Transpl Int (1996) 9 [Suppl 1]: S455–S459




**J. Torras**
**K. Soto**
**M. Riera**
**I. Herrero**
**J. Valles**
**J. M. Cruzado**
**J. Alsina**
**J. M. Grinyo**

# Changes in renal hemodynamics and physiology after normothermic ischemia in animals supplemented with eicosapentaenoic acid

J. Torras · K. Soto · M. Riera · I. Herrero · J. M. Cruzado · J. Alsina
Nephrology Service, Hospital of Bellvitge, Fundació August Pi i Sunyer, Department of Medicine, University of Barcelona, Ciutat Sanitària i Universitària de Bellvitge

J. Valles
LASA Laboratories, Barcelona, Spain

J. M. Grinyo (✉)
Nephrology Service, Hospital of Bellvitge, Ciutat Sanitària i Universitària de Bellvitge, Feixa Llarga s/n, 08097 L'Hospitalet de Llobregat, Barcelona, Spain
Tel. +34-3-3356111-2405
Fax +34-3-2631595

**Abstract** In order to determine whether treatment of animals with an n-3 fatty acid, eicosapentaenoic acid (EPA), could modify renal hemodynamics and physiology after normothermic ischemia, we studied 42 Spraque Dawley rats orally supplemented with either olive oil or a purified lysine salt of EPA for 4 weeks. Four experimental groups were established. Three groups were treated with increasing doses of EPA: 20 mg/kg per day (EPA 20), 40 mg/kg per day (EPA 40) and 80 mg/kg per day (EPA 80), and one group was supplemented with isovolumetric olive oil (OLI). A control group that received neither EPA nor ischemia was also studied. On day 28, right nephrectomy was performed, followed by 30 min of left renal warm ischemia. Basal arterial pressure and renal blood flow (RBF) were monitored in two kidneys before arterial occlusion and continuously thereafter throughout the experiment in one kidney using an electronic transducer and a flowmeter. From 60 to 120 min after the end of ischemia, urine output (μl/min), glomerular filtration rate (GFR, μl/min), measured by inulin clearance, and fractional reabsortion of sodium (FRNa) were determined every 20 min. Renal plasma flow (RPF, ml/min) and renal vascular resistance (VR, mm Hg/ml per min) were calculated. RPF was estimated as RBF (1–hematocrit). Before ischemia, the mean RPF and RBF were higher in EPA-fed than in olive oil-fed animals and after ischemia showed a significantly greater increase in EPA-fed animals than in olive oil-fed animals. Mean VR was lower in EPA-fed animals than in olive oil-fed animals, both before arterial occlusion and after ischemia. Mean urine output was similar in the OLI and EPA 20 groups, and significantly higher in the EPA 40 and EPA 80 groups than in the control group. GFR was significantly lower in the OLI and EPA 20 groups than in the control group. Finally, the EPA 40 group showed a similar and the EPA 80 group a slightly higher GFR than the control group. We conclude that EPA supplementation provides protection from renal ischemic-reperfusion injury, and this effect is more evident at higher EPA doses.

**Key words** Eicosapentaenoic acid supplementation · Renal ischemic · Reperfusion injury

## Introduction

Increased dietary or parenteral intake of fish oils rich in n-3 fatty acids affects a large number of biochemical and functional parameters producing a beneficial effect on the course of some lipid, inflammatory and cardiovascular diseases [1]. Recently, n-3 fatty acids have been shown to have protective effects on renal hemodynam-

