SINPE
Società Italiana di Nutrizione Artificiale e Metabolismo

26-28 ottobre 2023

# INCREASE MALNUTRITION AWARENESS: CHALLENGE FOR THE FUTURE

CONGRESSO NAZIONALE

## Could disease activity and protein intake affect serum amino acids concentration in adult patients with Crohn's disease?


**Dr Iolanda Cioffi, PhD RD**

Dept. of Food, Environmental and Nutritional Sciences – DeFENS, Università degli Studi di Milano, Milan
Dept. of Clinical Medicine and Surgery, Federico II University Hospital, Naples


# BACKGROUND (1)

- **Crohn's disease (CD) is an immune-mediated inflammatory bowel disease (IBD) characterized by a remitting–relapsing course.**

- **Chronic inflammation along with malabsorption and/or inadequate dietary intake can facilitate the development of malnutrition in CD patients, impairing their quality of life.**

- **Among nutrients, amino acids (AAs), the building blocks of protein macromolecules, may play critical roles in the intestinal manifestations of disease, due to their involvement in many metabolic and immune functions.**

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# BACKGROUND (2)

- **AA concentrations can vary in serum and tissues with developmental stage, diseases, nutritional state, endocrine status, and physical activity.**

- **Variations in AA concentrations have been observed in patients with IBD and in different other conditions such as liver diseases, heart failure, type 2 diabetes etc.**

- **Some AAs alterations, including reduced levels of Gln, Trp and His have been found in active IBD patients, suggesting that AA profiles might be altered in this population and might reflect disease activity as well as patients' nutritional status.**



Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# AIMS OF THE STUDY

**The present study aimed to explore serum AA concentrations in adult patients with CD, looking into their variations due to disease activity and protein content of diet. Finally, the link between AAs and inflammatory markers was also assessed.**

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# METHODS (1)

- This is a secondary analysis of the cross-sectional REECD (Resting Energy Expenditure evaluation in subjects with Crohn's Disease) study.

- Adult patients aged 18–65 years with diagnosis of CD were consecutively recruited from July 2016 to March 2018 at the Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy.

- Exclusion criteria:

- use of corticosteroids in the last 3 months
- history of acute or chronic liver or kidney disease
- current enteral (i.e., tube feeding) or parenteral nutrition
- presence of fistulae, ileostomy, or colostomy; presence of extensive small bowel resections (residual small bowel <2 meters)
- pregnancy or lactation; unstable body weight in the last month; and unable or unwilling to give informed consent.

- Socio-demographic data, disease duration, previous surgery, smoking habits, drug treatment, location and disease behavior (according to Montreal classification) were collected.

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# METHODS (2)

- Disease activity was clinically defined using the Crohn's Disease Activity Index (**CDAI)** score classifying patients in the active **(≥150)** and quiescent phases **(< 150)**.

- Blood samples were collected to analyze serum AA profiles and inflammatory markers (IL-6, Il-1β, TNF-α).

| Essential AAs (EAAs) | Non-essential (NEAAs) | Non-proteogenic AAs |
|---|---|---|
| • Valine (Val)<br>• Isoleucine (Ile)<br>• Leucine (Leu)<br>• Methionine (Met)<br>• Lysine (Lys)<br>• Phenylalanine (Phe)<br>• Tryptophan (Trp)<br>• Threonine (Thr)<br>• Histidine (His) | • Arginine (Arg)<br>• Glycine (Gly)<br>• Alanine (Ala)<br>• Serine (Ser)<br>• Tyrosine (Tyr)<br>• Cystine (Cys)<br>• Asparagine (Asn)<br>• Gutamine (Gln)<br>• Aspartic Acid (Asp)<br>• Glutamate (Glu) | • Ornithine (Orn)<br>• Citrulline (Cit)<br>• Taurine (Tau) |

- Participants underwent anthropometry (body weight, height and BMI) and were instructed to fill in a 3-day food record to assess their protein intake.

