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ORIGINAL ARTICLE

Igor Sukhotnik · Habib Helou · Jorge Mogilner
Michael Lurie · Aleksander Bernsteyn
Arnold G. Coran · Eitan Shiloni

Oral arginine improves intestinal recovery following ischemia-reperfusion injury in rat

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Abstract Arginine and nitric oxide are critical to the normal physiology of the gastrointestinal tract and maintain the mucosal integrity of the intestine in various intestinal disorders. In the present study, we evaluate the effects of oral arginine (ARG) supplementation on intestinal structural changes, enterocyte proliferation, and apoptosis following intestinal ischemia–reperfusion (IR) in the rat. Male Sprague–Dawley rats were divided into three experimental groups: sham rats underwent laparotomy and superior mesenteric artery mobilization, IR rats underwent superior mesenteric artery occlusion for 30 min following by 24 h of reperfusion, and IR-ARG rats were treated with enteral arginine given in drinking water (2%) 48 h before and following IR. Intestinal structural changes, enterocyte proliferation, and enterocyte apoptosis were determined 24 h following IR. A nonparametric Kruskal–Wallis ANOVA test was used for statistical analysis with $p < 0.05$ considered statistically significant. IR rats demonstrated a significant decrease in bowel weight in duodenum and jejunum, mucosal weight in jejunum and ileum, and villus height in jejunum and ileum compared with control animals. IR rats also had a significantly lower cell proliferation index in jejunum and ileum and a higher apoptotic index in ileum compared with control rats. IR-ARG animals demonstrated greater duodenal and jejunal bowel weight; duodenal, jejunal, and ileal mucosal weight; and jejunal and ileal cell proliferation index compared with IR animals. In conclusion, oral ARG administration improves mucosal recovery following IR injury in the rat.

Keywords Ischemia–reperfusion · Intestine · Arginine · Rat

I. Sukhotnik (✉) · J. Mogilner · M. Lurie · A. Bernsteyn
Department of Pediatric Surgery B,
Rappaport Faculty of Medicine, Technion,
Bnai Zion Medical Center, 47 Golomb St., 4940,
Haifa, 31048, Israel
E-mail: igor-dr@internet-zahav.net
Tel.: +972-4-8256815/+972-4-8359647
Fax: +972-4-8359620

H. Helou · E. Shiloni
Carmel Medical Center, Haifa, Israel

A. G. Coran
Section of Pediatric Surgery, C.S. Mott Children's Hospital
and University of Michigan Medical School,
Ann Arbor, MI, USA

Introduction

The precise mechanisms of intestinal ischemia–reperfusion (IR) injury have not been elucidated completely. Restoration of blood flow after a period of intestinal ischemia is necessary to maintain cell function and viability; however, the reintroduction of oxygen can initiate a cascade of events that exacerbate tissue injury via the formation of reactive oxygen species [7, 23]. Necrosis has been assumed to be synonymous with epithelial cell death after an ischemic insult. However, more recent reports have noted that apoptosis is a significant, and perhaps the principal, contributor to cell death after IR. Recent evidence has shown that ischemia and/or IR induces apoptosis in several tissues, such as the brain and the liver. Recently an effect of IR on apoptosis of enterocytes in intestine has been demonstrated [6, 12].

Therapy of IR injury should include the pharmacologic agents that inhibit or affect IR pathophysiologic pathways. Selected agents or drug combinations may offer novel, scientifically relevant, and practical approaches to alleviating intestinal IR injury. Arginine (ARG) is a nonessential amino acid processed metabolically by the urea cycle [4]. It plays an important role in many physiologic and biologic processes, including release of several hormones, collagen synthesis during wound healing, immune response, tumor biology, and the regulation of inflammation [10, 17]. L-arginine is converted to nitric oxide (NO) by the enzyme nitric oxide synthase [22]. There is growing interest in the potential roles of ARG and NO as