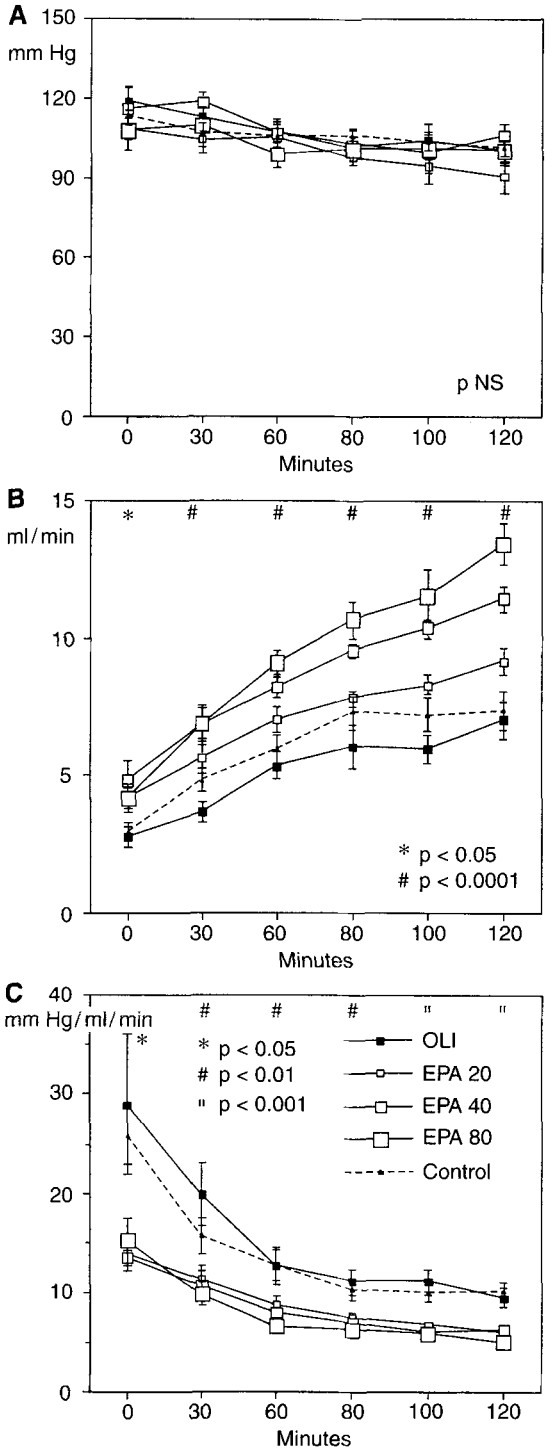


**Fig. 1 A–C** Effects of dietary supplementation with a lysine salt of eicosapentaenoic acid on arterial pressure (**A**), renal plasma flow (**B**), and renal vascular resistance (**C**) in rats with moderate acute renal failure (30 min)

ics and on blood pressure in renal transplant patients [2]. Also, n-3 fatty acids have been shown to be effective in ischemic experimental models [3–6]. Thus, in a stroke model, animals prefed with an n-3 fatty acid-enriched diet showed smaller infarcts than control animals [3]. In myocardial infarct models, it has been demonstrated that fish oils increase post-ischemic blood flow [4, 5]. However, there is a discrepancy in results concerning infarct size, a reduction being reported in some studies [4] and no modification in others [5].

To date, there are few studies on the effects of fish oils in renal ischemic models [6]. Warm or cold ischemia are present under various conditions in clinical nephrology and transplantation, for example in ischemic or atheromatous renal diseases, or during preservation in renal transplantation. So, the need to dispose of therapeutic or protective drugs in these situations is evident.

The aim of this study was to determine whether chronic animal supplementation with a purified salt of eicosapentaenoic acid (EPA), a fish-oil n-3 fatty acid, could modify the renal hemodynamics and physiology after normothermic ischemia of the kidney. In addition, we sought to determine if these effects were dose dependent.

## Material and methods

### Materials

A purified lysine salt of EPA (purity > 95 %, bioavailability > 90 %) soluble in water was obtained from Lasa Laboratories SAE (Barcelona, Spain).

### Animal preparation and surgical technique

Young male Sprague-Dawley rats (6 weeks old; 100 g body weight) were obtained from our own animal facility. They were housed four per cage in a light-controlled room with a 12-h light/dark cycle and had access to food and water ad libitum.

EPA or olive oil were given by daily gavage. Animals were weighed twice a week and EPA or olive oil dose schedules adjusted as appropriate. Animals were randomly allocated to four dietary groups. These comprised a group supplemented with isovolumetric olive oil (2 ml/kg) plus α tocopherol 0.08 mg/ml (OLI group, $n = 9$), and three groups receiving EPA: 20 mg/kg per day (2 ml/kg) plus α tocopherol 0.04 mg/ml (EPA 20, $n = 7$), 40 mg/kg per day (2 ml/kg) plus α tocopherol 0.08 mg/ml (EPA 40, $n = 7$), and 80 mg/kg per day (2 ml/kg) plus α tocopherol 0.16 mg/ml (EPA 80, $n = 8$). Alpha tocopherol was added to retard autoxidation of EPA and to prevent cell membrane oxidation by EPA.

