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# Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications

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**Abstract**

Arginine, an amino acid that is nutritionally essential for the fetus and neonate, is crucial for ammonia detoxification and the synthesis of molecules with enormous importance (including creatine, nitric oxide, and polyamines). A significant nutritional problem in preterm infants is a severe deficiency of arginine (hypoargininemia), which results in hyperammonemia, as well as cardiovascular, pulmonary, neurological, and intestinal dysfunction. Arginine deficiency may contribute to the high rate of infant morbidity and mortality associated with premature births. Although hypoargininemia in preterm infants has been recognized for more than 30 years, it continues to occur in neonatal intensive care units in the United States and worldwide. On the basis of recent findings, we propose that intestinal citrulline and arginine synthesis (the major endogenous source of arginine) is limited in preterm neonates owing to the limited expression of the genes for key enzymes (e.g., pyrroline-5-carboxylate synthase, argininosuccinate synthase and lyase), thereby contributing to hypoargininemia. Because premature births in humans occur before the normal perinatal surge of cortisol (an inducer of the expression of key arginine-synthetic enzymes), its administration may be a useful tool to advance the maturation of intestinal arginine synthesis in preterm neonates. Additional benefits of cortisol treatment may include the following: 1) allowing early introduction of enteral feeding to preterm infants, which is critical for intestinal synthesis of citrulline, arginine, and polyamines as well as for intestinal motility, integrity, and growth; and 2) shortening the expensive stay of preterm infants in hospitals as a result of accelerated organ maturation and the restoration of full enteral feeding. Further studies of fetal and neonatal arginine metabolism will continue to advance our understanding of the mechanisms responsible for the survival and growth of preterm infants. This new knowledge will be beneficial for designing the next generation of enteral and parenteral amino acid solutions to optimize nutrition and health in this compromised population. 

*Keywords:* Amino acids; Cortisol; Intestine; Metabolism; Nutrition; Peterm infants

## 1. Introduction

Premature births, a leading cause of infant morbidity and mortality in the United States and worldwide, occur in approximately 10% of all pregnancies and cost more than $2 billion annually to the American health care system [1,2]. Immature infants may have numerous complications including hyperammonemia, respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, and sepsis [3–5]. Preterm infants account for the majority of all neonatal deaths. A significant metabolic problem in the preterm infant is a severe deficiency of arginine (hypoargininemia) [6], which was initially identified over 30 years ago [7,8]. Knowledge of arginine biochemistry and nutrition is very beneficial for optimizing neonatal survival and health in this compromised population.

The small intestine is almost the exclusive source of citrulline for endogenous synthesis of arginine in mammals [9]. Using the neonatal pig, an excellent model for studying infant nutrition, we have recently shown that endogenous synthesis of arginine is crucial for maintaining arginine homeostasis in milk-fed neonates [10]. Further, both metabolic and molecular studies indicate that the underdevelopment of intestinal arginine synthesis may be primarily responsible for hypoargininemia in preterm neonates [11,12]. The major objective of this review is to examine current information related to arginine deficiency in preterm infants as well as underlying biochemical mechanisms and nutritional implications.

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## 2. Metabolic roles of arginine

Young mammals including preterm infants have a particularly high requirement for arginine [13], partly because of the abundance of arginine in tissue proteins and the multiple pathways for arginine utilization. Besides serving as a building block for tissue proteins, arginine plays a critical role in ammonia detoxification [14,15]. This amino acid is an allosteric activator of N-acetylglutamate synthase, the enzyme catalyzing the synthesis of N-acetylglutamate from glutamate and acetyl-CoA. N-acetylglutamate is an allosteric activator of carbamoylphosphate synthase–I (CPS-I), the first enzyme of the urea cycle that converts ammonia and bicarbonate into carbamoylphosphate [15]. In addition, arginine is a physiological precursor for the synthesis of important molecules such as nitric oxide (NO), creatine, and polyamines [6]. NO is the endothelium-derived relaxing factor, a neurotransmitter, a mediator of immune response, and a signal transduction molecule [16]. Recent evidence suggests that NO plays an important role in regulating homeostasis of the fetus and neonate [17,18]. Creatine plays a major role in energy metabolism in skeletal muscle and neuronal cells [9]. Polyamines regulate gene expression, signal transduction, ion channel function, DNA and protein synthesis, apoptosis, as well as cell proliferation and differentiation [6]. Moreover, arginine stimulates the secretion of growth hormone and insulin in mammals including preterm infants [19], thereby playing an important role in regulating protein, lipid, and carbohydrate metabolism. Arginine deficiency results in hyperammonemia as well as cardiovascular, pulmonary, intestinal, immunological, and neurological dysfunction, particularly in preterm infants [6,13]. Therefore, arginine is considered to be a nutritionally essential amino acid for neonates, particularly under such stress conditions as infection and premature birth [6,13,20].

