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Increased Serum Levels of L-Arginine in Ulcerative Colitis and Correlation with Disease Severity

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Abstract

Background—L-arginine (L-Arg) is a semi-essential amino acid that is the substrate for both nitric oxide and polyamine synthesis. Cellular uptake of L-Arg is an active transport process that is subject to competitive inhibition by L-ornithine (L-Orn) and L-lysine (L-Lys). We investigated L-Arg utilization in patients with ulcerative colitis (UC).

Methods—Serum was collected from 14 normal controls and 22 UC patients with pancolitis of moderate or severe activity by histopathology score. The Mayo Disease Activity Index (DAI) and endoscopy sub-score were assessed. Serum amino acid levels were measured by high performance liquid chromatography. Arginine availability index (AAI) was defined as $[L-Arg]/([L-Orn] + [L-Lys])$.

Results—Serum L-Arg levels were significantly associated with histopathologic grade ($P = 0.001$). L-Arg levels were increased in subjects with severe colitis when compared to those with moderate colitis or normal mucosa. L-Orn + L-Lys levels were also increased in severe colitis, so that AAI was not significantly increased. L-Arg levels were also strongly associated with the endoscopy sub-score ($P < 0.001$). There was a strong correlation between DAI and L-Arg levels ($r = 0.656$, $P < 0.001$).

Conclusions—Serum L-Arg levels correlate with UC disease severity, but availability is not increased, due to competitive inhibition by L-Orn and L-Lys. Our findings suggest that L-Arg uptake by cells in the inflamed colon is defective, which may contribute to the pathogenesis of UC. Studies delineating the mechanism of uptake inhibition could enhance our understanding of UC or lead to novel treatment options.

Keywords

L-arginine; ulcerative colitis; nitric oxide; disease activity index

INTRODUCTION

Ulcerative colitis (UC) is characterized by recurring episodes of immune-mediated inflammation limited to the mucosal layer of the colon. The mechanisms underlying this

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abnormal immune system response are being actively investigated and the hope is that new therapeutics may arise from better understanding of UC pathophysiology. There is growing literature suggesting that L-arginine (L-Arg) metabolism in the alimentary tract is altered in animal models of ischemic colitis and inflammatory bowel disease.¹ In a rat model, L-Arg supplementation attenuated the degree of tissue damage in intestinal ischemia and promoted healing of intestinal mucosa.² We have shown that in mouse models of infectious (*Citrobacter rodentium*) colitis and dextran sulfate sodium-induced colitis, L-Arg supplementation is beneficial and inhibition of polyamine synthesis is deleterious.^{3, 4}

L-Arg is a semi-essential amino acid that is important in protein synthesis and plays a key role in the intermediate metabolism of nitrogen by participating in the urea cycle. It can be synthesized by the body and hence dietary arginine is not absolutely essential to nitrogen balance in normal adults.⁵ Total body arginine, however, may become depleted under stressful conditions.^{6, 7} L-Arg is a substrate for four enzymes, some of which exist as multiple isoforms: the arginases, the nitric oxide (NO) synthases (NOS), arginine:glycine amidinotransferase, and arginine decarboxylase.⁸ In addition to its role in protein synthesis and nitrogen disposal, L-Arg serves as a precursor to glutamine, proline, and ornithine, with the latter serving as the substrate for the synthesis of polyamines.^{9, 10} Notably, polyamines are associated with mucosal protection in the gastrointestinal tract.^{11, 12}

Cellular uptake of L-Arg is an active transport process via arginine transporters, also known as y^+ transporters.¹³ Cationic amino acid transporters (CAT) 1 – 4 have been described. CAT2 is the inducible transporter that is upregulated in macrophages upon immune activation,^{14, 15} while CAT1 is constitutively expressed.¹⁵ The potential importance of CAT3 and CAT4 is poorly understood.^{16, 17} CAT proteins are subject to competitive inhibition by L-ornithine (L-Orn) and L-lysine (L-Lys).^{16–18}

