Plasma-Free Amino Acid Profiles in Crohn's Disease: Relationship With the Crohn Disease Activity Index

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ABSTRACT

We aimed to clarify the relationship between plasma-free amino acid (PFAA) profiles and the Crohn's disease (CD) activity index (CDAI) in patients with CD.

METHODS: We measured fasting PFAA concentrations in 29 patients with CD and their correlation with disease activity.

RESULTS: In all patients, significant correlations were noted between CDAI and concentrations of valine, methionine, leucine, histidine, tryptophan, alanine, tyrosine, total amino acids (TAAs), nonessential amino acids (NEAAs), essential amino acids (EAAs), and branched-chain amino acids (BCAAs). In patients with the ileo-colonic type of CD, significant correlations were noted between CDAI and valine, histidine, tryptophan, glutamine, TAA, NEAA, EAA, and BCAA. In ileal type, significant correlations were observed between CDAI and threonine, valine, histidine, serine, and glycine. In colonic type, significant correlations were noted between CDAI and valine, histidine, tryptophan, TAA, NEAA, EAA, and BCAA.

CONCLUSIONS: In patients with CD, plasma amino acids appear to be associated with disease activity.

KEYWORDS: Amino acids, Crohn disease, disease activity, CDAI

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Introduction

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract characterized by recurrent inflammation at any location along its length. The disease typically manifests as bowel strictures, abscess formation, and fistulas. Malnutrition is one of the most significant problems in patients with CD, necessitating nutritional support for modulating intestinal inflammation.[1,2]

CD is characterized by damage of the epithelium and impaired the immune response of the bowel, function of the intestinal mucosal barrier, and absorption and/or wasting in the intestine of nutritional particles such as amino acids (AAs). Then, disturbances of metabolic homeostasis would be related to the pathogenesis of chronic inflammatory disorders such as CD.

The main role of AAs is to provide the building block for proteins, which are essential for life. Specific AAs belonging to the group of essential AAs (EAAs), such as glutamine (Gln) and arginine (Arg), have immunomodulatory effects during metabolic stress, especially when the gut is involved in systemic inflammation. Some AAs, such as glycine (Gly), histidine (His), cysteine (Cys), and taurine (Tau), are candidates for anti-inflammatory properties in intestinal epithelial cells.[3] Recently, the anti-inflammatory activity of certain AAs has been reported,[4,5] and antagonistic effects of AAs on intestinal inflammation have also been demonstrated.[6–15]

Gln is an immunomodulatory agent. It is the preferred energy source for intestinal epithelial cells, it protects the function of the intestinal mucosal barrier, and it serves as the basic nutritional element for cellular immune function.[16] It has been shown that the combination of Gln and Arg suppresses the release of pro-inflammatory cytokines from colonic tissues of patients with CD.[17] Gln supplementation increased plasma concentrations of threonine (Thr), citruline (Cit), and His; and Arg enhanced the ratio of AAs in rats with dextran sulfate sodium (DSS)-induced colitis.[12] Gly prevents chemical-induced colitis by inhibiting the induction of inflammatory cytokines and chemokines.[18] Plasma His and tryptophan (Trp) concentrations were significantly lower in patients with inflammatory bowel disease (IBD) than in healthy controls.[19]

These observations strongly suggest that plasma AA concentrations are closely associated with disease activity in CD. However, plasma-free AA (PFAA) profiles have not been well investigated in patients with CD. The goal of this study was to clarify the relationship between PFAAs and the CD activity index (CDAI)[20,21] and to supplement patients with possibly beneficial AAs.

Patients and Methods

Patients

A total of 29 patients with CD were enrolled (6 women, 23 men; mean age, 41.0 years; 17 ileo-colonic type, 7 ileal type, 5 colonic

Table 1. Clinical characteristics of patients with the ileo-colonic, ileal, and colonic types of CD (n=29).

