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Arginyl-Glutamine Dipeptide or Docosahexaenoic Acid Attenuates Hyperoxia-induced Small Intestinal Injury in Neonatal Mice

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Abstract

Background and Objective—Supplementation studies of glutamine, argi-nine, and docosahexaenoic acid (DHA) have established the safety of each of these nutrients in neonates; however, the potential for a more stable and soluble dipeptide, arginyl-glutamine (Arg-Gln) or DHA with anti-inflammatory properties, to exert benefits on hyperoxia-induced intestinal injury has not been investigated. Arg-Gln dipeptide has been shown to prevent retinal damage in a rodent model of oxygen-induced injury. The objective of the present study was to investigate whether Arg-Gln dipeptide or DHA could also attenuate markers of injury and inflammation to the small intestine in this same model.

Methods—Seven-day-old mouse pups were placed with their dams in 75% oxygen for 5 days. After 5 days of hyperoxic exposure (P₇–P₁₂), pups were removed from hyperoxia and allowed to recover in atmospheric conditions for 5 days (P₁₂–P₁₇). Mouse pups received Arg-Gln (5 g · kg⁻¹ · day⁻¹) or DHA (5 g · kg⁻¹ · day⁻¹) or vehicle orally started on P₁₂ through P₁₇. Distal small intestine (DSI) histologic changes, myeloperoxidase (MPO), lactate dehydrogenase (LDH), inflammatory cytokines, and tissue apoptosis were evaluated.

Results—Hyperoxic mice showed a greater distortion of overall villus structure and with higher injury score ($P < 0.05$). Arg-Gln dipeptide and DHA supplementation groups were more similar to the room air control group. Supplementation of Arg-Gln or DHA reduced hyperoxia-induced MPO activity ($P < 0.05$). Supplementation of Arg-Gln or DHA returned LDH activity to the levels of control. Hyperoxia induced apoptotic cell death in DSIs, and both Arg-Gln and DHA reversed this effect ($P < 0.05$).

Conclusions—Supplementation with either Arg-Gln or DHA may limit some inflammatory and apoptotic processes involved in hyperoxic-induced intestinal injury in neonatal mice.

Keywords

arginine; dipeptide; docosahexaenoic acid; glutamine; intestinal injury

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Necrotizing enterocolitis (NEC) is one of the most common and devastating diseases found in premature infants in neonatal intensive care units, with a 10% to 50% mortality rate (1). Previous studies have demonstrated that intestinal barrier malfunction plays an important role in the development of NEC (1,2). Only 1 layer of intestinal epithelial cells separates a vast array of microbes and antigens from a greatly immunoreactive subepithelium. Disruption of this barrier can lead to severe inflammatory conditions such as bacterial translocation, NEC, and sepsis (2–5). Oxidative stress is one of the risk factors implicated in the pathogenesis of NEC (6). Premature infants often need high concentrations of oxygen. Hyperoxia can be considered to be an oxidative stress condition that affects the development of the intestine (7). In addition, premature infants and critically ill neonates often do not obtain optimal nutrients via the intestinal tract, which predisposes them to mucosal breakdown and inflammation.

Glutamine (Gln), the most abundant amino acid in the human body, contributes to the maintenance of the intestinal barrier functions and intercellular junctions (8–11). Gln supplementation in humans and animals has been demonstrated to decrease the proinflammatory response (12–14) and prevents cytokine-induced apoptosis in intestinal epithelial cells (15,16). Low plasma concentrations of Gln have been associated with a higher incidence of NEC (17); however, Gln undergoes hydrolysis in aqueous solution, and therefore, free Gln is not a component of parenteral amino acid solutions (18).

Arginine (Arg) is the substrate for nitric oxide synthesis, a potent vasodilator and anti-inflammatory mediator known to influence mucosal integrity, gut healing, and blood pressure regulation (19). Arg also serves as a precursor for the synthesis of other amino acids, including glutamine (20). The hospitalized premature infant experiences numerous stresses that increase the use of critical amino acids while simultaneously exceeding endogenous biosynthetic pathways (20). Although free Arg is provided in parenteral amino acid solutions, the concentration administered may be insufficient to meet the needs of critically ill premature or very-low-birth-weight infants. Low plasma Arg concentrations are associated with NEC (17,21). Arg supplementation reduced the incidence of NEC in premature infants (22) and attenuated NEC-induced intestinal tissue damage in mice (23).