After 4 weeks on this diet, animals were anesthetized with ketamine hydrochloride (75 mg/kg), diazepam (5 mg/kg) and atropine (0.5 mg/kg) intramuscularly and placed on a heating pad to maintain body temperature at 37 °C. The abdominal cavity was opened, and vascular polyethylene catheters were implanted into the abdominal aorta and vena cava via the femoral vessels. The arterial catheter was used for continuous monitoring of arterial pressure using an electronic pressure transducer (Nihon Kohden Co., Germany) and blood sampling. The venous catheter was used throughout the experiment for the infusion of fluid (Ringer's lactate

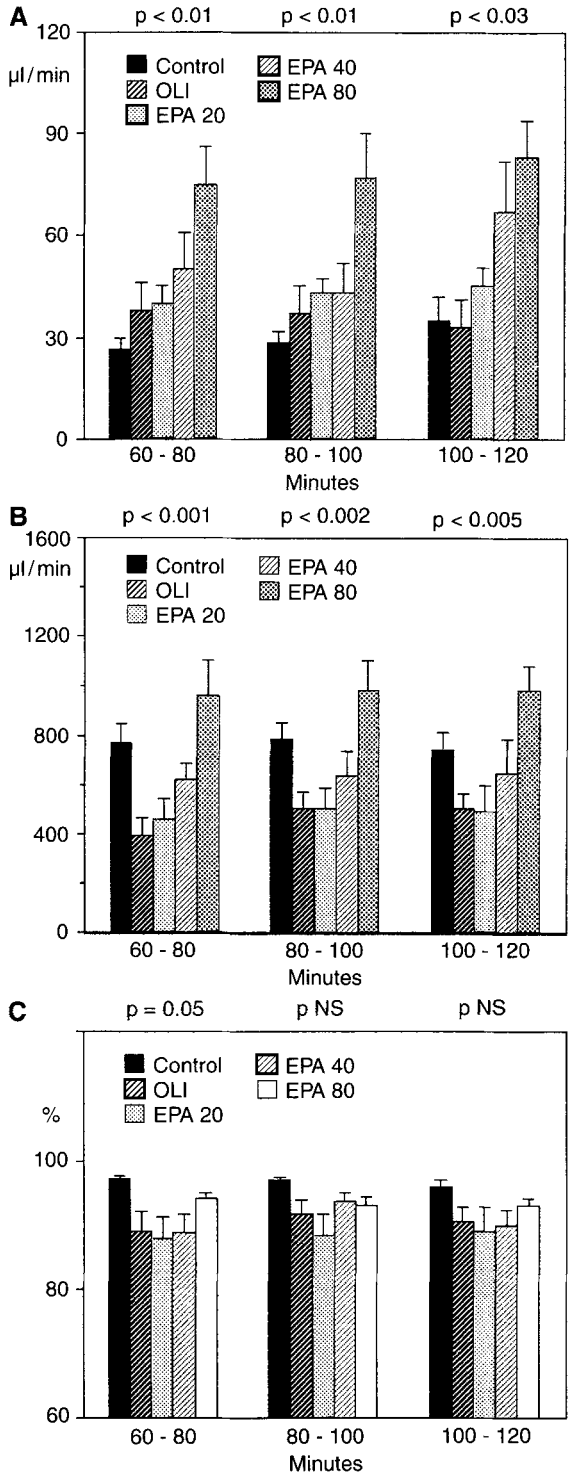


**Fig. 2 A–C** Effects of dietary supplementation with a lysine salt of eicosapentaenoic acid on urine output (**A**), GFR (**B**), and FRNa (**C**) in rats with moderate acute renal failure (30 min)

1.5 ml/h per 100 g) and inulin (0.65 mg/ml of serum) (Polyfructosan, Laevosan, Linz, Austria). The left ureter was cannulated for the collection of urine with polyethylene tubing.

The left renal artery was exposed and an electronic flow probe (Transonic Systems, USA) was placed around the vessel to measure basal renal blood flow (RBF) before arterial occlusion and continuously thereafter throughout the experiment. The left renal artery was occluded for 30 min with a nontraumatic clamp to induce a moderate acute renal failure [7] and, meanwhile, contralateral nephrectomy was performed. A 120-min reperfusion period was then monitored, allowing a 1-h equilibration for inulin, followed by three 20-min urine collections for inulin and electrolyte measurement. At the mid point of these 20-min periods, a blood sample (300 µl) was drawn for inulin and electrolyte measurement. Two additional blood samples (200 µl) were drawn just before ischemia and at the end of the experiment for hematocrit measurement. After every blood sample withdrawal, an equal volume of blood obtained from a pool of rats was reinfused.

