10.5152/turkjnephrol.2021.21637

Original Article

Turkish Journal of Nephrology

Histopathologic and Electron Microscopic Investigation of the Effects of Parenteral Nutrition Combined with Starvation on Kidney Tissue of Rabbits

Semra Gürünlüoğlu[1], Mehmet Gül[2], Harika Gözükara Bağ[3]

[1]Pathology Laboratory, Malatya Training and Research Hospital, Malatya, Turkey
[2]Department of Histology and Embryology, Inönü University School of Medicine, Malatya, Turkey
[3]Department of Biostatistics and Medical Informatics, Inönü University School of Medicine, Malatya, Turkey

ABSTRACT

Objective: To conduct a histopathologic examination of the effects of a combination of parenteral nutrition (PN) and starvation on rabbits' kidney tissue using light microscopy and transmission electron microscopy.
Methods: Four groups consisting of equal numbers of adult female and male New Zealand rabbits were formed (n = 14 each). Rabbits in the PN + oral feeding group were provided with half of their daily caloric needs from rabbit feed and the other half through PN. Rabbits in the PN + starvation group received a full dose of PN daily and received no feed. Rabbits in the half-starvation group were provided with rabbit feed covering only half their daily caloric needs. Rabbits in the control group were provided with all their caloric needs from rabbit feed. After 10 days, all the rabbits were weighed, anesthetized, and euthanized, and their kidney tissue samples were collected. Histopathologic examination was performed by a surgical pathologist blinded to the experimental groups.
Results: The kidney tissue samples of rabbits in the PN + starvation group showed mild tubular dilatation, mild tubular degeneration, moderate renal inflammation, mild interstitial fibrosis, and increased apoptosis. The destructive effects were considerably milder in the PN + oral feeding group.
Conclusion: PN combined with starvation can cause devastating damage to the kidneys. The damage can be minimized by combining PN with enteral nutrition.
Keywords: Kidney, parenteral nutrition, starvation, pathology, impairment

Corresponding author: Semra Gürünlüoğlu ✉ casemra@yahoo.com
Received: January 26, 2021 **Accepted:** March 14, 2021

Cite this article as: Gürünlüoğlu S, Gül M, Gözükara Bağ H. Histopathologic and electron microscopic investigation of the effects of parenteral nutrition combined with starvation on kidney tissue of rabbits. *Turk J Nephrol*. 2021; 30(4): 269-278.

INTRODUCTION

The best way to obtain the nutrients that the body needs is undoubtedly through the alimentary tract.[1] However, in certain situations, the alimentary tract cannot be used. In such situations, the nutritional needs are met through the intravenous administration of fluids, electrolytes, vitamins, and other nutritional substances, known as parenteral nutrition (PN).[1,2] In many diseases, and in cases of surgical operations after which the alimentary tract cannot be used for some time, PN is lifesaving.[1-3] However, PN may also exert negative effects such as liver disease and cardiotoxic effects.[4-9]

The aim of this study was to conduct a histopathologic examination of the effects of PN combined with starvation on rabbits' kidney tissue using light microscopy and transmission electron microscopy.

METHODS

This randomized animal experimental study was approved by the Experimental Animal Ethics Committee of İnönü University (No: 2020/13-1). The study was conducted at the Experimental Animal Production and Research Center of İnönü University.

Table 1. Parenteral Nutrition Solution Formula (Total Liquid Volume 230 mL/kg/day and Total Calories 204 kcal/kg/day)

Ingredient	Volume (mL)		Calories (kcal)		Calories (%)	
	PN + Starvation	Oral feeding + PN	PN+ Starvation	Oral Feeding + PN	PN + Starvation	Oral Feeding + PN
20% Medium long-chain fat emulsion*	38	19	72.2	36.1	35	17.4
10% Compound amino acids	88	44	34.8	17.4	17	8.9
30% Glucose	80	40	97	48.5	48	24
3% Sodium chloride	13	6.5				
10% Potassium chloride	3	1				
10% Calcium gluconate	3	1				
Water-soluble vitamins†	1/2 ampoule	1/4 ampoule				
Fat-soluble vitamins‡	1/2 ampoule	1/4 ampoule				
Trace elements‖	1	0.5				
Total	**230**	**115**	**204**	**102**	**100**	**50.3**

*Medium/long-chain fat emulsion (250 mL) composition: soybean oil 25 g, medium-chain triglycerides 25 g and lecithin 3 g, Linoleic acid 13 g, α-Linolenic acid 1.5 g, 3 g egg phospholipids.
†Water-soluble vitamins composition: vitamin B 1 0.5 mg, vitamin B 2 0.7 mg, nicotinamide 8 mg, vitamin B6 0.2 mg, pantothenic acid 3 mg, vitamin C 20 mg, biotin 12 µg, folic acid 80 µg, vitamin B 100 µg.
‡Fat-soluble vitamins composition: vitamin A 50 µg (165 IU), vitamin D 20.25 µg (10 IU), vitamin E 0.455 µg (0.5 IU), and vitamin K 7.5 µg.
‖Trace elements: chromic chloride $6H_2O$ 5.33 µg/mL, copper chloride $2H_2O$ 0.34 mg/mL, ferric chloride $6H_2O$ 0.54 mg/mL, manganese chloride $4H_2O$ 99.0 µg/mL, potassium iodide 16.6 µg/mL, sodium fluoride 0.21 mg/mL, sodium molybdate $2H_2O$ 4.85 µg/mL, sodium selenite anhydrous 6.90 µg/mL, zinc chloride 1.36 mg/mL.
Values in bold are the volume, calories and percentage of PN administered intravenously to each rabbit in the PN groups.

Before starting the study, power was analyzed and it was calculated that in order to achieve Power: 0.90, a total number of at least 56 animals for 4 groups had to be included in the study. A total of 56 adult New Zealand rabbits were randomly assigned to 4 groups of 14 consisting of equal numbers of females and males. Rabbits in a PN + starvation group were left completely unfed and provided with a full dose of PN (230 mL/kg/day) covering all their daily caloric needs (204 kcal/kg/day). Rabbits in a PN + oral feeding group were provided with rabbit feed pellets (TV-01 rabbit feed; DSA Agrifood Products, Kırıkkale, Turkey), covering half of their daily caloric needs, and half a dose of PN (115 mL/kg/day) covering the other half of their caloric needs (102 kcal/kg/day). Rabbits in a half-starvation group were provided with rabbit pellets (TV-01 rabbit feed; DSA Agrifood Products) meeting half their daily caloric requirements (102 kcal/kg/day). Rabbits in a control group were fed rabbit pellets covering all their daily caloric requirements (204 kcal/kg/day).