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

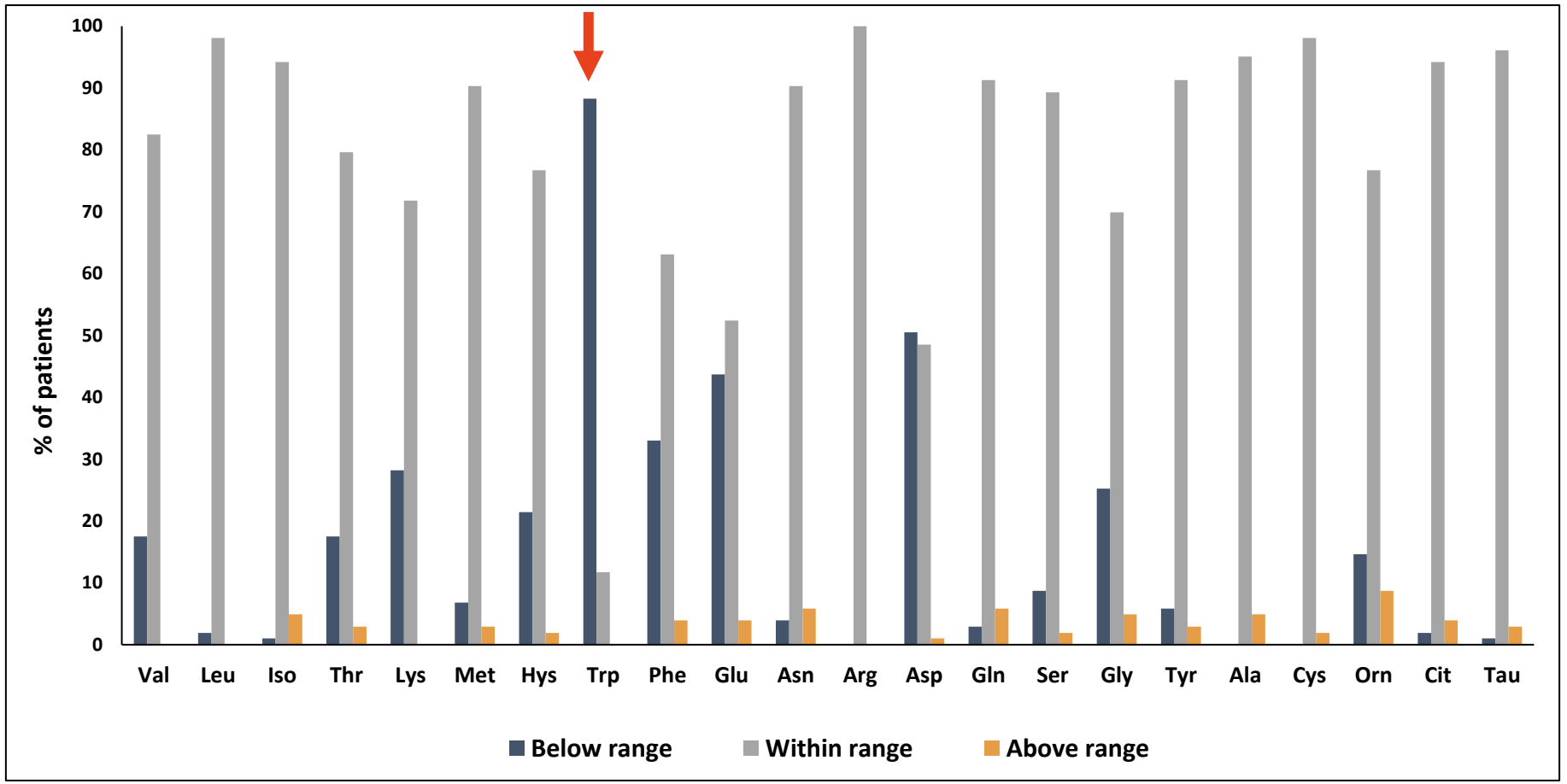
# RESULTS – Demographic and clinical characteristics

TABLE 1 Demographic and clinical characteristics of CD patients.