A group of rats with a similar body weight (control group, $n = 11$) that had been fed neither olive oil nor EPA were used as controls. These animals were experimentally managed in the same way as the study groups but without warm ischemia.

### Analytical methods and estimations

Glucose was measured enzymatically by the hexokinase/glucose-6-phosphate dehydrogenase method (Boehringer Mannheim, Germany), and polyfructosan was measured after acid hydrolysis by including glucose-6-phosphate isomerase in the assay [8]. Sodium levels were measured by flame photometry (Ciba Corning, Spain). Hematocrit was measured using an automated method (288 blood gas system, Ciba Corning).

Renal plasma flow (RPF, ml/min) was calculated from the formula RPF = RBF(1–hematocrit) and renal vascular resistance (VR, mm Hg/ml per min) from the formula VR = arterial pressure/RBF. Urine output was measured by collecting urine into preweighed tubes (µl/min). Glomerular filtration rate (GFR, µl/min) was evaluated from inulin clearance using the formula GFR = urine output × urine inulin/plasma inulin. Finally, fractional sodium reabsortion (FRNa) was calculated from the formula: FRNa = 100 × (1 – urine Na/perfusate Na × perfusate inulin/urine inulin).

### Statistical analysis

For comparisons between more than two groups, one-way analysis of variance was performed followed by Fisher's procedure for multiple pairwise comparisons. When a nonparametric test was needed, the Kruskal Wallis analysis was used. All $P$-values were two tailed and a $P$-value of $< 0.05$ was considered statistically significant. Data are presented as mean ± SEM.

## Results

Mean arterial pressure was similar in all groups throughout the experiment (Fig. 1 A). As shown in Figure 1 B, basal mean RPF was similar in the control and OLI groups, and both were significantly lower than mean RPF in the EPA groups, suggesting a state of chronic vasodilatation. After ischemia, the mean RPF increased progressively in all groups. The EPA groups

showed a significantly greater increase in mean RPF with time during the experiment than the OLI group. There was a dose-dependent effect in this increase in mean RPF. This increase was probably due to adaptive mechanisms to contralateral nephrectomy and to the high fluid infusion. RBF followed a similar pattern (data not shown). Concerning VR, EPA-fed animals showed lower mean values than olive oil-fed animals, both before arterial occlusion and after ischemia (Fig. 1 C).

Urine output showed similar mean values in the control, OLI and EPA 20 groups. The EPA 40 and EPA 80 groups showed significantly higher mean values than the other groups (Fig. 2 A). Concerning GFR (Fig. 2 B), the OLI and EPA 20 groups showed a significantly lower mean value than the control group, the EPA 40 group showed a mean value similar to the control group, and the EPA 80 group showed a slightly but not significantly higher mean value than control group. Mean FRNa showed no conclusive results (Fig. 2 C): in the 60–80-min period there was a significantly higher mean value in the control group than in the OLI, EPA 20 and EPA 40 groups but not compared to the EPA 80 group which showed similar values, but in the two other periods there were no significant differences between the groups.

## Discussion

The results of this study demonstrated that chronic supplementation of rats with a purified lysine salt of EPA increased renal blood and plasma flow, and decreased renal vascular resistance in the basal state and after warm ischemia, compared with rats fed with olive oil. In addition, kidney function, evaluated as the GFR and the urine output, was also protected in rats supplemented with EPA. These protective effects of EPA on overall renal function were dose-dependent.

Our positive results are quite similar to those of other studies in animal models of cerebral or cardiac ischemia [3–5], suggesting the potential preventive role of fish oils rich in n-3 fatty acids in ischemic states. Our results also agree with those reported in a study in uninephric conscious dogs in which prefeeding with fish oils ameliorated the course of acute ischemic renal failure [6]. However, in this study there was no modification of RBF or VR either during feeding with fish oils or after ischemia, in contrast to our results. There are some possible mechanisms that could explain these beneficial effects [1, 5, 9]: decreased formation of vasoconstricting and proaggregatory prostaglandins from the endothelium (mainly thromboxane $B_2$); enhanced release of nitric oxide/endothelium-derived relaxing factor; decreased polymorphonuclear cell recruitment within the ischemic zone during reperfusion, due to reduced production of leukotriene $B_4$, platelet activating factor and interleukin-1; reduced production of PDGF-like protein; and modulation of the receptor characteristics of vasoactive substances [10]. Concerning the effects on the kidneys, it is well known that EPA exerts important effects on renal function in normal humans [11] and favorably influences renal function and prostanoid production in experimental animals and transplanted patients receiving cyclosporin [2, 12].