## 3. Arginine deficiency in preterm infants

Arginine deficiency represents a significant metabolic problem in the preterm infant [21–24] (Fig. 1). Snyderman et al. [21] first reported that plasma arginine concentration (34 $\mu$mol/L) is remarkably low in preterm infants fed enterally 2 g protein/kg body wt/day (estimated protein requirements for term infants). Subsequently, Heird et al. [7] and other investigators [8,25–27] discovered that life-threatening hyperammonemia occurred in preterm infants maintained on total parenteral nutrition (TPN) and could be effectively treated by intravenous administration of L-arginine. This finding suggests that hyperammonemia in the neonates results from hypoargininemia rather than an insufficiency of urea cycle enzymes. Batshaw and Brusilow [25] and Batshaw et al. [26] further demonstrated that life-threatening hypoargininemia (plasma [arginine] = 32 $\mu$mol/L) and hyperammonemia syndrome occurred in more than 50% of the preterm infant population.

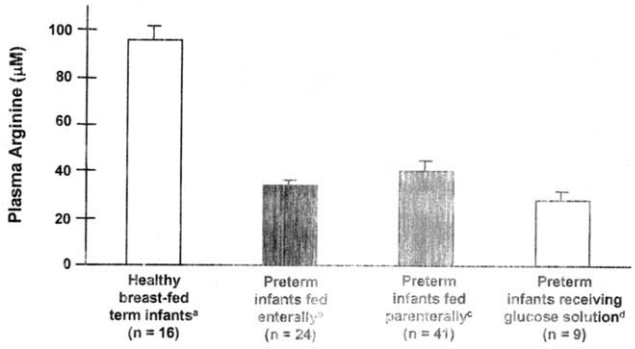


Fig. 1. Arginine deficiency in preterm infants. Values are means ± SEM, with the number of infants given in parentheses. Blood was obtained at day 3 of life for preterm infants receiving parenteral nutrition or glucose infusion. [a]Breast-fed, healthy, term infants [24]. [b]Preterm infants fed enterally 2 g protein/kg body wt/day [21]. [c]Preterm infants parenterally fed Trophamine solution [22]. [d]Preterm infants receiving standard intravenous infusion of 10% glucose solution [23].

Recent studies have shown that hypoargininemia continues to occur in preterm infants in the United States and worldwide [22,23,28–30]. For example, in very low–birth weight infants (<29 weeks of gestation) maintained entirely on parenteral feeding of Freeamine III (Kendall-McGaw Laboratories, Irvine, CA), the mean plasma concentration of arginine remained critically low (19 $\mu$mol/L) at day 3 of life [29]. Preterm infants (<30 weeks of gestation) receiving a standard intravenous infusion of 10% glucose solution are also low in arginine (plasma [arginine] = 28 $\mu$mol/L) [23] (Fig. 1). Zamora et al. [22] recently studied preterm infants (<32 weeks of gestation) maintained primarily on parenteral feeding of Trophamine (Kendall-McGaw Laboratories), which contains the highest amount of arginine among all current parenteral nutrition solutions [31]. These authors reported that the mean plasma concentration of arginine was only 40 $\mu$mol/L at day 3 of life [22]. This value of plasma arginine concentration is <50% of normal plasma concentrations of arginine in healthy, breast-fed, term infants (95.3 $\mu$mol/L) [24] (Fig. 1), and approaches the critical value of plasma arginine concentration (32 $\mu$mol/L) at which hyperammonemia syndrome frequently occurs in the preterm infant population [7,25–27,32]. Hypoargininemia in preterm infants was associated with increased severity of respiratory distress syndrome (the most frequent pulmonary disease) and decreased systemic oxygenation [31]. We [28] and Zamora et al. [30] also found that arginine deficiency was associated with an increased incidence of necrotizing enterocolitis (the most common severe intestinal disease) in preterm infants. These recent findings that hypoargininemia during the first days of life is associated with cardiovascular, pulmonary, and intestinal dysfunction in preterm infants are significant, in view of the recent report that the majority of deaths in preterm infants occur within the first 3 days of life [3]. Importantly, increasing provision of exogenous arginine prevents hyperammonemia and necrotizing enterocolitis in preterm infants [33], persistent pulmonary hypertension (a

potentially lethal condition) in infants with the mean plasma arginine level of 12.5 μmol/L [34], as well as hyperammonemia and death in parenterally fed neonates [20].

Cigarette smoking is a risk factor for spontaneous abortion, and women who smoke have a 2-fold higher risk of delivering a low–birth weight infant who is either premature or small for gestational age [35]. Recently, plasma concentrations of amino acids were measured in maternal and fetal plasma from smoking and nonsmoking mothers [36,37]. Significantly, low levels of plasma arginine (<75% of normal) were found in the fetuses of women who smoked [36]. This defect appears to persist throughout gestation, as concentrations of both citrulline and arginine in the umbilical artery plasma of the newborn infants of smokers were much lower than those of nonsmokers [37]. The low availability of arginine was associated with reduced umbilical blood flow, impaired fetal nutrient delivery, and intrauterine growth retardation [36,37].

