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A Distinct Pattern of Circulating Amino Acids Characterizes Older Persons with Physical Frailty and Sarcopenia: Preliminary Results from the BIOSPHERE Study

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Abstract: Physical frailty and sarcopenia (PF&S) are hallmarks of aging that share a common pathogenic background. Perturbations in protein/amino acid metabolism may play a role in the development of PF&S. In this preliminary study, 68 community-dwellers aged 70 years and older, 38 with PF&S and 30 non-sarcopenic, non-frail controls (nonPF&S), were enrolled. A panel of 37 serum amino acids and derivatives was assayed by UPLC-MS. Partial Least Squares Discriminant Analysis (PLS-DA) was used to characterize the amino acid profile of PF&S. The optimal complexity of the PLS-DA model was found to be three latent variables. The proportion of correct classification was $76.6 \pm 3.9\%$ ($75.1 \pm 4.6\%$ for enrollees with PF&S; $78.5 \pm 6.0\%$ for controls). Older adults with PF&S were characterized by higher levels of asparagine, aspartic acid, citrulline, ethanolamine, glutamic acid, sarcosine, and taurine. The profile of nonPF&S individuals was defined by higher levels of α -aminobutyric acid and methionine. Distinct profiles of circulating amino acids and derivatives characterize older individuals with PF&S. The dissection of these patterns may provide novel insights into the role played by protein/amino acid perturbations in the disabling cascade and possible new targets for interventions.

Keywords: aging; muscle; protein; metabolism; metabolomics; profiling; biomarkers; multi-marker; physical performance; multivariate

1. Introduction

Over the last decades, sarcopenia, the progressive and generalized decline in skeletal muscle mass and function with age, has become a “blockbuster” condition in geriatrics, given its increasing prevalence in a globally aging world and its clinical relevance [1–4]. Indeed, this condition is associated with a broad spectrum of negative health outcomes, including disability, loss of independence, institutionalization, and mortality [5,6]. Frailty has been defined as a geriatric “multidimensional syndrome characterized by decreased reserve and diminished resistance to stressors” and is often envisioned as a pre-disability condition [7]. Sarcopenia overlaps with the clinical picture of frailty, especially in its physical domain, and may represent both the biological substratum of physical frailty (PF) and the pathophysiologic basis upon which the negative health outcomes of PF develop [8,9]. The two conditions have therefore been merged into a new entity (i.e., PF & sarcopenia; PF&S) [10] that was operationalized in the context of the “Sarcopenia and Physical Frailty IN older people: multi-component Treatment strategies” (SPRINTT) project [11,12].

Although the pathophysiology of PF&S is complex and multifactorial, the central role of muscle wasting suggests that biomarkers related to sarcopenia may be used to support the diagnosis, track the evolution of PF&S, unveil the underlying mechanisms, and identify meaningful targets for interventions [13,14].

Dietary protein intake and circulating amino acids play a pivotal role in muscle plasticity and trophism [15], but also modulate several physiologic processes (including inflammation, insulin sensitivity, redox homeostasis) that may be involved in age-related muscle atrophy and dysfunction [16,17]. Hence, perturbations of protein-amino acid metabolism may represent a major mechanism in sarcopenia [18,19].

Amino acid profiling, especially when coupled with multivariate statistical analysis, may represent a powerful analytical approach to evaluate the role of protein-amino acid networks in the PF&S condition [20]. Recently, distinct amino acid signatures were associated with muscle mass in older adults with functional limitation [21] and low muscle quality in the Baltimore Longitudinal Study of Aging [22]. Moreover, reduced non-fasting plasma concentrations of the branched-chain amino acids leucine and isoleucine were observed in Norwegian older community-dwellers with sarcopenia [23], while higher proline concentrations were independently associated with sarcopenia in elderly Japanese people [24]. Finally, low plasma levels of essential amino acids (EAAs) characterized the amino acid profile of severely frail Japanese older people compared with non-frail peers [25].

The “BIOMarkers associated with Sarcopenia and Physical frailty in Elderly pErsons” (BIOSPHERE) study was designed to determine and validate a panel of biomarkers for PF&S through multivariate statistical modeling [26]. In the present work, we present preliminary results obtained through the simultaneous analysis of an array of circulating amino acids and derivatives coupled with Partial Least Squares Discriminant Analysis (PLS-DA). This innovative approach allowed identifying distinct patterns of circulating amino acids and derivatives that characterize older adults with and without PF&S. This may represent a first relevant step towards the integration of specific biochemical measurements into the assessment of PF&S both in research and clinical settings.