	ALL (N=29)	ILEO-COLONIC TYPE (N=17)	ILEAL TYPE (N=7)	COLONIC TYPE (N=5)	*P* VALUE[a]
Age, y	18-55 (41.0)	18-55 (32.4)	33-49 (41.0)	27-32 (29.0)	.98
Gender, female/male	6/23	3/14	2/5	1/4	.28
Height, cm	168.2 ± 8.9	167.5 ± 10.0	169.2 ± 4.8	169.0 ± 9.6	.75
Body weight, kg	58.4 ± 9.9	56.2 ± 10.0	62.6 ± 6.2	60.0 ± 11.8	.39
Body mass index, kg/m^2	20.6 ± 2.8	19.9 ± 2.4	22.0 ± 3.1	20.9 ± 3.0	.43
Disease duration, y	1-19 (5.4)	1-15 (6.1)	2.3-19 (5.8)	1-7 (2.8)	.19
CDAI	160.1 ± 74.6	160.2 ± 85.0	151.3 ± 51.1	151.7 ± 75.5	.95
Treatment					
5-aminosalicylic acid, %	29 (100)	17 (100)	7 (100)	5 (100)	1.00
Prednisone, %	3 (10.3)	2 (11.2)	1 (14.3)	1 (25.0)	.28
Azathioprine, %	6 (20.7)	3 (17.6)	2 (28.5)	1 (25.0)	.29
Anti TNF-α agents, %	20 (70.0)	13 (76.5)	3 (42.9)	4 (80.0)	.28

Abbreviations: CDAI, Crohn's disease activity index; TNF-α, tumor necrosis factor α.
Data are presented as the mean ± SD. Age and disease duration are expressed as median (25th-75th percentiles).
[a]Mann-Whitney *U* test.

type). The mean disease duration was 10.3 years (range, 3-30 years). The patients were evaluated with the CDAI at the time of blood sampling.[20] The contributors to CDAI index consists of 8 factors, such as number of loose stools, abdominal pain, general well-being, presence of complications, taking antidiarrhea drug, presence of an abdominal mass, hematocrit, and percentage deviation from standard weight, each summed after adjustment with a weighting factor based on the past 7 days. Plasma samples for AA analysis were obtained in the morning before breakfast using EDTA as an anticoagulant. The study protocol was approved by the Institutional Review Board at Iwate Medical University and performed in accordance with the Declaration of Helsinki.

Measurement of plasma AAs

Fasting plasma concentrations of the 20 AAs—Thr, valine (Val), methionine (Met), isoleucine (Iso), leucine (Leu), phenylalanine (Phe), His, Trp, lysine (Lys), aspartic acid (Asp), serine (Ser), asparagine (Asn), glutamic acid (Glu), Gln, proline (Pro), Gly, alanine (Ala), Cys, tyrosine (Tyr), arginine (Arg)—were measured by high-performance liquid chromatography. Subsequently, the concentrations of total AAs (TAAs), nonessential AAs (NEAAs), EAAs, and branched-chain AAs (BCAAs) were also calculated.

Statistical analyses

Patient characteristics data are presented as the mean ± SD. The AA levels are expressed as median (25th-75th percentiles). Within-group comparisons were analyzed by nonparametric comparisons with the Mann-Whitney *U* test. Multiple comparisons were performed using the Benjamini-Hochberg procedure. Correlations were evaluated with Pearson correlation coefficient test. A *P* value of $<.05$ was considered statistically significant.

Results

Patient characteristics

Table 1 summarizes the clinical characteristics of patients with the ileo-colonic, ileal, and colonic types of CD. A total of 17 patients had the ileo-colonic type (3 women, 14 men; mean age, 32.4 years), 7 patients had the ileal type (2 women, 5 men; mean age, 41.0 years), and 5 patients had the colonic type of CD (1 woman, 4 men; mean age, 29.0 years). All of the patients were treated with oral mesalamine (750-3000 mg/d). Two patients with the ileo-cecal type and patient with the colonic type also received oral prednisone (5-25 mg/d). Three patients with the ileo-colonic type, 2 patients with the ileal type and 1 patient with the colonic type were given oral azathioprine (50 mg/d). In addition, 13 patients with the ileo-colonic type, 3 patients with the ileal type, and 4 patients with the colonic type were treated with anti–TNF-α (tumor necrosis factor α) agents. No statistical differences in age, sex, disease duration, and CDAI were observed among the 3 groups.

Comparison of AA profiles among types of CD

The AA profiles were not significantly different among patients with the ileo-colonic, ileal, and colonic types of CD (Table 2).

Table 2. Comparison of AA profiles in patients with the ileo-colonic, ileal, and colonic types of CD (n = 29).