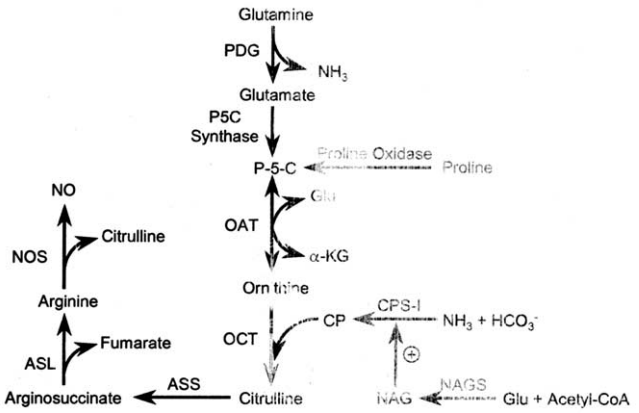


Fig. 2. Arginine synthesis in enterocytes. PDG = phosphate-dependent glutaminase; P5CS = pyrroline-5-carboxylate synthase; OAT = ornithine aminotransferase; OCT, ornithine carbamoyltransferase; ASS = argininosuccinate synthase; ASL = argininosuccinate lyase; CPS I = carbamoylphosphate synthase-I; NAGS = N-acetylglutamate (NAG) synthase; NOS = nitric oxide synthase. Arginine is an allosteric activator of NAGS. Adapted from Wu and Morris [9].

## 4. The essential role of the small intestine in endogenous arginine synthesis in neonates

Although arginine is formed via the urea cycle in periportal hepatocytes, an exceedingly high activity of cytosolic arginase in the liver rapidly hydrolyzes it to urea and ornithine. Thus, the liver is not a site for nutritionally significant net arginine synthesis [9]. This concept is substantiated by the clinical finding that patients receiving liver transplants to correct inherited defects in urea cycle enzymes regain the capacity for hepatic urea synthesis but not for extrahepatic arginine synthesis [38]. Furthermore, there is no net synthesis of citrulline from either glutamine or proline by either the liver or the kidney, because pyrroline-5-carboxylate (P5C) synthase is absent from these two organs [10,39], and because hepatic ornithine aminotransferase (OAT) activity is restricted to the perivenous hepatocytes, which have neither CPS-I nor ornithine carbamoyltransferase (OCT) activity [40]. On the basis of our current knowledge of mammalian arginine metabolism, the enterocytes (epithelial absorptive cells of the small intestine) are the major cells responsible for the net synthesis of citrulline and arginine from glutamine and proline in neonates [9,41]. In support of this view, we found that plasma citrulline concentration decreases by 77% in preterm infants with intestinal dysfunction (necrotizing enterocolitis) compared with healthy infants [42]. Both arterial and enteral glutamine is used for intestinal synthesis of citrulline. However, the small intestine does not take up a significant quantity of arterial glutamate and proline [44], and therefore only the enteral source of these two amino acids provides nitrogen and carbon for citrulline production by enterocytes [9].

Arginine synthesis from glutamine and proline involves phosphate-dependent glutaminase (PDG), proline oxidase, P5C synthase, OAT, OCT, CPS-I, argininosuccinate synthase (ASS), and argininosuccinate lyase (ASL) (Fig. 2). Glutamine and proline are abundant amino acids in fetal and neonatal plasma, as well as in milk [43–45], which is consistent with their role as major substrates for intestinal synthesis of citrulline and arginine. All arginine-synthetic enzymes occur in enterocytes of most mammals, probably with the exception of cats and ferrets [9]. PDG, P5C synthase, proline oxidase, OAT, OCT, and CPS-I are mitochondrial enzymes, whereas ASS and ASL are located in the cytosol, indicating the complex compartmentation of intestinal and whole-body arginine synthesis. Mammalian cDNAs for all of these enzymes have been cloned [46–49], which will facilitate future studies of the molecular regulation of arginine metabolism.

Citrulline generated in the small intestine is not taken up by the liver and is used by extrahepatic tissues for arginine synthesis [9]. Thus, arginine becomes a nutritionally essential amino acid for adult rats after resection of the small intestine [39], and selective inhibition of intestinal citrulline synthesis in vivo severely decreases growth in young rats [50]. In adult animals, the conversion of intestine-derived citrulline into arginine occurs mainly in the kidney [14,51]. However, in neonates whose renal ASL activity is only minimally developed, we have shown that most of the citrulline synthesized in enterocytes is converted locally into arginine by ASS and ASL [10,52,53]. The near absence of arginase activity from neonatal enterocytes maximizes the intestinal release of arginine into systemic circulation [52,54].

