

# Can arginine and ornithine support gut functions?

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## Abstract

Arginine and ornithine are precursors of nitric oxide and polyamines, respectively. These metabolites intimately participate in permeability and adaptive responses of the gut. The liver possesses high arginase activity as an intrinsic part of urea synthesis and would consume most of the portal supply of dietary arginine. The gut reduces this possibility by converting dietary arginine to citrulline, which effectively bypass the liver and is resynthesised to arginine in the kidney. Dietary ornithine supplementation, in the form of ornithine  $\alpha$ -ketoglutarate (OKG) can be considered as an arginine precursor. Several supplementation studies have shown both amino acids to promote growth hormone and insulin secretion with anabolic effects in post-operative patients. Their intermediary metabolites (for example, glutamine, proline) may also be of benefit in trauma metabolism. Specific effects of either amino acid on the gut are poorly reported. One recent animal study showed improved morphology after OKG administration, perhaps through increased polyamine secretion. Generation of nitric oxide from arginine has two facets. Excess production from high dose arginine potentiated the effects of experimentally induced sepsis, whereas low doses improved survival. These considerations suggest that the role of enteral diet supplementation with arginine or OKG should be urgently examined for any benefits it may have on mucosal barrier function.

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For many years, arginine and related compounds (ornithine and citrulline) have been considered solely as intermediate metabolites in the process of nitrogen detoxification – that is, in the context of ureagenesis. There is renewed interest, however, in these amino acids because ureagenesis may have an important role in pH homeostasis<sup>1</sup> and also because arginine becomes an essential amino acid during growth and catabolic states.<sup>2</sup> Ornithine is not a constituent of proteins, but is clearly important in the regulation of nutritional state as a precursor of aliphatic polyamines. In addition, in the form of the  $\alpha$  ketoglutarate salt, ornithine generates multiple metabolic effects, which do not result solely to the additive action of the two moieties of this molecule.<sup>3</sup> There is, however, a paucity of data on the role of arginine or its metabolites in the maintenance of gut function and morphology; this indicates possible future research directions.

## Metabolism of arginine and related compounds in the gut

Arginine synthesis and catabolism in specific tissues is conditioned by the presence of arginosuccinase and arginase respectively, but only periportal hepatocytes and, to some extent, certain brain areas, possess all the enzymes required for arginine recycling and urea synthesis.<sup>1</sup> The gut acts as a user of arginine because it possesses arginase (isoenzyme II) and ornithine carbamoyltransferase.<sup>4</sup> The gut thus releases urea and citrulline.<sup>5</sup> In addition, enterocytes express ornithine decarboxylase<sup>6</sup> and an NADPH<sub>2</sub> dependent arginine deiminase<sup>7,8</sup> which respectively lead to local production of aliphatic polyamines and nitric oxide.

Despite the high ornithine decarboxylase activity in enterocytes, however, most of the ornithine produced from arginine is released into the portal blood stream, and polyamine formation accounts for a small part of arginine consumption.<sup>8</sup>

After <sup>14</sup>C-ornithine is given by the enteral route, <sup>14</sup>C-proline, <sup>14</sup>C-glutamate, and <sup>14</sup>C-polyamines are detected,<sup>9</sup> as expected, but, in contrast with arginine administration, no citrulline is produced. This could suggest a degree of metabolic compartmentalisation, arginine flux being directed preferentially towards ornithine and citrulline production. Indeed, ornithine translocase, ornithine carbamoyltransferase, and citrulline translocase function as a multienzyme complex<sup>1</sup> (Fig 1). Figure 2 summarises arginine and ornithine pathways in the gut.

## Arginine and related compounds in artificial nutrition

Arginine has multiple biological properties, including the ability to stimulate anabolic hormone secretion: intravenous and enteral arginine administration increases both insulin and human growth hormone secretion.<sup>12</sup> Several studies (reviewed in references 2 and 13) show that arginine given to patients as well as in various experimental stress models, acts by improving nitrogen balance, accelerating wound healing, and restoring depressed immunity (Table). These effects are seen whether arginine is given orally<sup>14,15</sup> or parenterally.<sup>16</sup>

Ornithine shares with arginine the ability to stimulate human growth hormone secretion.<sup>3</sup> In addition, ornithine as its  $\alpha$ -ketoglutarate salt (OKG) generates various molecules (for example, glutamine)<sup>17</sup> which play a key part in the control of protein metabolism.<sup>18</sup> OKG has been shown to improve nitrogen balance in various acute and chronic malnutrition states (see reference 3 for a review). OKG increases

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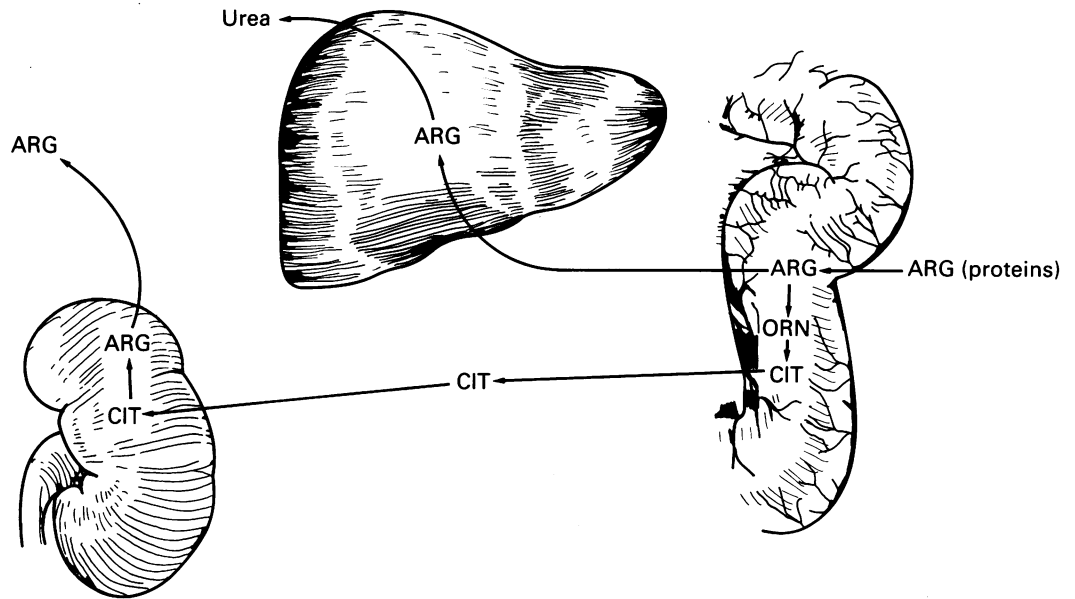