One role of L-Arg that has generated great interest for both scientists and clinicians is its function as the substrate for the synthesis of NO.¹⁹ In the past twenty years, many studies have shown that NO plays an important role in immune regulation and in governing the vascular response to stress.¹ Therefore, investigators have devoted efforts toward manipulating these functions in an attempt to enhance host responses to infection or regulate inflammation. In recent years, L-Arg supplementation or restriction has been proposed and studied in several disorders including asthma; vascular diseases; mitochondrial encephalopathy, lactic acidosis, and stroke (MELAS); glutaric aciduria type I; and disorders of creatine metabolism.^{8, 20–23} Additionally, L-Arg supplementation has been shown to alleviate intrauterine growth retardation²⁴ and to have a protective effect against necrotizing enterocolitis in premature infants.²⁵ In the latter study, these infants were found to have deficiencies in glutamine and L-Arg, which are essential to intestinal integrity.²⁶

Because of its importance in epithelial function, we hypothesized that L-Arg utilization is dysregulated in ulcerative colitis (UC). Our aim was to gain insight into total body levels of L-Arg and its availability in UC. Therefore we determined if serum L-Arg levels are altered in UC, and if these levels are associated with indices of disease severity.

MATERIALS AND METHODS

Patients

Subjects were recruited prospectively for serum collection prior to routine colonoscopy for either colorectal cancer screening, evaluation for possible inflammatory bowel disease, or surveillance of known UC. Body mass index (BMI) and serum albumin level were recorded for study subjects at the time of enrollment. Serum samples were stored at -80°C in the Vanderbilt Tissue Repository until use in a coded and de-identified manner. All subjects

underwent overnight fast and received polyethylene glycol electrolyte solution (GoLYTELY®) for bowel preparation prior to colonoscopy. Subjects with known hepatic dysfunction, renal insufficiency, or L-Arg supplementation were excluded from the study.

After reviewing all available serum samples in the tissue repository, 14 subjects with normal histopathologic findings were included to serve as controls for this study, and 22 patients with pancolitis of moderate or severe activity by pathology score were also included. Tissue biopsies were reviewed by the Department of Pathology at Vanderbilt University, and graded accordingly as normal, quiescent, mild, moderate, or severe activity. The Mayo Disease Activity Index (DAI) was determined by standard measures (0 to 12 scale), which included number of bowel movements per day, the presence or absence of blood, endoscopy scoring, and physician's global assessment.²⁷ Endoscopic assessment was performed by a single gastroenterologist specializing in inflammatory bowel disease (DAS) and graded on a scale of normal, mild disease (erythema, decreased vascular pattern, mild friability), moderate disease (marked erythema, lack of vascular pattern, friability, erosions), or severe disease (spontaneous bleeding, ulceration).

Amino Acid Measurements

Serum L-Arg, L-Orn, and L-Lys levels were determined by high performance liquid chromatography (HPLC) at the Vanderbilt Hormone Assay Core. Amino acid concentrations were determined by a reverse-phase HPLC technique with a precolumn derivatization using Waters AccQFluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate or AQC). The amino acids were subjected to LC separation on a 3.9 × 300mm Nova-pak C18 column (Waters part number WAT011695, Milford MA). Eluents chosen were Eluent A with 19% sodium acetate, 7% phosphoric acid, and 2% Triethylamine and 72% water, pH 5.8, and Eluent B with 60% acetonitrile and 40% water. The areas under the peaks were integrated using Millenium software, version 3.2 provided by the Waters Chromatography Corporation, Milford, MA.

Statistics

While serum L-Arg was the primary marker of interest, L-Arg availability is dependent on serum levels of L-Orn and L-Lys since all three amino acids are taken up by cells via the same cationic amino acid transport system.¹⁸ We defined the arginine availability index (AAI) as serum [L-Arg]/([L-Orn] + [L-Lys]).^{9, 20} Results were expressed as means ± SEM. Statistical analysis was performed with SPSS V. 15 (Chicago, IL) and GraphPad Prism 5.0 (San Diego, CA).

D'Agostino & Pearson omnibus testing was performed to assess for normality in the groups. Data were then analyzed by one-way ANOVA and the Tukey post-hoc multiple comparisons test. Pearson's correlation coefficient was used to test for strength of association between DAI and serum arginine level. $P < 0.05$ values were considered statistically significant.

