

Citrulline: Is it Ready for Primetime. Its Uses and Limitations in Neonatal Medicine

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Abstract

Citrulline is a non-protein amino acid produced almost exclusively by the gut and present only in small amounts in the diet. Since the gut is the main source of citrulline, it could be used as a potential biomarker of intestinal function. Necrotizing Enterocolitis is an intestinal dysfunction in neonates leading to significant morbidity and mortality. This review discusses the various aspects of intestinal injury and the association of citrulline with bowel disorders, as well as recent developments with citrulline in the pediatric population.

As citrulline is directly related to small bowel length, it has been recently shown that its levels are an efficient marker when the active mass of the bowel is affected. This could be used as a prognostic marker for parenteral nutrition weaning and development of enteral tolerance. Lower levels of citrulline are found in preterm neonates with necrotizing enterocolitis and such neonates demonstrate a more prolonged course of the disease. The concomitant increase in citrulline levels along with clinical improvement in neonates and progression of enteral feeds suggest that citrulline levels may be a sensitive marker of intestinal recovery.

Studies have shown that citrulline levels are well correlated with the length of the bowel as well as intestinal function. Citrulline levels used as a sensitive biomarker for intestinal absorptive function would be clinically useful in diagnosis of necrotizing enterocolitis and detection of bowel function and recovery from intestinal disorders such as necrotizing enterocolitis, although more studies are needed in newborns affected with these diseases.

Keywords: Citrulline; Neonates; Necrotizing enterocolitis; Short bowel; Parenteral nutrition; Nitric oxide

Introduction

L-Citrulline is a naturally occurring non-essential amino acid, an intermediate of the urea cycle and a potent hydroxyl radical scavenger. It is a more effective precursor of arginine and nitric oxide production than arginine. Its unique metabolism has prompted suggestions that plasma citrulline could be a reliable marker of gut function [1]. Initially viewed only as an intermediary product in the urea cycle, citrulline is now one of the most researched amino acid, especially of interest in nutrition and intestinal injury.

Citrulline: Biology and Dynamics of the Gut

Most of the circulating plasma citrulline is derived from glutamine conversion through the glutamate to ornithine to citrulline pathway in the enterocyte [2]. Glutamine is supplied both from the arterial blood (25-33%) and intestinal lumen (66%) [3]. Citrulline thus produced by the enterocyte enters the urea cycle as an intermediate in the production of arginine, an amino acid that has key roles in protein synthesis, ammonia detoxification, nitric oxide production [4]. Arginine can also be a precursor for enterocyte synthesis of citrulline (Figure 1).

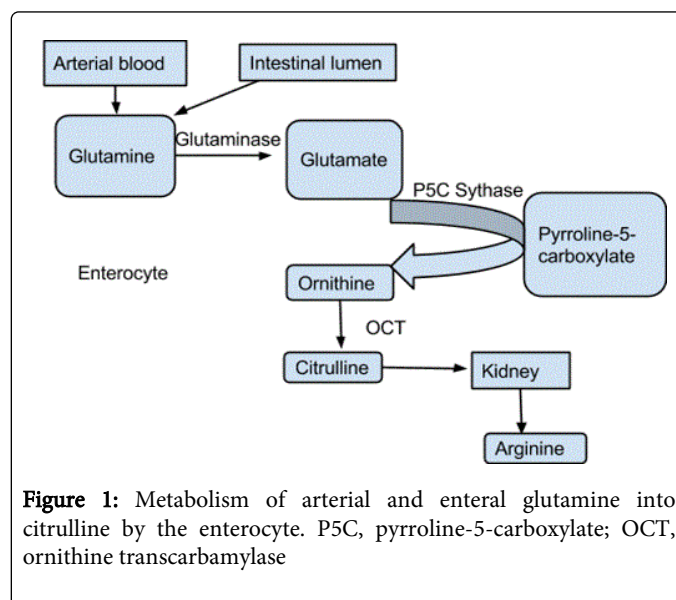


Figure 1: Metabolism of arterial and enteral glutamine into citrulline by the enterocyte. P5C, pyrroline-5-carboxylate; OCT, ornithine transcarbamylase

