

Comparison of the effects of intradialytic parenteral nutrition alone and combined with anabolic steroid in patients undergoing hemodialysis therapy

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Aim: It has been shown that androgen anabolic steroids and intradialytic parenteral nutrition (IDPN) may have advantages in malnourished hemodialysis patients. We aimed to compare the effects of IDPN and IDPN with added anabolic steroid.

Materials and methods: Twenty hemodialysis patients who had albumin of <3.5 g/dL were included. Of those, 10 patients received 1000 mL of IDPN at each hemodialysis session 3 days per week, whereas the other 10 patients received IDPN plus nandrolone (100 mg intramuscularly) once every 2 weeks. Nutritional parameters were recorded at baseline and after 6 months.

Results: In patients who received IDPN alone, significant elevations were found in albumin, total protein levels, and body weight ($P < 0.05$) after 6 months when compared with baseline values. In patients who received IDPN plus nandrolone, significant elevations were found in the same parameters ($P < 0.05$). Except for total cholesterol ($P = 0.034$), there were no differences in nutritional parameters between the 2 groups.

Conclusion: Patients receiving IDPN for 6 months exhibited a significant increase in albumin levels. Adding nandrolone to IDPN treatment achieved no additional increase in these parameters; however, an increase in total cholesterol in the IDPN plus nandrolone group may be unfavorable at long-term follow-up.

Key words: Hemodialysis, malnutrition, intradialytic parenteral nutrition, anabolic steroids

Introduction

The prevalence of protein-calorie malnutrition remains as high as 30% to 40%. Protein-calorie malnutrition, a reflection of dietary protein intake in the stable hemodialysis (HD) patient, has been shown to be highly associated with increased morbidity and mortality in patients with end-stage renal disease. It remains one of the major factors that adversely affect patient outcome (1-3). Furthermore, recent studies have shown that a low concentration of serum albumin and low levels of creatinine, cholesterol, and blood urea nitrogen were all associated with increased risk of mortality (4-6).

Inadequate nutritional intake is considered the most common cause of malnutrition in HD patients. Thus, nutritional support can prevent or ameliorate malnutrition, improve quality of life, and reduce morbidity and mortality (7-12). There are many techniques to provide nutrients to maintain dialysis patients who have inadequate food intake. These techniques include dietary counseling, food supplements, enteral tube feeding, intradialytic parenteral nutrition (IDPN), and total parenteral nutrition (7-10). For malnourished hemodialysis patients who failed to respond to dietary counseling or food supplements, IDPN may be the best choice of treatment (11,12). It was shown that IDPN promotes

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a great increase in whole-body protein synthesis and a significant decrease in whole-body proteolysis, along with a significant increase in muscle-protein synthesis (13,14).

In HD patients with malnutrition, therapeutic options other than enteral or parenteral nutrition include recombinant growth hormone and anabolic androgen steroids (AASs) (15). Stimulation of protein anabolism in muscle may be attempted via use of AASs (16,17). Nandrolone decanoate and oxandrolone are the most commonly used AASs in clinical practice. Although positive effects have been documented with nandrolone decanoate and oxandrolone in patients with burns, cancer, human immunodeficiency virus infection, and chronic obstructive pulmonary diseases, data are limited in HD patients (18-20). Although there are many studies in which individual effects of IDPN and anabolic steroids were evaluated, to our knowledge, there is no study comparing combined IDPN and androgen treatment to IDPN alone. Therefore, we aimed to assess the effects of adding nandrolone decanoate to IDPN versus IDPN alone in the present study.

Materials and methods

Twenty patients (10 female, 10 men; mean age: 66.4 ± 6.9 years) who had been on regular (3 times/week) hemodialysis for at least 6 months were enrolled in the study. Patients were selected for IDPN by a renal dietitian and a nephrologist in accordance with the criteria listed in Table 1 (21-23). Patients with diabetic nephropathy, chronic liver disease, congestive heart failure, malignancies, or chronic infections were

excluded. Patients using statins were excluded from the study because of the risk of hepatotoxicity when used together with nandrolone decanoate.