AAS, NMOL/ML	ALL (N = 29)	ILEO-COLONIC TYPE (N = 17)	ILEAL TYPE (N = 7)	COLONIC TYPE (N = 5)	*P* VALUE[a]	NORMAL RANGE
EAAs						
Thr	103.4 (90.6-120.1)	101.4 (92.1-120.1)	118.7 (117.5-142.7)	82.9 (82.5-110.1)	.81	(66.5-188.9)
Val	191.0 (156.2-232.2)	189.4 (160.1-226.3)	192.8 (173.6-243.3)	146.8 (133.8-232.2)	.92	(147.8-307.0)
Met	23.8 (21.1-28.3)	22.8 (21.1-28.3)	24.6 (26.3-30.0)	19.3 (18.4-23.8)	.50	(18.9-40.5)
Iso	63.4 (52.9-70.5)	57.8 (51.2-69.2)	69.1 (61.1-77.7)	64.4 (59.3-70.8)	.21	(43.0-112.8)
Leu	99.1 (88.1-129.3)	99.0 (88.1-127.4)	111.7 (97.6-134.1)	102.5 (80.9-133.9)	.57	(76.6-171.3)
Phe	51.4 (44.3-60.6)	50.6 (43.9-61.1)	54.1 (48.5-56.8)	55.3 (44.3-68.8)	.24	(42.6-75.7)
His	73.7 (65.1-81.1)	73.7 (65.1-79.7)	74.4 (71.3-78.6)	50.2 (47.0-88.9)	.81	(59.0-92.0)
Trp	42.4 (24.4-52.6)	42.4 (29.0-48.9)	49.1 (32.1-51.9)	22.4 (19.3-53.6)	.73	(37.0-74.9)
Lys	157.9 (130.9-191.7)	153.0 (118.4-181.1)	189.5 (155.5-216.5)	137.0 (132.5-162.0)	.70	(108.7-242.2)
NEAAs						
Asp	3.1 (2.6-3.7)	3.0 (2.6-3.4)	3.2 (2.3-3.3)	3.6 (3.0-4.5)	.95	(<2.4)
Ser	113.5 (102.5-125.0)	115.6 (105.4-124.1)	125.0 (107.7-129.6)	105.5 (101.5-109.7)	.91	(72.4-164.5)
Asn	43.4 (37.5-47.3)	42.6 (37.5-46.4)	48.1 (39.2-52.7)	38.9 (38.7-45.2)	.72	(44.7-96.8)
Glu	44.8 (37.5-54.6)	41.5 (38.7-52.4)	45.3 (22.1-62.2)	44.8 (38.1-63.8)	.29	(12.6-62.5)
Gln	518.7 (453.7-561.7)	517.6 (455.4-553.7)	556.5 (493.8-617.6)	510.8 (434.4-540.0)	.36	(422.1-703.8)
Pro	168.5 (123.7-204.3)	144.8 (116.0-195.7)	202.4 (168.6-214.2)	123.7 (119.3-168.5)	.77	(77.8-272.7)
Gly	247.9 (210.3-321.2)	247.9 (210.3-294.6)	321.2 (249.3-373.1)	201.5 (201.0-223.3)	.62	(151.0-351.0)
Ala	360.6 (285.4-463.0)	319.0 (262.1-463.0)	422.0 (367.7-533.2)	311.6 (296.7-367.1)	.49	(208.7-522.7)
Cys	30.1 (21.7-36.7)	30.5 (23.0-36.7)	30.8 (21.7-37.5)	29.4 (21.7-30.1)	.94	(13.7-28.3)
Tyr	52.1 (46.1-60.7)	51.9 (46.2-59.7)	54.0 (47.7-60.9)	49.7 (33.4-61.9)	.69	(40.4-90.3)
Arg	68.2 (59.5-76.8)	68.2 (59.5-76.1)	79.8 (67.9-82.6)	61.4 (47.8-61.7)	.64	(53.6-133.6)
TAAs	2781.7 (2242.0-3096.9)	2553.1 (2244.6-2984.2)	3086.0 (2890.5-3209.3)	2242.0 (2135.5-2847.4)	.70	(2068.2-3510.3)
NEAAs	1844.7 (1600.2-2158.5)	1757.3 (1590.2-2091.0)	2137.9 (2046.9-2178.7)	1614.4 (1572.9-1826.6)	.62	(1381.6-2379.4)
EAAs	851.4 (691.6-942.3)	840.1 (758.0-923.8)	884.8 (836.4-993.2)	691.6 (627.6-943.9)	.75	(660.0-1222.3)
BCAAs	348.6 (297.2-433.2)	346.8 (303.6-424.2)	396.9 (322.9-450.6)	308.6 (285.5-441.2)	.69	(265.8-579.1)

Abbreviations: AAs, amino acids; BCAAs, branched-chain amino acids; EAAs, essential amino acids; NEAAs, nonessential amino acids; NS, nonsignificant; TAAs, total amino acids.
Data are presented as median (25th-75th percentiles).
[a]Multiple comparisons were performed using the Benjamini-Hochberg procedure.