## 5. Nutritional significance of intestinal arginine synthesis in neonates

Arginine has recently been recognized to be remarkably deficient in the milk of humans as well as pigs and many

other animal species [40,43,45,55,56]. Recent evidence also indicates that arginine concentration remains low in current pediatric TPN solutions [20,22,28,30]. Thus, endogenous synthesis of arginine is expected to play a crucial role in maintaining arginine homeostasis in neonates fed enterally or parenterally. Our published work indicates that the small intestine of the term piglet is capable of producing nutritionally significant quantities of citrulline and arginine to meet arginine requirements during the first week of life [10,52]. On the basis of arginine needs for tissue protein accretion and arginine intake from the milk, endogenous synthesis of arginine has been estimated to provide ≥60% of total daily arginine requirements in 7-day-old pigs [13]. Our recent study in fetal pigs has also shown that uterine uptake of arginine is insufficient to meet arginine requirements for fetal accretion during the late stage of gestation (days 110–114 of gestation) [57]. Thus, on the basis of indirect evidence, endogenous synthesis of arginine likely plays a crucial role in regulating arginine homeostasis in the rapidly growing fetus and neonate. There is also more direct evidence supporting the idea that endogenous synthesis of arginine is essential for maintaining arginine homeostasis in neonates. First, in 4-day-old suckling pigs, inhibition of intestinal OAT (the enzyme interconverting P5C into ornithine; Fig. 2) for 12 hours decreased plasma concentrations of ornithine, citrulline, and arginine by 59%, 52% and 76%, respectively [10]. Second, both OAT deficiency in human infants and OAT gene knock-out in mice resulted in hypoargininemia, hyperammonemia, retarded growth, and death during the neonatal period [58]. Third, an inherited deficiency of intestinal P5C synthase has recently been reported to cause hypoargininemia, hyperammonemia, retarded growth, and progressive neurological dysfunction in human infants [59].

Our recent work has shown that serum citrulline levels are 25% lower in preterm infants compared with term neonates [60], suggesting limited synthesis of this nonprotein amino acid by the former. On the basis of the current concept that the endogenous synthesis of arginine (occurring primarily in enterocytes of the small intestine) is crucial for maintaining arginine homeostasis in neonates [9,14], we have suggested that the major reason why parenterally fed preterm infants are deficient in arginine is limited expression of key intestinal arginine-synthetic enzymes [13]. Other reasons for low arginine synthesis in TPN-supported neonates may be 1) low plasma glutamine concentrations due to the absence of glutamine from current commercial TPN solutions, 2) limited uptake of arterial proline and glutamate by the small intestine for intestinal arginine synthesis, 3) the lack of enteral provision of glutamine/glutamate and proline for intestinal arginine synthesis, and 4) gut atrophy. Even if substrates are fully available to the mucosa of the small intestine, low activities of key enzymes (e.g., P5C synthase, ASS, and ASL) will still limit citrulline and arginine synthesis by enterocytes. This view is supported by the finding that arginine was also remarkably deficient in enterally fed preterm infants [21], as in TPN-supported preterm neonates [7,25–27,30]. Thus, an important strategy for enhancing endogenous arginine synthesis in preterm infants will likely be to promote the maturation of the intestinal arginine-synthetic pathway.

## 6. The essential role of the small intestine in fetal arginine synthesis

The fetal and neonatal pig is a well established animal model for the human fetus and infant, because of similarities in anatomy, development, nutrition, and physiology between the pig and the human [31,61,62]. In addition, amino acid composition is similar between the fetal pig and the human fetus [57]. Although there are several basic differences in pregnancy between pigs and humans, which include time of implantation, type of placentation, gestational length, and number of offspring [61], intestinal villus development and cytodifferentiation in fetal pigs occur at relative times in gestation similar to those in the human fetus [63]. Thus, the pig has been used extensively as an animal model for studying prenatal and postnatal intestinal development and metabolism of the human [20,64,65]. Because of the nature of nutritional and developmental research, which often involves invasive tissue collections and surgical procedures, it is neither ethical nor practical to conduct these experiments with the human fetus or infant. During recent years we have developed and used a porcine model for studying fetal amino acid metabolism during pregnancy and intestinal arginine metabolism in neonates [65–69].