Malnutrition was defined by scoring body weight (>10% below ideal body weight) in addition to total protein and albumin concentrations; only patients having at least 2 abnormal values were included. The last abnormal results were recorded as baseline value. Hemodialysis was performed 3 times a week for 4 h with Fresenius FX80 low flux membrane and a glucose-containing dialysate (2 g/L). The flow rates for blood and dialysate were 300 mL/min and 500 mL/min, respectively. All patients received erythropoietin replacement therapy for at least 3 months prior to the study. Parameters such as dialysis modality, treatment time, and filter used remained unchanged during the study period. The study patients were administered IDPN throughout each hemodialysis session (4 h per session 3 times a week), and these solutions were infused at a rate of 250-300 mL/h. Each individual was assigned randomly to receive either 1000 mL of IDPN supplement (each containing amino acids = 42.5 g, glucose = 125 g, lipid emulsion = 50 g, and total nonprotein calories = 925 kcal) (N = 10 patients) or IDPN plus 100 mg of nandrolone intramuscularly once every 2 weeks (N = 10 patients). Urea, creatinine, albumin, total protein, cholesterol, hemoglobin, C-reactive protein, Kt/V [ratio of dialyzer clearance (in milliliters per minute) multiplied by time (in minutes) to patient water volume (in liters)], protein catabolic rate, calcium, phosphorus, and body weight (kg) were recorded at baseline and after 6 months. All blood samples were taken before dialysis during the entire study period. Kt/V dialysis doses were

Table 1. Criteria for initiation of intradialytic parenteral nutrition (IDPN).

Initiate IDPN if patient meets any 3 of the following criteria.

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1. Serum albumin concentration of <3.5 g/dL.
 2. Unintentional loss of body weight of >10% or current body weight of <90% of ideal body weight.
 3. Dietary history showing a decreased daily protein intake of <1 g/kg or daily calorie intake of <25 kcal/kg.
 4. Protein catabolic daily rate of <1 g/kg.

AND

- Inability to increase oral intake with oral supplements,
 - Not a candidate for enteral tube feeding,
 - All possible attempts have been made to achieve adequate dialysis.
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calculated using the Daugirdas formula as stated in the 2006 Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines.

Statistical analysis

All results were expressed as mean \pm standard error of the mean (SEM). Data were assessed using nonparametric Wilcoxon signed ranks and chi-square tests. Paired 2-sample t-tests were used to

determine changes from baseline in the secondary endpoints. The level of significance was set at $P < 0.05$.

Results

There was no difference in baseline parameters between patients receiving IDPN alone and IDPN plus AASs (Table 2). Dialysis was adequate for all the patients on the basis of Kt/V.

Table 2. Patient demographics at baseline in 2 study groups (mean \pm SEM).

Characteristic	IDPN alone	IDPN plus nandrolone
Number of patients	10	10
Number of men	5	5
Number of women	5	5
Mean age (years)	66.7	66.1
Mean duration of HD (months)	6.5	6.8
Weight (kg)	57 \pm 8	58 \pm 1
Serum albumin (g/dL)	3.0 \pm 0.2	3.06 \pm 0.1
Serum total protein (g/dL)	5.7 \pm 1.0	5.6 \pm 1.1
Kt/V	1.48 \pm 0.2	1.51 \pm 0.1
Urea reduction ratio (%)	75.2 \pm 6.7	76.1 \pm 5.9
Protein catabolic rate	0.8 \pm 0.1	0.78 \pm 0.2
Hemoglobin (g/dL)	10.7 \pm 0.8	10.9 \pm 0.7
Serum urea (mg/dL)	92.8 \pm 6.5	89 \pm 6.8
Serum creatinine (mg/dL)	6.4 \pm 1.1	6.6 \pm 1.2
Serum C-reactive protein (mg/L)	2.7 \pm 0.9	2.9 \pm 1.1
Serum potassium (mEq/L)	4.4 \pm 0.3	4.3 \pm 0.2
Serum calcium (mEq/L)	9.8 \pm 0.1	9.9 \pm 0.1
Serum phosphate (mEq/L)	5.4 \pm 0.4	5.3 \pm 0.3
Serum cholesterol (mg/dL)	176 \pm 58.5	178 \pm 62.1
Alkaline phosphatase (units/L)	199.4 \pm 84.9	192.3 \pm 79.8
Aspartate transaminase (units/L)	25.1 \pm 11.5	25.1 \pm 11.5
Drugs used during the study period:		
rHuEPO (IU/HD session)	2480 \pm 250	2520 \pm 220
Calcium acetate (no. of patients)	4	5
Calcitriol IV (no. of patients)	3	3
Carnitine IV (no. of patients)	10	10

Between 2 groups, P was greater than 0.05 for all values.