Docosahexaenoic acid (DHA), a long-chain fatty acid, is most abundant in the brain and retina. Although there is considerable evidence suggesting that extremely premature and very-low-birth-weight infants have increased DHA requirements in the immediate postnatal period, the provision of adequate DHA remains a critical gap for this population (24). Moreover, parenteral lipid emulsions contain high concentrations of proinflammatory omega-6 fatty acids and negligible concentrations of preformed DHA (25). The lack of anti-inflammatory omega-3 long-chain fatty acids, such as preformed DHA, may predispose the neonate to acute inflammatory conditions (24). Previous investigations suggest that omega-3 fatty acids may ameliorate inflammation via their action on transcription factors such as nuclear factor-kappa B (NF- κ B) and peroxisome proliferator-activated receptor- γ (26). Lu et al (27) found that DHA supplementation blocked platelet-activating-factor-induced apoptosis in intestinal epithelial cells and reduced the incidence of NEC in neonatal rats (28).

Arginyl-glutamine (Arg-Gln) dipeptide is an aqueous stable source of glutamine with greater solubility compared with each individual amino acid (29). There is a theoretical benefit of enhanced absorption of the dipeptide compared with single amino acids by virtue of oligopeptide transporters found in both small and large intestines (30).

We have shown that intraperitoneal administration of Arg-Gln dipeptide reduced abnormal retinal neovascularization and vascular leakage in a mouse model of oxygen-induced

retinopathy (31); however, the effects of enteral supplementation of the dipeptide Arg-Gln on the small intestine are unknown. We hypothesized, therefore, that Arg-Gln dipeptide or DHA would protect the neonatal intestine from oxygen-induced injury by reducing inflammation and apoptosis.

METHODS

Animals and Procedures

All of the procedures were approved by the institutional animal care and use committee of the University of Florida. C57BL/6/J timed-pregnant mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Hyperoxic Exposure and Recovery

We conducted 2 separate but related studies (see the online-only figure, which shows the time course of hyperoxic-exposure/recover study, at <http://links.lww.com/MPG/A74>). To directly compare the effects of hyperoxic exposure on tissue injury with the room air condition, 1 group of 7-day-old mouse pups ($n = 8$) were placed with their nursing dams in a 75% oxygen atmosphere for 5 days, whereas a separate group of age-matched pups ($n = 10$) were maintained in room air for 5 days to serve as normoxic controls. On postnatal day 12 (P₁₂), pups were removed from oxygen and both groups were immediately euthanized.

We next investigated the potential for enteral nutrition support, as Arg-Gln or DHA, to attenuate hyperoxic-induced tissue injury. Seven-day-old mice ($n = 31$) were placed in a 75% oxygen atmosphere for 5 days. The pups were then removed from oxygen and allowed to recover in room air for 5 days (P₁₂–P₁₇). From P₁₂ to P₁₇, animals were assigned to receive daily supplementation of either Arg-Gln ($5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$; $n = 12$) or DHA ($5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$; $n = 12$) by oral gavage twice per day. Water with 5% dextrose served as a vehicle. A third group of animals ($n = 7$), designated as hyperoxia, received the vehicle orally twice per day. The physiologic dose of Arg-Gln and the high dose of DHA were chosen based on the previous studies (32–34). A group of age-matched animals maintained in room air served as controls ($n = 6$). On postnatal day 17 (P₁₇), all of the pups were euthanized by injection of a lethal dose of a combination of ketamine (70 mg/kg body weight) and xylazine (15 mg/kg body weight).

