

# Effects of n-3 fatty acid, fructose-1,6-diphosphate and glutamine on mucosal cell proliferation and apoptosis of small bowel graft after transplantation in rats

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## Abstract

**AIM:** To evaluate the effects of n-3 fatty acids (n-3FA), fructose-1,6-diphosphate (FDP) and glutamine (GLN) on mucosal cell proliferation and apoptosis of small bowel graft.

**METHODS:** One hundred and ninety-six inbred strain Wistar rats were grouped as donors and recipients, and underwent heterotopic small bowel transplantation (SBT). n-3FA, FDP and GLN were administered via gastric tube as well as venous infusion for 10 days before and after surgery, respectively. The proliferation and apoptosis of mucosal cells were analyzed with flow cytometry and *in situ* cell death detection kits.

**RESULTS:** Apparent apoptosis and minor proliferation of mucosal cells of small bowel graft after transplantation were observed. A higher mucosal cell proliferative index and lower apoptotic index were found in all small bowel grafts after supplying with n-3FA, FDP and GLN.

**CONCLUSION:** Nutritional support with n-3FA, FDP and GLN promotes mucosal cell proliferation significantly, and prevents mucosal cell from undergoing apoptosis with different degrees. These regulatory effects on the apoptosis alter the structure and absorption function of transplanted small bowel favorably.

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## INTRODUCTION

The atrophy and malabsorption of transplanted small intestine are the main obstacles to small bowel transplantation (SBT). The effects of n-3 fatty acids (n-3FA), fructose-1, 6-diphosphate (FDP) and glutamine (GLN) on mucosal cell

proliferation and apoptosis of small bowel graft were evaluated. The mechanisms of mucosal atrophy and malabsorption of small bowel graft were studied at cellular and molecular levels.

## MATERIALS AND METHODS

### Animals

One hundred and ninety-six inbred male Wistar rats (180-310 g) purchased from Shanghai Animal Center of Chinese Academy of Medical Sciences were housed in laminar-flow cabinets under specific pathogen-free (SPF) condition. All studies on rats were conducted in accordance with the "Guideline for the Care and Use of Laboratory Animals" by National Institute of Health. The protocol approved by Shanghai Medical Experimental Animal Care Committee was followed during study. The rats were divided into 5 groups with 10 rats in each group. Group (1) was treated with non-essential amino acids. Group (2) was treated with FDP, group (3) with n-3FA, group (4) with GLN and group (5) with n-3FA+FDP+GLN. A modified heterotopic SBT was utilized<sup>[1]</sup>. End to side anastomosis between graft abdominal aorta and recipient abdominal aorta was performed. The left kidney of recipient was resected. The portal vein of graft was anastomosed to the left renal vein of the recipient. Fistulizations of the distal and proximal ends of graft were performed through left abdominal wall.

### Administration of special nutrition

In group (3) and (5), n-3FA (Sigma Company, USA), 1 mL·d<sup>-1</sup>, was given via gastric tube to both donor and recipient 10 days before and after operation, respectively. All animals received TPN. The dosage of FDP was 1 g·kg<sup>-1</sup>·d<sup>-1</sup> (Foscama Company, Italy). The nutritional solution in group (4) contained 2 % GLN (Ajinomoto company, Japan). L-alanine, glycine, L-proline and L-serine were applied to the nutritional solution of group (1), (2) and (3). The nitrogen and calorie in the nutritional solution were equal in each group.

### Specimen collection

Prior to transplantation, 5 cm of jejunum distal to the treitz ligament of graft was resected as baseline samples. Ten days postoperative TPN support, 5 cm of proximal jejunum of the graft in the recipient was resected for examination.

### Preparation of specimen

**Flow cytometry** The mucosae of the specimen were scissored into tiny pieces in saline and filtered to get cell suspension. The nucleus was stained by hypotonic propidium iodide at 4 °C over night.

