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Metabolomic profiling of amino acid alterations in anorexia nervosa: implications for appetite regulation and therapeutic strategies

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Abstract. – OBJECTIVE: Anorexia nervosa (AN), a severe psychiatric disorder primarily affecting adolescents and young adults, is characterized by extreme dietary restriction and distorted body image. While the psychological aspects of AN are well-documented, its intricate metabolic underpinnings remain less explored. We think that metabolomic analysis of hair samples emerges as a promising tool to unveil the complex physiological alterations in AN.

This study aims to comprehensively profile amino acid concentrations in hair samples from AN patients and healthy controls. Additionally, it seeks to elucidate potential correlations between amino acid alterations and appetite dysregulation in AN, thereby shedding light on the physiological basis of this debilitating disorder.

PATIENTS AND METHODS: A total of 25 AN patients and 25 age-matched healthy controls were recruited for this study. Hair samples were collected, and metabolites were extracted and analyzed using high-resolution liquid chromatography-mass spectrometry. Clinical data and biochemical markers were also gathered to characterize participants' demographic and clinical profiles.

RESULTS: Metabolomic analysis revealed significant alterations in amino acid concentrations in AN patients compared to healthy controls. Notably, deficiencies in essential amino acids (EAAs) and branched-chain amino acids (BCAAs) were observed, highlighting potential contributors to muscle wasting and appetite dysregulation. Further analysis identified specific amino acids as robust biomarkers capable of distinguishing AN patients with high sensitivity and specificity.

CONCLUSIONS: This study unveils the complex metabolic disturbances associated with AN and underscores the role of amino acid dysregulation in the disorder's pathophysiology. The identified biomarkers hold promise for diagnostic screening and potential therapeutic interventions, opening avenues for personalized approaches in AN treatment. Ultimately, this research contributes to our understanding of chronic disorders through the lens of metabolomics and the chemosensory underpinnings of appetite regulation.

Key Words:
Anorexia Nervosa, AN, Metabolomic, Chemoreceptors, EAA, BCAA, Valine, Leucine, APC.

Introduction

Anorexia nervosa (AN) is a debilitating psychiatric disorder characterized by extreme dietary restriction, distorted body image, and an intense fear of gaining weight[1]. The disorder primarily affects adolescents and young adults, with a higher prevalence among females, and has a significant impact on physical health, psychological well-being, and overall quality of life[2]. Despite its well-documented psychological aspects, AN is associated with complex physiological changes contributing to its etiology and maintenance[3]. The regulation of appetite and energy homeostasis is intricate and involves the interplay of various neuroendocrine, metabolic, and behavioral

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factors[4,5]. Metabolomic analysis, a powerful tool that examines the comprehensive profile of small molecule metabolites in biological samples, has emerged as a promising approach to unraveling the underlying metabolic alterations in AN[6,7]. This approach has been successfully employed in various fields, including clinical diagnostics, drug development, and nutrition research[8]. Metabolomic analysis of biological samples, such as blood, urine, and hair, can offer insights into disease-specific metabolic perturbations and help uncover potential biomarkers for various disorders[9]. The utilization of hair metabolomics offers a unique advantage in the study of AN[10]; hair metabolomics captures long-term metabolic changes[11]. Indeed, hair strands grow at an average rate of 1 cm per month, preserving metabolites over extended periods[12]. This temporal dimension is particularly relevant for AN, a disorder characterized by chronic and persistent metabolic alterations[13].

Recent advances in mass spectrometry and liquid chromatography have enabled high-throughput and high-resolution metabolomic analysis of hair samples[11]. By profiling the metabolites present in hair, researchers can gain insights into the long-term biochemical processes associated with AN and potentially uncover novel metabolic pathways linked to the disorder[14,15]. These investigations have identified disturbances in lipid metabolism, amino acid metabolism, and energy production in AN patients[16]. Amino acids, as central components of protein synthesis and energy metabolism, play a pivotal role in maintaining overall health[17]. Their levels are tightly regulated by intricate metabolic pathways, and any disruptions can have profound effects on physiological processes[18].

Recent research[19] has proposed that individuals with Anorexia Nervosa (AN), may also experience alterations in their perception of chemosensory stimuli. These alterations change in the response of taste receptors and regulators of appetite to essential amino acids (EAAs) and branched-chain amino acids (BCAAs)[20].

Amino acids, as central players in this pathway, serve as building blocks for proteins and enzymes, participate in neurotransmitter synthesis, and contribute to energy production through gluconeogenesis and the citric acid cycle[21]. Dysregulation of amino acid metabolism has been implicated in a range of metabolic disorders, including diabetes, obesity, and cardiovascular diseases[22]. Our study has two main objectives: firstly, to comprehensively profile the amino acid concentrations in hair samples from AN patients and healthy controls; and secondly, to elucidate potential correlations between amino acid alterations and appetite dysregulation in AN. By examining the metabolic profile of AN patients, we aim to identify specific amino acids that are significantly altered and understand how these perturbations contribute to the complex metabolic milieu associated with the disorder. Understanding the link between amino acid metabolism and appetite regulation, as influenced by the chemosensory pathway, could provide valuable insights into the physiological basis of AN. By elucidating the metabolic pathways involved, we may uncover novel therapeutic targets for the treatment of AN. Moreover, this study contributes to the broader field of metabolomics, shedding light on the potential of hair metabolomics as a tool to investigate complex and chronic disorders with a deeper appreciation of the chemosensory underpinnings of appetite regulation.