To assess the role of fetal organs in arginine synthesis from glutamine, we measured P5C synthase activity (a rate-controlling enzyme in arginine synthesis from glutamine/glutamate) in the small intestine, liver, kidney, skeletal muscle, heart, lung, and brain of 90- and 114-day-old fetal pigs (length of gestation in pigs is 114 days), as we had done for postnatal pigs [10,70]. P5C synthase activity was found only in enterocytes of the fetal small intestine, as in neonatal pigs [10]. In contrast to P5C synthase, proline oxidase was widely distributed in fetal tissues. In fetal pigs, as in newborn and postweaning pigs [71], proline oxidase activity was greatest in the small intestine and was predominantly located in enterocytes. The kidney of fetal pigs, like adult pigs, did not contain OCT or CPS-I activity. Thus, there is no synthesis of citrulline from proline in fetal kidneys. Because hepatic OAT activity is restricted to the perivenous hepatocytes which have no CPS-I or OCT activity [15,40], there is no conversion of P5C into citrulline in the fetal liver. As in adults, an exceedingly high activity of cytosolic arginase in the fetal liver [62] precludes net arginine synthesis by this organ. These results suggest that the small intestine is the exclusive organ for synthesizing citrulline and arginine from both glutamine and proline in fetal pigs. Arginase activity is negligible in enterocytes of fetal pigs, as reported for newborn pigs and mice [53,54,72].

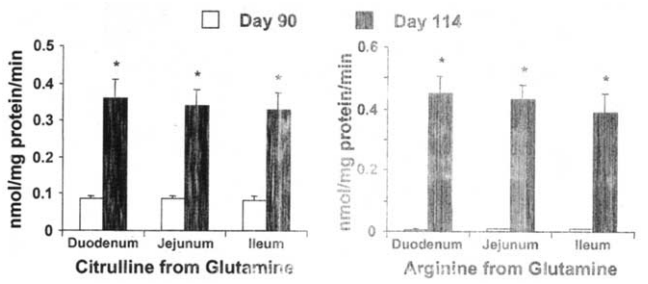


Fig. 3. Citrulline and arginine synthesis from glutamine in enterocytes from the duodenum, jejunum and ileum of fetal pigs. Data are means ± SEM, $n = 6$ gilts. Enterocytes were used for the measurement of citrulline and arginine synthesis in the presence of 2 mmol/L glutamine, as previously described [53]. *$P < 0.01$: different from 90-day-old fetal pigs.

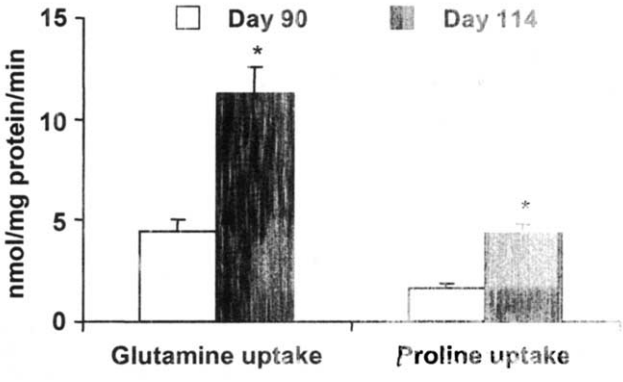


Fig. 4. Uptake of glutamine (Gln) and proline (Pro) by jejunal enterocytes of fetal pigs. Data are means ± SEM, $n = 6$ gilts. Uptake of 2 mmol/L [U-$^{14}$C] glutamine or 2 mmol/L [$^{14}$C]proline was measured as previously described [71]. *$P < 0.01$: different from 90-day-old fetal pigs.

## 7. Underdevelopment of intestinal synthesis of citrulline and arginine in preterm neonates

Results of our recent studies indicate that rates of citrulline synthesis from glutamine in enterocytes of 90-day-old fetal pigs were much lower than those for 114-day-old fetal pigs, and there was little synthesis of arginine in enterocytes of preterm pigs (Fig. 3). The near absence of intestinal arginine synthesis in preterm neonates is striking and is in sharp contrast to the high rate of intestinal arginine synthesis in term neonates [52]. As the gut of the preterm infant becomes more mature, its capacity to produce citrulline will increase. For example, we found that in premature infants, the average serum concentration of citrulline increased ($P < 0.05$) from 14 μmol/L on day 7 of life to 20 μmol/L on day 14, and to 34 μmol/L on day 21 [28]. In contrast to the differential expression of digestive enzymes and intestinal transporters, rates of citrulline or arginine synthesis from glutamine (expressed per milligram of protein) were similar in enterocytes from the duodenum, jejunum, and ileum of fetal pigs (Fig. 3), suggesting similar underdevelopment of arginine-synthetic enzymes among different segments of the preterm small intestine. Similar results were obtained when 2 mmol/L of proline was used as a precursor (our unpublished data). Thus, when compared with term neonates, the small intestine of preterm neonates cannot produce full amounts of citrulline and arginine. The underdevelopment of the intestinal synthesis of citrulline and arginine, a crucial event for postnatal metabolism and growth [13], contributes substantially to hypoargininemia in preterm neonates.