Main Points

- Parenteral nutrition (PN) may cause damage to the kidney tissue.
- The damage may be severe when PN is combined with starvation.
- A combination of parenteral and oral nutrition minimizes the damage.

The parenteral and oral nutrition protocols were adopted from a previous study, with some modifications.[6] The PN protocol is shown in Table 1. The PN formula contained 10% amino acids (w/v) (Primene®; E. Baxter, Istanbul, Turkey), trace elements (Addamel® N; Fresenius Kabi, Uppsala, Sweden), 20% (w/v) lipids (Lipofundin® MCT/LCT 20%; B. Braun, Melsungen, Germany), and 30% glucose (dextrose 30%; Polifleks®; Polifarma Pharmaceuticals, Tekirdag, Turkey). The rabbit pellets contained 18.75% protein, 6.38% cellulose, 2.28% fat, 6.2% ash, 2.45% minerals, 0.98% lysine, 0.36% methionine, and 12% starch, providing 240 kcal per 100 g.

In the 2 PN groups, central catheters were placed in the rabbits' internal jugular veins in sterile conditions. During the venous cutdown procedure, 25-35 mg/kg ketamine (Alfamine 10%; Ata Fen Veterinary Supplies, Izmir, Turkey) and 3-7 mg/kg xylazine (Alfazyne 2%; Alfasan International, Woerden, The Netherlands) were administered intramuscularly. A polyethylene catheter (20 G Cut Down Catheter, UPS Medical Instruments Co., Ltd., Balgat, Ankara, Turkey) was placed up to 2-cm-long in a supine position inside the internal jugular vein. The proximal end of the catheter was delivered from the skin of the back and fixed through a subdermal tunnel.

During the experiment, the rabbits were kept in a 12-h light and dark cycle at 20-21°C and sufficient relative humidity (45-50%).

Table 2. The Scoring Criteria of Kidney Tissue Damage

Score	Tubular Dilatation	Tubular Degeneration	Inflammation	Interstitial Fibrosis
0	None	None	None	None
1	Mild, <25%	Mild, <25%	Mild	Mild, <25%
2	Moderate, 25-50%	Moderate, 25-50%	Mild, diffuse or moderate, local	Moderate, 25-50%
3	Severe, >50%	Severe, >50%	Severe, diffuse	Severe, >50%

All rabbits were provided with water ad libitum. All rabbits were weighed at the beginning and at the end of the experiment, and their weights were recorded. The experiment lasted 10 days. All rabbits were then anesthetized and euthanized.

Histopathologic Methods

Collection and Preparation of Kidney Tissue Samples: After the rabbits were euthanized, the abdomen was immediately incised, and the right kidney was removed in 1 piece. A sample of the middle portion between the 2 poles about 2-3 cm long and 1 cm thick, including the cortex and the medulla, was excised, washed with serum saline, and placed in 10% formalin for 24 hours. A 2-mm-wide and 1-mm-thick piece of tissue from the same location was excised, washed with serum saline, and placed in 2.5% glutaraldehyde for 24 hours for electron microscopic examination.

The kidney tissue samples were then cut into 3- to 5-mm-thick slices, and standard tissue processing was performed on 1 piece of each sample. The samples were subsequently buried in paraffin blocks. Then, 3- to 5-µm-thick sections were taken from each paraffin block. The sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT) according to routine protocols and immunohistochemically analyzed with anti-caspase-3 antibodies according to the manufacturer's protocol.

Immunohistochemical Methods: The tissue sample sections were deparaffinized and placed on adhesive slides. They were then placed in citrate buffer (pH 7.6; Thermo Scientific, Fremont, CA, USA), and antigen retrieval was performed in a pressure cooker (Retriever 2100; Aptum Biologics, Southampton, UK) at 121°C for 15 minutes. The sections were then left to cool, the citrate buffer was removed, and phosphate-buffered saline (PBS) was added. The slides were washed with PBS at every stage except during protein blocking and primary antibody application. The following steps were performed at room temperature, and a horse radish peroxidase kit (Thermo Scientific) for immunohistochemistry staining was used according to the manufacturer's protocol. First, 3% hydrogen peroxide was dripped for 10 minutes. Second, a protein-V blocking solution was dripped for 5 minutes, followed by incubation with caspase-3 primary antibody (anti-caspase-3 antibody [ABM1C12] ab208161; Abcam, Cambridge, UK; dilution rate 1 : 300) at room temperature for 1 hour. Third, the glass slides were incubated for 10 minutes in a biotinylated goat anti-polyvalent secondary antibody. Streptavidin peroxidase was then dripped and incubated for 10 minutes. Aminoethyl carbazole chromogen (Thermo Scientific) was dripped on to the slides and incubated for 15 minutes. Finally, the slides were placed in distilled water, counter-stained with Mayer's hematoxylin, and mounted.

Scoring and Histopathological Evaluation of Stained Kidney Tissue Sample Sections: Histopathologic examination was performed by a surgical pathologist blinded to the experimental groups. Ten cortical areas of the sections not adjacent to each other were observed under a light microscope (Eclipse E200MV R; Nikon corporation, Tokyo, Japan) at 400× magnification. Different degrees of tubulointerstitial changes were found in the PN groups. The changes were tubular epithelial degeneration, mainly in the proximal tubules, tubular dilatation in both the proximal and distal tubules, interstitial inflammation, and fibrosis. The kidney damage scoring systems described by Zhao et al.[10] and Kar et al.[11] were used. Tubular dilatation was scored as 0 (no tubular dilatation), 1 (dilatation of <25% of the tubules), 2 (dilatation of 25-50% of the tubules), or 3 (dilatation of >50% of the tubules). Tubular degeneration was scored as 0 (no degeneration), 1 (slight degeneration), 2 (moderate degeneration), or 3 (severe degeneration). Active chronic inflammation in the interstitial area was scored as 0 (no inflammation), 1 (mild focal inflammation), 2 (mild diffuse or moderate focal inflammation), or 3 (severe diffuse inflammation). Fibrosis in the interstitial area observed in the MT sections was scored as 0 (no fibrosis), 1 (fibrosis covering<25% of the field), 2 (fibrosis covering 25-50% of the field), or 3 (fibrosis covering >50% of the field). The scoring system is shown in Table 2.