Chemosensory Function

Chemosensory signals provide us with multiple cues during social interactions[23]. Chemosensory alterations can have effects on response to starvation, indeed individuals with anorexia nervosa (AN) exhibit degrees of chemosensory dysfunction[19]. Studies[24] on animals in which the availability of suitable food is limited show EAAs regulate food intake. This response is triggered by the detection of nutrients within a brain region, the anterior piriform cortex (APC)[25-27]. When meals with low essential amino-acid content are consumed, their concentrations decrease in plasma and brain[28]. As the concentration of intracellular EAAs decreases, the corresponding transfer RNA (tRNA) becomes deacylated[29]. Subsequently, the enzyme general control nonderepressible kinase 2 (GCN2) phosphorylates eukaryotic initiation factor 2 (eIF2), resulting in a slowdown of protein synthesis. This favors the translation of mRNA, regulating gene expression[30,31]. This regulatory process takes longer than the rapid decrease in protein synthesis. Following studies[24] on mice, both APC and GCN2 have been identified as EAA chemosensors. APC exhibits sensory function when EAA levels are depleted[32], with GCN2 playing a pivotal role by detecting meals lacking in EAA, binding to deacylated tRNA, and phosphorylating eIF2, consequently impairing protein synthesis[33]. Numerous studies[24,34] highlight that the reduced food intake of EAAs involves mechanisms in both the hypothalamus and the APC, and research on mice showed a direct connection

between these two regions. The hypothalamus contains appetite regulators, like neuropeptide Y (NPY)[35]. Starvation triggers an increase in ghrelin, the hunger-stimulating hormone, which activates NPY[36]. Recent research[37] involved subjecting mice to a valine-deficient diet, resulting in significant reductions in food intake and body weight. When a diet rich in valine is reintroduced, normal food intake levels return. This suggests that the taste of a valine-deficient diet might deter mice from consuming an EAA-deficient diet[37]. Similar results were observed in pigs[38], a valine-deficient diet reduced food intake, and this effect was enhanced when an excessive dose of leucine was administered. This could be attributed to the adverse effects of an imbalanced diet in branched-chain amino acids (BCAA), including valine, leucine, and isoleucine, leading to an anorexic response as a protective measure. This response occurs rapidly, likely to safeguard against neuronal pathologies resulting from the imbalance of these three BCAAs[37]. Recent studies[38] involving piglets and pigs have focused on optimizing body weight gain through the careful consideration of dietary valine-to-lysine ratios[38]. Additionally, research has explored diets centered around valine, isoleucine, and tryptophan to enhance growth performance, while also examining the potential negative impacts on growth associated with high concentrations of leucine and lysine[39]. Tryptophan, a precursor of serotonin, a neurotransmitter known for its role in regulating food intake, is a key player in these studies[37,38]. It is well-established that an imbalance in branched-chain amino acids (BCAA) can impede the brain's ability to absorb tryptophan. This is due to the shared transporters between large neutral amino acids, such as tryptophan, and BCAA. Consequently, this imbalance can lead to reduced serotonin synthesis and, consequently, a decrease in food intake[40]. This finding could hold significant implications for individuals with eating disorders, including anorexia nervosa (AN)[41]. Therefore, there is potential for further investigation into the amino acid pathways involved in these processes.