regulators of cell proliferation and apoptosis in general, and in the gastrointestinal tract in particular. Several studies have suggested that ARG and NO maintain the mucosal integrity of the intestine. Dietary supplementation with ARG accelerates ulcer healing in experimental ulcerative ileitis [19] and stimulates small intestinal mucosal recovery following experimental radiation enteritis [8]. In a recent study, we have shown that oral ARG decreases intestinal injury caused by lipopolysaccharide endotoxemia in a rat [18]. The mechanisms of these positive effects are still unclear; however, a stimulating effect of appropriate amounts of NO on enterocyte proliferation and a suppressive effect on enterocyte death via apoptosis may be considered smong them.

The role of ARG following intestinal IR is unclear. Exogenous ARG might stimulate mucosal recovery following IR through growth hormone release or production of polyamines [3]. However, ARG might also exacerbate reperfusion injury of intestine following ischemic insult by overproduction of NO and free oxygen radicals. In a recent study, Schleiffer et al. have shown that pretreatment with L-arginine decreases mucosal intestinal permeability, improves gut barrier function, and ameliorates survival after IR injury in rats [16].

The purpose of the present study was to evaluate the effect of oral ARG supplementation on structural mucosal changes in the small bowel induced by IR injury in the rat and to evaluate the mechanisms by which ARG influences intestinal recovery, including its effect on enterocyte proliferation and death via apoptosis.

Materials and methods

Animals

The experimental protocol was approved by the "Guide for the Care and Use of Laboratory Animals," Rappaport Faculty of Medicine, Technion (Haifa, Israel). Male Sprague–Dawley rats weighing 250–300 g were acclimatized at 21°C on 12-h day and night cycles for a minimum of 1 week before experimentation. The rats had free access to water and were pair-fed with standard chow. Rats were fasted for 24 h before the experiment but were allowed free access to water.

Experimental design

Animals were randomly assigned to one of three experimental groups of 15 rats each: (1) sham rats (group A), (2) IR rats, which underwent IR injury by 30 min of mesenteric artery occlusion and 24 h of reperfusion, and (3) IR-ARG animals, which were pretreated with ARG given in drinking water (2%) for 3 days, followed by IR injury.

Operative procedure

Following overnight fasting, the rats were anesthetized with intraperitoneal pentobarbital (40 mg/kg). The abdomen was opened through a midline incision. In rats undergoing a sham operation (group A), simple mobilization of the superior mesenteric artery (SMA) without its clamping was performed. In IR rats (groups B and C), the SMA was occluded by an atraumatic microvascular clamp for 30 min followed by a reperfusion period of 24 h. Before closure of the abdomen, the rats were resuscitated with a 3-ml intraperitoneal injection of warm 0.9% saline. For all operations, the abdominal cavity was closed in two layers with a running suture of Dexon S polyglycolic acid 3/0. Rats were allowed free access to water and food. After 24 h, the rats were anesthetized with intraperitoneal pentobarbital (75 mg/kg) and were sacrificed by open pneumothorax.

Intestinal mucosal parameters

The remaining small bowel was excised quickly, washed with cold isotonic saline, and divided into three segments: duodenum, jejunum, and terminal ileum. Each segment was weighed and cut longitudinally, and the diameter was measured at three equidistant places as described by Dowling [5]. Mucosa was scraped using a glass slide, collected, and weighed. DNA and protein were extracted from the mucosa of jejunum and ileum using TRIzol reagent as described by Chomczynski [1] and were calculated as μg/cm bowel length/100 g body weight. Histological sections were prepared from the proximal jejunum and distal ileum. The samples of intestinal tissues were fixed in a 10% formaldehyde solution (2–3% methanol) and then were embedded in paraffin wax, using standard techniques. Sections (5 μm each) were cut and stained with hematoxylin and eosin. The villus height and crypt depth for each specimen were measured using an objective mounted micrometer (100× magnification) and an optical microscope (10×100 magnifications). Villus height and crypt depth data are derived from six rats in each group, and each measurement consists of the mean of five villi and crypts.