We have conducted studies to determine whether the marked difference in intestinal citrulline and arginine synthesis between 90- and 114-day-old fetal pigs may result from changes in glutamine and proline uptake or in the activities of arginine-synthetic enzymes. We measured the uptake of glutamine and proline by fetal enterocytes and the activities of all arginine-synthetic enzymes using our established methods [71]. For facilitating comparison, data on citrulline and arginine synthesis, as well as glutamine and proline uptake, are expressed on the same scale (nmol/mg protein/min). In jejunal enterocytes from 90- and 114-day-old fetal pigs, rates of glutamine uptake (Fig. 4) were approximately 60- and 35-fold greater than rates of citrulline synthesis, respectively. Similar results were obtained for proline uptake by fetal enterocytes (Fig. 4). These findings clearly indicate that glutamine and proline uptake is not a major limiting factor for citrulline and arginine synthesis in enterocytes of the preterm or term piglets. Consistent with this suggestion are our results on the activities and gene expression of intestinal arginine-synthetic enzymes [11,12]. For example, activities of glutaminase, proline oxidase, OAT, CPS-I, and OCT were only 35–50% lower in the small intestine of 90-day-old fetal pigs compared with term piglets [11,12]. The activities of intestinal P5C synthase, ASS, and ASL were low or negligible at day 90 of gestation, but were the greatest at term (Fig. 5). Thus, in enterocytes of preterm neonates, low P5C synthase activity may limit the conversion of glutamine into citrulline, and negligible expression of ASS and ASL may account for little synthesis of arginine from glutamine- or proline-derived citrulline.

Although the fetal kidney cannot synthesize citrulline from glutamine or proline, this organ may be a site for converting intestine-derived citrulline into arginine because of the presence of ASS and ASL, which are located in the

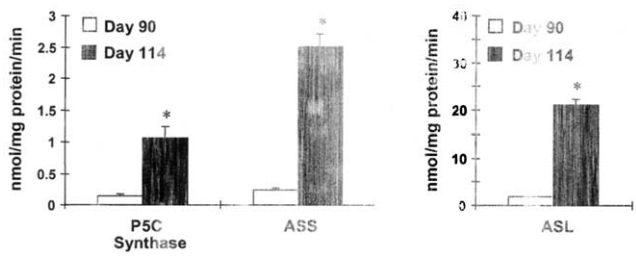


Fig. 5. Activities of pyrroline-5-carboxylate (P5C) synthase, argininosuccinate synthase (ASS) (panel A) and argininosuccinate lyase (ASL) (panel B) in jejunal enterocytes of fetal pigs. Data are means ± SEM, $n = 6$ gilts. Enzymatic activities were measured as previously described [53]. * $P <$ 0.01: different from 90-day-old fetal pigs.

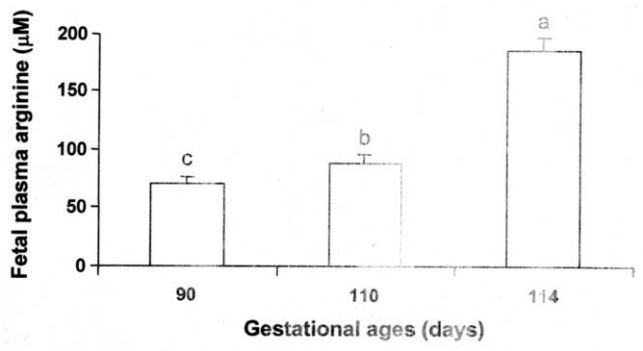


Fig. 6. Concentrations of arginine in plasma of preterm and term piglets obtained through hysterectomy. Data are means ± SEM, $n = 8$. Blood was withdrawn from umbilical artery of preterm (days 90 and 110 of gestation) and term (day 114 of gestation) piglets and plasma was analyzed for arginine [44]. Means with different letters (a–c) are different ($P < 0.01$).

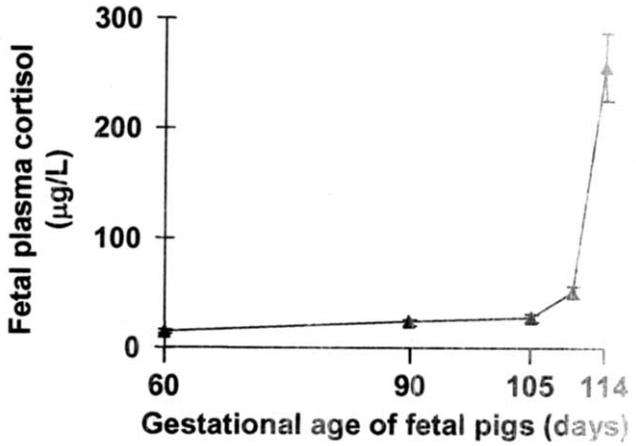


Fig. 7. Fetal cortisol surge during the perinatal period. Data are means ± SEM, $n = 6$ gilts. Umbilical arterial blood was withdrawn from fetal pigs as previously described [67]. Plasma was analyzed for concentrations of cortisol using an immunoassay kit [65].

proximal tubules of the renal cortex [51]. Whereas the activities of intestinal arginine-synthetic enzymes including ASS and ASL are greatest at term birth, renal ASL activity is only minimally developed in term newborns (e.g., piglets and mice) [52,54]. The available evidence suggests that expression of fetal renal ASL is not up-regulated by glucocorticoid treatment [73]. Thus, an important strategy for enhancing endogenous arginine synthesis in the preterm neonate is to promote the maturation of fetal intestinal arginine-synthetic enzymes.