Metabolism Pathways of AA

Valine-deficient, threonine-deficient, and lysine-deficient diets cause mice to cease eating before reaching satiety[42-44] rats can detect EAA-deficient diets within just 20-30 minutes, resulting in meal termination[45]. However, when exposed to an EAA-balanced diet after such an experience, their eating rate increases rapidly[46]. Hence, the biochemical and neurological mechanisms responsible for detecting the presence of EAAs are remarkably swift[24]. The anterior piriform cortex (APC) has been identified as the brain region responsible for sensing EAAs. An EAA-imbalanced diet can rapidly reduce the levels of limiting amino acids in the APC by up to 56% in just 21 minutes[28]. A deficiency in EAAs leads to an accumulation of deacylated tRNA, resulting in such low levels of aminoacylated tRNA that new protein synthesis cannot be initiated[29]. This rapid mechanism of recognizing EAA-deficient diets involves an essential enzyme in initiating new protein synthesis: the GCN2 enzyme, which phosphorylates eIF2[47]. To further support the role of uncharged tRNA in detecting EAA-deficient diets, micro-injections of tRNA synthetase inhibitors (L-amino alcohols) were performed in the rat APC, resulting in reduced food intake after 20 minutes, simulating the effects of an EAA-deficient diet[48]. L-amino alcohols inhibit the synthesis of charged tRNA, favoring the synthesis of uncharged tRNA. The pyramidal cells of layer II in the APC serve as the output neurons. These cells are excitatory glutamatergic neurons regulated by GABAergic proteins[49]. In the absence of new protein synthesis, inhibitory proteins critical for controlling the APC's output circuit are quickly lost from the neural membrane[50]. Consequently, the APC cannot maintain its normal balance between stimulatory and inhibitory neurons within the circuit. This leads to the liberation of excitatory glutamatergic neurons, which transmit signals to various brain areas involved in heightened motor activity and the rejection of EAA-deficient diets, resulting in reduced food intake and impaired growth[34]. Leucine activates the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. This EAA stimulates ribosomal protein S6 kinase (S6K1) and inhibits eukaryotic translation initiation factor 4E binding protein-1 (4EBP1)51. mTOR pathway regulation depends on leucine transport[52], and it triggers protein synthesis and cell growth in the presence of EAA-balanced diets. As mentioned earlier, valine-deficient diets decrease food intake, and this effect is exacerbated when leucine intake is elevated[26]. High levels of leucine cause excessive mTOR signaling, adversely affecting normal growth by reducing food intake[53]. However, since BCAAs (valine, leucine, isoleucine) share the same transporters, valine has been shown to hinder the transport of leucine across the blood-brain barrier, mitigating the excessive stimulation of mTOR[39]. Excessive leucine concentrations stimulate the catabolism

of all BCAAs (valine, leucine, isoleucine) and not just leucine[39]. A deficiency in these amino acids can alter the expression of growth hormone-insulin-like growth factor-1 (*GH-IGF-1*). GH promotes the secretion of IGF-1, but this process appears to be contingent on the availability of valine, which can, in turn, inhibit the expression of IGF-1. Consequently, as dietary leucine levels increase, food intake decreases[54,55]. However, adding valine and isoleucine to the diet can counteract the negative effects of high leucine concentrations[39]. It is crucial, though, to maintain an appropriate balance and not overconsume leucine. Problems related to food intake and growth are associated with an unbalanced intake of valine, leucine, and isoleucine. An EAA-balanced diet not only increases appetite but also enhances growth. Therefore, a direct signaling mechanism operates to maintain a balanced EAA profile in the diet[52]. The metabolomic pathways of BCAA, leucine, valine, and isoleucine are represented in Figure 1.

Patients and Methods

Study Design and Participant Recruitment

The study was designed to investigate the metabolic alterations in anorexia nervosa (AN) through hair metabolomic analysis. A total of 25 female AN patients and 25 age-matched healthy female controls were recruited from clinical centers in Italy. The AN patients met the DSM-V criteria for the disorder and were clinically diagnosed by experienced psychiatrists specializing in eating disorders[56]. Healthy controls were selected based on normal or underweight BMI and matched for age and gender. All participants provided written informed consent, and the study was conducted following ethical guidelines.

Clinical Data Collection

Clinical data were collected from both AN patients and healthy controls to characterize their demographic and clinical characteristics. The data included age, height, weight, BMI, presence of comorbidities, dietary behaviors, biochemical markers (glycemia, azotemia, creatinine, uric acid, sodium, potassium, calcium, magnesium, vitamin D3, cholesterol, triglycerides, thyroid-stimulating hormone, glutamic-oxalacetic transaminase, glutamate pyruvate transaminase), and other relevant parameters.

Hair Sample Collection and Preparation

Hair samples were collected from participants by cutting a 1 cm diameter hair strand, starting from the scalp base and extending 4-5 cm towards the tip. Each hair sample was cleaned, dried, and cut into 1 cm long pieces. These hair pieces were placed in glass vials and subjected to metabolite extraction[11].

Metabolite Extraction

Metabolites were extracted from the hair samples using a multi-step procedure[57]. First, 30 mg of hair pieces were immersed in 2 mL of methanol in glass vials. The vials were vortexed and incubated at 50°C for 1 hour. After cooling