HD = hemodialysis, IDPN = intradialytic parenteral nutrition, Kt/V = ratio of dialyzer clearance (in milliliters per minute) multiplied by time (in minutes) to patient water volume (in liters).

In patients who received IDPN, significant elevations were found in hemoglobin ($P = 0.007$), urea ($P = 0.04$), creatinine ($P = 0.03$), albumin ($P = 0.005$), total protein levels ($P = 0.005$), and body weight ($P = 0.005$) after 6 months when compared with baseline values (Table 3).

In patients who received IDPN plus nandrolone decanoate, significant elevations were found in hemoglobin ($P = 0.005$), urea ($P = 0.04$), creatinine ($P = 0.03$), albumin ($P = 0.005$), total protein ($P = 0.005$), and body weight ($P = 0.005$) after 6 months when compared baseline values. Furthermore, the most significant difference seen in the group receiving nandrolone decanoate was a distinct elevation in total cholesterol concentration at month 6 when compared to baseline ($P = 0.005$) (Table 4). Except for total cholesterol ($P = 0.034$), there were no differences in the nutritional parameters between the 2 groups.

The intradialytic infusion of IDPN solutions was well tolerated. Despite infusion rates as high as 300 mL/h, known side effects of rapid amino acid infusions such as headache, flushing, or fever were

not seen in any of the subjects. Of the 20 patients, 6 had nausea, but this side effect was tolerated by the patients.

Discussion

Malnutrition is common in hemodialysis patients and is a powerful predictor of morbidity and mortality. Although much progress has been made in recent years in identifying the causes and pathogenesis of malnutrition in hemodialysis patients, as well as in recognizing the link between malnutrition and morbidity and mortality, no consensus has been reached concerning its management (15). Along with such conventional interventions as nutritional counseling, oral nutritional supplements, and IDPN, novel preventive and therapeutic strategies, such as appetite stimulants, growth hormones, AASs (nandrolone decanoate and oxandrolone), and antiinflammatory drugs, have been tested with contradictory and inconclusive results (19,20,24-30).

Inconsistent results were found in recent studies evaluating the effects of IDPN in patients undergoing

Table 3. Comparison of parameters at baseline and after 6 months in patients who received IDPN alone.

Characteristic	Baseline	After 6 months	P-values
Weight (kg)	57 ± 8	60.1 ± 6.5	0.005
Serum albumin (g/dL)	3.0 ± 0.2	3.7 ± 0.4	0.005
Serum total protein (g/dL)	5.7 ± 1.0	6.6 ± 0.9	0.005
Kt/V	1.48 ± 0.2	1.46 ± 0.17	0.38
Urea reduction ratio (%)	75.2 ± 6.7	72.2 ± 7.6	0.41
Protein catabolic rate	0.8 ± 0.1	1.1 ± 0.2	0.28
rHuEPO (IU/HD session)	2480 ± 250	2000 ± 210	0.61
Hemoglobin (g/dL)	10.7 ± 0.8	11.8 ± 0.7	0.007
Serum urea (mg/dL)	92.8 ± 6.5	112.4 ± 7.4	0.04
Serum creatinine (mg/dL)	6.4 ± 1.1	7.91 ± 2.1	0.03
Serum C-reactive protein (mg/L)	2.7 ± 0.9	2.94 ± 1.2	0.57
Serum potassium (mEq/L)	4.4 ± 0.3	4.2 ± 0.3	0.36
Serum calcium (mEq/L)	9.8 ± 0.1	9.6 ± 0.2	0.34
Serum phosphate (mEq/L)	5.4 ± 0.4	5.1 ± 0.5	0.45
Serum cholesterol (mg/dL)	176 ± 58.5	184 ± 46.81	0.07
Alkaline phosphatase (units/L)	199.4 ± 84.9	201 ± 68	0.56
Aspartate transaminase (units/L)	25.1 ± 11.5	26 ± 12	0.42

Table 4. Comparison of parameters at baseline and after 6 months in patients who received IDPN plus nandrolone.