The mucosal damage of the small bowel or intestinal injury grade was graded using the Park score [10].

Crypt cell proliferation and villus cell apoptosis

Standard 5-bromodeoxyuridine (5-BrdU) labeling reagent (Zymed Laboratories, San Francisco, CA) was injected intraperitoneally at a dose of 1 ml/100 g body weight 2 h before sacrifice. Crypt cell proliferation was assessed using biotinylated monoclonal anti-BrdU antibody system provided in a kit form (Zymed Laboratories, San Francisco, CA). An index of proliferation was determined as the ratio of crypt cells staining positively for BrdU per five crypts.

The terminal deoxyuridine nick-end labeling (TUNEL) immunohistochemical assay was used to identify apoptotic cells using the I.S. Cell Death Detection kit (Boehringer Mannheim, Mannheim, Germany). The apoptotic index (AI) was defined as the number of apoptotic TUNEL-positive cells per five villi.

All measurements were performed by a qualified pathologist blinded to the source of intestinal tissue.

Statistical analysis

The data are expressed as the mean ± SEM. A non-parametric Kruskal–Wallis ANOVA test was used for statistical analysis with $p < 0.05$ considered statistically significant.

Results

Body weight changes

One rat in group A (sham) and three rats in groups B (IR) and C (IR-ARG) died within 3–4 h following operation. The remaining rats recovered from the surgery uneventfully. About 90% of animals suffered from appetite loss, diarrhea, and weight loss. There was no significant difference in final body weight in any experimental group (Fig. 1).

Intestinal mucosal parameters

There was no difference in bowel diameter in duodenum, jejunum, or ileum between any of the three experimental groups. IR injury resulted in a significant decrease in bowel weight in duodenum (19%, $p < 0.05$) and jejunum (13%, $p < 0.05$); mucosal weight in duodenum (24%, $p < 0.05$), jejunum (22%, $p < 0.05$), and ileum (25%, $p < 0.05$); and ileal mucosal DNA (36%, $p < 0.05$) and protein (40%, $p < 0.05$) compared with control animals (Table 1). Following oral ARG administration, IR rats demonstrated a significant increase in duodenal (24%, $p < 0.05$) and jejunal (20%, $p < 0.05$) bowel weight; duodenal (50%, $p < 0.05$), jejunal (43%, $p < 0.05$), and ileal (33%, $p < 0.05$) mucosal weight; ileal mucosal DNA (38%, $p < 0.05$); and jejunal (70%, $p < 0.05$) and ileal (33%, $p < 0.05$) mucosal protein compared with IR-untreated animals.

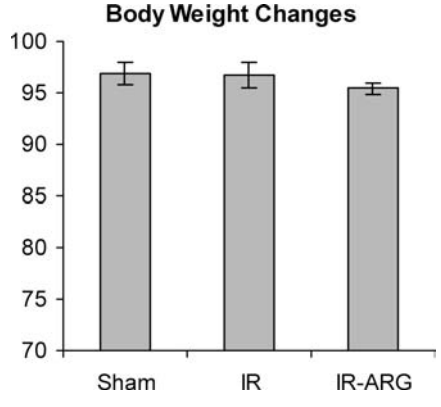

Fig. 1 Body weight changes expressed as percentage of preoperative weight (mean ± SEM) in control (sham) and IR rats untreated or treated with oral ARG (*IR* ischemia-reperfusion, *ARG* arginine)

Table 1 Effect of oral arginine on macroscopic bowel appearance, mucosal DNA, and protein following intestinal IR in rat (*IR* ischemia–reperfusion, *ARG* arginine)

