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Association of Plasma and Muscle Amino Acid Profiles with Resting Energy Expenditure and Inflammation in Stable Chronic Obstructive Pulmonary Disease

Dr Salman Khan[1], Dr Sara Yar Khan[2], Dr Naseema Ahmed Jan[3], Dr Nisar Khan[4], Dr Rakhshanda Naheed[5], Dr Muhammad Razaq[6]

[1]Associate Professor, Department of General Medicine, MMMTH, MTI, Gomal Medical College, Dera Ismail Khan, Pakistan
[2]Assistant Professor, Department of Biochemistry, Peshawar Medical and Dental College, Peshawar, Pakistan.
[3,5]Assistant Professor, Department of Physiology, Loralai Medical College, Loralai, Pakistan
[4]Assistant Professor, Department of Biochemistry, Muhammad College of Medicine, Peshawar, Pakistan
[6]Associate Professor, Department of Biochemistry, Jinnah Medical College, Peshawar, Pakistan

[*]**Corresponding Author:** Dr Sara Yar Khan,
Assistant Professor, Department of Biochemistry, Peshawar Medical and Dental College, Peshawar, Pakistan.
Email: asad_sarwar77@hotmail.com

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ABSTRACT

Background
The objective was to investigate thepotential changes in muscular and amino acid plasma levels between 'COPD patients' and identical age control volunteers.
Methodology
The study involved 121 participants, including 32 health individuals and 89 COPD patients, aged 63±2 and 65±3.2 years, respectively. Fasting arterial blood was taken from the radial artery, and blood gas assessment was performed.'Blood samples were also taken from a large draining vein in the cubital fossa to measure LBP levels.
Results
The study found that COPD patients had higher plasma LBP levels andREE/FFM (Resting Energy Expenditure/Fat-Free Mass) and LBP levels. A strong association was observed between' LBP and REE/FFM. The overall plasma amino acid was reduced in COPD patientsprimary due to lower levels of nonessential amino acids such as asparagine (ASN), glutamine (GLN), alanine (ALA), and glutamic acid (GLU). Adverse relationships were discovered between LBP and SumAA (total amino acid concentration) and between LBP and individual amino acids like GLN, ASN, and ALA.
Conclusion
There were signs of disruptions in 'intermediate metabolism among individuals with stable severe COPD, as evidenced by different changes in muscle and plasma AA levels'. Muscular AA changes in 'COPD patients' differ from those in illnesses linked to acute muscular wasting. Our research revealed a connection between inflammation and the noted drop in plasma AA levels.

Keywords: COPD, Resting Metabolic Rate, Amino Acid, inflammation

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) significantly affects global health, causing respiratory symptoms and systemic issues like skeletal muscle metabolism. Muscle wasting in COPD patients reduces exercise tolerance and quality of life, highlighting its importance in disease management. [1, 2]. Inflammation in COPD patients, marked by increased cytokines like TNF-α and IL-6, promotes muscle protein degradation, contributing to an imbalance between muscle protein synthesis and degradation that leads to muscle atrophy [3].

Amino acids are vital for muscle metabolism, and changes in their levels can indicate disruptions, as seen in muscle-wasting conditions like COPD. [4]. Studies have shown that COPD patients exhibit lower levels of essential and non-essential AAs in plasma, suggesting a state of AA depletion [5]. This depletion may be linked to systemic inflammation and increased energy expenditure associated with the catabolic processes.

'Resting energy expenditure (REE)' is frequently associated with increased levels in 'COPD', further contributing to muscle loss. Hypermetabolism or increased metabolic rate at rest has been observed in individuals with severe COPD, possibly due to improved work of breathing and systemic inflammation.The relationship between REE and muscle mass is well-documented, with higher REE leading to negative energy balance and subsequent muscle atrophy. Investigating the association between REE and AA levels may shed light on the metabolic shifts driving muscle wasting in COPD. [6].

In COPD, the acute-phase response (APR) triggered by systemic inflammation, marked by elevated lipopolysaccharide-binding protein (LBP), correlates with disease severity and may contribute to muscle wasting by redirecting amino acids from muscle protein synthesis to acute-phase protein production [7]. Several studies have highlighted the role of inflammatory markers such as LBP in modulating AA availability and muscle metabolism. In this context, it is crucial to investigate how plasma and muscle AA levels correlate with REE and inflammatory markers, as these relationships may offer potential therapeutic targets for managing muscle wasting in COPD [8].