In the H&E sections, we also evaluated the presence of apoptotic cells as round or oval-shaped cells with dark eosinophilic/dense cytoplasm and purple dense pyknotic/fragmented nuclei or fragmented apoptotic cells forming apoptotic bodies.[12] All tubular epithelial cells in 8 areas were counted.[13] under a microscope at 400× magnification. The apoptotic cells in the same areas were also counted. A total of 1600-1750 cells were counted for each kidney, and the ratio of apoptotic cells to total renal tubular cells was obtained as an apoptotic score.[13]

The caspase-3-stained sections were evaluated by counting positively stained renal tubular epithelial cells in 8 areas observed under a microscope at 400× magnification. The ratio

of stained cells to the total cells was then calculated for each kidney.[11]

Transmission Electron Microscopic Examination

The kidney tissue samples were fixed in 2.5% glutaraldehyde in 0.1 M PBS and postfixed in 1% osmium tetroxide. They were subsequently dehydrated in a series of acetone concentrations (30%, 50%, 70%, 90%, and 100%) and then embedded in epoxy resin (Araldite CY212; Agar Scientific, Stansted, UK). The resin blocks of kidney tissue were then cut into 80-nm sections using a microtome (Leica Ultracut R Ultramicrotome; Leica Microsystems, Vienna, Austria). Finally, the sections were contrasted with uranyl acetate and lead citrate and examined under a Zeiss transmission electron microscope (Libra 120; Carl Zeiss NTS, Oberkochen, Germany).

Statistical Analysis

The normality of continuous data was evaluated using the Shapiro–Wilk test. The results were expressed as medians and ranges. Group comparisons were performed using the Kruskal–Wallis test, and pairwise comparisons were performed using the Conover post-hoc test. In all analyses, a two-sided *P*-value of <.05 was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for the analyses.

RESULTS

Weight Changes

Table 3 shows the statistical analysis results of the weight changes in the 4 groups. The difference in weight change between the 2 PN groups and the control group was not statistically significant. The half-starvation group showed a statistically significant decrease in weight.

Histopathologic Results

Mild tubular dilatation was found in the PN + starvation group. Considerably milder dilatation was noted in the PN + oral feeding group. No noteworthy dilatation was observed in the half-starvation and control groups (Table 4, Figures 1A, 2A, and B).

Mild tubular degeneration was found in the PN + starvation group, while considerably milder degeneration was observed in the PN + oral feeding group. There was no notable degeneration in the half-starvation and control groups (Table 4, Figures 1B and 2C).

Moderate inflammation was found in the PN + starvation group. Considerably milder inflammation was observed in the PN + oral feeding group. There was no noteworthy inflammation in the half-starvation and control groups (Table 4, Figures 1C, 3A and B).

Mild interstitial fibrosis was observed in the PN + starvation group. No significant fibrosis was noted in the other 3 groups (Table 4, Figures 1D and 3C).

The apoptosis score in the PN + starvation group was significantly higher than in the other 3 groups. The difference was statistically significant. The apoptosis score in the PN + oral feeding group was higher than in the half-starvation and control groups. This difference was also statistically significant (Table 5, Figure 2C).

Table 3. Statistical Analysis of the Change in Weights of Rabbits in Study Groups During the Study Period

Groups	Pre-PN weight (g)	After-PN weight (g)	*P*
Control (*n* = 14)	3390.28 ± 107.52	3392.98 ± 119.29	.931
Half-starvation (*n* = 14)	3349.54 ± 155.59	3183.84 ± 124.05	**<.001**
PN + starvation (*n* = 14)	3408.92 ± 106.22	3392.93 ± 85.73	.226
Oral feeding + PN (*n* = 14)	3374.64 ± 108.58	3367.07 ± 137.65	.631

P value which is written in bold is statistically significant.

Table 4. Histopathological Evaluation Results

Groups	Tubular Dilatation	Tubular Degeneration	Inflammation	Interstitial Fibrosis
Control (*n* = 14)	0 (0-0)[a]	0 (0-1)[a]	0 (0-0)[a]	0 (0-0)[a]
Half-starvation (*n* = 14)	0 (0-0)[a]	0 (0-1)[a,b]	0 (0-0)[a]	0 (0-0)[a]
Oral feeding + PN (*n* = 14)	0.5 (0-1)[b]	0.5 (0-1)[b]	0.5 (0-1)[b]	0 (0-1)[a]
PN + starvation (*n* = 14)	1 (1-2)[c]	1 (1-3)[c]	2 (1-3)[c]	1 (0.5-2)[b]
P	**<.001**	**<.001**	**<.001**	**<.001**

*The difference between the groups with different superscript letters are found to be statistically significant.
P values which are written in bold are statistically significant.