As mentioned above, citrulline is a nonprotein amino acid synthesized from glutamine/glutamate and proline only in the intestine. Given that serum citrulline levels are not influenced by body mass index or creatinine clearance, plasma concentrations of citrulline may be a useful indicator for intestinal mass or adaptation [74]. In a recent study, we found that in infants with short-bowel syndrome (SBS) ($n = 24$), serum citrulline level was positively correlated with percent enteral calories at the time of measurement ($R = 0.84$; $P < 0.01$) and with the length of bowel measured at surgery ($R = 0.74$; $P < 0.01$) [60]. Serum citrulline level was 30 ± 2 μmol/L in SBS infants weaned off parenteral nutrition, 20 ± 2 μmol/L in those who would subsequently be weaned off parenteral nutrition, and 11 ± 2 μmol/L in those who would remain TPN dependent ($P < 0.01$) [60]. A serum citrulline level ≥19 μmol/L had a sensitivity of 100% and specificity of 70% for being off of TPN or coming off TPN [60]. Impaired intestinal synthesis of citrulline (the precursor of arginine) likely accounts for the reduced availability of citrulline in preterm infants.

In preterm piglets, low rates of intestinal arginine synthesis are associated with low plasma arginine concentrations (Fig. 6). Indeed, plasma arginine concentrations in preterm piglets are less than 50% of those in term piglets (Fig. 6), as reported for preterm infants (Fig. 1). This result suggests a similarity in arginine metabolism between preterm piglets and infants. Because uterine uptake of citrulline or arginine is similar between 110- and 114-day-old fetal pigs [57], lower plasma arginine concentrations in preterm piglets, in comparison with term piglets, likely results from lower endogenous synthesis of citrulline and arginine in preterm neonates.

## 8. A fetal cortisol surge coincides with the induction of intestinal P5C synthase, ASS, and ASL during the perinatal period

Cortisol (hydrocortisone) is the major circulating glucocorticoid in humans and pigs [75]. Concentrations of cortisol in fetal plasma gradually increase during late gestation and peak at term in both humans [75] and pigs [76]. We found that in pigs, concentrations of cortisol in fetal plasma do not change significantly between days 90 and 105 of gestation but increase gradually from day 105 of gestation (Fig. 7). In contrast to fetal pigs, concentrations of cortisol in the maternal plasma of pregnant pigs do not change significantly during the last 20 days of gestation [77]. Thus, the cortisol surge in fetal pigs just before birth results from its synthesis by the fetal adrenal gland rather than its transport from the maternal circulation. Interestingly, in pigs, the fetal cortisol surge during the perinatal period (Fig. 7) coincided with the induction of intestinal P5C synthase, ASS, and ASL (Fig. 5), as well as intestinal arginine synthesis (Fig. 3). These results suggest that the prenatal cortisol surge may be the regulatory gateway to maturation of the fetal intestinal arginine-synthetic pathway. A selective inhibition of fetal cortisol synthesis (e.g., by metyrapone [65]) may provide direct compelling evidence to test this novel hypothesis.

## 9. Glucocorticoid regulation of arginine-synthetic enzymes in the small intestine

Because of the recognition that the hepatic urea cycle plays a vital role in ammonia detoxification, much effort has

been devoted to study the regulation of PDG, CPS I, OCT, ASS, and ASL and arginase in mammalian liver [14,47,48,73]. Available evidence indicates that glucocorticoids enhance the expression of urea cycle enzymes in hepatocytes of postnatal rats [14,73]. Our recent studies have also demonstrated that administration of cortisol stimulates the intestinal expression of P5C synthase, ASS, and ASL in neonatal pig enterocytes [66,78,79]. Furthermore, a cortisol surge mediates the enhanced expression of intestinal P5C synthase and ASL in weanling pigs [80]. These results suggest a crucial role for glucocorticoids in regulating maturation of intestinal arginine synthesis. In support of this view, dexamethasone (a synthetic glucocorticoid) treatment of preterm infants increases plasma concentrations of both citrulline and arginine [81]. At present, little is known about glucocorticoid regulation of arginine-synthetic enzymes in the developing fetal small intestine or about the underlying molecular mechanisms. Because of the very limited survival time of fetal-pig enterocytes in culture, it will be necessary to use intestinal cell lines (e.g., the IEC-6 cell [82] and the IPEC-1 cell [83] to define mechanisms of the action of cortisol on the intestinal expression of P5C synthase, ASS, and ASL genes. The IEC-6 cell and the IPEC-1 cell are well-characterized nontransformed intestinal epithelial cell line derived from rat small-intestine crypt cells and the newborn pig small intestine, respectively. Both cell types have glucocorticoid receptors and respond to cortisol or dexamethasone (a synthetic glucocorticoid) treatment with regards to morphological and functional maturation [82–84].