| | Total | Men | Women |
|---|---|---|---|
| N, (%) | 103 (100) | 62 (60.2) | 41 (39.8) |
| Age (years), mean [SD] | 39.9 [13.9] | 38.7 [13.4] | 41.6 [14.5] |
| Body weight (kg), mean [SD] | 65.5 [12] | 70.6 [10.5] | 57.8 [9.58] |
| BMI, n (%) | | | |
| <18.5 kg/m² | 6 (5.8) | 1 (1.6) | 5 (12.2) |
| 18.5–24.9 kg/m² | 74 (71.8) | 48 (77.4) | 26 (63.4) |
| 25–29.9 kg/m² | 18 (17.5) | 10 (16.1) | 8 (19.5) |
| >30 kg/m² | 5 (4.9) | 3 (4.8) | 2 (4.9) |
| Previous surgery, n (%) | 54 (52.4) | 29 (46.8) | 25 (61) |
| Disease duration (years), median [range] | 6.50 [1–30] | 6.42 [1–30] | 6.58 [1–23] |
| Currently smoking habits n (%) | | | |
| Yes | 22 (21.4) | 11 (17.7) | 11 (26.8) |
| No | 63 (61.2) | 40 (64.5) | 23 (56.1) |
| Ex-smoker | 18 (17.5) | 11 (17.7) | 7 (17.1) |
| Clinical activity, n (%) | | | |
| CDAI <150 | 55 (53.4) | 36 (58.1) | 19 (46.3) |
| >150 CDAI <450 | 48 (46.6) | 26 (19.4) | 22 (53.7) |
| Montreal age at diagnosis, n (%) | | | |
| A1: < 16 y | 18 (17.5) | 11 (17.7) | 7 (17.1) |
| A2: 17–40 y | 70 (68.0) | 43 (69.4) | 27 (65.9) |
| A3: > 40 y | 15 (14.6) | 8 (12.9) | 7 (17.1) |
| Montreal disease location, n (%) | | | |
| L1: Ileum | 36 (35) | 20 (32.2) | 16 (39.0) |
| L2: Colon | 10 (9.7) | 10 (16.1) | 0 |
| L3: Ileum and colon | 54 (52.4) | 30 (48.4) | 24 (58.5) |
| L4: Upper GI tract | 3 (2.9) | 2 (3.2) | 1 (2.4) |
| Montreal disease behavior, n (%) | | | |
| B1: Inflammatory | 32 (31.1) | 24 (38.7) | 8 (19.5) |
| B2: Stricturing | 54 (52.4) | 32 (51.6) | 22 (53.7) |
| B3: Penetrating | 17 (16.5) | 6 (9.7) | 11 (26.8) |
| Perianal disease, n (%) | 19 (18.4) | 12 (19.4) | 7 (17.1) |
| Medications, n (%) | | | |
| None | 30 (29.1) | 19 (30.6) | 11 (26.8) |
| 5-ASA | 17 (16.5) | 11 (17.7) | 6 (14.6) |
| IMMs | 15 (14.6) | 8 (12.9) | 7 (17.1) |
| Biologics | 41 (39.8) | 24 (38.7) | 17 (41.5) |

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Percentage of patients with AA levels below, above or within the range

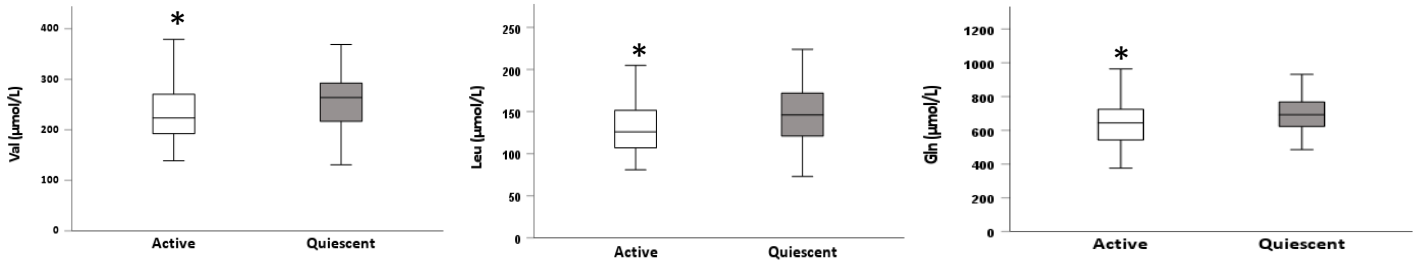



**Data are expressed as percentage of patients resulting below (blue column), above (orange column) or within (grey column) the reference range considered by the laboratory.**