**TdT-mediated X-dUTP nick end labeling (TUNEL method)** The specimen was sectioned in 3 μm-thick and dehydrated through 70-90 % ethanol in series. After digestion with proteinase K (20 μg·mL<sup>-1</sup>), the work solution of TUNEL was added. The reaction was terminated with ddH<sub>2</sub>O. The positive nucleus was stained with brownish yellow. Ten fields were selected randomly from each section under light microscope.

Two thousand mucosal cells were examined from each section. The apoptotic index (AI) was calculated with the formula: AI = (number of positive cells/total number of cells examined)×100 %.

### Flow cytometry analysis

Epics XL flow cytometer (Coulter Company, USA) was used in the analysis. The proportions of cells in stage G<sub>1</sub>, S and G<sub>2</sub> were calculated in 10 000 cells examined. The proliferative index (PI) indicating the proliferation of mucosal cells was calculated using the formula: PI = [(S+G<sub>2</sub>)/(G<sub>1</sub>+S+G<sub>2</sub>)]×100 %.

### Statistical analysis

Newman-Keuls' *s q* test and Student' *t* test were used. The statistical analysis software package stata 5.0 was used for the tests, and *P*<0.05 was considered statistically significant.

## RESULTS

### Flow cytometry

All the data from flow cytometry study are showed in Table 1.

**Table 1** Changes of proliferation of graft mucosal cells in each group ten days before and after SBT and TPN ( $\bar{x}\pm s$ )

Group	Stage of cell cycle	Before SBT and TPN (%)	After SBT and TPN (%)	<i>P</i>
(1)	G <sub>1</sub>	94.73±2.33	94.08±2.15	0.7440
	G <sub>2</sub>	0.38±0.31	1.38±1.44	0.3296
	S	4.88±2.42	4.55±0.92	0.8480
	PI	5.25±2.29	5.85±2.26	0.7682
(2)	G <sub>1</sub>	96.22±2.96 <sup>b</sup>	86.14±4.08 <sup>b</sup>	0.0011 <sup>b</sup>
	G <sub>2</sub>	0.88±1.20 <sup>a</sup>	3.06±1.12	0.0140 <sup>a</sup>
	S	2.90±1.78 <sup>b</sup>	10.78±3.06 <sup>b</sup>	0.0007 <sup>b</sup>
	PI	3.78±2.96 <sup>b</sup>	13.84±4.05 <sup>b</sup>	0.0011 <sup>b</sup>
(3)	G <sub>1</sub>	90.94±4.37 <sup>b</sup>	83.12±2.59 <sup>b</sup>	0.0069 <sup>b</sup>
	G <sub>2</sub>	1.96±1.12 <sup>a</sup>	3.86±0.96 <sup>a</sup>	0.0497 <sup>a</sup>
	S	7.10±3.98 <sup>a</sup>	13.02±3.39 <sup>a</sup>	0.0211 <sup>a</sup>
	PI	9.06±4.39 <sup>b</sup>	16.88±2.59 <sup>b</sup>	0.0071 <sup>b</sup>
(4)	G <sub>1</sub>	95.52±1.57 <sup>b</sup>	81.03±4.98 <sup>b</sup>	0.0002 <sup>b</sup>
	G <sub>2</sub>	0.63±0.60 <sup>b</sup>	5.93±1.62 <sup>b</sup>	0.0016 <sup>b</sup>
	S	4.02±1.57 <sup>b</sup>	13.52±4.70 <sup>b</sup>	0.0011 <sup>b</sup>
	PI	4.65±1.48 <sup>b</sup>	18.97±5.01 <sup>b</sup>	0.0002 <sup>b</sup>
(5)	G <sub>1</sub>	90.48±3.21 <sup>b</sup>	80.52±0.95 <sup>b</sup>	0.0053 <sup>b</sup>
	G <sub>2</sub>	2.52±2.06	5.02±1.43	0.0674
	S	6.96±2.96 <sup>a</sup>	14.22±1.77	0.0148 <sup>a</sup>
	PI	9.48±3.24 <sup>b</sup>	19.24±0.94 <sup>b</sup>	0.0057 <sup>b</sup>

<sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01 vs before SBT.