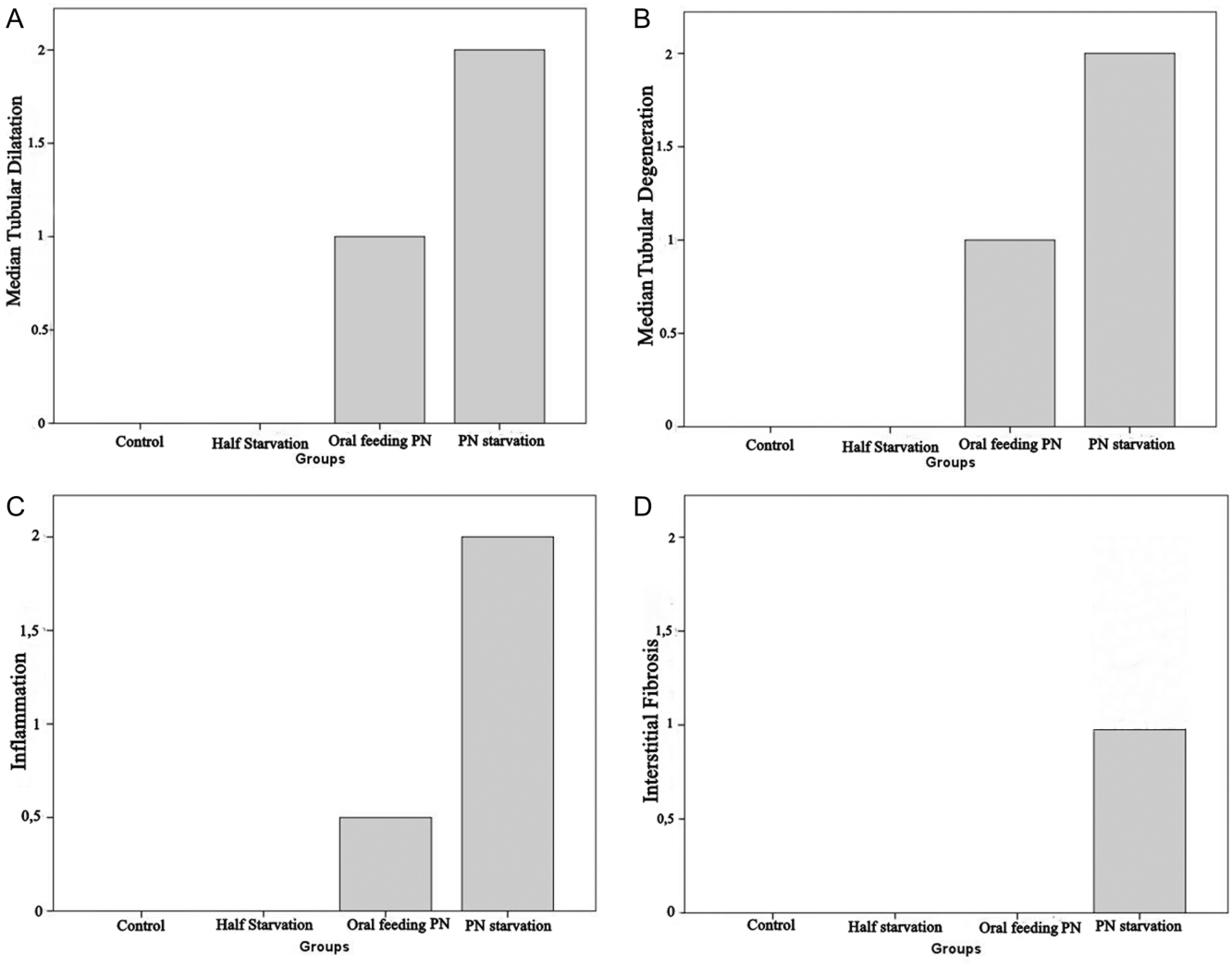

Figure 1. a-d. Histopathologic evaluation results of the 4 groups. (A) Mean tubular dilatation, (B) Mean tubular degeneration, (C) Mean inflammation, (D) Mean interstitial fibrosis.

Caspase-3 activity was higher in the PN + starvation group than in the other 3 groups. The difference was statistically significant. The caspase-3 activity in the PN oral feeding group was higher than in the half-starvation and control groups. This difference was also statistically significant (Table 5, Figure 4A and B).

Transmission Electron Microscopy Findings

The ultrastructural appearance of kidney sections in the PN+ oral feeding, half-starvation, and control groups was normal (Figure 5A). Conversely, significant ultrastructural changes were detected in renal tubular epithelial cells in the PN + starvation group, including intracytoplasmic edema, vacuolization, lysosome accumulation, and damage in cristae, dilatation, and degeneration in mitochondria. Furthermore, electron-dense material accumulation was observed along the basement membranes of tubule epithelial cells (Figure 5B, C, and D).

DISCUSSION

Since the first known intravenous nutritional supplementation attempt by Cristopher Wren in 1658, PN has developed significantly.[2] With the development of surgical treatments in the early 1900s, preoperative and postoperative nutrition support became ever more important.[2,14] Under normal circumstances, the ideal way to obtain nutrients is through the gastrointestinal tract. However, in some cases, especially after gastrointestinal surgery, this pathway may not be usable for a certain period.[1,3] In such cases, PN positively affects the success of the treatment.[1] However, despite its great benefits, PN can cause various complications.[2,4-9] Previous studies have reported complications related to the composition of PN formulas,[8,15] including infection and technical complications,[2] liver disease, intestinal pathogenic bacterial colonization, heavy metal accumulation in the organs, vascular endothelial damage, and cardiotoxic effects associated with complications of sepsis and hyperglycemia.[2,4-6,8,9,15,16] A previous experimental study reported