The studyaims 'to explore the changes in plasma and muscle AA levels in stable and COPD patients and their association with the REE and LBP levels by comparing COPD patients with age-matched health controls'. We seek to identify specific AA alteration and their link to muscle wasting. Understanding this metabolic disturbance may aid in developing stragedies to preserve muscle mass and improve the quality of life in COPD patients.

MATERIALS AND METHODS

A cross-sectional study was conducted at Gomal Medical College and its affiliated hospital from January 2023 to January 2024, involving121 participants (89 COPD and 32 healthy controls). Identical age of individuals, aged 63±2 years, and 89 individuals, aged 65±3.2 years (mean ± SEM). COPD patients were selected based on the American Thoracic Society's [9] standards force expiratory volume in one second (FEV1), < 55% of predicted value.

The study's sample size was calculated via power analysis to detect differences in plasma and muscle amino acid levels, requiring at least 30 participants per group. This led to a final sample of 121 (89 COPD patients and 32 healthy controls) after a 10% increase for potential dropouts.

Ethical Committee of Gomal Medical College and its affiliated hospital, and written informed consent was obtained from all participants before the study commenced. Each patient had inhaler therapy with steroids (n = 24), β2-agonists (n = 72), and anticholinergic medications (n = 25).

Inclusion and exclusion criteria: male patients aged 60-70 years with a confirmed diagnosis of COPD and stable clinical status were included. Exclusion criteria were past medical records of distal arteriopathy, cancer, hormone-related, liver, or kidney disease, as well as anticoagulant medication use. Individuals who had used corticosteroids within 6 months. The participants were considered stable in clinical terms at the beginning of the trial if they had not experienced an illness or a worsening condition for at least four weeks.

Data collection procedure: fasting blood samples were collected from the radial artery for plasma amino acid analysis and arterial blood gases. Venous blood was drawn from the cubital fossa to measure LBP

levels using 'enzyme-linked immunosorbent assay (ELISA)'. Muscle biopsies were performed with the use of local anesthesia; a sample was taken from the anterior tibialis muscle by evacuated tubes containing 50 units of heparin. Within one hour of collection, the plasma was separated from the blood cells by centrifugation at 1000 x g for 5 minutes. And the samples were stored at -80°C for subsequent amino acid analysis using high-performance liquid chromatography (HPLC) [10].

Height and body weight (BW) were measured, and BMI was calculated as BW divided by height squared. 'Fat-free mass (FFM) was measured using deuterium dilution, with fat mass (FM) obtained by subtracting FFM from BW'. Resting energy expenditure (REE) was measured after an overnight fast using indirect calorimetry.

After one week of the muscle biopsy, 'REE, FFM, BW, and lung function tests' were performed. The 'blood samples and muscle biopsy specimens' were collected on the gathered, around (8:00 and 10:00) morning.

Laboratory Investigations: 'Plasma and muscle amino acid levels were measured using HPLC'. The total concentration of amino acids (SumAA), essential amino acids (SumEAA), and non-essential amino acids (SumNEAA) was calculated. LBP levels were quantified using ELISA, and REE was measured using indirect calorimetry under fasting conditions.

Data were analyzed using SPSS version 23.0, employing non-parametric methods for non-normally distributed data, and Pearson correlation coefficients to assess relationships between LBP levels, REE normalized by fat-free mass, and plasma and muscle amino acid concentrations. A stepwise linear regression model was used to identify key factors influencing plasma amino acid concentrations, with statistical significance set at $p < 0.05$.

RESULTS

In the context of normocapnia, pulmonary function and metabolic measures indicated significant airway obstruction in individuals with COPD. Key findings include; Pulmonary Function, COPD patients exhibited considerable airway obstruction, identified air trapping, mild hyperinflation, and reduced diffusion capacity for carbon monoxide (DLCO). Oxygen saturation and arterial tension levels were 'slightly diminished compared to the control group, where all pulmonary function parameters were within the normal range'.