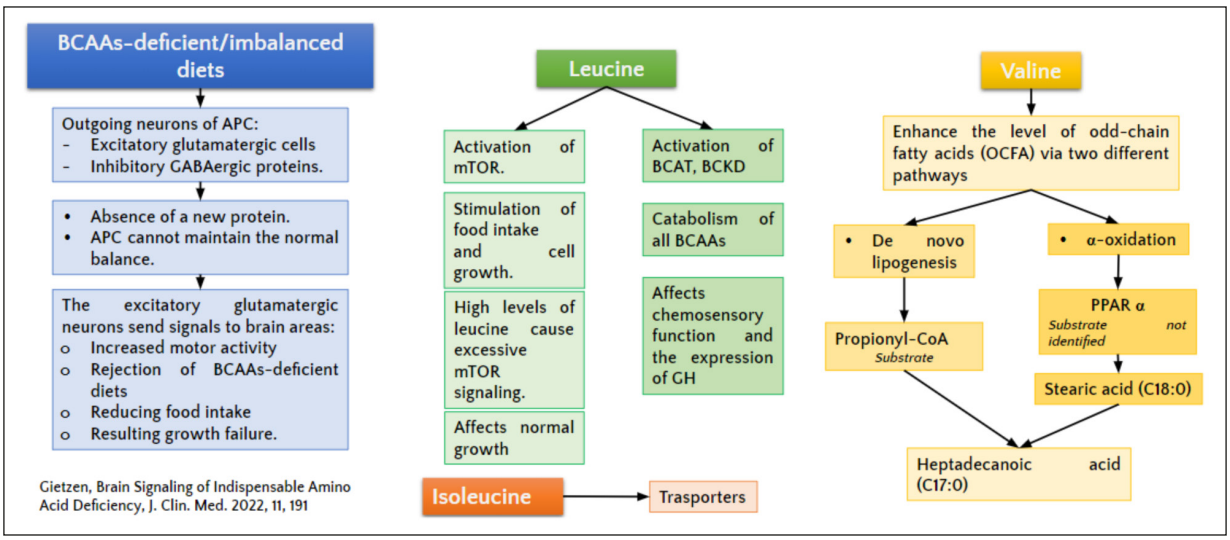

Figure 1. Representation of metabolic pathways of BCAA, leucine, valine, and isoleucine[52].

to room temperature, 2 mL of acetonitrile was added, and the vials were vortexed, followed by centrifugation at 13,000 g for 10 minutes. The organic layers were collected, combined, and dried under N2 gas. The remaining hair residue was further processed by adding water and adjusting the pH to extract both acidic and basic metabolites. The obtained extracts were then reconstituted in H_2O:CH_3OH (70:30).

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

Metabolomic analysis was performed using liquid chromatography (LC) coupled with mass spectrometry (MS)[58]. LC separation was carried out using a Phenomenex Jupiter C18 column (50 x 2.1 mm, 5 µm particle size). Binary gradient elution with mobile phases consisting of water with 0.2% formic acid (mobile phase A) and acetonitrile (mobile phase B) was employed. The LC system was coupled to an HCT Ultra high-capacity ion trap mass spectrometer equipped with an electrospray ionization (ESI) source. The mass spectrometer was operated in both positive and negative ion modes.

Data Analysis

The acquired mass spectrometry data were processed and analyzed using the SANIST software suite, a tool specifically designed for metabolomic data analysis[59]. Statistical comparisons between AN patients and healthy controls were conducted using appropriate tests, and *p*-values were calculated to assess the significance of differences in amino acid concentrations (level of significance $p < 0.05$). Ratios of specific amino acids were calculated to provide insights into their interplay and potential implications for appetite regulation.

Statistical Analysis

In the context of our research, we employed multivariate analysis techniques, specifically Principal Component Analysis (PCA) and Receiver Operating Characteristic (ROC) analysis, with a significance threshold set at p-value < 0.05 to evaluate the robustness of our findings. The ratio of patients with Anorexia Nervosa (AN) to healthy controls (CTR) was calculated based on amino acid concentrations in hair, expressed in nanograms per milligram. Additionally, Partial Least Squares Regression (PLSR) was utilized to identify biomarkers most strongly correlated with anorexia nervosa by comparing patient and control data. Furthermore, we conducted ROC analysis on selected metabolites, presenting maximum sensitivity and specificity values, associated cut-off points, and the area under the curve (AUC). These methodological approaches provided a comprehensive insight into significant differences in metabolic profiles between individuals with anorexia nervosa and healthy controls, contributing to our understanding of the pathophysiology of this condition.

Results

The metabolomic analysis of hair samples from anorexic patients and healthy controls revealed significant alterations in amino acid concentrations that exhibited a key role in the metabolic disturbances associated with anorexia nervosa (AN).

Amino Acid Concentrations

Comparative analysis of amino acid concentrations in hair samples showed distinct differences between AN patients and healthy controls (Table I). Notably, propionyl-carnitine and carnitine concentrations were significantly lower in anorexic patients compared to controls ($p < 0.001$), indicating potential disruptions in lipid metabolism. Propionyl-carnitine and carnitine are involved in fatty acid metabolism, and their reduced levels may suggest altered lipid utilization in anorexic individuals. With the results obtained, it was possible to define the true metabolomic profiles of anorexic patients and of controls. Their comparison enables the discrimination of an affected person from a healthy one, as shown in Figure 2.

Table I. Amino Acid Concentrations in AN Patients and Healthy Controls. The ratio is between healthy controls (CTR), where we found the amino acid in nanograms on milligrams of hair, and anorexia nervosa patients (AN).