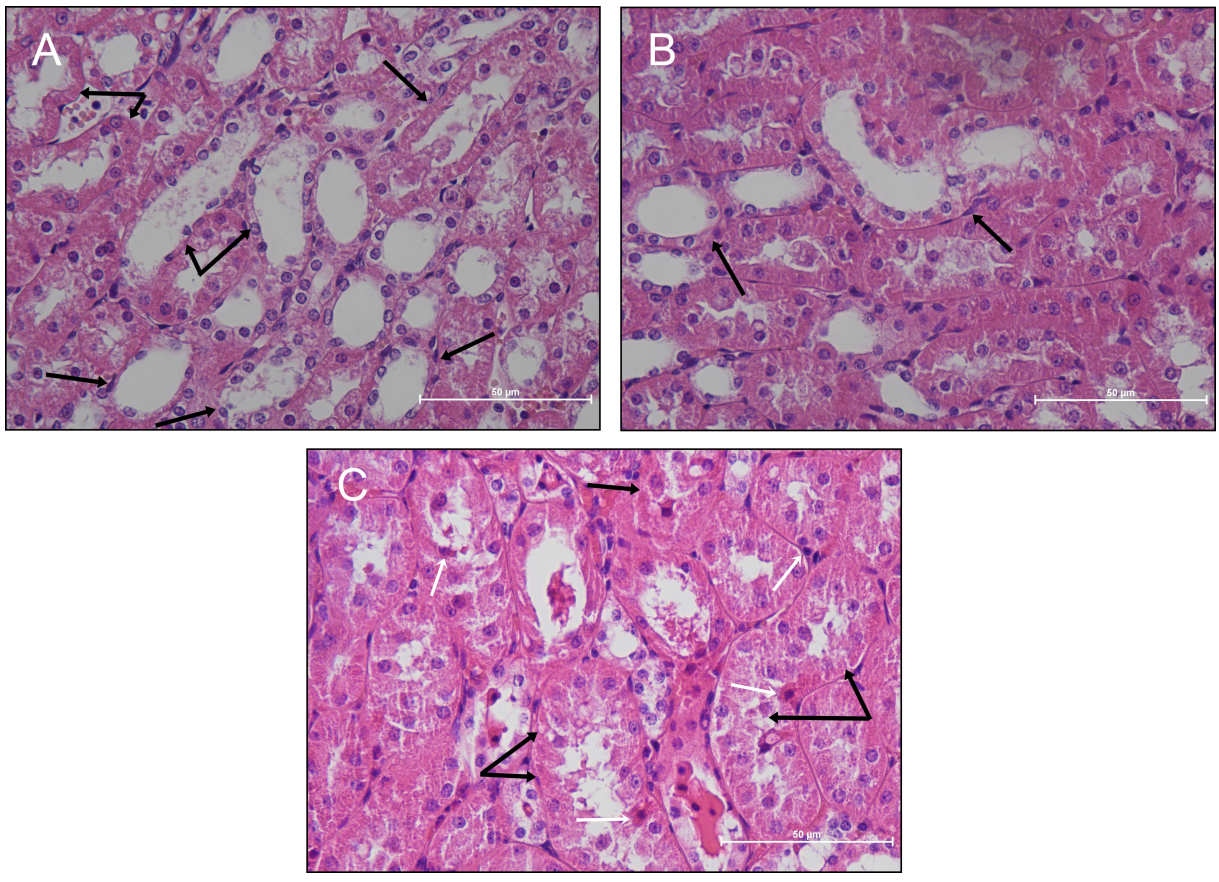

Figure 2. a-c. Tubular dilatation and degeneration in kidney tissue samples (H&E, 400× magnification). (A) Dilated tubules covering about 25-50% of the field (black arrows), in a section belonging to the PN + starvation group. (B) Tubular dilatation (scored 1), including tubules covering less than 25% of the field (black arrows), in a section belonging to the PN + oral feeding group. (C) Moderate (scored 2) tubular degeneration in a section belonging to the PN + starvation group (black arrows). Apoptotic cells are shown with white arrows.

devastating cardiac effects linked to PN when combined with starvation.[6] This suggests that the toxic effects expected from PN can be devastating when it is combined with starvation.

The renal complications occurring during PN administration have often been associated with metabolic changes.[17] The negative effects on the kidneys include glucose, acid–base, fluid, and electrolyte imbalance, nephrolithiasis, and kidney function impairment.[17] Moukarzel et al.[18] observed a glomerular filtration rate reduction in pediatric patients receiving long-term PN, which correlated with the duration of PN, even in the absence of underlying diseases and nephrotoxic medications. Similarly, Buchman et al.[19] found a reduction in creatinine clearance that correlated with the duration of PN. Lauverjat et al.[20] reported renal function impairment due to chronic dehydration in patients receiving long-term PN. Tabel et al.[21] observed renal function impairment in preterm infants receiving PN. Pironi et al.[22] also reported renal function impairment due to long-term PN administration.

The aforementioned studies investigated the effects of PN on kidney function from a biochemical perspective. No previous study has examined the histopathologic changes in kidney tissue caused by PN. In our experimental study, the histopathologic effects of PN were investigated using light microscopy and transmission electron microscopy. We found that PN combined with starvation caused mild tubular dilatation, mild tubular degeneration, moderate renal inflammation, mild interstitial fibrosis, and an increased apoptosis rate in rabbit renal tissue. These effects were significantly less destructive in the PN + oral feeding group. In light of these findings, we speculate that oral nutrition protects against the devastating effects of PN on the kidneys. Our results are consistent with Messova et al.[23] who investigated the kidney function of pediatric patients with various degrees of intestinal failure receiving PN for 3 years, and found that PN did not cause significant kidney function impairment if combined with oral nutrition.

PN exerts systemic effects that may damage multiple organs. In particular, it has been found to cause major metabolic changes, including hyperglycemia, hypoglycemia, hypercapnia, acid–base imbalance, hyperlipidemia, liver complications, and manganese toxicity.[24] It has also been reported that the ingredients in PN provide the basis for oxidative stress.[25] An experimental