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Differences in AA concentrations between Active vs. Quiescent CD patients

## Val, Leu and Gln decreased in active vs. quiescent CD patients




## Glu, Asp and Gly increased in active vs. quiescent CD patients

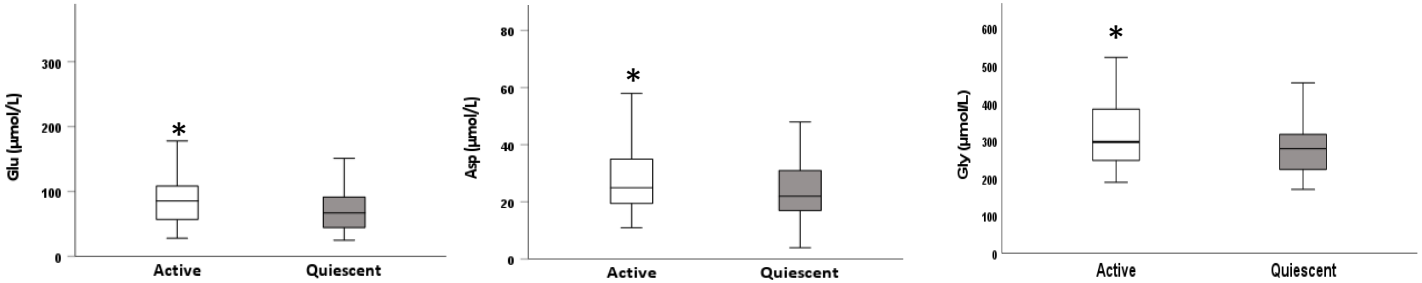



TABLE 2 Serum amino acids concentration in patients with CD according to disease activity.

| | All | Active | Quiescent | Reference (35, 36) |
|---|---|---|---|---|
| EAA, µM/L | | | | |
| Valine | 233 (79) | 223.5 (84)* | 264 (76) | *187–411* |
| Leucine | 139 (57) | 127 (59.3)* | 146 (53) | *82–258* |
| Isoleucine | 74 (26) | 73.5 (26) | 74 (25) | *42–111* |
| Threonine | 146 (65) | 147.5 (64.3) | 145 (67) | *109–268* |
| Lysine | 176 (60) | 169.5 (51) | 183 (66) | *150–286* |
| Methionine | 28 (10) | 26.5 (10) | 29 (10) | *21–44* |
| Histidine | 88 (22) | 84 (24.8) | 90 (18) | *72–131* |
| Tryptophan | 6 (3) | 6 (3) | 7 (3) | *10–30* |
| Phenylalanine | 64 (17) | 60 (17.3) | 67 (14) | *59–112* |
| BCAA | 445 (149) | 421 (156) | 484 (154) | – |
| Total EAAs | 908 (264) | 862 (253) | 957 (263) | – |
| NEAA, µM/L | | | | |
| Glutamic acid | 73 (45) | 85.5 (52.3)* | 67 (50) | *67–223* |
| Asparagine | 60 (15) | 59.9 (15.8) | 60 (16) | *43–87* |
| Arginine | 101 (34) | 95.5 (37) | 107 (28) | *36–172* |
| Aspartic acid | 23 (16) | 25 (15.8)* | 22 (14) | *24–102* |
| Glutamine | 664 (141) | 644 (187.5)** | 693 (42) | *432–871* |
| Serine | 147 (42) | 147.5 (37.3) | 146 (42) | *111–297* |
| Glycine | 287 (113) | 296.5 (139)* | 280 (98) | *240–460* |
| Tyrosine | 62 (20) | 61 (21.8) | 62 (17) | *45–107* |
| Alanine | 379 (132) | 376.5 (143.5) | 397 (129) | *193–597* |
| Cysteine | 31 (12) | 30.5 (12.5) | 31 (11) | *5–55* |
| Total NEAAs | 1864 (417) | 1853 (469) | 1890 (316) | – |
| Other metabolites, µM/L | | | | |
| Ornithine | 93 (12) | 99.5 (46.8) | 91 (44) | *63–133* |
| Citrulline | 33 (11) | 33 (10.8) | 33 (14) | *15–57* |
| Taurine | 151 (70) | 150 (57) | 155 (78) | *50–270* |

Data are expressed as median and interquartile range. BCAA, branched-chain amino acid; EAAs, essential amino acids; NEAAs, non-essential amino acids. $^{**}p < 0.01$; $^{*}p < 0.05$.