**G<sub>1</sub> stage** No change of the proportion of graft mucosal cells in G<sub>1</sub> stage in group (1) was observed after transplantation. Most cells remained at G<sub>1</sub> stage. However, the proportions of graft mucosal cells in G<sub>1</sub> stage in the rest groups after transplantation decreased significantly.

**G<sub>2</sub> stage** Changes of proportion of graft mucosal cells in G<sub>2</sub> stage of group (1) and (5) after transplantation were not obvious. The proportions of graft mucosal cells in G<sub>2</sub> stage in the rest groups increased with great statistical significance.

**S stage** Following transplantation the change of proportion of graft mucosal cells in S stage was increased significantly in all groups except group (1).

**PI** Compared with the baseline before transplantation, the PI of graft mucosal cells changed significantly in all groups except group (1).

### Apoptosis in situ

**Group (1)** After SBT, the number of graft mucosal cells with apoptosis was increased. The AI was increased significantly after transplantation (Table 2). The AI in group (1) was significantly higher than those in other groups.

**Group (2)** The AI of graft mucosal cells increased after SBT and TPN with significant difference (Table 2). The AI in group (2) was higher than those in other groups with significance.

**Group (3)** The AI of graft mucosal cells after transplantation increased slightly without significance compared with baseline (Table 2). The AI was similar among groups (3), (4) and (5) without any significant difference.

**Group (4)** The AI of graft mucosal cells in group (4) was similar to that in group (5), decreased slightly after SBT without significant difference (Table 2).

**Group (5)** No change of the AI of graft mucosal cells was observed after SBT (Table 2).

**Table 2** Change of AI of graft mucosal cells in each group ten days before and after SBT and TPN ( $\bar{x}\pm s$ )

Group	Before SBT and TPN (%)	After SBT and TPN (%)	<i>P</i>
(1)	25.50±5.43 <sup>b</sup>	40.50±3.24 <sup>b</sup>	0.0041 <sup>b</sup>
(2)	24.63±1.70 <sup>a</sup>	30.88±2.50 <sup>a</sup>	0.0447 <sup>a</sup>
(3)	24.25±1.32	25.63±1.38	0.2141
(4)	25.00±2.68	23.63±0.85	0.4863
(5)	23.75±0.87	24.00±1.68	0.8130

<sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.01 vs before SBT.

## DISCUSSION

Rejection of graft will not occur in organ transplantation between two individuals from same strain of inbred animals. Thus, the effect of special nutrition on the transplanted small intestine could be observed in our research without the influence of transplant immunological factors.

Our results indicated that the proliferation of graft mucosal cells after SBT in group (1) was obviously less active than that before SBT. Most cells were rested in stage G<sub>1</sub>. The ratios of cells in stage S over stage G<sub>2</sub> decreased, which suggested that DNA synthesis in graft mucosal cells after SBT reduced, and that mucosal cells were at rest stage instead of active proliferation. These changes in cytodynamics might be the underling cause of cytopathy of graft with the characteristics of atrophy and malabsorption.

Compared with the results of group (1), after SBT the proliferation of graft mucosal cells in rest groups was more active. The ratios of cells in stage S over stage G<sub>2</sub> increased, which suggested that n-3FA, FDP and GLN contributed to the proliferation and repair of graft mucosal cells, and were helpful in preventing atrophy and maintaining the normal structure and function of intestinal mucus.

The number of apoptotic cells and AI of graft mucosal cells in groups (1) and (2) after SBT and TPN increased significantly compared with those before SBT and TPN. On the contrary, those of groups (3), (4) and (5) did not change obviously. The AI in groups (2), (3), (4) and (5) after SBT and TPN decreased significantly in comparison with that in group (1). The result suggested that n-3FA, FDP and GLN could inhibit the apoptosis of graft mucus after SBT to a certain degree.