Tissue Preparation

At sacrifice, blood was collected from the retro-orbital sinus using a microtube. The distal small intestines (DSIs) were removed, and a 1-cm DSI section next to cecum was fixed in 10% neutral-buffered formaldehyde for histologic analysis. The plasma and the rest of the DSIs were snap frozen in liquid nitrogen immediately upon removal and stored at -80° for later analysis.

Intestinal Morphology

The intestinal injury was evaluated using a semiquantitative scoring system ranging from 0 to 4 modified by Arumugam et al (35).

Myeloperoxidase Activity

Myeloperoxidase (MPO) activity, an indicator of neutrophil accumulation and a marker of tissue injury, was determined by a standard enzymatic procedure, as previously described (36).

Lactate Dehydrogenase Activity

Lactate dehydrogenase (LDH) activities in plasma and tissue cytoplasmic fractions were determined spectrophotometrically using a commercially available assay (Sigma-Aldrich, St Louis, MO) according to the manufacturer's instructions. The concentration of LDH was expressed as optical density per milligram (OD/mg) of protein.

Enzyme-linked Immunosorbent Assays for Interleukin-6 and CXCL1/KC

To evaluate proinflammatory cytokines in the DSI, interleukin (IL)-6 levels were determined using the OptEIA mouse IL-6 enzyme-linked immunosorbent assay (ELISA) (BD Biosciences, San Diego, CA) whereas KC levels were quantified with the DuoSet mouse CXCL1/KC ELISA (R&D Systems; Minneapolis, MN). In both assays, absorbance was determined at 450 nm and concentrations were calculated from a linear standard curve. Cytokine concentrations were expressed as picograms per milligram of protein.

Cell Death Detection ELISA

DNA fragmentation in DSI homogenized supernatant was detected by an ELISA that is specific for histone-associated cytosolic DNA. The apoptosis ELISA was performed by using a cell death detection ELISA kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Statistical Analysis

GraphPad Prism (GraphPad Software Inc, La Jolla, CA) was used to analyze the results. When the data passed the normality test, they were represented as mean $\pm$ SEM. One-way analysis of variance was used to evaluate differences among groups on P₁₇ data, and followed by a post-hoc Tukey test. An unpaired Student t test was used on the P₁₂ data comparisons. If the data failed the normality test, then they were evaluated and represented as median and interquartile ranges and a nonparametric test was used: a Kruskal-Wallis test for P₁₇ data with the Dunn test for individual contrasts and a Mann-Whitney test for P₁₂ data, respectively. A value of $P < 0.05$ was considered significant.

RESULTS

Effects of Hyperoxia and Nutritional Intervention on DSI Structure and Development

To compare intestinal morphology among the dietary groups, DSI villus morphology was evaluated using light microscopy on hematoxylin and eosin (H&E) stained slides (Fig. 1A). Five days of oxygen exposure did not result in a significant morphology change on P₁₂ (data not shown); however, with a 5-day exposure to hyperoxic conditions followed by a 5-day recovery period in room air on P₁₇, DSIs from the hyperoxia group showed a greater distortion of overall villus structure (Fig. 1A) and higher injury score (Fig. 1B, $P < 0.05$) than the other groups. Animals with either Arg-Gln dipeptide or DNA supplementation were more similar to the room air control group (Fig. 1).

Effects of Hyperoxia and Nutritional Intervention on Biochemical Markers of Inflammation and Tissue Injury

Hyperoxia-induced inflammation and neutrophil activation, as indicated by MPO activity, was also moderated by Arg-Gln dipeptide and DHA. To determine whether the DSI injury in response to hyperoxic exposure was associated with neutrophil activation, DSI MPO activity was measured (Fig. 2). On P₁₇, hyperoxia led to an induction of MPO activity (0.015 U/mg protein; interquartile range 0.0023–0.040 U/mg) compared with room air controls (0.0019 U/mg protein; range 0.0014–0.0024 U/mg). Arg-Gln or DHA supplementation brought

MPO back close to control levels, with DHA being more effective ($P < 0.05$) than Arg-Gln. MPO activities did not differ in DSI on P₁₂ (data not shown).