Amino Acid	Healthy Controls [Ng/Mg]	AN Patients [Ng/Mg]	Ratio CTR/AN	*p*-value
Propionyl-Carnitine	1.45784	0.00788	185.01	1.76E-21
Carnitine	0.08468	0.00396	21.38	6.12E-14
Leucine/Isoleucine	3.47448	1.33596	2.60	6.08E-08
Valine	9.04788	3.92512	2.30	4.38E-07

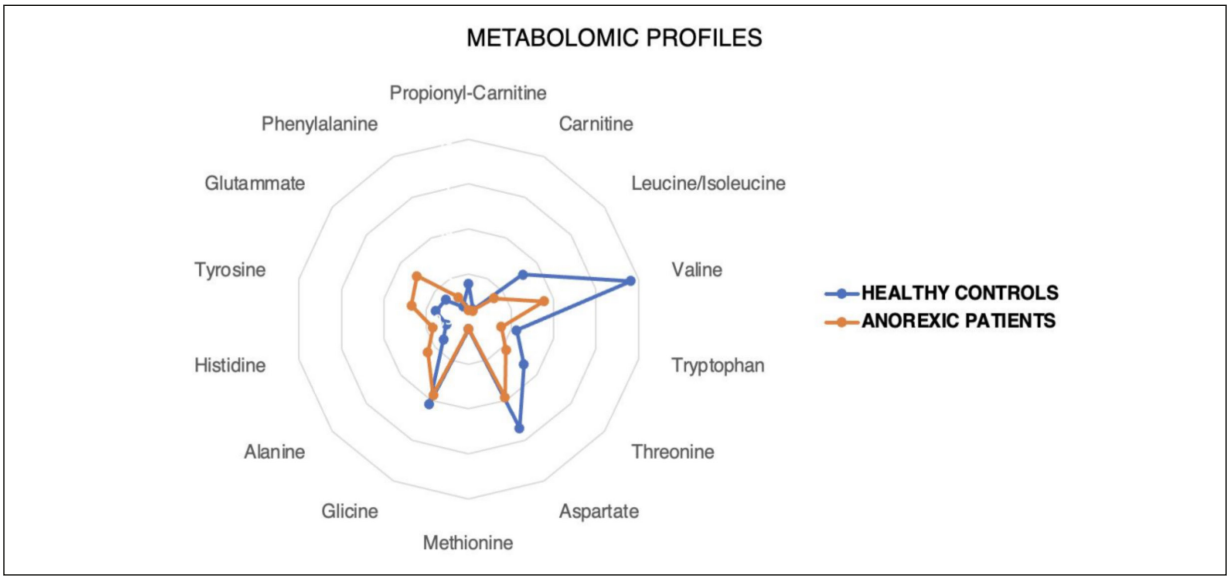

Figure 2. Metabolomic profiles of anorexic patients and controls.

Leucine, isoleucine, and valine are branched-chain amino acids (BCAAs) crucial for muscle maintenance and protein synthesis[60]. AN patients exhibited significantly lower concentrations of leucine ($p < 0.001$), isoleucine ($p < 0.001$), and valine ($p < 0.001$) compared to controls, suggesting compromised protein metabolism and muscle preservation in AN. This observation aligns with the known catabolic state associated with anorexia[61].

Alanine, tyrosine, and phenylalanine concentrations were found to be higher in AN patients than in controls. Alanine, a key player in carbohydrate metabolism[62], may reflect increased protein degradation or altered metabolic pathways linked to protein catabolism in AN. Tyrosine and phenylalanine are precursors of neurotransmitters[63], and their elevated levels might contribute to the neurochemical imbalances often observed in AN. Figure 3 and Figure 4 illustrate the alterations in amino acid concentrations in anorexic patients compared to healthy controls. Figure 3 shows the different metabolomic profiles between patients and controls, while principal component analysis (Figure 4) shows that patients and controls clustered in two distinct groups. Significant differences were observed for propionyl-carnitine, carnitine, leucine/isoleucine, valine, and other amino acids. These alterations point to potential metabolic disruptions associated with anorexia nervosa.

Essential Amino Acid Deficiency

An important finding is the significant deficiency of essential amino acids (EAAs) in AN patients. The total concentration of EAAs was notably lower in AN patients (9.81 ng/mg) compared to healthy controls (18.72 ng/mg), resulting in a ratio of 1.91 (CTR/AN). EAAs play a pivotal role in regulating appetite and food intake, and their deficiency in anorexic patients may contribute to appetite dysregulation and compromised nutritional status[64].

Tryptophan Ratio

The ratio of tryptophan to valine, leucine, and isoleucine was elevated in AN patients (0.27) compared to controls (0.19), resulting in a ratio of 0.70 (CTR/AN). This finding suggests that anorexic individuals preferentially transport tryptophan over these BCAAs due to their shared transporters[40].

The observed alterations in amino acid concentrations provide insights into the metabolic disruptions in AN. The deficiencies in BCAAs and EAAs point to the importance of these amino acids in maintaining muscle mass and regulating appetite.

These findings have potential clinical implications for AN treatment. Restoring BCAA and EAA levels through targeted supplementation may support muscle preservation and address appetite dysregulation[65]. Further investigations are warranted to explore the mechanisms underlying these metabolic alterations and to develop personalized interventions for AN patients[66-68] (Table II).