Parameters	Sham ($n=15$)	IR ($n=15$)	IR-ARG ($n=15$)
Bowel weight (mg/cm/100 g)			
Duodenum	31 ± 3	25 ± 1[a]	31 ± 1[b]
Jejunum	23 ± 1	20 ± 1[a]	24 ± 1[b]
Ileum	20 ± 1	20 ± 1	21 ± 1
Mucosal weight (mg/cm/100 g)			
Duodenum	11 ± 1	8 ± 1[a]	12 ± 1[b]
Jejunum	9 ± 0.5	7 ± 0.6[a]	10 ± 1[b]
Ileum	8 ± 0.6	6 ± 0.6[a]	8 ± 0.4[b]
Mucosal DNA (μg/cm/100 g)			
Jejunum	9.1 ± 0.9	7.5 ± 0.8	9.1 ± 1.3
Ileum	9.6 ± 05	6.1 ± 1.0[a]	8.4 ± 0.7[b]
Mucosal protein (μg/cm/100 g)			
Jejunum	30 ± 6	20 ± 2[a]	34 ± 3[b]
Ileum	30 ± 4	18 ± 2[a]	24 ± 2[a,b]

[a] $p < 0.05$ IR vs. sham, [b] $p < 0.05$ IR-ARG vs. IR

Microscopic bowel appearance

Villus height significantly decreased in jejunum (33%, $p < 0.05$) and ileum (34%, $p < 0.05$) in IR rats compared with sham animals (Table 2). Crypt depth showed a pattern similar to that of the villus height. IR rats demonstrated a lower crypt depth in jejunum (19%, $p < 0.05$) and ileum (24%, $p < 0.05$) compared with sham animals. Oral ARG attenuated this negative effect of ischemia and reperfusion on mucosal architecture. IR-ARG rats showed greater villus height in jejunum (59% increase, $p < 0.05$) and ileum (49% increase, $p < 0.05$), and greater crypt depth in jejunum (27% increase, $p < 0.05$) and ileum (28% increase, $p < 0.05$) compared with IR-nontreated animals (Fig. 2).

Table 2 Effect of oral arginine on microscopic bowel appearance and mean intestinal injury following intestinal IR in rat (*IR* ischemia-reperfusion, *ARG* arginine)

Parameters	Sham ($n=15$)	IR ($n=15$)	IR-ARG ($n=15$)
Mean intestinal injury score			
Jejunum	0	0.8 ± 0.2[a]	0.6 ± 0.4
Ileum	0.4 ± 0.2	1.8 ± 0.5[a]	0.8 ± 0.4
Villus height (μm)			
Jejunum	521 ± 48	350 ± 26[a]	557 ± 65[b]
Ileum	345 ± 37	229 ± 22[a]	341 ± 37[b]
Crypt depth (μm)			
Jejunum	208 ± 23	168 ± 7[a]	214 ± 24[b]
Ileum	177 ± 18	133 ± 6[a]	170 ± 20[b]