Extracellular appearance of LDH or LDH release is used to detect cell damage. On P₁₇, LDH activity in plasma was elevated significantly in the hyperoxia group. These changes were reversed by either Arg-Gln or DHA supplementation (Fig. 3A, all $P < 0.05$). LDH tissue activity relates to glycolysis (37). Tissue LDH activities were reduced in the hyperoxia group compared with air control mice in DSI (Fig. 3B, $P < 0.05$), suggesting that LDH was inactivated by hyperoxic exposure. Supplementation of Arg-Gln or DHA reversed this effect with an increase in LDH activity in DSIs by 39.3% and 55.8%, respectively (Fig. 3B, $P < 0.05$). Differences were not found in proinflammatory cytokines, IL-6, and cytokine-induced neutrophil chemoattractant-1 among the groups (data not shown).

Effects of Hyperoxic Exposure and Nutritional Intervention on DSI Apoptosis

To determine whether hyperoxia-induced DSI injury is associated with apoptosis, histone-associated DNA fragmentation, a marker of apoptosis, was evaluated in the cytoplasm using a quantitative cell death ELISA (Fig. 4). There was an increase in cell death on P₁₂ (Fig. 4A, 0.918 OD/mg protein, range 0.72–2.28 OD/mg protein, $P < 0.05$) compared with room air controls (4.79 OD/mg, range 2.89–6.68 OD/mg protein). On day 17, apoptotic cell death was also higher in hyperoxia group (Fig. 4B, 10.84 OD/mg, range 7.90–14.03 OD/mg) than in normoxia animals (3.50 OD/mg, range 1.30–7.90 OD/mg, $P < 0.05$), whereas Arg-Gln or DHA-supplemented animals had a level of DNA fragmentation similar to air controls. Our data also suggested a developmental effect on DSI apoptosis when comparing cell death of day 12 to day 17.

DISCUSSION

The model, although not an established model for NEC, was chosen based on our previous work (31) showing the benefits of Arg-Gln dipeptide on oxygen-induced retinopathy in this mouse model. In this study, we wanted to determine whether other organs could be affected and whether the injury to intestine could be ameliorated as well. The tissue injury occurs secondary to the hyperoxia and then relative hypoxia. It has been reported that hyperoxia altered villus structure and decreased nitric oxide synthase II protein concentration in rat pups (7), which may lead to the disruption of the intestinal barrier function and make the infant intestine more susceptible to bacterial insult. Therefore, the pathogenesis of our study model could be the response to the oxidative stress and then relative hypoxia, with damage of barrier function followed by inflammation.

Our study demonstrates that 5 days of hyperoxic oxygen exposure followed by 5 days of recovery in atmospheric conditions result in a villus injury in the DSI. Supplementation of Arg-Gln or DHA on P₁₇ prevented morphologic evidence of villus injury. Arg-Gln dipeptide or DHA also prevented leukocyte infiltration and enzymatic tissue destruction, determined by myeloperoxidase levels. Although extracellular appearance of LDH or LDH release is used to detect cell damage, LDH tissue activity relates to glycolysis (37). LDH activity was increased in plasma in the hyperoxia group, suggesting cell damage caused by hyperoxia; however, LDH activity was reduced in DSI in hyperoxia, suggesting that LDH is inactivated by hyperoxic exposure and the slowing down of glycolysis. Animals supplemented with Arg-Gln or DHA had normal LDH activity levels. Evaluation of apoptotic markers showed that histone-associated DNA fragmentation was higher in the hyperoxia group than in normoxic animals, whereas dipeptide Arg-Gln or DHA supplementation reduced hyperoxia-induced cell death.

A limitation of our study is that we did not investigate the serum Arg or Gln levels before or after administration of dipeptide and do not have direct evidence that dipeptide was hydrolyzed. Studies from other researchers demonstrate that the serum Gln level was higher in alanyl-glutamine dipeptide-supplemented rats, suggesting that oral administration of Gln in dipeptide form is an efficient way to provide Gln (38). This effect may be attributed to the mechanism that enterocytes have a more efficient transport system for the absorption of dipeptides than free amino acids (39,40). In our study, we assumed that Arg-Gln was absorbed by the intestinal epithelial cells and used by the small intestine as a donor for amide nitrogen in nucleotide synthesis, thus playing an important role in preventing intestinal tissue injury from hyperoxia.