GLN might influence the graft in several ways, in which it promotes the proliferation of graft mucosal cells and inhibits the cell apoptosis. (1) GLN offers the energy and metabolic substrate which are necessary to the proliferation of intestine mucosal cells<sup>[2-15]</sup>, including carbon chains for energy releasing

(30 mol ATP/mol) and nitrogen for synthesis of amino acid, protein and nuclear acid. (2) GLN also participates in the synthesis of glutathione (GSH). Administration of exogenous GLN will result in high level of GSH. It is indicated in some experiments that GSH could antagonize the oxidation injury to the biomembrane caused by oxygen-derived free radicals, and improve the survival of cells<sup>[16-19]</sup>. (3) GLN may indirectly stimulate the secretion of hormones which are beneficial to the nutrition of intestine, and improve the hormone environment of intestinal mucosal cells. This may help establish normal physiological metabolism and the balanced proliferation and apoptosis of intestinal mucosal cells<sup>[20]</sup>. (4) The apoptosis of host cells could be induced by bacterial and viral infections<sup>[21]</sup>. GLN may improve the structure and function of the transplanted intestine and reduce bacterial translocation, which could prevent infection and reduce apoptosis<sup>[22-32]</sup>.

It is not clear why FDP could promote proliferation of mucosal cells of intestinal graft and inhibit apoptosis. The possible reasons might include: (1) As a kind of potent energy substrate, FDP can offer plenty of ATP quickly whether it is on normal or stress occasion, such as ischemia or anoxia<sup>[33,34]</sup>. So, FDP can meet the energy need of proliferation of intestinal mucosal cells with quick metabolism. (2) As a promoter of energy synthesis, FDP may activate pyruvate kinase and phosphofructokinase on stress occasion, such as ischemia or anoxia. These enzymes may promote the metabolism of carbohydrate to offer adequate energy<sup>[33]</sup>, which is helpful to cell proliferation. The products of zymolysis could be decomposed in the energy-producing process<sup>[35]</sup>. Thus tissue damage caused by these products could be avoided and the inducing factors of apoptosis were reduced. (3) FDP could reduce the retention of intracellular calcium at stress, and inhibit endogenous calcium dependent endonuclease which is important to nuclear changes in apoptosis<sup>[36]</sup>. (4) The cell injuries during ischemia/reperfusion and cold storage of graft are associated with apoptosis<sup>[37-39]</sup>. FDP could inhibit the production of toxic oxygen group by activated polymorphonuclear leukocyte and scavenge the hypoxanthine accumulated in the tissues during ischemia and reperfusion<sup>[40]</sup>. Thus, the apoptosis caused by these factors could be reduced<sup>[41]</sup>.

The mechanism of n-3FA in improving the proliferation of mucosal cell of intestinal graft and inhibiting apoptosis is not clear. It may involve the following factors: (1) As a kind of energy substrate, n-3FA could offer energy for the proliferation of mucosal cells. (2) n-3FA could be esterified into phospholipid and neutral lipid which are essential to neogenetic mucosal cells as important component of structure membrane<sup>[42-45]</sup>. (3) n-3FA could inhibit the production of IL-1 $\alpha$ , IL-1 $\beta$  and TNF secreted by monocytes. It is indicated that IL-1 $\beta$  and TNF can promote apoptosis<sup>[46]</sup>. (4) Apoptosis might be one of the ways of cell death in rejection after allogeneic transplantation<sup>[47,48]</sup>. As a kind of immunosuppressant<sup>[49-51]</sup>, n-3FA could inhibit the apoptosis of graft cells through immunosuppression.

In conclusion, special nutrition with n-3FA, FDP and GLN after SBT can promote the proliferation of graft mucosal cells and inhibit apoptosis. Our results may provide new ways to treat intestinal atrophy and malabsorption after SBT.