[a] $p < 0.05$ IR vs. sham, [b] $p < 0.05$ IR-ARG vs. IR

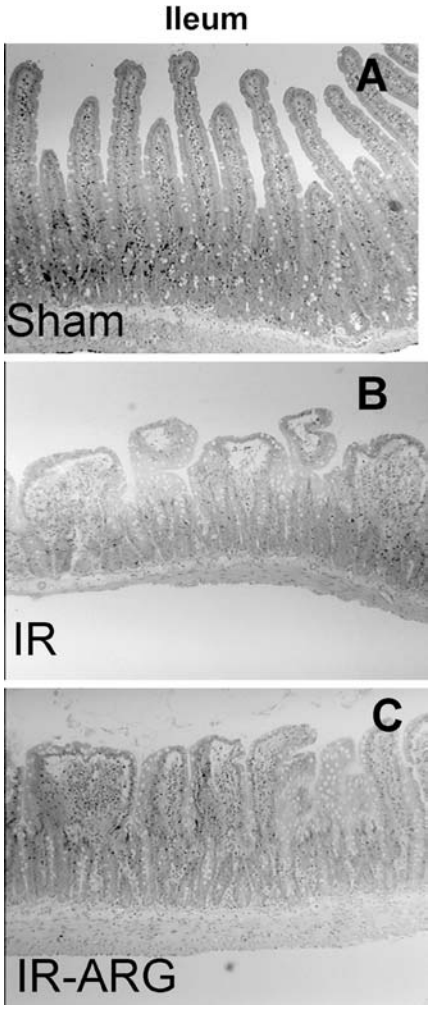

Fig. 2 Low-power photomicrographs of the full-thickness hematoxylin-eosin stained sections of distal ileum in **(a)** sham, **(b)** ischemia–reperfusion, and **(c)** ischemia–reperfusion rats treated with oral ARG. Distal ileum section from sham rats shows the normal architecture of the intestinal epithelium. Distal ileum from IR rats demonstrates subepithelial space at villus tip, inflammatory cell infiltration extending through the wall, shortening and loss of villi, and reduction of crypt depth. Distal ileum of IR-ARG rats shows, similar to IR rats, signs of intestinal injury but increasing villus height and crypt depth

In the small bowel segments from sham rats, the mean intestinal injury grade ranged from 0 in jejunum to 0.4 in ileum without significant variation. IR injury (group B) led to significant increase in the mean intestinal injury grade in jejunum (0.8 ± 0.2 vs. 0, $p < 0.05$) and ileum (1.8 ± 0.5 vs. 0.4 ± 0.2, $p < 0.05$) compared with sham animals. Oral ARG did not significantly change the injury grade in either jejunum or ileum compared with IR animals (Table 2, Fig. 2).

Cellular proliferation and apoptosis

A significant decrease in enterocyte proliferation occurred following IR injury (group B) in jejunum (15%, $p < 0.05$) and ileum (23%, $p < 0.05$) when compared with sham animals (group A) (Table 3). ARG-treated animals (group C) showed a significant increase in the enterocyte proliferation rate in jejunum (39%, $p < 0.05$) and ileum (48%, $p < 0.05$) compared with IR animals (group B).

The frequency of apoptotic cells was increased in the animals after IR insult in ileum (tenfold, $p < 0.05$) compared with sham animals (Table 3). The AI decreased following ARG administration in ileum (threefold, $p < 0.05$) compared with IR-untreated animals. Although not statistically significant, the trend was the same in jejunal apical villi in IR-ARG animals compared with IR rats.

Discussion

The mechanisms of intestinal injury following an ischemia–reperfusion event include nonspecific damage induced by the ischemia per se and damage caused by reactive oxygen species following reperfusion [7, 23]. The identification of factors that prevent mucosal injury during reperfusion might suggest new therapeutic strategies for maintaining mucosal integrity of the gastrointestinal tract and in improving the outcome in patients following IR event. One such mediator might be NO, which is synthesized endogenously by nitric oxide synthase from ARG [22]. Several studies have suggested that endogenous formation of NO maintains the mucosal integrity of the intestine and protects the gut from injuries from bloodborne toxins and tissue-destructive mediators [8, 18, 19].

It has been reported previously that at low concentrations, NO may have a protective physiologic function, whereas high NO production may cause intestinal injury.

The positive effect of inhaled NO in preventing pulmonary damage induced by intestinal IR has been reported by several investigators [20]. There are few controversial studies concerning the effects of NO and its precursor ARG on intestinal recovery following IR. In a recent study, Khanna and coworkers have demonstrated that intraluminal nitroglycerin, which is an exogenous NO donor, produces several beneficial local and systemic effects in a rat model of intestinal IR [9].