Table 3. Correlation between the CDAI score and amino acid concentrations in patients with the ileo-colonic, ileal, and colonic types of CD (n = 29).

AAS	ALL (N = 29)		ILEO-COLONIC TYPE (N = 17)		ILEAL TYPE (N = 7)		COLONIC TYPE (N = 5)	
	R	*P* VALUE[a]	*R*	*P* VALUE[a]	*R*	*P* VALUE[a]	*R*	*P* VALUE[a]
EAAs								
Thr	−.36	.054	−.34	.17	−.44	.32	−.83	.07
Val	−.58	.0009	−.57	.0174	−.47	.29	−.90	.0349
Met	−.46	.0124	−.44	.07	−.17	.71	−.87	.053
Iso	−.27	.15	−.29	.25	.17	.71	−.50	.39
Leu	−.47	.0105	−.47	.055	.03	.94	−.95	.0124
Phe	−.37	.0483	−.29	.25	.30	.50	−.95	.0115
His	−.64	.0002	−.73	.0008	−.39	.38	−.77	.12
Trp	−.65	.0001	−.77	.0003	−.18	.70	−.80	.10
Lys	−.39	.03	−.46	.07	.02	.59	−.68	.33
NEAAs								
Asp	.36	.06	.31	.23	−.01	.98	.69	.19
Ser	−.38	.0444	−.35	.17	−.85	.0164	−.29	.64
Asn	−.06	.76	−.0017	.99	.00	.99	−.53	.35
Glu	−.24	.21	−.22	.39	.07	.88	−.77	.13
Gln	−.34	.06	−.57	.0164	.23	.62	−.24	.69
Pro	−.28	.13	−.34	.18	−.01	.98	−.23	.70
Gly	−.23	.23	−.19	.45	−.57	.18	−.13	.83
Ala	−.52	.0042	−.62	.0073	−.07	.88	−.85	.06
Cys	−.24	.20	−.35	.17	.03	.94	−.50	.39
Tyr	−.51	.0049	−.42	.09	−.42	.34	−.99	.0017
Arg	−.22	.24	−.44	.07	−.26	.59	.56	.33
TAAs	−.56	.0015	−.63	.0063	−.26	.58	−.68	.19
NEAAs	−.48	.0082	−.56	.02	−.23	.62	−.47	.42
EAAs	−.61	.0004	−.66	.0037	−.24	.59	−.92	.0230
BCAAs	−.53	.0031	−.52	.0327	−.24	.61	−.90	.0328

Abbreviations: AAs, amino acids; BCAAs, branched-chain amino acids; EAAs, essential amino acids; NEAAs, nonessential amino acids; NS, nonsignificant; TAAs, total amino acids.
[a]Pearson correlation coefficient test.

Relationship between CDAI scores and concentrations of AAs

All patients with CD. Significant correlations were noted between CDAI scores and concentrations of Val, Met, Leu, His, Trp, Ala, Tyr, TAA, NEAA, EAA, and BCAA (Table 3). Concentrations of 5 EAAs, Val, Met, Leu, His, and Trp were significantly correlated with CDAI scores (Figure 1A to E). No significant correlations were observed between CDAI scores and concentrations of the other AAs.

Ileo-colonic type. Significant correlations were noted between CDAI scores and concentrations of Val, His, Trp, Gln, Ala, TAA, EAA, and BCAA in patients with the ileo-colonic type of CD (Table 3). No significant correlations were observed between CDAI scores and concentrations of the other AAs.