Our results provide insight into the mechanisms by which Arg-Gln dipeptide and DHA may support normal intestinal development in infants exposed to hyperoxic conditions. Gln has been shown to attenuate sepsis-induced lung injury by suppressing NF- κ B activation and reducing the production of proinflammatory cytokines (41–43). Gln may also preserve metabolic function by suppressing cellular apoptosis and protecting against oxidant-induced damage by preserving cellular glutathione stores (42). Gln is necessary to maintain intestinal tight junction stabilization and barrier function and is a signal to intestinal cells to enhance cell survival via mitogen-activated protein kinases (44). Rodent models of ischemia-reperfusion-mediated gut injury also demonstrated that Gln minimized mucosal injury by preserving the actin cytoskeleton (45). Arg is a potent vasodilator and anti-inflammatory mediator known to influence mucosal integrity, gut healing, and blood pressure regulation (19). Decreased concentration of nitric oxide is proposed as one of the possible cellular mechanisms for NEC. Arg can act as a substrate for the production of nitric oxide in the tissues, and Arg supplementation may help in preventing NEC (22,23).

Previous studies suggest that extremely premature, very-low-birth-weight infants and individuals under stress conditions have increased DHA requirements (24). DHA may ameliorate inflammation via their action on transcription factors such as NF- κ B and peroxisome proliferator-activated receptor- γ (26). DHA supplementation blocked platelet-activating factor-induced apoptosis in intestinal epithelial cells (27) and also blocked platelet-activating factor-induced TLR4 and platelet-activating factor receptor mRNA expression. These effects may, in part, explain the protective effect of polyunsaturated fatty acid on neonatal NEC in neonatal rats (28).

Several studies in premature infants have established the safety and efficacy of Arg and Gln when administered as individual nutrients. For example, in various studies in premature or very-low-birth-weight infants, Gln supplementation, either parenterally or enterally administered, has been reported to reduce hospital costs (46), decrease morbidity (47), and reduce time on mechanical ventilation (48). Moreover, plasma Gln levels may be lower in premature infants who develop NEC (17). Other studies have suggested long-term benefits of enteral glutamine supplementation (49). Similarly, plasma Arg levels are reduced or low in infants with NEC (17,48). Arg supplementation has been reported to decrease the incidence of all stages of NEC (22). The common suggestion in each of these reports is that premature infants are deficient in Gln, Arg, or both; however, high doses of Arg showed harmful effects in an intestinal cell culture model (50) and a rat intestinal ischemia/reperfusion injury model (45). Our study suggested a safe dose for Arg-Gln dipeptide supplementation in this mouse model.

To our knowledge, the effects of Arg-Gln dipeptide or DHA supplementation in a model of hyperoxia-induced intestinal injury have not been evaluated. Our data suggest that Arg-Gln and DHA may affect clinically relevant outcomes in extremely premature infants experiencing significant physiologic stress. In light of our findings, the lack of these