The discovery of Pyrroline 5 carboxylate synthase (P5CS) in 1983 [5] has contributed to our understanding of citrulline metabolism. This enzyme reduces glutamate to pyrroline 5 carboxylate, a critical step in the biosynthesis of ornithine which in turn leads to citrulline synthesis. Mammalian P5CS is exclusively located in small bowel enterocytes [6]. The degree of differential expression of this enzyme

may account for the observed differences in citrulline production associated with intestinal length. It has a greater activity in the duodeno-jejunal area as compared to ileum which in turn is more than in colon. Recent studies however demonstrate that the enzyme in the colon becomes more important in intestinal adaptation after small bowel loss [7]. Citrulline does not appear to be greatly influenced by non-intestinal disorders other than renal failure with plasma citrulline levels being inversely correlated with renal function when creatinine clearance falls below 50 ml/min [8]. Citrulline is synthesized and catabolized in situ, in the liver without a net contribution of citrulline to the plasma citrulline pool [2]. Thus, the functional small intestine represents the only source of circulating citrulline with renal excretion its normal route for clearance. This unique one-way route strengthens its claim to be a potential clinical marker for intestinal integrity [9].

Despite the above known facts of citrulline two factors must be considered when assuming that the intestine is the main endogenous source of citrulline. First, intestinal metabolism can vary according to species; hence the data from animal studies cannot be directly interpreted in humans and should be interpreted with caution. Secondly, intestinal metabolism changes during development and is especially with weaning among the humans.

Plasma citrulline assessment as a biomarker in intestinal disorders

Healthy patients with normal intestinal mucosa and renal function have citrulline levels between 30-50 $\mu\text{mol/L}$ with a median of 40 $\mu\text{mol/L}$ [10]. Although length of small bowel is a predictive indicator of enteral tolerance [11], the precise determination of bowel length is difficult especially if the bowel is inflamed as in necrotizing enterocolitis and/or has extensive adhesions. The technical difficulty of precise measurements and the changing surface areas of the intestine, characteristic of small bowel adaptation, potentially limits the use of intestinal length as a completely reliable marker for intestinal function.

Studies among adult populations have found a significant correlation between post-absorptive plasma citrulline level and remnant small bowel length. In chronic villous atrophy, citrulline levels are decreased to less than 20 $\mu\text{mol/L}$ in patients with proximal destructive lesions and decreased further in patients with extensive (proximal and distal) impairment in intestinal mucosa [10]. In Crohn's disease, citrulline levels are normal because there is no enterocyte damage [12]. Studies have shown that lower citrulline levels are associated with reduced enterocyte function in short bowel syndrome [1], acute mucosal enteropathy, intestinal graft versus host disease [13] and radiation enteritis [14]. Plasma citrulline concentration appears to be a quantitative biomarker of small bowel mass integrity in HIV positive enteropathy [15]. Crenn et al [16] showed that plasma citrulline is a reliable biomarker of enterocyte functional mass in HIV patients receiving anti-retroviral therapy. Despite positive correlation of fasting citrulline and degree of decreased enterocyte mass, Luo et al. [17] failed to show such a relation between fasting citrulline levels and intestinal absorptive capacity in patients with short bowel syndrome.

Intestinal adaptation following massive enterectomy refers to the process of more efficient intestinal absorption per unit length and involves intestinal hypertrophy, slight lengthening and increased villous diameter and height [18]. In short bowel patients, the ability of the enterocytes to generate citrulline depends on the duration of the intestinal adaptation phase. Another interesting observation is that

short bowel syndrome patients beyond the intestinal adaptation period of 24 months are capable of generating significantly more citrulline compared to healthy subjects and non-adapted short bowel syndrome patients [19]. Many adult studies have concluded that citrulline measurements may give an accurate estimate of the functional remnant small bowel length independent of the etiology and the presence or absence of inflammation.

Citrulline: uses in pediatric and neonatal population

Correlation with bowel length in pediatric population

Adult plasma citrulline levels are achieved within weeks of life in term infants without bowel disease. Mean plasma citrulline level of 34 $\mu\text{mol/L}$ is reported at 21 days of life in preterm infants without bowel disease. In pediatric population, small bowel syndrome patients have shown significant correlation between plasma citrulline levels post-surgery and the remnant bowel length [20,21]. Crenn et al. [1] demonstrated a strong correlation ($r=0.83$, $p<0.01$) between fasting plasma citrulline and remnant bowel length. But this observation was made in relatively large proportion of patients that had remnant bowel lengths ranging 0-50 cm, which could potentially be over-estimate the sensitivity and specificity of fasting citrulline levels. Peters et al has also shown that fasting citrulline levels do not differentiate between transient and permanent intestinal failure in patients with bowel length between 50-100 cm [1,19]. More recently, Papadia et al. [12] reported a quadratic correlation between fasting citrulline levels and remnant small bowel length, corroborating the original findings of Crenn et al. [1]. Steltz et al. [22] observed that similar to adults, citrulline levels decrease in pediatric patients with decreased enterocyte function and/or stimulation even without bowel loss.