## 10. Benefits of glucocorticoid treatment to preterm neonates

Prenatal administration of glucocorticoids advances the maturation of key fetal organs, including the small intestine and the lungs [5]. Our recent studies with neonatal pigs indicate that an increase in plasma cortisol levels within a physiological range promotes intestinal polyamine synthesis and growth and does not result in body weight loss [66]. Likewise, prenatal or postnatal administration of glucocorticoids improves gut maturation and function [85], reduces the risk of respiratory distress syndrome, and possibly decreases the incidence of necrotizing enterocolitis in preterm infants [86]. Although increasing intravenous provision of arginine has been shown to effectively treat hyperammonemia in preterm infants, it does not promote intestinal maturation in preterm infants [25–27]. Importantly, an increase in plasma glucocorticoid levels within physiologic range (e.g., perinatal cortisol surge) does not promote intestinal arginase expression in the fetus or neonate [87], suggesting a feasibility of the antenatal treatment of cortisol to effectively increase arginine provision in vivo. Glucocorticoids stimulate the activity of intestinal ornithine decarboxylase for the synthesis of polyamines [66], which are essential for protein synthesis, as well as intestinal cell proliferation, differentiation, and function [88]. Antenatal corticosteroid treatment may not only advance the maturation of the small intestine and other vital organs of the preterm neonate [85], but may also promote intestinal synthesis of citrulline and arginine and thus the endogenous arginine supply [81, 87]. An increase in the availability of circulating arginine to preterm neonates will maintain the hepatic urea cycle in an active state and prevent cardiovascular, pulmonary, intestinal, immunological, and neurological dysfunctions [6,13]. Therefore, additional benefits of the corticosteroid treatment may include the following: 1) allowing early introduction of enteral feeding to preterm infants, which is critical for intestinal polyamine synthesis [88] as well as intestinal motility, integrity, and growth [64, 89]; 2) decreasing the incidence of necrotizing enterocolitis in preterm infants [86]; and 3) shortening the expensive stay of preterm infants in hospitals due to accelerated organ maturation and restoration of full enteral feeding [5]. Other “trophic” strategies aimed at increasing ODC and polyamine synthesis in the intestine include administration of growth hormone or glucagon-like peptide-2 [90].

## 11. Other possible determinants of low arginine levels in premature infants

Preterm infants may be associated with multiple organ dysfunction. Thus, the leakage of arginase from injured tissues (e.g., liver, intestine, heart, lungs, and kidneys) may result in elevated plasma levels of arginase [30,91], the major enzyme responsible for initiating arginine catabolism in the whole body. Red blood cells of preterm infants may also be a significant source of arginase I in plasma. In addition, creatine synthesis from arginine, a major pathway for arginine utilization [6], may be increased in preterm infants relative to the plasma flux of arginine. Furthermore, because preterm infants may often be subject to immunological challenge, elevated levels of cytokines may stimulate the expression of arginase and NO synthase to promote arginine catabolism [9], thereby contributing to hypoargininemia. Future studies are necessary to address these potentially important questions.

## 12. Conclusions

Enterocytes are the major cells responsible for the endogenous synthesis of citrulline and arginine, and this metabolic pathway is crucial for maintaining arginine homeostasis in both the fetus and neonate. Strikingly, synthesis of citrulline from glutamine or proline was low and there was little conversion of citrulline into arginine in enterocytes of preterm neonates because of limited expression of the genes for intestinal P5C synthase, ASS, and ASL. The perinatal cortisol surge may be the regulatory

gateway to the maturation of key enzymes of the fetal intestinal arginine-synthetic pathway and therefore its absence due to premature delivery may be responsible for the limited endogenous synthesis of arginine. The latter, along with a possible increase in whole-body arginine catabolism, may contribute substantially to hypoargininemia in preterm infants. Future studies will be required to define the molecular mechanisms for glucocorticoid and other hormonal regulation of arginine-metabolic enzymes in the developing fetal small intestine and other tissues. Such work will not only greatly advance the field of neonatal nutrition but also will provide a knowledge base to design novel preventive and therapeutic interventions to optimize survival and health in preterm neonates.

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