ETHICAL CONSIDERATIONS

The study protocol was approved by the Institutional Review Board at Vanderbilt University. Written informed consent was obtained from all subjects before entry into this study.

RESULTS

Patient Characteristics

To test the hypothesis that ulcerative colitis is associated with dysregulation of L-Arg availability, we elected to analyze serum amino acid levels in human subjects with histologically-proven pancolitis. We chose patients with pancolitis for this first study on amino acid levels because we considered them most likely to have homeostatic imbalances compared to patients with more limited disease, as has been reported with micronutrients.^{28, 29} The total number of subjects included in the analysis was 36. In addition to 14 normal controls, there were 14 UC subjects with moderate pancolitis, and 8 UC subjects with severe pancolitis, based on histopathologic findings. The baseline characteristics of the study participants are presented in Table 1. The mean age of subjects with moderate pancolitis was 34.7 years and for those with severe pancolitis was 52.1 years; the mean age of the control subjects was intermediate at 48.2 years. Commonly used clinical indicators of nutritional status were assessed (Table 1). There were no significant differences in BMI between the subject groups. Serum albumin levels were modestly decreased in both the moderate and severe colitis groups when compared to normal controls. However, the mean values, as well as all individual values for albumin in the colitis patients were still in the normal range (3.5 – 5.0 for our clinical laboratory).

While the majority of UC patients were on 5-aminosalicylate therapy, there was no significant difference between the moderate and severe UC group. It should be noted that 3 subjects in our study presenting for evaluation of possible UC who were on either 5-aminosalicylate, corticosteroid, or immunodulator therapy were found to have normal histologic and endoscopic findings. Parameters of gender, smoking status, alcohol use, and immunomodulator treatment were not significantly different among the three groups.

Serum Amino Acid Levels and Ulcerative Colitis Severity Assessed by Histopathology

For our initial analyses, we selected patients for this study based on histopathology in order to directly assess the effect of graded tissue injury on L-Arg homeostasis. Furthermore, we chose subjects with moderate or severe pancolitis, to increase the likelihood of detecting abnormalities in our study. By ANOVA, serum L-Arg levels were significantly associated with histopathologic grade ($P = 0.001$). More specifically, when we stratified the data based on colitis severity (Figure 1), serum L-Arg concentrations were significantly elevated in subjects with severe colitis when compared to normal control subjects ($P = 0.001$). Patients with only moderate colitis did not exhibit increased L-Arg levels compared to control subjects, and these levels were also less than those in the severe colitis group ($P = 0.006$; Figure 1).

Because L-Orn and L-Lys are known to compete with L-Arg for cellular uptake by the y^+ transport system, we also measured the levels of these two amino acids. ANOVA revealed an overall association of disease severity with levels of [L-Orn] + [L-Lys] ($P = 0.034$). Similar to what was observed with L-Arg, the levels of L-Orn + L-Lys were increased in severe colitis ($P = 0.03$; Figure 2). The mean serum amino acid levels for L-Arg, and for L-Orn + L-Lys are shown in Table 2. When the relative arginine availability was calculated for each subject, this revealed that there was no significant increase in AAI in the severe colitis group when compared to either the moderate colitis or normal control group; the mean values are shown in Table 2. These data suggest that while the concentration of L-Arg is increased in the serum, its availability is limited by the presence of L-Orn and L-Lys, resulting in no net increase in cells and tissues.

Serum Arginine and Other Clinical Indicators of Colitis Severity

Because clinical scores are often used in drug trials and are employed to assess biomarkers, we analyzed the relationship between L-Arg levels and the parameters of the endoscopy sub-score and the full disease activity index (DAI), which includes the endoscopy score. ANOVA revealed a significant association of serum L-Arg levels with the endoscopy sub-score ($P < 0.001$). As shown in Figure 3, L-Arg levels were significantly higher in the severe group when compared with either the normal, mild, or moderate endoscopy score groups ($P < 0.001$ for each comparison). However, as with the histopathology data above, AAI was not increased in the severe colitis group (data not shown).