nutrients in the diet of this population in the immediate postnatal period is particularly relevant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Emami CN, Petrosyan M, Giuliani S, et al. Role of the host defense system and intestinal microbial flora in the pathogenesis of necrotizing enterocolitis. *Surg Infect (Larchmt)*. 2009; 10:407–17. [PubMed: 19943775]
2. Hackam DJ, Upperman JS, Grishin A, et al. Disordered enterocyte signaling and intestinal barrier dysfunction in the pathogenesis of necrotizing enterocolitis. *Semin Pediatr Surg*. 2005; 14:49–57. [PubMed: 15770588]
3. Soeters PB, Luyer MD, Greve JW, et al. The significance of bowel permeability. *Curr Opin Clin Nutr Metab Care*. 2007; 10:632–8. [PubMed: 17693749]
4. Berkes J, Viswanathan VK, Savkovic SD, et al. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut*. 2003; 52:439–51. [PubMed: 12584232]
5. Farhadi A, Banan A, Fields J, et al. Intestinal barrier: an interface between health and disease. *J Gastroenterol Hepatol*. 2003; 18:479–97. [PubMed: 12702039]
6. Deulofeut R, Critz A, Adams-Chapman I, et al. Avoiding hyperoxia in infants < or = 250 g is associated with improved short- and long-term outcomes. *J Perinatol*. 2006; 26:700–5. [PubMed: 17036032]
7. Giannone PJ, Bauer JA, Schanbacher BL, et al. Effects of hyperoxia on postnatal intestinal development. *Biotech Histochem*. 2007; 82:17–22. [PubMed: 17510810]
8. Li N, Lewis P, Samuelson D, et al. Glutamine regulates Caco-2 cell tight junction proteins. *Am J Physiol Gastrointest Liver Physiol*. 2004; 287:G726–33. [PubMed: 15130874]
9. Seth A, Basuroy S, Sheth P, et al. L-Glutamine ameliorates acetaldehyde-induced increase in paracellular permeability in Caco-2 cell monolayer. *Am J Physiol Gastrointest Liver Physiol*. 2004; 287:G510–7. [PubMed: 15331350]
10. Potsic B, Holliday N, Lewis P, et al. Glutamine supplementation and deprivation: effect on artificially reared rat small intestinal morphology. *Pediatr Res*. 2002; 52:430–6. [PubMed: 12193680]
11. DeMarco VG, Li N, Thomas J, et al. Glutamine and barrier function in cultured Caco-2 epithelial cell monolayers. *J Nutr*. 2003; 133:2176–9. [PubMed: 12840174]
12. Li N, Liboni K, Fang MZ, et al. Glutamine decreases lipopolysaccharide-induced intestinal inflammation in infant rats. *Am J Physiol*. 2004; 287:G726–33.
13. Huang Y, Li N, Liboni K, et al. Glutamine decreases lipopolysaccharide-induced IL-8 production in Caco-2 cells through a non-NF- κ B p50 mechanism. *Cytokine*. 2003; 22:77–83. [PubMed: 12849706]
14. Liboni K, Li N, Neu J. Mechanism of glutamine-mediated amelioration of lipopolysaccharide-induced IL-8 production in Caco-2 cells. *Cytokine*. 2004; 26:57–65. [PubMed: 15050605]
15. Evans ME, Jones DP, Ziegler TR. Glutamine prevents cytokine-induced apoptosis in human colonic epithelial cells. *J Nutr*. 2003; 133:3065–71. [PubMed: 14519785]
16. Larson SD, Li J, Chung DH, et al. Molecular mechanisms contributing to glutamine-mediated intestinal cell survival. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293:G1262–71. [PubMed: 17916648]

17. Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. *J Pediatr*. 2000; 137:785–93. [PubMed: 11113834]
18. Khan K, Hardy G, McElroy B, et al. The stability of l-glutamine in total parenteral nutrition solutions. *Clin Nutr*. 1991; 10:186–92. [PubMed: 16839917]
19. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev*. 1991; 43:109–42. [PubMed: 1852778]
20. Wu G, Jaeger LA, Bazer FW, et al. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *J Nutr Biochem*. 2004; 14:442–51. [PubMed: 15302078]
21. Zamora SA, Amin HJ, McMillan DD, et al. Plasma l-arginine concentrations in premature infants with necrotizing enterocolitis. *J Pediatr*. 1997; 131:226–32. [PubMed: 9290608]
22. Amin HJ, Zamora SA, McMillan DD, et al. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. *J Pediatr*. 2002; 140:425–31. [PubMed: 12006956]
23. Akisu M, Ozmen D, Baka M, et al. Protective effect of dietary supplementation with l-arginine and l-carnitine on hypoxia/reoxygenation-induced necrotizing enterocolitis in young mice. *Biol Neonate*. 2002; 81:260–5. [PubMed: 12011570]
24. Lapillonne A, Jensen CL. Reevaluation of the DHA requirement for the premature infant. *Prostagl Leukotr Essen Fatty Acids*. 2009; 81:143–50.
25. Driscoll DF, Bistran BR, Demmelmair H, et al. Pharmaceutical and clinical aspects of parenteral lipid emulsions in neonatology. *Clin Nutr*. 2008; 27:497–508. [PubMed: 18582994]
26. Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie*. 2009; 91:791–5. [PubMed: 19455748]
27. Lu J, Caplan MS, Li D, et al. Polyunsaturated fatty acids block platelet-activating factor-induced phosphatidylinositol 3 kinase/Akt-mediated apoptosis in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G1181–90. [PubMed: 18356536]
28. Lu J, Jilling T, Li D, et al. Polyunsaturated fatty acid supplementation alters proinflammatory gene expression and reduces the incidence of necrotizing enterocolitis in a neonatal rat model. *Pediatr Res*. 2007; 61:427–32. [PubMed: 17515866]
29. Furst P. New developments in glutamine delivery. *J Nutr*. 2001; 131:2562S–8. [PubMed: 11533314]
30. Adibi SA, Phillips E. Evidence for greater absorption of amino acids from peptides than from free form in the human intestine. *Clin Res*. 1968; 16:466.
31. Neu J, Afzal A, Pan H, et al. The dipeptide Arg-Gln inhibits retinal neovascularization in the mouse model of oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci*. 2006; 47:3151–5.
32. Meier H, Hoag WG, Mcburney JJ. Chemical characterization of inbred-strain mouse milk. I. Gross composition and amino acid analysis. *J Nutr*. 1965; 85:305–8. [PubMed: 14261842]
33. Motoki T, Naomoto Y, Hoshiba J, et al. Glutamine depletion induces murine neonatal melena with increased apoptosis of the intestinal epithelium. *World J Gastroenterol*. 2011; 17:717–26. [PubMed: 21390141]
34. Beharry S, Ackerley C, Corey M, et al. Long-term docosahexaenoic acid therapy in a congenic murine model of cystic fibrosis. *Am J Physiol Gastrointest Liver Physiol*. 2007; 292:G839–48. [PubMed: 17095751]
35. Arumugam TV, Arnold N, Proctor LM, et al. Comparative protection against rat intestinal reperfusion injury by a new inhibitor of sPLA2, COX-1 and COX-2 selective inhibitors, and an LTC4 receptor antagonist. *Br J Pharmacol*. 2003; 140:71–80. [PubMed: 12967936]
36. Li N, Russell WM, Douglas-Escobar M, et al. Live and heat-killed *Lactobacillus rhamnosus* GG: effects on proinflammatory and anti-inflammatory cytokines/chemokines in gastrostomy-fed infant rats. *Pediatr Res*. 2009; 66:203–7. [PubMed: 19390478]
37. Bin-Jaliah I, Dallak M, Haffor AS. Effect of hyperoxia on the ultra-structural pathology of alveolar epithelium in relation to glutathione peroxidase, lactate dehydrogenase activities, and free radical production in rats, *Rattus norvegicus*. *Ultrastruct Pathol*. 2009; 33:112–22. [PubMed: 19479651]
38. Cruzat VF, Tirapegui J. Effects of oral supplementation with glutamine and alanyl-glutamine on glutamine, glutamate, and glutathione status in trained rats and subjected to long-duration exercise. *Nutrition*. 2009; 25:428–35. [PubMed: 19056244]