2. Materials and Methods

2.1. Study design and population

BIOSPHERE was conceived as a cross-sectional, case-control study [26]. The study protocol was approved by the Ethics Committee of the Università Cattolica del Sacro Cuore (Rome, Italy; protocol number: 8498/15). After obtaining written informed consent, 200 older persons, 100 cases (individuals with PF&S) and 100 non-physically frail, non-sarcopenic (nonPF&S) controls aged 70+ were enrolled. Participant recruitment and assessment were described in detail elsewhere [26]. Briefly, candidates were diagnosed with PF&S when presenting the following parameters: (a) low appendicular muscle mass (aLM), as determined by dual X-ray absorptiometry (DXA) using the cut-points recommended by the Foundation for the National Institutes of Health (FNIH) sarcopenia project [27]; (b) low physical performance, defined as a summary score on the Short Physical Performance Battery (SPPB) [28] between 3 and 9; and (c) absence of major mobility disability, operationalized as inability to walk 400m in 15 min without sitting, the use of a walker, help from another person or stopping to rest for more the 60 s at a time [29]. Inclusion and exclusion criteria are summarized in Supplementary Table 1 in Appendix A. Thirty-eight cases and 30 controls were considered for this preliminary analysis.

2.2. Measurement of appendicular lean mass by DXA

Whole-body DXA scans were obtained on a Hologic Discovery A densitometer (Hologic, Inc. Bedford, MA). Scan acquisition and analysis were performed according to manufacturer's directions. Candidates were considered to be eligible if presenting with an aLM to body mass index (BMI) ratio (aLM_{BMI}) <0.789 or <0.512 in men and women, respectively. When the aLM_{BMI} criterion was not met, candidates were tested with the alternative criterion (i.e., crude aLM <19.75 kg in men and <15.02 kg in women) [27].

2.3. Blood sample collection

Blood samples were collected in the morning by venipuncture of the median cubital vein after overnight fasting, using commercial collection tubes (BD-Vacutainer). For serum separation, samples were left at room temperature for 20 min and subsequently centrifuged at $1000\times g$ for 10 min at $4^{\circ}C$. Aliquots of serum were subsequently stored at $-80^{\circ}C$ until analysis.

2.4. Amino acids profiling

Thirty-seven amino acids and derivatives (alanine, α -aminobutyric acid, aminoadipic acid, anserine, arginine, asparagine, aspartic acid, β -alanine, β -aminobutyric acid, carnosine, citrulline, cystathionine, cystine, ethanolamine, γ -aminobutyric acid, glycine, glutamic acid, histidine, isoleucine, 4-hydroxyproline, leucine, lysine, methionine, 1-methylhistidine, 3-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine, valine) were measured in serum through a ultraperformance liquid chromatography / mass spectrometry (UPLC/MS) validated methodology. Briefly, 50 μL of sample were mixed with 100 μL 10% (w/v) sulfosalicylic acid containing an internal standard mix (50 μM) (Cambridge Isotope Laboratories, Inc., Tewksbury, MA) and centrifuged at $1000\times g$ for 15 min. Ten μL of the supernatant were transferred into a vial containing 70 μL of borate buffer to which 20 μL of AccQ Tag reagents (Waters Corporation, Milford, MA) were subsequently added. Samples were then vortexed for 10 s and heated at $55^{\circ}C$ for 10 min. The chromatographic separation was performed by ACQUITY H-Class (Waters Corporation) using a CORTECS UPLC C18 column 1.6 μm 2.1 \times 150 mm (Waters Corporation) eluted at a flow rate of 500 $\mu L/min$ with a linear gradient (9 min) from 99 to 1 water 0.1% formic acid in acetonitrile 0.1% formic acid. MS was an ACQUITY QDa single quadrupole equipped with electrospray source operating in positive mode (Waters

Corporation). Analytical process was monitored using amino acids controls (level 1 and level 2) manufactured by the MCA laboratory of the Queen Beatrix Hospital (The Netherlands).

2.5. Statistical Analysis

All analyses were performed using in-house routines running under Matlab R2015b environment (The MathWorks, Natick, MA).

2.5.1 Descriptive statistics

Differences in demographic, anthropometric, clinical and functional characteristics between cases and controls were assessed via t-test statistics and χ^2 tests, for continuous and categorical variables, respectively. Both tests were two-sided, with statistical significance set at $p < 0.05$.