Machine Learning Algorithms

We employed a machine learning model, specifically Partial Least Squares Regression[69], to explore the potential utility of classification models

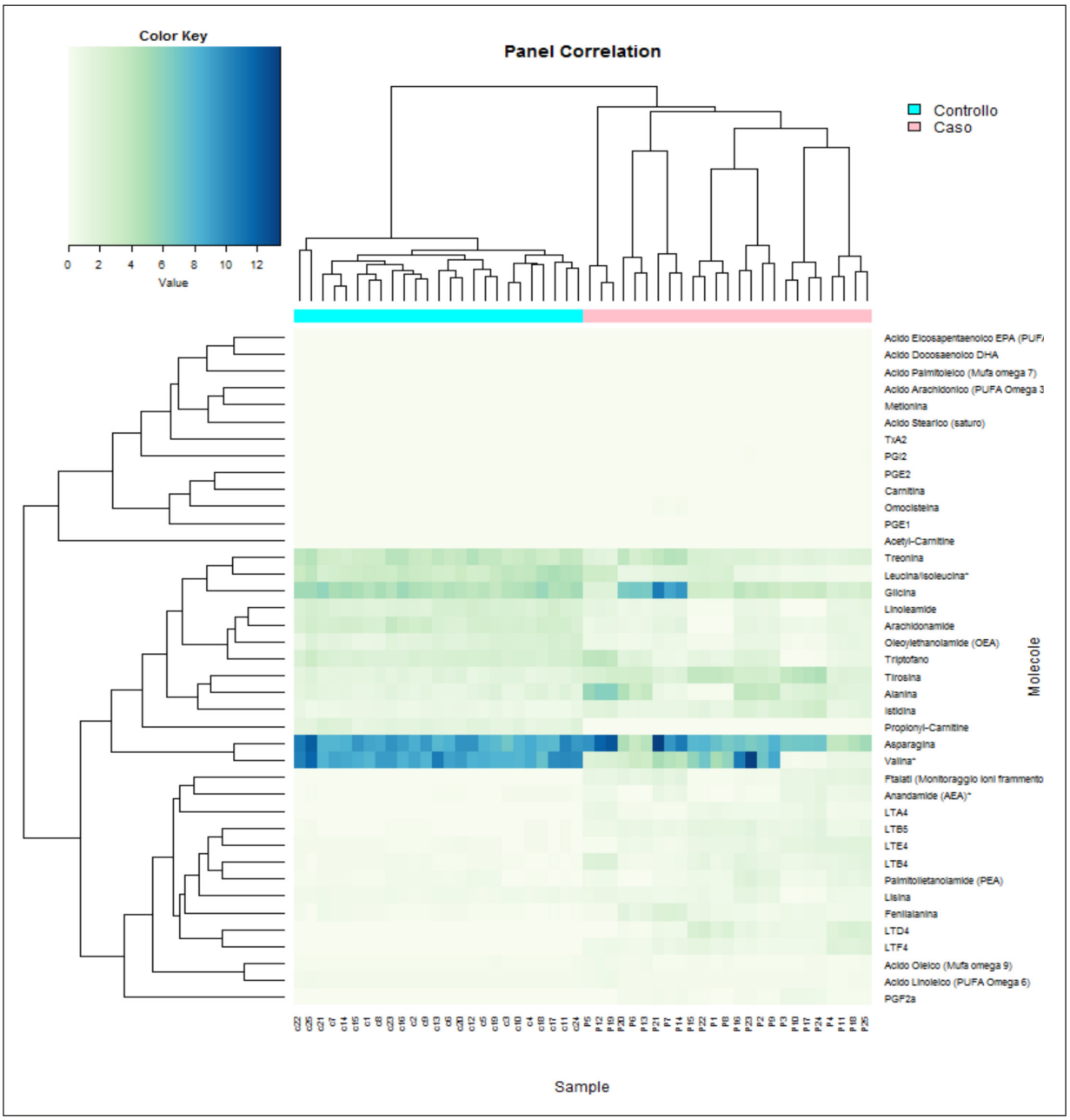

Figure 3. Heat map to compare metabolomic profiles of patients and controls. Patients are shown in red, and controls are shown in light blue.

in the context of AN, as shown in Table III. In AN context, these features represent molecules, allowing us to identify those of utmost importance. To ensure the robustness of our findings, we conducted 100 iterations of analysis[70]. In each iteration, we randomly divided the samples into a training set (comprising 75% of the data) and a testing set (comprising 25% of the data). The outcomes of this analysis were promising, with the model consistently achieving a high correlation of 98%. The top 10 molecules that consistently emerged as the most important throughout these iterations hold a particular interest in the context of AN. These molecules serve as potential markers, demonstrating their exceptional ability to distinguish between different groups within our dataset. However, it is important to note that while these molecules are effective classifiers within our dataset, further investigations are required to elucidate the nature of this variation.

Table II. ROC analysis of selected metabolites. The maximum sensitivity and specificity values and their correlated cut-off are shown. Then, we were able to calculate the area under the curve (AUC).