Because we had consistently observed that serum L-Arg levels were correlated with disease severity, we next sought to determine if such a non-invasive measurement could be predictive of a composite measurement of clinical status, the disease activity index (DAI), which incorporates symptom parameters, clinical signs, endoscopic findings and physician assessment. We therefore plotted serum L-Arg against the DAI (Figure 4), and found that there was a strong correlation when assessed by Pearson's correlation test ($r = 0.656$, $P < 0.001$).

DISCUSSION

In this study we found increased serum concentrations of L-Arg in patients with severe UC as assessed by histopathology. However, relative arginine availability, as calculated by the AAI, was not increased in these subjects due to the concomitant increase in serum L-Orn and L-Lys, which have been demonstrated to act as competitive inhibitors for L-Arg transport by CAT1 or CAT2 located at the cell membrane (Figure 5).^{9, 13, 17, 18} Our findings indicate that while there is a high circulating level of L-Arg, there is a functional deficiency of L-Arg availability in UC. We suggest that the unchanged L-Arg availability in UC patients, due to increased L-Orn + L-Lys, reduces the ability of the tissues to respond to metabolic stress. As illustrated in Figure 5, this inability to import sufficient L-Arg is likely to contribute to cellular dysfunction such as impairments in epithelial restitution, protein translation, phagocytosis, and innate immunity, as well as dysregulation of apoptosis.

It should be noted that there were some limitations to our cross-sectional study of serum L-Arg in ulcerative colitis. Our findings demonstrate an association between disease severity (using either pathology score or DAI) and serum L-Arg levels. However, a potential causal role for alterations in L-Arg availability in UC could not be directly assessed. In addition, alterations in nutritional status could affect serum amino acid levels. However, our study patients with UC did not appear to be catabolic or nutritionally deficient, since their BMI was not decreased, and serum albumin levels were in the normal range. Furthermore, when assessed by Pearson's correlation test, there was no association between albumin level and either serum L-Arg ($P = 0.410$) or serum L-Orn + L-Lys ($P = 0.402$). The sample size of our study was limited by the selection of only patients with pancolitis and not localized UC disease. We measured serum L-Arg levels rather than tissue levels, because L-Arg is in constant flux between the intracellular and extracellular space, making serum levels a more reliable means to measure L-Arg availability.¹⁰ UC patients were significantly more likely to be on 5-aminosalicylates than normal subjects, which cannot be avoided due to the near universal treatment of UC patients with these first-line agents.

Relevant to the current study, in an animal model of colitis induced by *Citrobacter rodentium*, we found serum L-Arg to be decreased compared to uninfected mice, which we attributed to consumption due to marked transmural inflammation, bacteremia, and massive induction of iNOS.³ However, we have also found that serum L-Arg levels are significantly increased in mice treated with dextran sulfate sodium (DSS) (unpublished observations).

Experimental DSS colitis in rodents is highly reproducible and shares features with human UC,³⁰ especially the epithelial crypt damage that characterizes the model. Interestingly, in our laboratory, L-Arg supplementation in both *C. rodentium*- and DSS-treated mice was shown to improve colitis.^{3, 4}

Delineation of factors that inhibit uptake of L-Arg could improve our understanding of UC or lead to novel treatment options. One potential explanation could be either altered expression or function of L-Arg transporters. In preliminary studies of mRNA expression of the inducible transporter, CAT2, we have found that levels are upregulated in UC subjects compared to control tissues. These findings suggest that it is unlikely that there is defective expression of arginine transporters in UC. Additionally, we have observed that mice with targeted deletion of CAT2 exhibit increased levels of serum L-Arg, and exacerbation of DSS colitis when compared to wild-type mice (manuscript in preparation). When taken together with the data presented in the current report, we speculate that there is a defect in CAT function in UC.