## REFERENCES

- 1 **Wu XT**, Li JS, Zhao XF, Zhuang W, Feng XL. Modified techniques of heterotopic total small intestinal transplantation in rats. *World J Gastroenterol* 2002; **8**: 758-762
- 2 **Windmueller HG**, Spaeth AE. Uptake and metabolism of plasma glutamine by the small intestine. *J Biol Chem* 1974; **249**: 5070-5079
- 3 **Zhang W**, Bain A, Rombeau JL. Insulin-like growth factor-I (IGF-I) and glutamine improve structure and function in the small bowel allograft. *J Surg Res* 1995; **59**: 6-12
- 4 **Klimberg VS**, Souba WW, Salloum RM, Holley DT, Hautamaki RD, Dolson DJ, Copeland EM 3rd. Intestinal glutamine metabolism after massive small bowel resection. *Am J Surg* 1990; **159**: 27-32
- 5 **Marks SL**, Cook AK, Reader R, Kass PH, Theon AP, Greve C, Rogers QR. Effects of glutamine supplementation of an amino acid-based purified diet on intestinal mucosal integrity in cats with methotrexate-induced enteritis. *Am J Vet Res* 1999; **60**: 755-763
- 6 **Fox AD**, Kripke SA, De Paula J, Berman JM, Settle RG, Rombeau JL. Effect of aglutamine supplemented enteral diet on methotrexate induced enteritis. *JPEN* 1988; **12**: 325-331
- 7 **Gu Y**, Wu ZH. The anabolic effects of recombinant human growth hormone and glutamine on parenterally fed, short bowel rats. *World J Gastroenterol* 2002; **8**: 752-757
- 8 **Colomb V**, Darcy-Vrillon B, Jobert A, Guihot G, Morel MT, Corriol O, Ricour C, Duee PH. Parenteral nutrition modifies glucose and glutamine metabolism in rat isolated enterocytes. *Gastroenterology* 1997; **112**: 429-436
- 9 **Cherbuy C**, Darcy-Vrillon B, Morel MT, Pegorier JP, Duee PH. Effect of germfree state on the capacities of isolated rat colonocytes to metabolize n-butyrate, glucose, and glutamine. *Gastroenterology* 1995; **109**: 1890-1899
- 10 **Wilmore DW**. Glutamine and the gut. *Gastroenterology* 1994; **107**: 1885-1901
- 11 **Minami H**, Morse EL, Adibi SA. Characteristics and mechanism of glutamine-dipeptide absorption in human intestine. *Gastroenterology* 1992; **103**: 3-11
- 12 **Dejong CH**, Kampman MT, Deutz NE, Soeters PB. Altered glutamine metabolism in rat portal drained viscera and hindquarter during hyperammonemia. *Gastroenterology* 1992; **102**: 936-948
- 13 **Rhoads JM**, Keku EO, Quinn J, Woosely J, Lecce JG. L-glutamine stimulates jejunal sodium and chloride absorption in pig rotavirus enteritis. *Gastroenterology* 1991; **100**: 683-691
- 14 **Klein S**. Glutamine: an essential nonessential amino acid for the gut. *Gastroenterology* 1990; **99**: 279-281
- 15 **Firmansyah A**, Penn D, Lebenthal E. Isolated colonocyte metabolism of glucose, glutamine, n-butyrate, and beta-hydroxybutyrate in malnutrition. *Gastroenterology* 1989; **97**: 622-629
- 16 **Yu JC**, Jiang ZM, Li DM. Glutamine: a precursor of glutathione and its effect on liver. *World J Gastroenterol* 1999; **5**: 143-146
- 17 **Yagi M**, Sakamoto K, Inoue T, Fukushima W, Muraoka K, Ii T, Iyobe T, Iwasa K, Hashimoto T, Shimizu K, Izumi R, Miyazaki I. Effect of a glutamine-enriched elemental diet on regeneration of the small bowel mucosa following isotransplantation of small intestine. *Transplant Proc* 1994; **26**: 2297-2298
- 18 **Scheppach W**, Dusel G, Kuhn T, Loges C, Karch H, Bartram HP, Richter F, Christl SU, Kasper H. Effect of L-glutamine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. *Gut* 1996; **38**: 878-885
- 19 **Xu Y**, Nguyen Q, Lo DC, Czaja MJ. c-myc-Dependent hepatoma cell apoptosis results from oxidative stress and not a deficiency of growth factors. *J Cell Physiol* 1997; **170**: 192-199
- 20 **Petronini PG**, Urbani S, Alfieri R, Borghetti AF, Guidotti GG. Cell susceptibility to apoptosis by glutamine deprivation and rescue: survival and apoptotic death in cultured lymphoma-leukemia cell lines. *J Cell Physiol* 1996; **169**: 175-185
- 21 **Thompson CB**. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; **267**: 1456-1462
- 22 **Schroeder P**, Schweizer E, Blomer A, Deltz E. Glutamine prevents mucosal injury after small bowel transplantation. *Transplant Proc* 1992; **24**: 1104
- 23 **Klimberg VS**, Souba WW, Dolson DJ, Salloum RM, Hautamaki RD, Plumley DA, Mendenhall WM, Bova FJ, Khan SR, Hackett RL, Bland KI, Copeland III EM. Prophylactic glutamine protects the intestinal mucosa from radiation injury. *Cancer* 1990; **66**: 62-68
- 24 **Zhang W**, Frankel WL, Singh A, Laitin E, Klurfeld D, Rombeau JL. Improvement of structure and function in orthotopic small bowel transplantation in the rat by glutamine. *Transplantation* 1993; **56**: 512-517
- 25 **Li JY**, Lu Y, Hu S, Sun D, Yao YM. Preventive effect of glutamine on intestinal barrier dysfunction induced by severe trauma. *World J Gastroenterol* 2002; **8**: 168-171
- 26 **Yoshida S**, Matsui M, Shirouzu Y, Fujita H, Yamana H, Shirouzu K. Effects of glutamine supplements and radiochemotherapy on