Body Composition: 'BMI was significantly lower in the patient group, primarily due to a reduced fat-free mass index (FFMI). In contrast, the control group's fat mass index (FMI) did not differ significantly'.

Plasma LBP Levels: COPD patients had elevated plasma LBP levels and increased resting energy expenditure (REE) per fat-free mass (FFM). 'A notable positive correlation was found between LBP and REE/FFM ($r = 0.38$, $p = 0.02$)'.

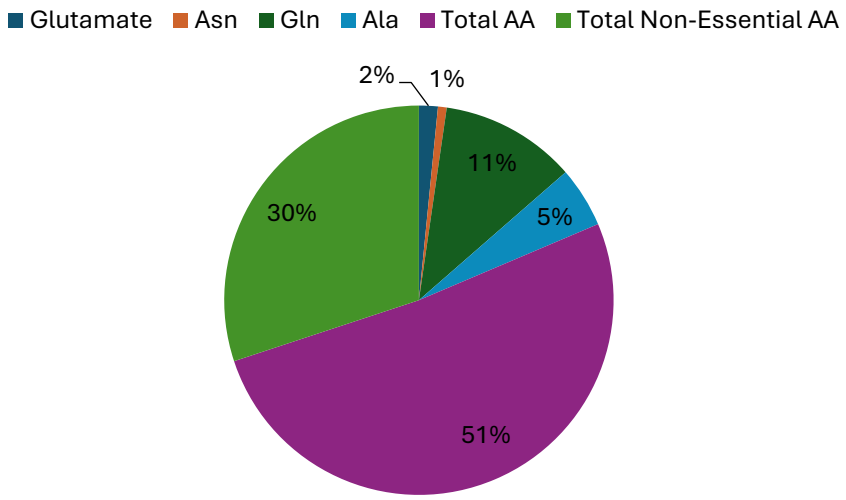

Figure 1: Plasma amino acid levels of COPD and control groups.

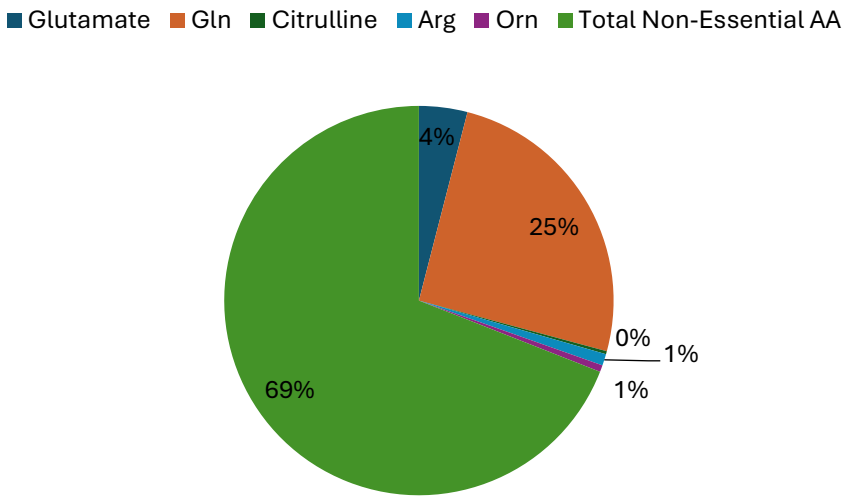

Figure 2: Muscle amino acid levels of COPD and control groups

Table 1. The concentrations of glutamate, asparagine (Asn), glutamine (Gln), and alanine (Ala) were significantly lower in COPD patients compared to controls ($p < 0.05$). This indicates a significant impairment in amino acid metabolism in these patients.

The total amino acid concentration was significantly reduced ($p = 0.004$), highlighting a deficiency that could adversely affect muscle maintenance and recovery.