Molecules	Cut-off	Sensibility	Specificity	AUC
Propionyl-Carnitine	0.5825	1	1	1
Carnitine	0.0255	1	1	1
PGE2	0.022	1	1	1
PGE1	0.007	1	1	1
Arachidonamide	1.7975	1	1	1
Linoleamide	1.5595	1	1	1
Leucine/Isoleucine	2.3875	0.88	1	0.94
OEA	1.308	1	0.84	0.92
Valine	7.3105	0.88	1	0.94
LTB4	0.475	0.88	1	0.94
LTB5	0.525	1	1	1
LTA4	0.149	1	1	1
LTF4	0.293	1	1	1
PGI2	0.0055	1	0.96	0.98

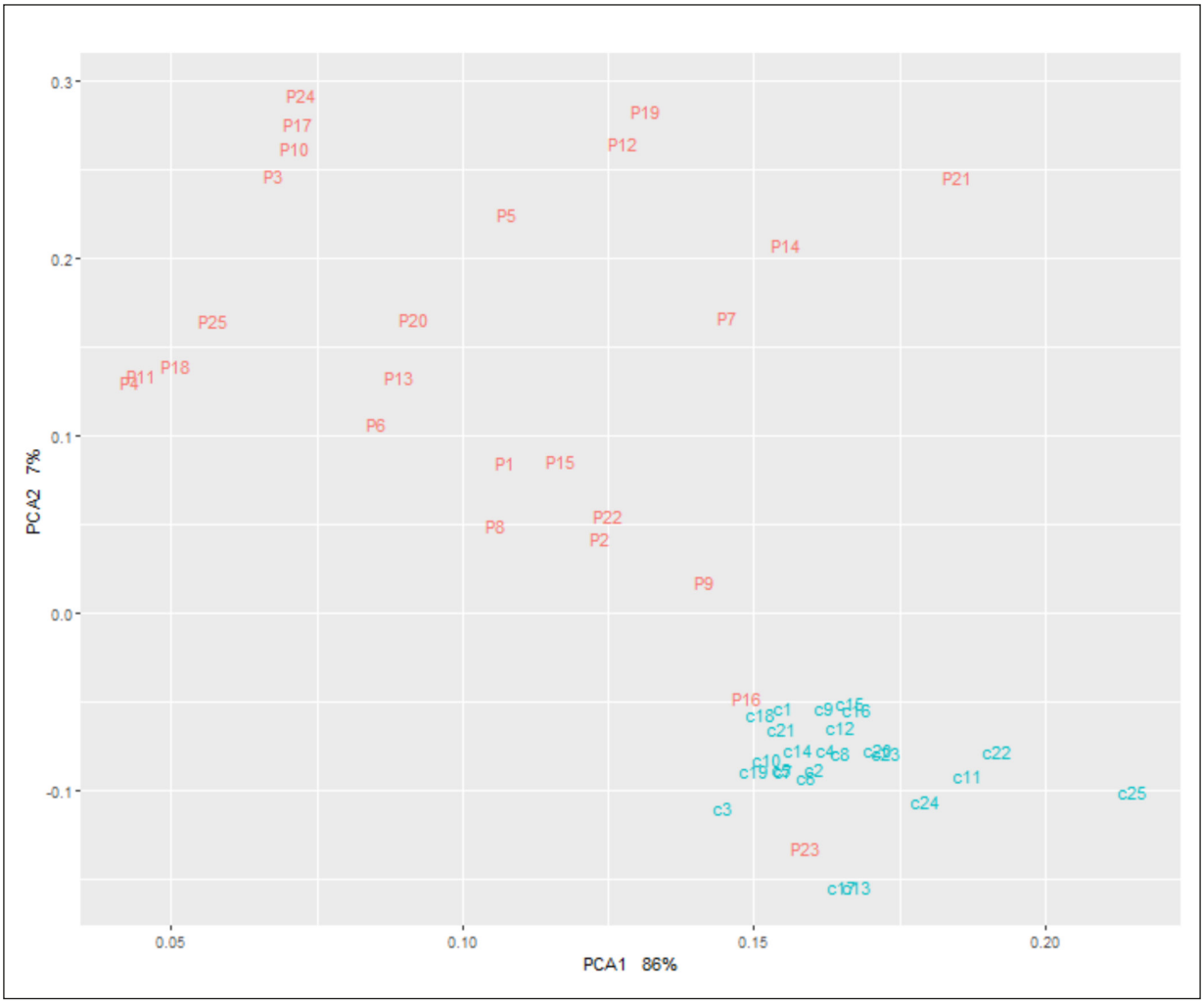

Figure 4. Principal component analysis (PCA). Metabolic Alterations in Amino Acid Concentrations. Patients are shown in red, and controls are shown in light blue.

