term effects on serum concentrations of albumin and protein homeostasis.

Acknowledgments

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