Characteristic	Baseline	After 6 months	P-values
Weight (kg)	58 ± 1.0	60.91 ± 6.6	0.005
Serum albumin (g/dL)	3.06 ± 0.1	3.81 ± 0.2	0.005
Serum total protein (g/dL)	5.6 ± 1.1	6.7 ± 1.12	0.005
Kt/V	1.51 ± 0.1	1.45 ± 0.1	0.38
Urea reduction ratio (%)	76.1 ± 5.9	74.16 ± 6.6	0.39
Protein catabolic rate	0.78 ± 0.2	1.13 ± 0.1	0.30
rHuEPO (IU/HD session)	2520 ± 220	2020 ± 200	0.04
Hemoglobin (g/dL)	10.9 ± 0.7	11.78 ± 0.68	0.005
Serum urea (mg/dL)	89 ± 6.8	108.8 ± 6.3	0.04
Serum creatinine (mg/dL)	6.6 ± 1.2	8.09 ± 1.3	0.03
Serum C-reactive protein (mg/L)	2.9 ± 1.1	2.76 ± 0.9	0.57
Serum potassium (mEq/L)	4.3 ± 0.2	4.3 ± 0.2	0.38
Serum calcium (mEq/L)	9.9 ± 0.1	9.7 ± 0.1	0.36
Serum phosphate (mEq/L)	5.3 ± 0.3	5.3 ± 0.2	0.44
Serum cholesterol (mg/dL)	178 ± 62.1	236 ± 58.7	0.005
Alkaline phosphatase (units/L)	192.3 ± 79.8	200 ± 88.1	0.51
Aspartate transaminase (units/L)	25.1 ± 11.5	25 ± 11	0.41

hemodialysis therapy. Some studies (31,32) reported an increase in serum albumin levels but an unchanged body weight. Contrary to these studies, Mortelmans et al. (33) reported an increase in body weight but unchanged serum albumin levels. This lack of consistency may be related to characteristics of patient population, frequency and duration of hemodialysis, content of IDPN, or duration of study. Finally, studies with fewer than 10 patients found either an increase in serum albumin level without change in body weight (14) or an increase in body weight without change in albumin level (34). In our study, the use of IDPN alone for 6 months significantly increased both body weight and serum albumin levels.

Stimulation of protein anabolism in muscle should be attempted by using AASs, a large family of testosterone-related hormones that vary in terms of chemical structure, mode of action, anabolic effects, and risk of undesired side effects. AAS administration induces an increase in the mRNA expression of skeletal muscle androgen receptor and intracellular use of amino acids derived from protein degradation and stimulates net synthesis of muscle

protein (32,33). The most commonly used AAS in clinical practice is nandrolone decanoate. In some previous studies (16,17,19), the dose of nandrolone decanoate was 100 mg once a week, while we used 100 mg nandrolone decanoate once every 2 weeks.

In a study by Gascon et al. (18), in the group receiving nandrolone but not erythropoietin, a significant increase in body weight, muscle mass, and hemoglobin level was observed. Similarly, in Johansen et al.'s study (19), subjects receiving nandrolone decanoate gained an average of 2.5 kg more lean body mass than those receiving a placebo with a concomitant increase in serum creatinine levels, suggesting that nandrolone caused increased muscle mass. Furthermore, Barton Pai et al. (20) reported an elevation in serum levels of albumin and hematocrit by nandrolone decanoate therapy.