Table 1: Comparison of Plasma Amino Acid Concentrations in COPD

'Parameters'	**'Patient group (n=89)'**	**'Control group (n=32)'**	**p-value**
Glutamate	82	119	0.04
Asn	38	48	0.03
Ser	131	132	NS
Gln	591	652	0.002
His	89	77	NS
Gly	232	244	NS
Thr	139	145	NS
Citrulline	65	59	NS
Arg	81	81	NS
Ala	263	365	0.001
Taurine	68	59	NS
Tyr	62	62	NS
Val	228	252	NS
Met	35	39	NS
Ile	72	78	NS
Phe	42	42	NS
Trp	52	54	NS
Leu	133	147	NS
Orn	84	72	NS
Lys	188	201	NS
Total AA	2686	2985	0.004
Total Essential AA	901	965	NS

Total Non-Essential AA	1574	1810	0.002

The data are presented as 'mean (SEM) in µmol/L'. 'SumAA = total of all amino acids, SumEAA = total of all essential amino acids, SumNEAA = total of all non-essential amino acids'.AA = Amino Acids, EAA = Essential Amino Acids, NEAA = Non-Essential Amino Acids, 'Asn = Asparagine, Ser = Serine, Gln = Glutamine, His = Histidine, Gly = Glycine,,Thr = Threonine, Tyr = Tyrosine, Val = Valine, Met = Methionine, Ile = Isoleucine, Phe = Phenylalanine, Trp = Tryptophan, Leu = Leucine, Orn = Ornithine, Lys = Lysine, NS = Not Significant'

Table 2 Some amino acids, such as glutamate and glutamine, exhibited significantly higher concentrations in COPD patients ($p < 0.05$), possibly reflecting compensatory mechanisms to combat muscle wasting.

Elevated levels of arginine (Arg) and citrulline were significantly higher ($p < 0.05$), suggesting adaptive responses aimed at enhancing nitric oxide synthesis and muscle perfusion, despite ongoing stress and inflammation.

Table 2: Comparison of Muscle Amino Acid Concentrations in COPD

'Parameters'	**'Patient group (n=89)'**	**'Control group (n=32)'**	**p-value**
Glutamate	1899	2731	0.002
Asn	153	154	NS
Ser	363	292	NS
Gln	11893	8955	0.003
His	243	182	NS
Gly	773	643	NS
Thr	501	489	NS
Citrulline	119	72	0.03
Arg	452	203	0.03
Ala	1245	1365	NS
Taurine	15940	326	NS
Tyr	67	66	NS
Val	287	287	NS
Met	31	28	NS
Ile	61	60	NS
Phe	62	58	NS
Trp	23	22	NS
Leu	114	124	NS
Orn	265	95	0.02
Lys	647	461	NS
Total AA	34500	32400	NS
Total Essential AA	1784	1572	NS
Total Non-Essential AA	32616	30545	'NS'

Data are presented as 'mean (SEM) in µmol/kg wet weight'. 'SumAA = total amino acids, SumEAA = total essential amino acids, SumNEAA = total non-essential amino acid, 'AA = Amino Acids', EAA = Essential Amino Acids, NEAA = Non-Essential Amino Acids, Asn = Asparagine, Ser = Serine, Gln = Glutamine, His = Histidine, Gly = Glycine, Thr = Threonine, Tyr = Tyrosine, Val = Valine, Met = Methionine, Ile = Isoleucine, Phe = Phenylalanine, Trp = Tryptophan, Leu = Leucine, Orn = Ornithine, Lys = Lysine, NS = Not Significant'

Table 3 A strong negative correlation between LBP and total amino acids ($r = -0.87$, $p < 0.05$) indicates that higher inflammation levels are significantly associated with lower total amino acid concentrations.

Significant negative correlations with glutamine (r = -0.72, p = 0.002) and asparagine (r = -0.63, p = 0.03) further emphasize the adverse effects of inflammation on these amino acids.
The negative correlations between REE/FFM and key amino acids like glutamine (r = -0.74, p = 0.003) highlight COPD patients' metabolic challenges, suggesting that amino acid availability for energy and recovery decreases as inflammation increases.