39. Ganapathy, V.; Brandsch, M.; Leibach, FH. Intestinal transport of amino ACIK and peptides.. In: Johnson, LR.; Alpers, DH., editors. *The Physiology of the Gastratintestinal Tract*. Raven Press; New York: 1994. p. 1773-93.
40. Grimble GK. The significance of peptides in clinical nutrition. *Annu Rev Nutr.* 1994; 14:419–47. [PubMed: 7946528]
41. Singleton KD, Wischmeyer PE. Glutamine's protection against sepsis and lung injury is dependent on heat shock protein 70 expression. *Am J Physiol Regul Integr Comp Physiol.* 2007; 292:R1839–45. [PubMed: 17234954]
42. Curi R, Lagranha CJ, Doi SQ, et al. Molecular mechanisms of glutamine action. *J Cell Physiol.* 2005; 204:392–405. [PubMed: 15795900]
43. Hubert-Buron A, Leblond J, Jacquot A, et al. Glutamine pretreatment reduces IL-8 production in human intestinal epithelial cells by limiting IkappaB alpha ubiquitination. *J Nutr.* 2006; 136:1461–5. [PubMed: 16702304]
44. Rhoads JM, Wu G. Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids.* 2009; 37:111–22. [PubMed: 19130170]
45. Kozar RA, Verner-Cole E, Schultz SG, et al. The immune-enhancing enteral agents arginine and glutamine differentially modulate gut barrier function following mesenteric ischemia/reperfusion. *J Trauma.* 2004; 57:1150–6. [PubMed: 15625443]
46. Van Marter LJ. Epidemiology of bronchopulmonary dysplasia. *Semin Fetal Neonatal Med.* 2009; 14:358–66. [PubMed: 19783238]
47. Atkinson SA. Special nutritional needs of infants for prevention of and recovery from bronchopulmonary dysplasia. *J Nutr.* 2001; 131:942S–6S. [PubMed: 11238791]
48. Pearson E, Bose C, Snidow T, et al. Trial of vitamin A supplementation in very low birth weight infants at risk for bronchopulmonary dysplasia. *J Pediatr.* 1992; 121:420–7. [PubMed: 1517921]
49. Thomas W, Speer CP. Prevention and treatment of bronchopulmonary dysplasia: current status and future prospects. *J Perinatol.* 2007; 27:S26–32.
50. Rhoads JM, Chen W, Gookin J, et al. Arginine stimulates intestinal cell migration through a focal adhesion kinase dependent mechanism. *Gut.* 2004; 53:514–22. [PubMed: 15016745]

FIGURE 1.

Effects of hyperoxia and nutritional intervention on distal small intestine (DSI) structure and development. A, Hematoxylin and eosin staining slides of DSI villi (scale bar = 40 μm, original magnification ×250) on P₁₇. B, Scatterplot shows DSI injury score on P₁₇ (* $P < 0.05$ vs air, $n = 6, 7, 12,$ and $5,$ respectively). The data are represented as mean ± SEM. DHA = docosahexaenoic acid.

FIGURE 2.

Effects of hyperoxia and nutritional intervention on myeloperoxidase (MPO) activities. On P₁₇, Arg-Gln or docosahexaenoic acid (DHA) supplementation reduced distal small intestine MPO close to control level (* $P < 0.05$, vs hyperoxia, $n = 6, 7, 12$, and 12 , respectively). The data are represented as median and interquartile ranges.

FIGURE 3.

Effects of hyperoxia and nutritional intervention on lactate dehydrogenase (LDH) activities on P₁₇. A, Plasma LDH activity was elevated in the hyperoxia group. These changes were reversed by either Arg-Gln or docosahexaenoic acid (DHA) supplementation (* $P < 0.05$ vs air, ‡ $P < 0.05$ vs hyperoxia; $n = 6, 6, 12,$ and $10,$ respectively). B, Distal small intestine LDH activity was decreased with hyperoxia and reversed by supplementation of Arg-Gln or DHA (* $P < 0.05$ vs air, ‡ $P < 0.05$ vs hyperoxia; $n = 6, 7, 12,$ and $12,$ respectively). The data are represented as mean $\pm$ SEM.

FIGURE 4.

Effects of hyperoxic exposure and nutritional intervention on distal small intestine (DSI) apoptosis. A, Cell death enzyme-linked immunosorbent assay in DSI on P₁₂ (* $P < 0.05$ vs air; $n = 9$ and 6 , respectively). B, Cell death enzyme-linked immunosorbent assay in DSIs on P₁₇ (* $P < 0.05$ vs air; $n = 5, 6, 10$, and 11 , respectively). The data are represented as median and interquartile ranges.