Defective L-Arg uptake can lead to diminished ability of the arginase/ODC pathway to generate polyamines (Figure 5). There may also be concomitant dysfunction of arginase or ODC enzymes in IBD. In fact, we have reported that pharmacologic inhibition of either arginase or ODC exacerbates colitis in mouse models.^{3, 4} In severe human colitis, a decrease in ODC activity has been reported.³¹ Thus, there may be an important combined deleterious effect of diminished L-Arg substrate for polyamine synthesis coupled with decreased ODC activity in subjects with severe colitis. This is especially important because polyamines have been shown to have many biological functions that may be relevant to amelioration of colitis, including inhibition of Th1 cytokine production and monocyte activation,³² stimulation of epithelial restitution by enhancing cell migration¹² and proliferation,¹¹ and regulation of apoptosis.³³

Our data suggest that serum L-Arg concentration may prove to be useful as a biomarker for UC disease severity, since we found that L-Arg levels were highly correlated with DAI. A larger prospective study would be required to determine if L-Arg levels are predictive of clinical outcome in UC. Our data also raise the question of the potential utility of L-Arg as an adjunctive treatment for humans with UC in the setting of a paradoxical increase in serum levels of L-Arg in severe colitis. It would be very intriguing to determine if L-Arg supplementation would allow for increased functional availability of L-Arg by overcoming the competitive inhibition by L-Orn and L-Lys, and thus have a benefit in UC as we have observed in mouse models of colitis.

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REFERENCES

1. Cross RK, Wilson KT. Nitric oxide in inflammatory bowel disease. *Inflamm Bowel Dis*. 2003; 9:179–189. [PubMed: 12792224]
2. Fotiadis C, Adamis S, Misiakos EP, et al. The prophylactic effect of L-arginine in acute ischaemic colitis in a rat model of ischaemia/reperfusion injury. *Acta Chir Belg*. 2007; 107:192–200. [PubMed: 17515270]
3. Gobert AP, Cheng Y, Akhtar M, et al. Protective role of arginase in a mouse model of colitis. *J Immunol*. 2004; 173:2109–2117. [PubMed: 15265947]