Figure 3: Arginine (ARG) metabolism into citrulline (CIT) in the gut seen as a means of protecting arginine from excessive degradation in the liver. ORN=ornithine.

an additional propylamine moiety to spermidine, thus forming spermidine. The source of the propylamine groups is S-adenosyl methionine.<sup>6</sup>

The functions of polyamines in mammalian cells are poorly understood, although the use of ornithine decarboxylase inhibitors such as difluoromethyl ornithine has established that polyamines are essential for cell growth<sup>6</sup> and protein synthesis.<sup>24</sup> In this way, it has been clearly shown that the action of ornithine on fibroblast growth in culture<sup>25</sup> and on protein synthesis by the liver<sup>26</sup> are both dependent upon polyamine synthesis.

With regard to the intestine, there are consistent data supporting an important role of polyamines in the control of hypo and hyperplasia of this tissue. Ornithine decarboxylase in the mucosa of the small intestine has high basal activity compared with most tissues.<sup>6</sup> Ornithine decarboxylase is associated with mature cells of the villus tip as well as proliferating crypt cells, suggesting that polyamines participate in both intestinal cell differentiation and proliferation.<sup>27</sup>

Intestinal polyamine content falls after a fast<sup>28</sup> and after an eight-day total parenteral nutrition programme in the rat,<sup>29</sup> but increases during refeeding.<sup>28</sup> The fall in the concentrations of polyamines during fasting results from a simultaneous decrease in polyamine synthesis (that is, a decrease in ornithine decarboxylase content) and an increase in their degradation (that is, an increase in diamine oxidase content). The reverse is true during refeeding.<sup>30</sup> The trophic effect of nutrients seems to be due in particular to glucose and to amino acids such as glycine and alanine.<sup>31 32</sup> This effect is strong in the jejunum and ileum, moderate in the duodenum, and small in the proximal colon.<sup>31</sup>

In the same way, during intestinal adaptation in response to jejunectomy<sup>33</sup> or to parasite (*Trichinella spiralis*) induced inflammation,<sup>34</sup> intestinal contents of ornithine decarboxylase

and polyamines increase<sup>34</sup> in parallel with the increase in mucosal proliferation indices.<sup>33</sup> This is clear in the distal segment of the intestine where polyamine content is lowest in the basal state.<sup>35</sup> With the administration of difluoromethylornithine the increase in ornithine decarboxylase and polyamines is suppressed and intestinal adaptation is abrogated.<sup>33</sup>

The real question is whether exogenous arginine can be a relevant precursor of polyamines. It is noteworthy that food contains polyamines and that polyamines are produced by the flora of the gastrointestinal tract.<sup>36</sup> Thus, the direct uptake by enterocytes of pre-formed polyamines could contribute to the polyamine cellular pool. Indeed, putrescine<sup>37</sup> and spermidine uptake<sup>38</sup> have been shown in isolated rat enterocytes. Interestingly, enterocytes from the distal quarter of the gut exhibit the highest rate of uptake.<sup>37</sup> The fact that a large fraction of metabolised arginine is transported out of the enterocyte as ornithine and citrulline may show that arginine metabolism is not responsible for relevant amounts of polyamines.

#### The nitric oxide pathway

The nitric oxide pathway is probably the most important recent discovery in the field of amino acid metabolism. Nitric oxide is produced from arginine by arginine deiminase. Although this enzyme has been identified in various cell types, the pathway seems to be located mainly in macrophages and endothelial cells,<sup>39</sup> where nitric oxide triggers cytotoxic activity of phagocytic cells and vascular smooth muscle relaxation of endothelial cells.<sup>40</sup> Nitric oxide activates guanylate cyclase, thereby forming cyclic GMP, which is its second messenger.<sup>39 40</sup> Experimental endotoxemia (lipopolysaccharide from *Escherichia coli*) leads to accumulation of  $\text{NO}_3^-$  in urine. Activation of the nitric oxide pathway is controlled by cytokines.<sup>41</sup> Taken together, the data

support a role for nitric oxide in the pathogenesis of septic shock syndrome. In this context, care must be taken with arginine supplementation in severely ill patients at risk of developing multiple organ failure. This dual effect of nitric oxide (and therefore arginine) is illustrated by a recent study from Alexander's group.<sup>42</sup> Septic guinea pigs supplemented with low doses of arginine recovered better than controls, whereas supplementation with high doses led to catastrophic results.

In conclusion, enterocytes contain high concentrations of polyamines, which play a crucial part in the control of cell multiplication and differentiation, and are able to synthesise nitric oxide, which emerges as a potent modulator of the response to inflammation. Despite these features, there is an extreme paucity of data on the action of arginine and ornithine on gut function. Such studies are urgently required.

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