Ileal type. Significant correlations were observed between CDAI scores and the concentration of Ser in patients with the ileal type of CD (Table 3). No significant correlations were

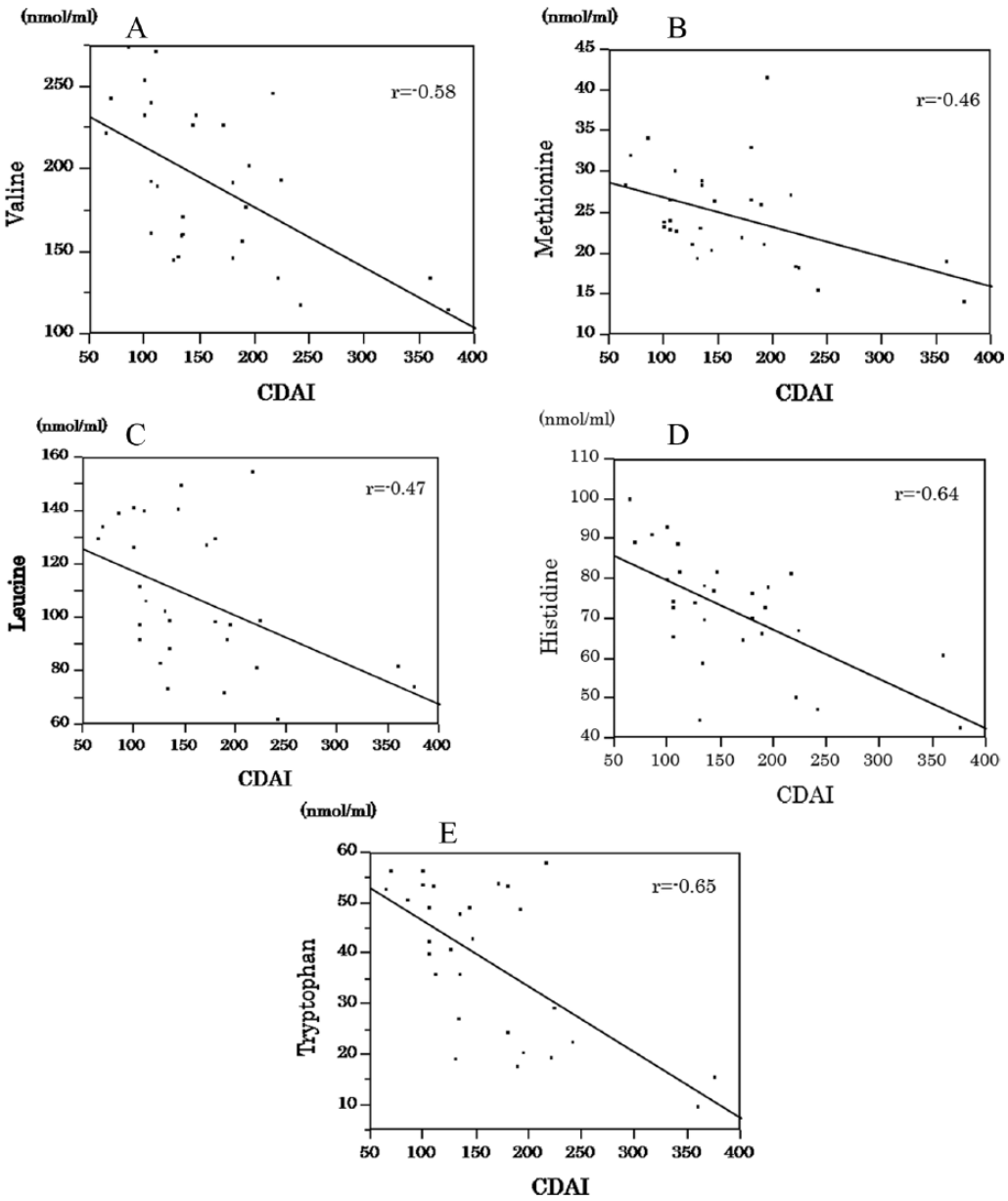

Figure 1. Correlation between CDAI and valine, methionine, leucine, histidine and tryptophan. (A) The concentration of valine was significantly correlated with the CDAI score in patients with CD. (B) The concentration of methionine was significantly correlated with the CDAI score in patients with CD. (C) The concentration of leucine was significantly correlated with the CDAI score in patients with CD. (D) The concentration of histidine was significantly correlated with the CDAI score in patients with CD. (E) The concentration of tryptophan was significantly correlated with the CDAI score in patients with CD.

observed between CDAI scores and concentrations of the other AAs.

Colonic type. In patients with the colonic type of CD, significant correlations were noted between CDAI scores and concentrations of Val, Leu, Phe, Tyr, EAA, and BCAA (Table 3). No significant correlations were observed between CDAI scores and concentrations of the other AAs.