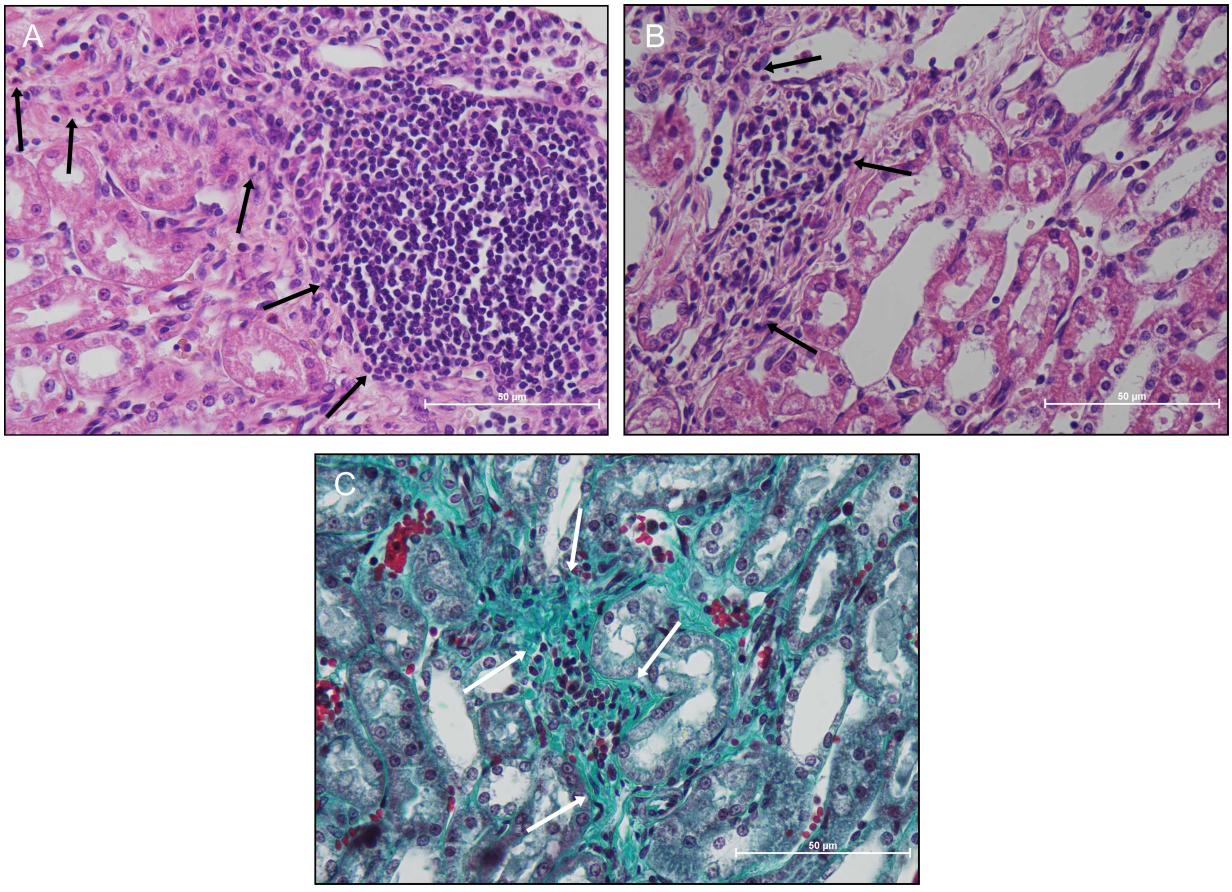

Figure 3. a-c. Inflammation and interstitial fibrosis in kidney tissue samples (400× magnification). (A) Moderate focal inflammation (scored 2) in a section belonging to the PN + starvation group (black arrows); H&E. (B) Considerably milder inflammation (scored 1) in a section belonging to the PN + oral feeding group (black arrows); H&E. (C) Mild interstitial fibrosis in a section belonging to the PN + starvation group (white arrows); Masson's trichrome.

study found that in the absence of oral nutrition, PN may cause failure of the hormonal mechanism that ensures the distribution of the nutritional content in the blood, which may result in hyperglycemia, low insulin levels, and oxidative stress.[6] A combination of hyperglycemia and oxidative stress may cause damage to the kidneys. Another experimental study found that during PN, hyperglycemia damaged liver, kidney, and heart tissue, while these effects were not observed when normoglycemia was achieved.[26] Wang et al.[27] found that hyperglycemia induced for 6 hours in rats caused damage to the renal tubules but not to the glomeruli. Weekers et al.[28] reported that hyperglycemia during PN administration led to acidosis, immune function impairment, and systemic inflammation, which could be corrected by administering exogenous insulin. In another experimental study, Jiang et al.[29] found that oxidative stress caused kidney damage and fibrosis. We also believe that oxidative stress may cause kidney damage. Schepens et al.[30] reported that in patients receiving PN at home while still obtaining nutrients through the alimentary tract, PN did not cause oxidative damage, although it led to increased oxidative stress. Similarly, Gürünlüoğlu et al.[6] observed no oxidative damage, and Messova et al.[23] found no kidney function impairment when PN was combined with oral nutrition.

Table 5. Apoptosis and Caspase-3 Expression Evaluation Results

Groups	Apoptosis	Caspase-3 activity
Control (*n* = 14)	0.001 (0-0.001)[a]	0.01 (0.01-0.01)[a]
Half-starvation (*n* = 14)	0.001 (0.001-0.002)[a]	0.01 (0.01-0.02)[a]
Oral feeding + PN (*n* = 14)	0.002 (0.001-0.003)[b]	0.02 (0.01-0.03)[b]
PN + starvation (*n* = 14)	0.024 (0.011-0.032)[c]	0.22 (0.13-0.38)[c]
P	**<.001**	**<.001**

*The difference between the groups with different superscript letters are found to be statistically significant.
P values which are written in bold fond are statistically significant.

Our study has certain limitations. As it was an animal experimental study, the observed effects may differ in humans. Moreover, although we evaluated kidney damage through histopathologic and electron microscopy findings, we did not examine the effects of PN on kidney function. Moreover, although we found that PN may cause severe damage to the kidneys when

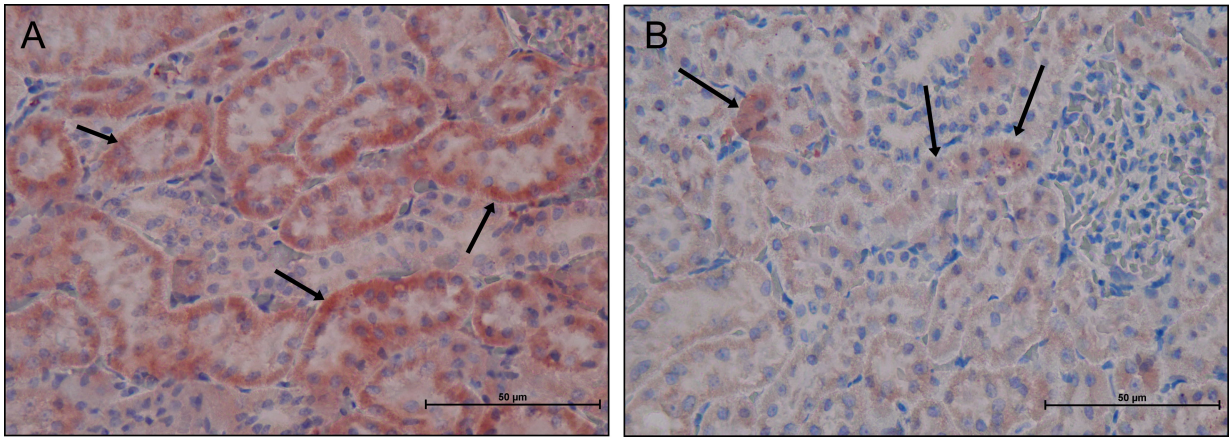

Figure 4. a,b. Caspase-3 activity in kidney tissue samples (400× magnification). (A) Renal tubular epithelial cells (some are indicated with black arrows) with high caspase-3 activity in a section belonging to the PN + starvation group. (B) Renal tubular epithelial cells with low caspase-3 activity (black arrows) in a section belonging to the PN + oral feeding group.