Although most studies have reported single citrulline levels in the pediatric population, some authors have suggested that a single assessment may not accurately reflect bowel energy absorptive capacity [19]. A non-invasive biomarker of bowel length and function is therefore needed to evaluate bowel adaptation in pediatric patients with significant bowel loss and aid in effectively advancing enteral nutrition.

Relationship with Parenteral Nutrition in pediatric patients with intestinal disorders

Long-term dependence of parenteral nutrition is a potentially life-threatening consequence of small bowel syndrome in children. Although parenteral nutrition can be lifesaving after surgery, parenteral nutrition related complications such as liver disease, catheter associated sepsis, thrombosis, metabolic bone disease and growth delay are leading causes of morbidity and mortality in the population. Cholestasis can develop fairly rapidly, presenting within the first month of exposure. The time course of enteral advancement can therefore be critical. Ionnou, et al., in exclusively parenterally fed neonates demonstrated stable, citrulline levels which increased when minimal enteral feeds were introduced. When the enteral feeds reached more than forty percent of total caloric intake, they were significantly higher than baseline values, increasing further when full feeds were established [23].

Previous studies have shown significant correlations between citrulline levels and parenteral nutrition dependence in the small bowel syndrome patients. Rhoads et al. [21] demonstrated citrulline levels of more than 19 $\mu\text{mol/L}$ were associated with enteral nutrition

tolerance while Fitzgibbons et al. [24] showed citrulline level of 15 $\mu\text{mol/L}$ may serve as a prognostic measure regarding the likelihood of future parenteral nutrition independence. Further, Bailly-Botuha et al. [20] observed that no patient with citrulline less than 11 $\mu\text{mol/L}$ was able to wean from parenteral nutrition. Crenn et al. [1] demonstrated, a fasting citrulline less than 20 $\mu\text{mol/L}$ was related to inability to wean home parenteral nutrition among adult patients from following an intestinal adaptation phase of 24 months.

Citrulline levels therefore can reliably distinguish patients that would successfully be weaned from parenteral nutrition [20,21,24]. Citrulline levels also correlate with the percent of enteral calories and with the rate of enteral advancement [24]. These observations warrant further investigation in prospective pediatric trials to determine impact of parenteral nutrition therapy on trends in citrulline levels and predict tolerance of enteral feeds.

Citrulline and necrotizing enterocolitis

Necrotizing enterocolitis is one of the leading causes of morbidity and mortality in premature infants [25]. The multifactorial pathophysiology of necrotizing enterocolitis is still poorly understood with prematurity being the most important risk factor. Many studies have demonstrated that citrulline as a marker of functional enterocyte mass, lower citrulline levels are expected in preterm neonates with necrotizing enterocolitis. Therefore it might be important to follow plasma citrulline levels in necrotizing enterocolitis to investigate the usefulness of citrulline as a marker of intestinal function.

Low plasma arginine concentrations have also been described in neonates with necrotizing enterocolitis. Zamora, et al. reported reduced arginine levels immediately before the onset of necrotizing enterocolitis, suggesting that the availability of L-arginine may be a factor limiting nitric oxide production, predisposing the immature gut to necrotizing enterocolitis [26]. Other investigators reported that not only plasma arginine levels but also plasma Asymmetric Dimethylarginine (ADMA) levels which are the endogenous nitric oxide synthase inhibitor, are low in preterm neonates with necrotizing enterocolitis and so is the arginine to ADMA ratio [27]. Becke et al.