The use of fish oils is increasing in humans. There are currently some ongoing studies in cardiovascular diseases, in IgA nephropathy and in the prevention of rejection in renal transplantation [1]. Their use in clinical ischemic states or in preservation during solid organ transplantation has not been tested yet. There is a time limitation in their use since all reported studies used long feeding protocols. Short treatment periods to study the protective effects of n-3 fatty acids on renal ischemia might be done. In this regard, a promising study has recently been reported which used the rapid administration of EPA by infusion of a trieicosapentaenoyl-glycerol emulsion into rabbits [13]. In this study, 6 h following infusion, the ex vivo formation of leukotriene $B_4$ by polymorphonuclear alls was markedly suppressed compared with preinfusion values, suggesting that the administered EPA had reached physiologically relevant levels. The use of EPA in ischemic states or preservation is therefore potentially possible and deserves further consideration.

**Acknowledgements** This work was supported in part by a grant from FISS (number 94/1296), by a grant from Sandoz SAE, Spain, and by a grant from LASA Laboratories. Marta Riera Oliva is a fellow of 'Fundació August Pi i Sunyer', Ciutat Sanitaria i Universitaria de Bellvitge. We thank Susana Miró for her technical help.

## References

1. Endres S, De Caterina R, Schmid EB, Kristensen SD (1995) n-3 Polyunsaturated fatty acids: update 1995. Eur J Clin Invest (in press)
2. Homan van der Heide JJ, Bilo HJG, Donker JM, Wilmik JM, Tegzess AM (1993) Effect of dietary fish oil on renal function and rejection in cyclosporin-treated recipients of renal transplants. N Engl J Med 329: 769–773
3. Lai ML, Hsu CY, Liu TH, He YY, Xu J, Navidi M, Sun G, Hogan EL (1993) Effects of fish oil supplementation on acute ischemic brain injury in the rat. Neurology 43: 1227–1232

4. Hock CE, Holahan MA, Reibel DK (1987) Effect of dietary fish oil on myocardial phospholipids and myocardial ischemic damage. Am J Physiol 252: H554–H560
5. Force Th, Malis ChD, Guerrero JL, Varadarajan GS, Bonventre JV, Weber PC, Leaf A (1989) n-3 Fatty acids increase postischemic blood flow but do not reduce myocardial necrosis. Am J Physiol 257: H1204–H1210
6. Neumayer HH, Heinrich Schmissas M, Haller H, Wagner K, Luft CF (1992) Amelioration of ischemic acute renal failure by dietary fish oil administration in concious dogs. J Am Soc Nephrol 3: 1312–1320
7. Gellai M, Jugus M, Fletcher T, DeWolf R, Nambi P (1994) Reversal of postischemic acute renal failure with a selective endothellin A receptor antagonist in the rat. J Clin Invest 93: 900–906
8. Schmidt FH (1961) Die enzymatische Bestimmung von Glukose und Fruktose nebeneinander. Klin Wochenschr 39: 1244–1247
9. Leaf A, Weber PC (1988) Cardiovascular effects of n-3 fatty acids. N Engl J Med 318: 549–557
10. Awazu M, Yared A, Swift LL, Hoover RL, Ichikawa I (1992) Dietary fatty acids modulate glomerular atrial natriuretic peptide receptor. Kidney Int 42: 265–271
11. Dusing R, Struck a, Gobel BO, Weisser B, Vetter H (1990) Effects of n-3 fatty acids on renal function and renal prostaglandin E metabolism. Kidney Int 38: 315–319
12. Torras J, Valles J, Sanchez J, Sabate I, Seron D, Carrera M, Castelao AM, Herrero I, Puig Parellada P, Alsina J, Grinyo JM, Prevention of experimental cyclosporine nephrotoxicity by dietary supplementation with LSL 90202, a lisine salt of eicosapentaenoic acid. Role of thomboxane and prostacyclin in renal tissue.
13. Sawazaki S, Nakamura N, Hamazaki T, Yamazaki K, Urazake M Yano (1992) Intravenous infusion of trieicosapentaenoyl glycerol and $LTB_4$ and $LTB_5$ production by leukocytes of rabbits. Am J Physiol 262: H1711–H1718