Table 3 Effect of oral arginine on enterocyte proliferation and apoptosis in a rat model of intestinal IR injury (*IR* ischemia-reperfusion, *ARG* arginine)

Parameters	Sham ($n = 15$)	IR ($n = 15$)	IR-ARG ($n = 15$)
Proliferation index (BRDU-positive cells/10 crypts)			
Jejunum	256 ± 14	217 ± 16^{a}	$301 \pm 20^{a,b}$
Ileum	222 ± 7	170 ± 9^{a}	$252 \pm 14^{a,b}$
Apoptotic index (TUNEL-positive cells/10 villi)			
Jejunum	0.4 ± 0.2	1.8 ± 0.1	0.5 ± 0.3
Ileum	0.2 ± 0.1	2.2 ± 0.9^{a}	0.7 ± 0.3^{b}

[a] $p < 0.05$ IR vs. sham, [b] $p < 0.05$ IR-ARG vs. IR

Luo et al. have shown that pretreatment with L-NAME, a specific inhibitor of NO production, exacerbates intestinal mucosal injury and increases intestinal permeability following bowel IR in the rat [13]. Ward et al. [21] have shown that L-arginine given intravenously prior to ischemia as well as intraluminal L-arginine given during the reperfusion period inhibited mucosal injury caused by IR. Cellular mechanisms of enterocyte turnover (enterocyte proliferation and apoptosis) were not studied in this experiment [21]. The mechanisms by which oral ARG preserved intestinal mucosa from IR injury is unclear; however, overproduction of NO from ARG may be considered as one of them. NO might attenuate various aspects of IR injury through scavenging the oxygen-derived free radical superoxide anion [15], inactivation of xantine oxidase and decrease in xantine oxidase/xantine dehydrogenase ratio [2], or by a direct inhibitory effect on neutrophil activation [11].

We designed the present study in order to evaluate the effects of oral ARG on intestinal recovery following IR injury in the rat and to determine the mechanisms by which ARG affects enterocyte turnover, including its effect on cell proliferation and death via apoptosis. Our data demonstrate that ischemia and reperfusion have obvious damaging effects on intestinal mucosa. The observed decreased bowel and mucosal weight, decreased mucosal DNA and protein, and decreased villus height and crypt depth in this model support this conclusion. This impaired effect was accompanied by a decrease in enterocyte production and increased enterocyte loss via apoptosis. Both mechanisms may be responsible for decreased enterocyte mass.

Results of the present study show that dietary ARG did not protect the intestinal mucosa from damage caused by IR. Both IR groups (treated with oral ARG and nontreated) manifested similar intestinal injury grade, suggesting similar degrees of intestinal damage. However, exposure to oral ARG significantly enhanced intestinal recovery following an IR event. This is evident from the significant increase in bowel and mucosal weight in all three segments, increased DNA content in ileum, and increased mucosal protein in jejunum and ileum. Increased mucosal weight without changes in mucosal surface area may suggest that mucosal hyperplasia rather than bowel enlargement or intestinal muscle hypertrophy is responsible for the increased intestinal mass. An increase in mucosal DNA and protein along with hypertrophy of the individual cells, which we have demonstrated morphometrically, is characteristic of tissues undergoing increased cell proliferation or repair. Histologically, marked increases in villus height and crypt depth in both jejunum and ileum suggest increased absorptive surface area and closely correlate with increased cell mass. The present data also suggest that ARG increases mucosal proliferation in functioning intestine, as demonstrated by the increased cell proliferation index. The cell apoptosis rate decreased significantly in ileum following oral ARG administration, which may represent an additional mechanism that maintains mucosal structure following IR. Our observations are consistent with the data of Raul and coworkers, who have shown that administration of ARG did not prevent ischemic damage but accelerated morphological repair and enhanced cell proliferation and polyamine content in a rat model of IR [14].

In conclusion, IR caused a marked intestinal mucosal injury in the rat. Decreased enterocyte proliferation and increased cell apoptosis may be responsible for this negative effect. Exposure to oral ARG did not prevent ischemic damage. However, exposure to oral ARG significantly improved intestinal recovery and accelerated the repair of the intestinal mucosa following IR.