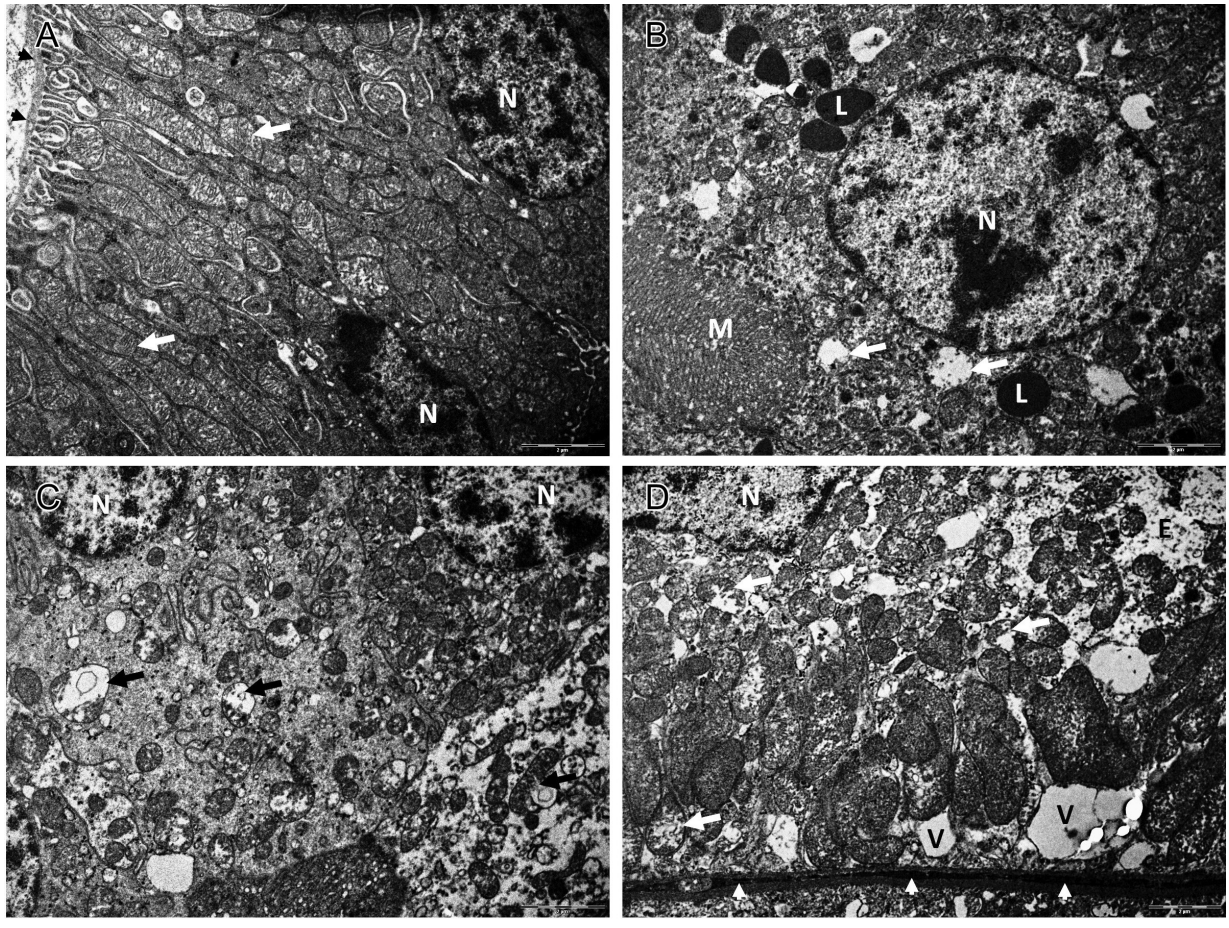

Figure 5. a-d. Transmission electron microscopic images of kidney tissue samples (uranyl acetate-lead citrate; scale bar: 2 µm, 6300× magnification). (A) PN+ oral feeding group: normal ultrastructural appearance of renal tubular epithelial cells, nuclei (N), mitochondria (arrow), and basement membrane (arrowhead). (B) PN + starvation group: renal tubular epithelial cell nuclei (N), total crista loss in mitochondria (arrow), lysosome (L), and microvilli (M). (C) PN + starvation group: renal tubular epithelial cell nuclei (N), and total crista damage in mitochondria (arrow). (D) PN + starvation group: renal tubular epithelial cell nuclei (N), crista damage in mitochondria (arrow), vacuole (V), intracytoplasmic edema (E), and electron-dense material accumulation along the basement membrane (arrowhead).

combined with starvation and that adding oral nutrition may minimize its destructive effects, we did not investigate the possible mechanisms of these effects. In our next study, we plan to explore the possible mechanisms by investigating the relationship between PN and oxidative stress biochemically and by modifying the PN composition.

CONCLUSIONS

In this study, we investigated the histopathologic changes in rabbit renal tissue caused by PN. We found that PN combined with starvation causes an increased apoptosis rate in renal tubular epithelial cells, tubular dilatation, tubular degeneration, interstitial inflammation, and fibrosis. We also found that continued oral feeding during PN application largely protects the kidneys from these negative effects.

Ethics Committee Approval: Ethics committee approval was received from the Experimental Animal Ethics Committee of Inonu University (2020/13-1).

Informed Consent: N/A.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – S.G.; Design - S.G.; Supervision -S.G.; Resource - S.G., M.G., H.G.B.; Materials - S.G., M.G., H.G.B.; Data Collection and/or Processing - S.G., M.G., H.G.B.; Analysis and/or Interpretation - S.G., M.G., H.G.B.; Literature Search - S.G., M.G., H.G.B.; Writing -S.G., M.G., H.G.B.; Critical Reviews -S.G.