- systemic immune and gut barrier function in patients with advanced esophageal cancer. *Ann Surg* 1998; **227**: 485-491
- 27 **Yoshida S**, Leskiw MJ, Schluter MD, Bush KT, Nagele RG, Lanza-Jacoby S, Stein TP. Effect of total parenteral nutrition, systemic sepsis, and glutamine on gut mucosa in rats. *Am J Physiol* 1992; **263**: E368-373
- 28 **Burke DJ**, Alverdy JC, Aoye E, Moss GS. Glutamine-supplemented total parenteral nutrition improves gut immune function. *Arch Surg* 1989; **124**: 1396-1399
- 29 **Rhoads JM**, Argenzio RA, Chen W, Graves LM, Licato LL, Blikslager AT, Smith J, Gatzky J, Brenner DA. Glutamine metabolism stimulates intestinal cell MAPKs by a cAMP-inhibitable, Raf-independent mechanism. *Gastroenterology* 2000; **118**: 90-100
- 30 **De Blaauw I**, Deutz NE, van der Hulst RR, von Meyenfeldt MF. Glutamine depletion and increased gut permeability in nonanorectic, non-weight-losing tumor-bearing rats. *Gastroenterology* 1997; **112**: 118-126
- 31 **Tremel H**, Kienle B, Weilemann LS, Stehle P, Furst P. Glutamine dipeptide-supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology* 1994; **107**: 1595-1601
- 32 **Scheppach W**, Loges C, Bartram P, Christl SU, Richter F, Dusel G, Stehle P, Fuerst P, Kasper H. Effect of free glutamine and alanyl-glutamine dipeptide on mucosal proliferation of the human ileum and colon. *Gastroenterology* 1994; **107**: 429-434
- 33 **Jurgens TM**, Hardin CD. Fructose-1,6-bisphosphate as a metabolic substrate in hog ileum smooth muscle during hypoxia. *Mol Cell Biochem* 1996; **154**: 83-93
- 34 **Trimarchi GR**, Arcadi FA, Imperatore C, Ruggeri P, Costa G. Effects of fructose-1,6-bisphosphate on microsphere-induced cerebral ischemia in the rat. *Life Sci* 1997; **61**: 611-622
- 35 **Hassinen IE**, Nuutinen EM, Ito K, Nioka S, Lazzarino G, Giardina B, Chance B. Mechanism of the effect of exogenous fructose 1,6-bisphosphate on myocardial energy metabolism. *Circulation* 1991; **83**: 584-593
- 36 **Pozzilli C**, Lenzi GL, Argentino C, Carolei A, Rasura M, Signore A, Bozzao L, Pozzilli P. Imaging of leukocytic infiltration in human cerebral infarcts. *Stroke* 1985; **16**: 251-255
- 37 **Takeuchi K**, Cao-Danh H, Friebs I, Glynn P, D'Agostino D, Simplaceanu E, McGowan FX, del Nido PJ. Administration of fructose 1,6-diphosphate during early reperfusion significantly improves recovery of contractile function in the postischemic heart. *J Thorac Cardiovasc Surg* 1998; **116**: 335-343
- 38 **Sun JX**, Farias LA, Markov AK. Fructose 1-6 diphosphate prevents intestinal ischemic reperfusion injury and death in rats. *Gastroenterology* 1990; **98**: 117-126