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Differences in serum AAs according to total intake of protein

Average intake of protein was 71.2 ± 22.5 g (17% of total energy intake).

When expressed as g/kg of body weight, it was 1.11 ± 0.36 g/kg/body weight.

To assess the effects of protein amount on serum AAs, we used as reference values the protein requirements suggested by the last ESPEN guideline in adults with IBD to split our sample:

- 1.0 g/kg/die in remission phase
- 1.2 g/kg/die in active phase

Using the above values, 47 (46%) out 103 patients did not meet the protein requirements.

Serum levels of Threonine, Lysine and Arginine were reduced in the group with unmet protein intake (UPI) compared to the one meeting the requirements (MPI).

TABLE 3 Serum amino acids concentration according to protein requirements in patients with CD.

| | UPI $N = 47$ | MPI $N = 56$ |
|---|---|---|
| EAA, μM/L | | |
| Valine | 224 (91) | 259 (76) |
| Leucine | 131 (48) | 140 (55) |
| Isoleucine | 72 (26) | 77 (29) |
| Threonine → | 134 (61)* | 156 (54) |
| Lysine → | 170 (41)** | 190 (68) |
| Methionine | 26 (8)* | 29 (10) |
| Histidine | 88 (23) | 89 (20) |
| Tryptophan | 6 (2.1) | 7 (4) |
| Phenylalanine | 61 (15)* | 66 (20) |
| BCAA | 424 (157) | 484 (144) |
| Total EAAs | 873 (231) | 948 (246) |
| NEAA, μM/L | | |
| Glutamic acid | 74 (42) | 70.5 (47) |
| Asparagine | 59 (15) | 60 (18) |
| Arginine → | 96 (43)* | 108 (38) |
| Aspartic acid | 23 (14) | 24.5 (17) |
| Glutamine | 684 (172) | 662 (130) |
| Serine | 145 (42) | 149 (40) |
| Glycine | 280 (151) | 291 (73) |
| Tyrosine | 61 (20) | 62.5 (20) |
| Alanine | 399 (104) | 367 (134) |
| Cysteine | 32 (13) | 29 (10) |
| Total NEAAs | 1892 (507) | 1846 (288) |
| Other metabolites, μM/L | | |
| Ornithine | 92 (44) | 93.5 (42) |
| Citrulline | 34 (11) | 33 (14) |
| Taurine | 149 (71) | 154.5 (72) |

BCAA, branched-chain amino acid; EAAs, essential amino acids; IQR, interquartile range; MPI, Met protein intake; NEAAs, non-essential amino acids; UPI, Unmet protein intake. $^{**}p<0.01$; $^{*}p<0.05$.

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Correlation between EAAs and CDAI