4. Cheng Y, Xu H, Forman JS, et al. Inhibition of colitis by the arginase-ODC pathway. *Gastroenterology*. 2003; 124:A473.
5. Rose WC. Amino acid requirements of man. *Fed Proc*. 1949; 8:546–552. [PubMed: 18146542]
6. Castillo L, Ajami A, Branch S, et al. Plasma arginine kinetics in adult man: response to an arginine-free diet. *Metabolism*. 1994; 43:114–122. [PubMed: 8289668]
7. Castillo L, Chapman TE, Sanchez M, et al. Plasma arginine and citrulline kinetics in adults given adequate and arginine-free diets. *Proc Natl Acad Sci U S A*. 1993; 90:7749–7753. [PubMed: 8356080]
8. Coman D, Yapfite-Lee J, Boneh A. New indications and controversies in arginine therapy. *Clin Nutr*. 2008; 27:489–496. [PubMed: 18640748]
9. Morris SM Jr. Enzymes of arginine metabolism. *J Nutr*. 2004; 134:2743S–2747S. discussion 2765S–2767S. [PubMed: 15465778]
10. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. *Biochem J*. 1998; 336(Pt 1): 1–17. [PubMed: 9806879]
11. Li L, Rao JN, Guo X, et al. Polyamine depletion stabilizes p53 resulting in inhibition of normal intestinal epithelial cell proliferation. *Am J Physiol Cell Physiol*. 2001; 281:C941–C953. [PubMed: 11502571]
12. Rao JN, Li L, Golovina VA, et al. Ca²⁺-RhoA signaling pathway required for polyamine-dependent intestinal epithelial cell migration. *Am J Physiol Cell Physiol*. 2001; 280:C993–C1007. [PubMed: 11245616]
13. Kakuda DK, Sweet MJ, MacLeod CL, et al. CAT2-mediated L-arginine transport and nitric oxide production in activated macrophages. *Biochem J*. 1999; 340(Pt 2):549–553. [PubMed: 10333501]
14. Martin L, Comalada M, Marti L, et al. Granulocyte-macrophage colony-stimulating factor increases L-arginine transport through the induction of CAT2 in bone marrow-derived macrophages. *Am J Physiol Cell Physiol*. 2006; 290:C1364–C1372. [PubMed: 16371438]
15. Nicholson B, Manner CK, Kleeman J, et al. Sustained nitric oxide production in macrophages requires the arginine transporter CAT2. *J Biol Chem*. 2001; 276:15881–15885. [PubMed: 11278602]
16. Closs EI, Simon A, Vekony N, et al. Plasma membrane transporters for arginine. *J Nutr*. 2004; 134:2752S–2759S. discussion 2765S–2767S. [PubMed: 15465780]
17. Verrey F, Closs EI, Wagner CA, et al. CATs and HATs: the SLC7 family of amino acid transporters. *Pflugers Arch*. 2004; 447:532–542. [PubMed: 14770310]
18. Closs EI, Basha FZ, Haberman A, et al. Interference of L-arginine analogues with L-arginine transport mediated by the y⁺ carrier hCAT-2B. *Nitric Oxide*. 1997; 1:65–73. [PubMed: 9701046]
19. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med*. 1993; 329:2002–2012. [PubMed: 7504210]
20. Morris CR, Poljakovic M, Lavrisa L, et al. Decreased arginine bioavailability and increased serum arginase activity in asthma. *Am J Respir Crit Care Med*. 2004; 170:148–153. [PubMed: 15070820]
21. Koga Y, Ishibashi M, Ueki I, et al. Effects of L-arginine on the acute phase of strokes in three patients with MELAS. *Neurology*. 2002; 58:827–828. [PubMed: 11889254]
22. Kolker S, Sauer SW, Okun JG, et al. Lysine intake and neurotoxicity in glutaric aciduria type I: towards a rationale for therapy? *Brain*. 2006; 129:e54. [PubMed: 16870879]
23. Braissant O, Henry H, Loup M, et al. Endogenous synthesis and transport of creatine in the rat brain: an in situ hybridization study. *Brain Res*. 2001; 86:193–201.
24. de Pace V, Chiossi G, Facchinetti F. Clinical use of nitric oxide donors and L-arginine in obstetrics. *J Matern Fetal Neonatal Med*. 2007; 20:569–579. [PubMed: 17674274]
25. Amin HJ, Zamora SA, McMillan DD, et al. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. *J Pediatr*. 2002; 140:425–431. [PubMed: 12006956]
26. Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. *J Pediatr*. 2000; 137:785–793. [PubMed: 11113834]
27. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005; 353:2462–2476. [PubMed: 16339095]

28. Lashner BA. Red blood cell folate is associated with the development of dysplasia and cancer in ulcerative colitis. *J Cancer Res Clin Oncol.* 1993; 119:549–554. [PubMed: 8392076]
29. Ringstad J, Kildebo S, Thomassen Y. Serum selenium, copper, and zinc concentrations in Crohn's disease and ulcerative colitis. *Scand J Gastroenterol.* 1993; 28:605–608. [PubMed: 8362213]
30. Gaudio E, Taddei G, Vetusch A, et al. Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects. *Dig Dis Sci.* 1999; 44:1458–1475. [PubMed: 10489934]
31. Ricci G, Stabellini G, Bersani G, et al. Ornithine decarboxylase in colonic mucosa from patients with moderate or severe Crohn's disease and ulcerative colitis. *Eur J Gastroenterol Hepatol.* 1999; 11:903–904. [PubMed: 10514125]
32. Zhang M, Caragine T, Wang H, et al. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. *J Exp Med.* 1997; 185:1759–1768. [PubMed: 9151701]
33. Shearer JD, Richards JR, Mills CD, et al. Differential regulation of macrophage arginine metabolism: a proposed role in wound healing. *Am J Physiol.* 1997; 272:E181–E190. [PubMed: 9124321]

Figure 1.

Serum concentrations of L-Arg in human subjects with normal mucosa and UC. Individual values for different patients are shown. For each group, the heavy solid line represents the mean and the error bars are the SEM. Serum L-Arg concentration was significantly increased in subjects with severe colitis when compared to those with moderate colitis ($P = 0.006$) or normal mucosa ($P = 0.001$).

Figure 2.

Serum concentrations of L-Orn + L-Lys in human subjects with normal mucosa and UC.