References

1. Chomszynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15:532–536
2. Cote CG, Yu FS, Zulueta JJ, Vosatka RJ, Hassoun PM (1996) Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide. Am J Physiol 271:L869–L874
3. Cynober L (1994) Can arginine and ornitine support gut functions? Gut 35(suppl 1):42–45
4. Cynober L, Le Boucher J, Vasson MP (1995) Arginine metabolism in mammals. Nutr Biochem 6:402–413
5. Dowling RH, Booth CC (1967) Structural and functional changes following small intestinal resection in the rats. Clin Sci 32:139–149
6. Fukuyama K, Iwakiri R, Noda T, Kojima M, Utsumi H, Tsunada S, Sakata H, Ootani A, Fujimoto K (2001) Apoptosis induced by ischemia–reperfusion and fasting in gastric mucosa compared to small intestinal mucosa in rats. Dig Dis Sci 46:545–549
7. Granger DN, Hollwarth ME, Parks DA (1986) Ischemia reperfusion injury: role of oxygen-derived free radicals. Acta Physiol Scand 5(suppl):47–48
8. Gurbuz AT, Kunzelman J, Ratzer EE (1998) Supplemental dietary arginine accelerates intestinal mucosal regeneration and enhances bacterial clearance following radiation enteritis in rats. J Surg Res 74:149–154
9. Khanna A, Rossman J, Caty MG, Fung HL (2003) Beneficial effects of intraluminal nitroglycerin in intestinal ischemia–reperfusion injury in rats. J Surg Res 114:15–24
10. Kirk SJ, Barbul A (1990) Role of arginine in trauma, sepsis and immunity. JPEN 14(suppl):226–229
11. Kubes P, Suzuki M, Granger DN (1991) Nitric oxide. An endogenous modulator of leukocyte adhesions. Proc Natl Acad Sci USA 88:4651
12. Luo CC, Shih HH, Chiu CH, Ma WC, Chung HY (2004) Reduced apoptosis in newborn compared to adult rat intestine after ischemia–reperfusion injury. Biol Neonate 85:90–93
13. Luo CC, Chen HM, Chiu CH, Lin JN, Chen JC (2001) Effect of *N*-nitro-L-arginine methyl ester on intestinal permeability following intestinal ischemia–reperfusion injury in a rat model. Biol Neonate 80:60–63
14. Raul F, Galluser, Schleiffer R, Gosse F, Hasselmann M, Seiler N (1995) Beneficial effects of L-arginine on intestinal epithelial resuscitation after ischemic damage in rats. Digestion 56:400–405
15. Rubanyi GM, Ho EH, Cantor EH, Lumma WC, Botelho LH (1991) Cytoprotective function of nitric oxide: inactivation of superoxide radicals produced by human leukocytes. Biochem Biophys Res Commun 181:1392–1397
16. Schleiffer R, Raul F (1996) Prophylactic administration of L-arginine improves the intestinal barrier function after mesenteric ischaemia. Gut 39:194–198

17. Suchner U, Heyland DK, Peter K (2002) Immune-modulatory actions of arginine in the critically ill. Br J Nutr 87(suppl 1):S121–S132
18. Sukhotnik I, Mogilner J, Krausz MM, Lurie M, Shiloni E (2004) Oral arginine reduces gut mucosal injury caused by lipopolysaccharide endotoxemia in a rat. J Surg Res 122:256–262
19. Sukumar P, Loo A, Magur E (1997) Dietary supplementation of nucleotides and arginine promotes healing of small bowel ulcers in experimental ulcerative ileitis. Dig Dis Sci 42:1530–1536
20. Terada LS, Mahr NN, Jacobson ED (1996) Nitric oxide decreases lung injury after intestinal ischemia. J Appl Physiol 81:2456–2460
21. Ward DT, Lawson SA, Gallagher CM, Conner WC, Shea-Donohue T (2000) Sustained nitric oxide production via L-sarginine administration ameliorates effects of intestinal ischemia–reperfusion. J Surg Res 89:13–19
22. Wu G, Morris SM (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17
23. Zimmerman BJ, Granger DN (1994) Mechanisms of reperfusion injury. Am J Med Sci 307:284–292