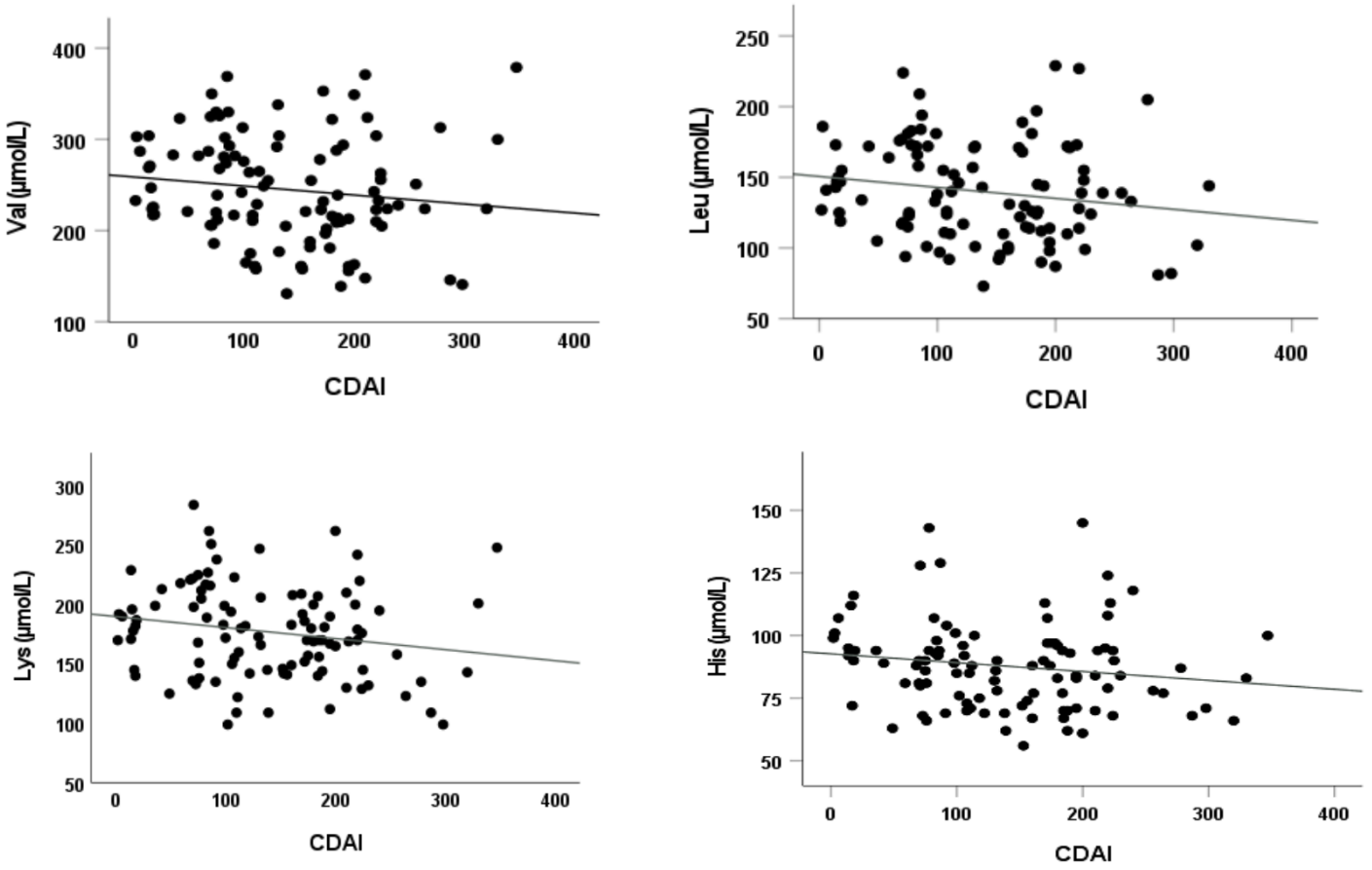



FIGURE 3
Correlations between serum essential AA levels and disease activity. Significant inverse Spearman's rank correlation coefficients between the following essential AAs valine (Val), leucine (Leu), lysine (Lys), and histidine (His) and disease activity (CDAI).

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Correlation between NEAAs and CDAI