- 39 **Farias LA**, Smith EE, Markov AK. Prevention of ischemic-hypoxic brain injury and death in rabbits with fructose-1,6-diphosphate. *Stroke* 1990; **21**: 606-613
- 40 **Chu SJ**, Chang DM, Wang D, Chen YH, Hsu CW, Hsu K. Fructose-1,6-diphosphate attenuates acute lung injury induced by ischemia-reperfusion in rats. *Crit Care Med* 2002; **30**: 1605-1609
- 41 **Raff MC**. Social controls on cell survival and cell death. *Nature* 1992; **356**: 397-400
- 42 **Teo TC**, DeMichele SJ, Selleck KM, Babayan VK, Blackburn GL, Bistrian BR. Administration of structured lipid composed of MCT and fish oil reduces net protein catabolism in enterally fed burned rats. *Ann Surg* 1989; **210**: 100-107
- 43 **Endres S**, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC, Schaefer EJ, Wolff SM, Dinarello CA. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989; **320**: 265-271
- 44 **Dinarello CA**, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MA Jr, O'Connor JV. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med* 1986; **163**: 1433-1450
- 45 **Thiele J**, Zirbes TK, Lorenzen J, Kvasnicka HM, Dresbach S, Manich B, Leder LD, Niederle N, Diehl V, Fischer R. Apoptosis and proliferation (PCNA labelling) in CML-a comparative immunohistological study on bone marrow biopsies following interferon and busulfan therapy. *J Pathol* 1997; **181**: 316-322
- 46 **Steller H**. Mechanisms and genes of cellular suicide. *Science* 1995; **267**: 1445-1449
- 47 **Krams SM**, Egawa H, Quinn MB, Villanueva JC, Garcia-Kennedy R, Martinez OM. Apoptosis as a mechanism of cell death in liver allograft rejection. *Transplantation* 1995; **59**: 621-625
- 48 **Ito H**, Kasagi N, Shomori K, Osaki M, Adachi H. Apoptosis in the human allografted kidney. Analysis by terminal deoxynucleotidyl transferase-mediated DUTP-biotin nick end labeling. *Transplantation* 1995; **60**: 794-798
- 49 **Prickett JD**, Robinson DR, Steinberg AD. Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZB x NZW F1 mice. *J Clin Invest* 1981; **68**: 556-559
- 50 **Kelley VE**, Ferretti A, Izui S, Strom TB. A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites, and suppresses lupus in MRL-lpr mice. *J Immunol* 1985; **134**: 1914-1919
- 51 **Kelley VE**, Kirkman RL, Bastos M, Barrett LV, Strom TB. Enhancement of immunosuppression by substitution of fish oil for olive oil as a vehicle for cyclosporine. *Transplantation* 1989; **48**: 98-102

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