Discussion

In this study, we analyzed the PFAAs profiles of patients with CD, with an emphasis on correlations with disease activity. In particular, concentrations of 5 EAAs—Val, Met, Leu, His, and Trp—were significantly correlated with CDAI scores in patients with CD, which would reflect the degree of inflammation (Figure 1A to E). These AAs belong to the EAAs, which are supplied in the diet. Furthermore, significant correlations were shown between CDAI scores and a certain proportion of EAAs and NEAAs in patients with CD. Nutritional deficiencies in patients with active CD would be the result of insufficient intake, malabsorption, and protein-losing enteropathy, as well as the metabolic disturbances induced by chronic disease.

In this study, Gln concentrations were correlated with CDAI in ileo-colonic CD. Gln plays a role in a variety of biochemical functions. Gln-enriched diets have been linked with the maintenance of gut barrier function and cell differentiation, suggesting that Gln may help protect the lining of the gastrointestinal mucosa.[22] The mucosal content of Gln has been shown to be

decreased in inflamed ileum and colon from patients with CD.[23] It has also been shown that Gln reduces the production of pro-inflammatory cytokines (IL-8 and IL-6) and enhances the production of the anti-inflammatory cytokine IL-10 in patients with CD.[17] In addition, it has been demonstrated that Gln supplementation improved outcomes in in vitro and in vivo experimental colitis models.[24] Gln administration failed to produce obvious biochemical or clinical benefit in patients with active IBD,[25] and according to a Cochrane Database System review, there is insufficient evidence to allow firm conclusions regarding the efficacy and safety of Gln for induction of remission in CD.[26]

In this study, the concentration of Trp was correlated with the CDAI score in patients with CD (Figure 1), especially those with the ileo-colonic type. Supplementation of L-Trp not only ameliorated clinical symptoms and increased weight gain but also improved histological scores and decreased the expression of pro-inflammatory cytokines in a porcine model of DSS colitis.[27]

In a rat model of DSS colitis, the administration of L-Tre, L-Pro, L-Cys, and L-Ser enhanced colonic protection and mucosal healing by increasing mucin synthesis and promoting gut microflora reequilibration.[28] Concentrations of AAs changed depending on the degree of colitis in DSS-treated mice, and serum levels of Gln, Trp, Tyr, Asn, and Gly were significantly lower than in control mice.[29] In patients with CD, the levels of Ala, Asp, Gly, Met, and Pro were significantly decreased in comparison with those of healthy volunteers.[30] Active CD is associated with depression of serum Trp.[31] However, AAs were not significantly different between ileo-colonic, ileal, and colonic type of CD in this study under the nutrition guidance of the dietician.

It has been shown that patients with ulcerative colitis displayed increased concentrations of 3-hydroxybutyrate, glucose, and Phe and decreased concentrations of lipids in serum.[32] In an IL-10 (−/−) cell transfer model of colitis recent studies, His prevented IL-8 secretion by intestinal epithelial cells[33] and reduced colitis lesions.[6] Trp metabolism has recently been highlighted as an immunological regulator,[34–36] and supplementation of His and Trp has been suggested as a therapeutic strategy for IBD.[21] The AA-based elemental diets would be useful by virtue of their low antigenicity,[37] resulting in the reduction in mucosal cytokine production such as IL-1, IL-6, IL-8, and TNF-α.[38] Then, disease activity and nutritional status were improved, particularly effective for poorly nourished with CD after administration of AAs, increases in the concentrations of His, Trp, and other plasma AAs in the remission group, whereas no increase in the level of AAs in the nonremission group.[39] Beneficial action of mucosal immunity from AAs in CD would be the ability to stimulate the integrity of the intestinal mucosal barrier and promote the restoration of the inflammatory reaction.[40]

There are several limitations in this study. First, we could not determine AA profiles specific to patients with CD because we did not enroll healthy subjects as controls. However, we believe that our data would aid in provisional supplementation for patients with active CD. Future research should evaluate the efficacy of the supplementation of the AA specifically depleted in active CD. Second, the low number of participants per study group was enrolled in this study; a threat to statistical validity would be worried. Third, most of the patients were treated with anti TNF-α agents in this study, a future study should investigate the AA profiles before and after administration of anti–TNF-α agents in patients with CD.

In conclusion, plasma concentrations of some AAs such as Val, Met, Leu, His, and Trp are closely associated with disease activity in patients with CD.

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Author Contributions

TC and KS designed the study, TC collected samples and analyzed the data. All authors were involved in drafting and revision of the manuscript and have approved the final manuscript for submission.

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