Acknowledgments: We would like to thank Kubilay Gürünlüoğlu for his valuable contributions in this study.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Dudrick SJ. Early developments and clinical applications of total parenteral nutrition. *JPEN J Parenter Enteral Nutr*. 2003;27(4):291–299. [CrossRef]
2. TeitelbaumDH, Coran AG. Nutritional Support. Grosfeld JL, O'Neill JA, Fonkalsrud EW, Coran AG eds. *Textbook of Pediatric Surgery*. Philadelphia: Mosby; 2006; Vol. 1, Chap.12; p: 194–220.
3. Ward N. Nutrition support to patients undergoing gastrointestinal surgery. *Nutr J*. 2003;2:18. [CrossRef]
4. Demircan M, Gürünlüoğlu K, Karaman A, Mızrak B. Damaging effects of total parenteral nutrition Formula on vascular endothelium. *J Pediatr Gastroenterol Nutr*. 2015;61(4):464–468. [CrossRef]
5. Buchman AL, Neely M, Grossie Jr VB, et al. Organ heavy-metal accumulation during parenteral nutrition is associated with pathologic abnormalities in rats. *Nutrition*. 2001;17(7-8):600–606. [CrossRef]
6. Gürünlüoğlu K, Gül M, Koçbıyık A, et al. Investigation of the cardiotoxic effects of parenteral nutrition in rabbits. *J Pediatr Surg*. 2020;55(3):465–474. [CrossRef]
7. Wildhaber BE, Lynn KN, Yang H, Teitelbaum DH. Total parenteral nutrition-induced apoptosis in mouse intestinal epithelium: regulation by the Bcl-2 protein family. *Pediatr Surg Int*. 2002;18(7):570–575. [CrossRef]
8. Wang H, Khaoustov VI, Krishnan B, et al. Total parenteral nutrition induces liver steatosis and apoptosis in neonatal piglets. *J Nutr*. 2006;136(10):2547–2552. [CrossRef]
9. Demircan M, Ergün O, Coker C, et al. Aluminium in total parenteral nutrition solutions produces portal inflammation in rats. *J Pediatr Gastroenterol Nutr*. 1998;26(3):274–278. [CrossRef]
10. Zhao J, Wang L, Cao AL, et al. HuangQi decoction ameliorates renal fibrosis via TGF-β/smad signaling pathway in vivo and invitro. *Cell Physiol Biochem*. 2016;38(5):1761–1774. [CrossRef]
11. Kar F, Hacioğlu C, Senturk H, Donmez DB, Kanbak G. The role of oxidative stress, renal inflammation, and apoptosis in post ischemic reperfusion injury of kidney tissue: the protective effect of dose-dependent boric acid administration. *Biol Trace Elem Res*. 2020;195(1):150–158. [CrossRef]
12. Archana M, Bastian, Yogesh TL, Kumaraswamy KL. Various methods available for detection of apoptotic cells—a review. *Indian J Cancer*. 2013;50(3):274–283. [CrossRef]
13. Yang B, Johnson TS, Thomas GL, et al. Apoptosis and caspase-3 in experimental anti-glomerular basement membrane nephritis. *J Am Soc Nephrol*. 2001;12(3):485–495. [CrossRef]
14. Holden WD, Krieger H, Levey S, Abbott WE. The effects of nutrition on nitrogen metabolism in the surgical patient. *Ann Surg*. 1957;146(4):563-77; discussion 577. [CrossRef]
15. Zhu X, Zhang X, Yu L, et al. Hepatic overexpression of GRP94 in a rabbit model of parenteral nutrition-associated liver disease. *Gastroenterol Res Pract*. 2015;2015:269831. [CrossRef]
16. Fernandes PC, Dolinger EJ, Abdallah VO, et al. Late onset sepsis and intestinal bacterial colonization in very low birth infants receiving long-term parenteral nutrition. *Rev Soc Bras Med Trop*. 2011;44(4):447–450. [CrossRef]
17. Dudley J, Rogers R, Sealy L. Renal consequences of parenteral nutrition. *Pediatr Nephrol*. 2014;29(3):375–385. [CrossRef]
18. Moukarzel AA, Ament ME, Buchman A, Dahlstrom KA, Vargas J. Renal function of children receiving long-term parenteral nutrition. *J Pediatr*. 1991;119(6):864–868. [CrossRef]
19. Buchman AL, Moukarzel A, Ament ME, et al. Serious renal impairment is associated with long-term parenteral nutrition. *JPEN J Parenter Enter Nutr*. 1993;17(5):438–444. [CrossRef]
20. Lauverjat M, Hadj Aissa A, Vanhems P, et al. Chronic dehydration may impair renal function in patients with chronic intestinal failure on long-term parenteral nutrition. *Clin Nutr*. 2006;25(1):75–81. [CrossRef]
21. Tabel Y, Oncul M, Akin IM, Karabulut AB, Gungor S. Effects of total parenteral nutrition on renal function in preterm neonate. *Pediatr Nephrol*. 1976;25:2010.
22. Pironi L, Lauro A, Soverini V, et al. Renal function in patients on long-term home parenteral nutrition and in intestinal transplant recipients. *Nutrition*. 2014;30(9):1011–1014. [CrossRef]
23. Messova A, Dziubak R, Köglmeier J. Renal function in children on long-term home parenteral nutrition. *Front Pediatr*. 2019;7:137. [CrossRef]

24. Btaiche IF, Khalidi N. Metabolic complications of parenteral nutrition in adults, part 1. *Am J Health Syst Pharm*. 2004;61(18):1938–1949. doi:[CrossRef])
25. Perrone S, Salvi G, Bellieni CV, Buonocore G. Oxidative stress and nutrition in the preterm newborn. *J Pediatr Gastroenterol Nutr*. 2007;45(suppl 3):S178–S182. [CrossRef]
26. Derde S, Vanhorebeek I, Ververs EJ, et al. Increasing intravenous glucose load in the presence of normoglycemia: effect on outcome and metabolism in critically ill rabbits. *Crit Care Med*. 2010;38(2):602–611. [CrossRef]
27. Wang J, Yue X, Meng C, et al. Acute hyperglycemia may induce renal tubular injury through mitophagy inhibition. *Front Endocrinol (Lausanne)*. 2020;11:536213. [CrossRef]
28. Weekers F, Giulietti AP, Michalaki M, et al. Metabolic, endocrine and immune effects of stress hyperglycemia in a rabbit model of prolonged critical illness. *Endocrinology*. 2003;144(12):5329–5338. [CrossRef]
29. Jiang JS, Chou HC, Yeh TF, Chen CM. Neonatal hyperoxia induces kidney fibrosis in rats. *Pediatr Neonatol*. 2015;56(4):235–241. [CrossRef]
30. Schepens MAA, Roelofs HMJ, Peters WH, Wanten GJA. No evidence for oxidative stress in patients on home parenteral nutrition. *Clin Nutr*. 2006;25(6):939–948. [CrossRef]