To our knowledge, although there are studies individually assessing conventional interventions and novel preventive and therapeutic interventions, this is the first study evaluating the superiority of adding AASs to IDPN treatment compared with IDPN alone. In our study, a significant increase

was seen in body weight, hemoglobin, and albumin levels after 6 months in both the IDPN and the IDPN plus AASs groups. The changes in the nutritional parameters were similar in these 2 groups. The absence of significant differences in hemoglobin elevations can be explained by using nandrolone in lower doses than the above-mentioned study. These data could support the hypothesis that ameliorating malnutrition and encouraging weight gain are adequate strategies for stable hemoglobin levels and decreased erythropoietin requirements. We should have used a control group that was not using IDPN or AASs for better assessment of these results, but we did not have enough malnourished hemodialysis patients to create a control group. In our study, serum creatinine levels significantly increased in both groups. However, these elevations were not different between the groups. Elevation in creatinine levels could be related to improved appetite and body weight rather than nandrolone decanoate.

The only observed difference between the IDPN plus AASs group and the IDPN alone group was a significant elevation in cholesterol level. This elevation

is an important finding, despite using a lower dose than previous studies. Considering this result, it should be thought that using nandrolone decanoate could lead to increased cardiovascular risk in HD patients (16). This result warrants further controlled clinical trials evaluating both long-term efficacy and the risk of dangerous side effects of AASs. Similarly, an important and significant warning comes from studies reporting complications associated to AAS usage in athletes, such as reduction in high density lipoprotein and elevation in cholesterol levels (17).

In conclusion, IDPN therapy, alone or in combination with nandrolone decanoate, improved both body weight and serum albumin concentration in malnourished hemodialysis patients in this study. According to our results, no additional benefit was provided by adding nandrolone to IDPN treatment; in fact, long-term usage of anabolic steroids could be harmful in HD patients due to significant increase in serum cholesterol levels. Thus, IDPN alone seems to be a safe and effective therapy in malnourished HD patients.

References

1. Ikizler T, Hakim R. Nutrition in end-stage renal disease. *Kidney Int* 1996; 50: 343-57.
2. Kopple JD. McCollum Award Lecture, 1996. Protein energy malnutrition in maintenance dialysis patients. *Am J Clin Nutr* 1997; 65: 1554-7.
3. Schoenfeld PY, Henry RR, Laird NM, Rixe DM. Assessment of nutritional status of the national cooperative dialysis study population. *Kidney Int* 1983; 23: 80-8.
4. Lowrie EG, Lew NL. Death risk in hemodialysis patients. The predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 1990; 15: 458-82.
5. Goldwasser P, Mittman M, Antignani A, Burrel D, Michel MA, Collier T et al. Predictors of mortality on hemodialysis. *J Am Soc Nephrol* 1993; 3: 1613-22.
6. Acchiardo SR, Moore LW, Latour PA. Malnutrition as the main factor in morbidity and mortality of hemodialysis patients. *Kidney Int* 1983; 24: 199-203.
7. Dukkipati R, Kopple JD. Causes and prevention of protein-energy wasting in chronic kidney failure. *Semin Nephrol* 2009; 29: 39-49.
8. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L et al. A proposed nomenclature and diagnostic criteria for protein energy wasting in acute and chronic kidney disease. *Kidney Int* 2008; 73: 391-8.
9. Shinaberger CS, Kilpatrick RD, Regidor DL, McAllister CJ, Greenland S, Kopple JD et al. Longitudinal associations between dietary protein intake and survival in hemodialysis patients. *Am J Kidney Dis* 2006; 48: 37-49.
10. Chazot C, Shahmir E, Matias B, Laidlaw S, Kopple J. Dialytic nutrition: provision of amino acids in dialysate during hemodialysis. *Kidney Int* 1997; 52: 1663-70.
11. Kopple JD, Bernard D, Messana J, Swartz R, Bergström J, Lindholm B et al. Treatment of malnourished CAPD patients with an amino acid based dialysate. *Kidney Int* 1995; 47: 1148-57.
12. Kopple JD. Therapeutic approaches to malnutrition in chronic dialysis patients: the different modalities of nutritional support. *Am J Kidney Dis* 1999; 33: 180-5.
13. Pupim L, Flakoll PJ, Broillette JR, Levenhagen DK, Hakim RM, Ikizler TA. Intradialytic parenteral nutrition improves protein and energy homeostasis in chronic hemodialysis patients. *J Clin Invest* 2002; 110: 483-92.