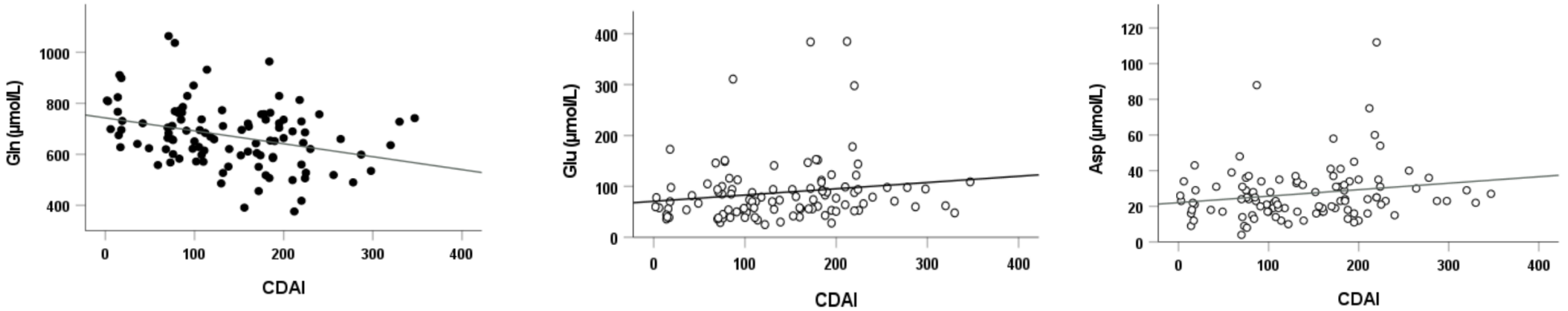



FIGURE 4
Correlations between serum non-essential AA levels and disease activity. Significant Spearman's rank correlation coefficients between the following non-essential AAs: glutamine (Gln) glutamate (Glu), and aspartic acid (Asp) and disease activity (CDAI).

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# RESULTS – Correlation between NEAAs and IL-1β

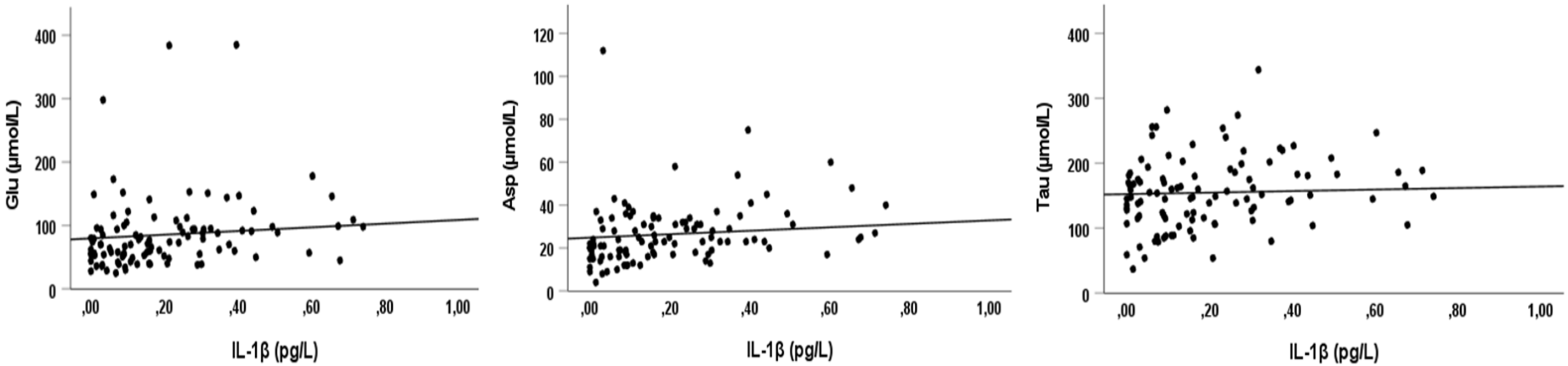



FIGURE 5
Correlations between Glu, Asp, Tau levels and IL-1β. Significant direct Spearman's rank correlation coefficients between glutamate (Glu), aspartic acid (Asp), and taurine (Tau) and a proinflammatory cytokine (interleukin-1beta).

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# CONCLUSIONS

- **This secondary analysis showed differences in specific AA concentrations between clinically active and quiescent CD patients as well as according to different protein intakes, especially for some EAAs.**
- **Levels of serum Trp were reduced in 90% of patients unrelated to CDAI.**
- **Glu and Asp values were positively correlated to CDAI and serum IL-1β, suggesting a potential link with disease activity.**
- **In light of these promising results, extensive research is needed to understand the mechanisms underpinning the relationship between AAs, disease activity and protein intake in patients with CD.**

Cioffi I et al. (2023) Front. Nutr. 10:1245574.
doi: 10.3389/fnut.2023.1245574

# Thanks for your attention!