Individual values for different patients are shown. For each group, the heavy solid line

represents the mean and the error bars are the SEM. Serum [L-Orn+L-Lys] was increased in

subjects with severe colitis when compared to those with moderate colitis ($P = 0.03$).

Figure 3.

Serum L-Arg levels plotted versus endoscopy score. For each group, the heavy solid line represents the mean and the error bars are the SEM. Levels were higher in the severe group when compared to either the normal, mild, or moderate endoscopy score groups ($P < 0.001$ for each comparison).

Figure 4. Spline graph of L-Arg concentration versus Disease Activity Index (DAI). [L-Arg] was strongly correlated with DAI, which was assessed by Pearson's correlation test ($r = 0.656$, $P < 0.001$). The association was almost linear.

Figure 5.

Pathways for utilization of L-Arg and potential biological effects relevant to colonic homeostasis. Solid lines represent known biochemical processes, and dotted lines indicate potential effects. L-Arg is imported into cells by the transport proteins CAT1 and CAT2. This process is competitively inhibited by L-Orn and L-Lys. Once inside cells, L-Arg can be utilized by two competing pathways. 1. The arginase pathway generates L-Orn, which is the substrate for ornithine decarboxylase (ODC) that produces the polyamines putrescine, spermidine, and spermine. Polyamines have been shown to play a role in wound repair and are potential regulators of immune response, apoptosis and phagocytosis. 2. The inducible nitric oxide synthase (iNOS) pathway produces high levels of nitric oxide, which can have a wide variety of effects in the innate immune response. L-Arg may also have important roles in modulating the additional cellular functions indicated.

TABLE 1

Demographic Features of the Study Subjects

Histopathology	Normal (n = 14)	Moderate Colitis (n = 14)	Severe Colitis (n = 8)	P value (overall)
Age in years, mean $\pm$ SD, (range)	48.2 $\pm$ 12.5 (25 – 67)	34.7 $\pm$ 13.5 [*] (18 – 62)	52.1 $\pm$ 15.1 (29 – 77)	0.009 ^a
Gender	4M:10F	7M:7F	6M:2F	0.122 ^b
Body Mass Index, mean $\pm$ SD	24.4 $\pm$ 2.9	24.9 $\pm$ 3.6	27.8 $\pm$ 3.8	0.130 ^a
Serum Albumin, mean $\pm$ SD	4.5 $\pm$ 0.3	4.0 $\pm$ 0.3 [§]	3.9 $\pm$ 0.3 [‡]	0.007 ^a
Smoking	1/14	0/14	0/8	1.000 ^b
Alcohol Consumption (> 5 drinks per week)	2/14	4/14	1/8	0.651 ^b
5-aminosalicylates	1/14	14/14	7/8	< 0.001 ^b
Corticosteroids	2/14	7/14	5/8	0.047 ^b
Immunomodulators	1/14	3/14	4/8	0.093 ^b

^aOne-way ANOVA and Tukey post-hoc comparisons test.

^bExact $r \times c$ Contingency Table.

^{*} $P = 0.031$ versus Normal and $P = 0.016$ versus Severe.

[§] $P = 0.014$ versus Normal

[‡] $P = 0.009$ versus Normal.

TABLE 2

Serum Amino Acid Levels and Arginine Availability Index (AAI) in the Study Subjects

Histopathology	Normal	Moderate Colitis	Severe Colitis
[L-Arg] μ M (mean $\pm$ SEM)	92.9 $\pm$ 4.6	100.9 $\pm$ 6.4	139.9 $\pm$ 14.2 ^{**§§}
[L-Orn + L-Lys] μ M (mean $\pm$ SEM)	298.1 $\pm$ 13.7	284.7 $\pm$ 16.8	354.3 $\pm$ 22.5 [§]
AAI	0.32 $\pm$ 0.02	0.37 $\pm$ 0.03	0.40 $\pm$ 0.04

^{**} $P = 0.001$ versus Normal

[§] $P < 0.05$

^{§§} $P < 0.01$ versus Moderate Colitis by One-way ANOVA and Tukey post-hoc comparisons test.