observed reduced amino acid levels in premature infants with necrotizing enterocolitis, especially glutamine and arginine [28]. There are only few studies that have correlated citrulline and necrotizing enterocolitis. Ionnou, et al. showed low plasma citrulline levels in preterm infants with NEC [23]. Celik et al. [29] showed that plasma citrulline and arginine levels were significantly lower in infants with necrotizing enterocolitis as compared to a control group while glutamine levels did not differ. Ioannou et al. [14] reported that plasma citrulline levels of necrotizing enterocolitis group increased after enteral feeding was started, but never reached the levels of control groups. The cut off level of citrulline to diagnose necrotizing enterocolitis was variable and was between of 13.5 $\mu\text{mol/l}$ [23] and 17.75 $\mu\text{mol/l}$ with similar sensitivity and specificity [29]. These two studies also compared citrulline levels of necrotizing enterocolitis and healthy preterm infants on day 7,14,21 and presented a progressive increase in relation between the citrulline levels and necrotizing enterocolitis with time. The limitation in both studies is that in preterm infants, compromised intestinal function can further diminish the already low citrulline levels. Inclusion of age-matched controls to trend citrulline levels in healthy preterm neonates would help in addressing this limitation. Another limitation in the study by Celik, et al. is that infants were started feeds at the earliest possible opportunity accelerating maturity of the gut and potentially impacting citrulline levels [29].

Neonates with lower citrulline levels at necrotizing enterocolitis presentation demonstrate a more prolonged course of the disease and receive parenteral for a longer period. The concomitant increase of citrulline levels along with the clinical improvement of the neonates and the progression of enteral feedings suggest that citrulline levels may be a sensitive marker of intestinal recovery during the course of necrotizing enterocolitis.

Two studies [23,29] though prospective performed are observational hence further studies are needed to measure citrulline levels as a guide to the clinical management of neonates, especially among preterm to predict starting, recovery and prognosis of necrotizing enterocolitis (Table 1).

Study	Design	Major Findings
Rhoads et al. [21]	Prospectively measured CIT in infants with SBS (n=24), compared to controls.	CIT correlated with bowel length, % enteral calories; CIT<19 $\mu\text{mol/L}$ predicts inability to achieve enteral autonomy
Bailly-Botuha et al. [20]	Prospectively measured CIT in infants and children with SBS	CIT correlated with intestinal length and energy supplied by PN; CIT increased with weaning of PN/increase in enteral feeds.
Fitzgibbons et al. [24]	Retrospectively reviewed serum CIT and GI outcomes of infant and pediatric patients in intestinal rehabilitation (n=27)	CIT correlated with bowel length and %enteral calories; CIT<15 $\mu\text{mol/L}$ predicts inability to achieve enteral autonomy
Ioannou et al. [23]	Prospectively measured serial CIT in infants with NEC (n=17) compared with controls	CIT levels are reduced in preterm infants with NEC; serially increased with enteral feeds.
Stultz et al. [22]	Retrospectively evaluated serial CIT levels in infants on TPN (n=18 infants with bowel loss vs. 18 infants with bowel dysfunction)	Decreased CIT levels in infants with bowel loss and bowel dysfunction; CIT increased with enteral feeding in group with bowel dysfunction only.
Celik et al. [29]	Prospectively measured serum CIT of preterm infants diagnosed with NEC (n=20), compared to 16 controls	Lower CIT and arginine levels in preterm infants with NEC vs. controls

Table 1: Studies evaluating citrulline as a marker of intestinal function in the infant population. CIT: serum citrulline levels; SBS: Short Bowel Syndrome; NEC: Necrotizing Enterocolitis; TPN: Total Parenteral Nutrition.

Conclusion

Currently there is no “gold standard” for functional assessment of intestinal function. Due to its unique metabolism, citrulline has recently emerged as a promising marker of enterocyte function. The ability to reliably predict disease process and to successfully wean from parenteral nutrition among neonates would be of significant benefit to the clinician. Citrulline measurement is simple and relatively inexpensive allowing it to be followed on serial basis. Serial citrulline determinations can help to monitor improvement of functional enterocyte mass during the course and resolution of intestinal diseases such as necrotizing enterocolitis. More studies are needed to test whether citrulline levels can serve not only as a marker for compromised intestinal function as in necrotizing enterocolitis but also as a guide in the feeding of the immature or compromised gut among preterm and term neonates. Also use of citrulline levels as a biomarker to identify patients who would likely be on long-term parenteral nutritional requirements, offers an assessment of intestinal rehabilitation potential, and possibly helps with the early identification of those who may ultimately benefit from intestinal transplantation. Furthermore, exploration of its potential as therapeutic intervention is also warranted.

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