Discussions

The present study was carried out to study metabolic alterations in anorexia nervosa (AN) through the analysis of amino acid concentrations in hair samples. The results provide valuable insights into the intricate biochemical changes associated with this complex and debilitating disorder. However, it is essential to consider the role of the chemosensory pathway in these metabolic changes. This pathway plays a pivotal role in shaping eating behaviors and is influenced by various amino acids. The observed alterations in amino acid concentrations underscore the extent of metabolic disturbances in AN and other metabolic disorders[71]. The reduced levels of propionyl-carnitine and carnitine are of particular interest, as they reflect potential disruptions in lipid metabolism[72]. These two molecules play a crucial role in fatty acid transportation into mitochondria for energy production[73]. The scarcity of propionyl-carnitine and carnitine in anorexic patients may suggest altered lipid utilization pathways, aligning with the energy conservation strategies often observed in individuals with AN[74]. The decreased concentrations of leucine, isoleucine, and valine in AN patients underscore the profound alterations in protein metabolism and muscle preservation in AN[75]. Branched-chain amino acids (BCAAs) are vital for maintaining muscle mass, and their deficiency in AN may contribute to muscle wasting and catabolism[76]. The reduction of BCAAs is a noteworthy finding, as it provides a biochemical basis for the skeletal muscle loss commonly seen in AN patients[77].

The elevated levels of alanine, tyrosine, and phenylalanine in AN patients indicate potential disturbances in amino acid metabolism[78]. Alanine's role in carbohydrate metabolism makes its increase in AN a plausible reflection of altered metabolic pathways associated with protein catabolism[62]. Similarly, the elevation of tyrosine and phenylalanine aligns with their role as precursors for neurotransmitter synthesis[63], suggesting that neurochemical imbalances might contribute to the neuropsychiatric features of AN. The marked deficiency of essential amino acids (EAAs) in AN patients carries significant implications[79]. EAAs are not only building blocks for proteins but also key regulators of appetite and food intake[80]. The notable decrease in total EAAs in AN patients points to a potential mechanism contributing to appetite dysregulation[81], and the overall malnourished state observed in AN. The increased ratio of tryptophan to valine, leucine, and isoleucine indicates preferential transport of tryptophan in AN patients. As these amino acids share the same transporters, this alteration might lead to an imbalance in the central nervous system's neurotransmitter production[82]. The preferential transport of tryptophan over the BCAAs could contribute to decreased appetite and the perpetuation of the catabolic state[83]. Finally, the metabolic profile of anandamide and oleoylethanolamide showed interesting results. According to the literature[84], anandamide is positively correlated with excessive physical activity. These data are supported by our findings. Indeed, anandamide was overexpressed in AN patients. On the contrary, oleoylethanolamide was more expressed in controls, while in literature seems to be positively correlated with weight loss[85].

Table III. Partial Least Squares Regression to identify the biomarkers more correlated to anorexia nervosa, comparing patients and controls data.

Molecules	Importance_mean
Propionyl-Carnitine	100
Carnitine	93.04
LTB5	92.93
Arachidonamide	92.11
PGE2	91.77
Linoleamide	87.63
LTA4	82.41
LTF4	82.30
Leucine/Isoleucine	82.01
OEA	78.58
PGI2	77.87
LTB4	77.28
PGE1	77.06
VALINE	71.64

While the findings of this study offer valuable insights into the metabolic alterations associated with AN, it is crucial to acknowledge the role of the chemosensory pathway in shaping the appetite and eating behaviors of individuals with this disorder. The deficiencies in BCAAs and EAAs underscore the potential benefits of targeted nutritional interventions that aim to restore these amino acid levels[86]. Such interventions could have a dual effect of preserving muscle mass and modulating appetite[65].

Limitations

However, it is essential to acknowledge certain limitations of the study. The cross-sectional design limits the ability to establish causality and does not capture the dynamic changes that might occur

over time. Additionally, the sample size is modest, warranting larger studies to validate these findings and explore potential factors influencing these metabolic alterations, such as disease duration, severity, and comorbidities.

Conclusions

The metabolomic analysis of hair samples from AN patients and healthy controls provides valuable insights into the metabolic disturbances associated with this complex disorder. The alterations in amino acid concentrations, especially EAAs and BCAAs, shed light on potential mechanisms underlying muscle wasting, appetite dysregulation, and neuropsychiatric features in AN. Further research is warranted to unravel the intricate interplay of these metabolic changes and to develop early diagnosis and targeted therapeutic strategies that address the multifaceted nature of anorexia nervosa.

Conflict of Interest
The authors declare no conflict of interest.

Informed Consent
All subjects gave their informed consent for inclusion before they participated in the study.

Ethics Approval
The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee of Azienda Sanitaria dell'Alto Adige, Bolzano, Italy (Approval No. 132-2020).

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Authors' Contributions
Conceptualization, M.B.; Methodology, S.C.; Investigation V.B. and L.D.R.; Writing- original draft preparation, K. Donato, and A.M.; Writing, review and editing, K. Dhuli, M.C.M., C.M., G.B., M.R.C., T.B. and P.C.; Project administration, M.B.; Funding acquisition, M.B. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials
All the data are within the test.

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