14. Pupim L, Flakoll PJ, Ikizler TA. Nutritional supplementation acutely increases albumin fractional synthetic rate in chronic hemodialysis patients. *J Am Soc Nephrol* 2004; 15: 1920-6.
15. Bossola M, Muscaritoli M, Tazza L, Giungi S, Tortorelli A, Rossi Fanelli F et al. Malnutrition in hemodialysis patients: what therapy? *Am J Kidney Dis* 2005; 46: 371-86.
16. Orr R, Fiatarone SM. The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders. Review of efficacy and safety. *Drugs* 2004; 64: 725-50.
17. Fouque D, Guebre-Egziabher F, Laville M. Advances in anabolic interventions for malnourished dialysis patients. *J Ren Nutr* 2003; 13: 161-5.
18. Gascon A, Belvis JJ, Derisa F, Iglesias E. Nandrolone decanoate is a good alternative for the treatment of anemia in elderly male patients on hemodialysis. *Geriatr Nephrol Urol* 1999; 9: 67-72.
19. Johansen KL, Mulligan K, Schambelan M. Anabolic effects of nandrolone decanoate in patients receiving dialysis. A randomized controlled trial. *JAMA* 1999; 281: 1275-81.
20. Barton Pai A, Chretien C, Lau AH. The effects of nandrolone decanoate on nutritional parameters in hemodialysis patients. *Clin Nephrol* 2002; 58: 38-46.
21. Owen WF, Lew NL, Liu Y, Lowrie EG, Lazarus JM. The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *N Engl J Med* 1993; 329: 1001-6.
22. Lazarus JM. Recommended criteria for initiating and discontinuing intradialytic parenteral nutrition therapy. *Am J Kidney Dis* 1999; 33: 211-6.
23. Cano N, Labastie-Coeyrehourq J, Lacombe P, Stroumza P, di Costanzo-Dufetel J, Durbec JP et al. Perdialytic parenteral nutrition with lipids and amino acids in malnourished hemodialysis patients. *Am J Clin Nutr* 1990; 52: 726-30.
24. Boccanfuso JA, Hutton M, McAllister B. The effect of megestrol acetate on nutritional parameters in a dialysis population. *J Ren Nutr* 2000; 10: 36-43.
25. Philips ME, Havard J, Howard J. Oral essential amino acids supplementation in patients on maintenance hemodialysis. *Clin Nephrol* 1978; 9: 241-8.
26. Schulman G, Wingard RL, Hutchison RL, Lawrence P, Hakim RM. The effects of recombinant human growth hormone and intradialytic parenteral nutrition in malnourished hemodialysis patients. *Am J Kidney Dis* 1993; 21: 527-34.
27. Biolo G, Ciochi B, Bosutti A, Situlin R, Toigo G, Guarnieri G. Pentoxifylline acutely reduces protein catabolism in chronically uremic patients. *Am J Kidney Dis* 2002; 40: 1162-72.
28. Juillard L, Guebre-Egziabher F, Fouque D. What is the benefit of the new European nutritional guidelines for dialysis? *Nephrol Ther* 2010; 1: S2-S6.
29. Bossola M, Tazza L, Giungi S, Rosa F, Luciani GJ. Artificial nutritional support in chronic hemodialysis patients: a narrative review. *Ren Nutr* 2010; 20: 213-23.
30. Riobó Serván P, Ortiz Arduan A. Efficacy of oral supplementation during dialysis in patients with chronic renal failure. *Endocrinol Nutr* 2011; 58: 236-42.
31. Heidland A, Kult J. Long-term effects of essential amino acids supplementation in patients on regular dialysis treatment. *Clin Nephrol* 1975; 3: 234-9.
32. Blondin J, Ryan C. Nutritional status: a continuous quality improvement approach. *Am J Kidney Dis* 1999; 33: 198-202.
33. Mortelmans AK, Duym P, Vandenbroucke J, De Smet R, Dhondt A, Lesaffer G et al. Intradialytic parenteral nutrition in malnourished hemodialysis patients: a prospective long-term study. *JPEN* 1999; 23: 90-5.
34. Siskind MS, Lien YH. Effect of intradialytic parenteral nutrition on quality of life in hemodialysis patients. *Int J Artif Organ* 1993; 16: 599-603.