Table 3: Correlation Coefficients of LBP with Amino Acid Concentrations

Variable	Correlation Coefficient (r)	p-value
LBP and SumAA	-0.87	< 0.05
LBP and GLN	-0.72	0.002
LBP and ASN	-0.63	0.03
LBP and ALA	-0.65	0.03
REE/FFM and GLN	-0.74	0.003
REE/FFM and ALA	-0.62	0.02
REE/FFM and SumAA	-0.60	0.007

DISCUSSION

In this investigation, we identified several abnormalities‘muscle and plasma amino acid (AA)levels in COPD patients’ toage-matched controls. Notably, levels of muscle glutamate (GLU)were found to be lower while glutamine (GLN), arginine (ARG), ornithine (ORN), and citrulline (CIT) levels were elevated. Conversely, GLN, alanine (ALA), GLU, and asparagine (ASN) plasma concentration was reduced. ‘Although the COPD patients were clinically stable during the trial, they exhibited signs of an acute-phase response, evidenced by elevated levels of lipopolysaccharide-binding protein (LBP)’. They increased resting energy expenditure (REE). Importantly, LBP levels demonstrated an inverse relationship with plasma AA levels.
The most significant change in muscle amino acid levels was the increased concentration of glutamine (GLN). As the body's most abundant amino acid, GLN plays a vital role in various physiological processes, fueling rapidly replicating cells like immune cells and enterocytes, and transporting ammonia (NH3)[11-13]. Previous studies have shown that GLN metabolism is altered during acute illness, particularly conditions like muscle trauma and sepsis, where muscle GLN levels typically decline.[14]. Karakioulaki et al 2020[15] Despite prior studies reporting decreased protein production in emphysema, ‘the unexpected increase in muscle GLN levels in COPD patients highlights a gap in understanding chronic muscle atrophy, warranting further investigation’. Consistent with Henrot et al in 2023 [2],‘the observed muscle efflux in COPD patients supports our finding of elevated muscle GLN levels alongside reduced plasma GLN, leading to an increased muscle-to-plasma GLN ratio’.
Our study found ‘elevated GLN, reduced GLU, and increased ARG, ORN, and CIT in muscle tissue, indicating impaired GLN transport and altered intracellular metabolism, with an unchanged muscle-to-plasma GLU ratio suggesting intact GLU transport’.‘The elevated levels of ARG, ORN, and CIT, key components of the urea cycle, suggest altered ammonia metabolism in COPD patients' muscles, warranting further investigation’[16].
Lower ALA, GLN, GLU, and ASN levels in arterial blood reduced overall plasma AA concentrations, affecting key metabolic processes. ‘In contrast, venous AA levels in COPD patients have been widely studied’ [17]. Holz et al 2020 [18]reported ‘normal plasma AA levels, except for elevated GLN and decreased LEU level’.Similarly, Tosun et al found ‘reduced plasma levels (ALA, GLN, GLU, leucine (LEU)), and valine (VAL) levels in COPD patients’ [19].
This investigation ‘explores the relationship between muscle and plasma AA levels, REE, and the acute-phase response indicated by LBP, noting increased REE in our cohort consistent with literature highlighting hypermetabolism in COPD patients linked to systemic inflammation’. [20].
LBP, a recognizedmarker of acute-phase response, is classified as a Type 1 acute-phase protein[21]. ‘Despite excluding participants with illness, our study found increased LBP levels, indicating that stable COPD patients can present an acute-phase response, with elevated TNF-α suggesting that AAs may be shuttled from muscles to the liver for acute-phase protein synthesis and gluconeogenesis’. [22].Our study

found higher muscle GLN levels in COPD patients compared to lower levels seen in acute inflammation, with no correlation between LBP and muscle GLN or AAs, although several plasma AAs negatively correlated with REE and LBP; regression analysis indicated LBP independently influenced associations, while GLN significantly correlated with REE corroboratedprevious finding in plasma 'venous'[23]. Regression analysis indicated that associations were independently influenced by LBP, except for GLN, which also correlated with REE; our findings suggest that COPD patients exhibit distinct alterations in muscle and plasma AA levels linked to systemic inflammation and hypermetabolism, warranting further research to clarify the underlying mechanisms and their clinical implications.

CONCLUSION

There were signs of disruptions in intermediate metabolism among individuals with stable severe COPD, as evidenced by different changes in 'muscle and plasma AA levels'. Muscular AA changes in 'COPD patients' differ from those in illnesses linked to acute muscular wasting. Our research revealed a connection between inflammation and the noted drop in plasma AA levels.

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