2.5.2 Partial Least Squares–Discriminant Analysis

The strategy for the the identification and validation of potential biomarkers for PF&S relied on the building of discriminant models to differentiate cases from controls. The approach chosen for the present study was based on PLS–DA [30], because of its versatility and ability to deal with highly correlated predictors. Briefly, PLS–DA is a classification method based on the PLS regression algorithm [31]. PLS–DA builds the linear relation between a set of responses Y and a matrix of predictors X by projecting the latter onto a low-dimensional space of latent (abstract) variables (LVs). LVs are characterised by having the highest covariance with the responses to be predicted. In order for the method to also be used in classification where the responses to be predicted are of a qualitative and not quantitative nature (in the present study, whether the participant is a case or a control), y is coded as a ‘dummy’ binary vector, assuming the value 1 if the participant has PF&S and 0 if he/she is a control. The classification of the individuals is then operated on the basis of the predicted value of y , adopting a threshold value of 0.5. If the predicted y is above that value, the individual is classified as belonging to PF&S group, while if it is below, he/she is predicted to be a control.

The statistical reliability of the PLS–DA model was subsequently verified by a double cross-validation procedure and by means of randomization tests [32]. The double cross-validation is a variant of standard cross-validation and includes an outer and an inner loop. The first loop mimics an external test set to be used for the validation of a PLS–DA model the optimal complexity of which is chosen on the basis of the error in the inner loop samples. The randomization test is used to obtain a non-parametric distribution of the figures of merit of the PLS–DA model under the null hypothesis to assess their statistical significance. Three figures of merit were considered in the present study: (i) the number of misclassifications (NMC); (ii) the area under the receiver operating characteristic (ROC) curve (AUROC); and (iii) the value of the discriminant Q^2 (DQ^2) [33]. NMC corresponds to the number of classification errors that occurred between groups (PF&S vs. nonPF&S). AUROC is a measure of a test’s discriminatory power. Its values range between 1 (perfect classification) and 0 (no discrimination). DQ^2 is a modification of the standard Q^2 and was introduced to cope with the peculiarities of classification problems addressed by regression methods [34]. DQ^2 is especially suitable for discriminating between groups in which the biological responses can be subtle and highly variable among individuals. As its regression analogue, DQ^2 assumes its highest values in the case of a perfect discrimination between classes. Different from standard Q^2 , DQ^2 is not bound to the 0–1 range of values (i.e., it can also be negative).

The identification of the experimental variables more important in the discrimination was obtained by inspecting the so-called variable importance in projection (VIP) indices [31] and rank product (RP) [35]. VIP scores indicate the contribution of each of the measured variables to the PLS model and are scaled so that a “greater than 1” rule can be used to assess statistical significance. RP

is an index accounting for the consistency of the selection of a variable in a resampling procedure. At each cross-validation split, metabolites are ranked in decreasing order of discriminant ability (here estimated as the absolute value of the PLS-DA regression coefficient), so that the variable with the highest discriminant power is given rank 1, the next largest 2 and so on. Eventually, RP is defined for each metabolite as the geometric mean of its ranks in all cross-validation segments; variables with the lowest rank products are those considered to be discriminant.

3. Results

3.1. Descriptive characteristics of the study population

The study population included 38 older adults with PF&S and 30 nonPF&S controls. The main demographic, anthropometric, clinical and functional characteristics of the study population according to the presence of PF&S are presented in Table 1. No differences between groups were observed with regard to age, gender distribution, number of comorbid conditions, and number of prescription medications. As expected, physical performance, as assessed by the SPPB, was lower in PF&S participants (SPPB score: 7.4 ± 1.5) relative to controls (11.3 ± 0.9) ($p < 0.0001$). Similarly, muscle mass was smaller in the PF&S group compared with nonPF&S enrollees. Indeed, the mean aLM_{BMI} was 0.554 ± 0.120 in PF&S participants and 0.795 ± 0.264 in controls ($p < 0.0001$), whereas absolute aLM was 16.2 ± 3.3 kg and 19.4 ± 3.9 kg in PF&S and nonPF&S older adults, respectively ($p = 0.004$).

Table 1. Main characteristics of BIOSPHERE participants according to the presence of physical frailty & sarcopenia (PF&S).

	PF&S (n=38)	nonPF&S (n=30)	p
Age, years (mean \pm SD)	76.4 ± 4.9	74.6 ± 4.3	0.1067
Gender (female), n (%)	25 (65.8)	16 (53.3)	0.4280
BMI, kg/m ² (mean \pm SD)	29.1 ± 4.4	26.7 ± 2.4	0.0112
SPPB (mean \pm SD)	7.4 ± 1.5	11.3 ± 0.9	<0.0001
aLM, kg (mean \pm SD)	16.2 ± 3.2	19.4 ± 3.9	0.0004
aLM_{BMI} (mean \pm SD)	0.554 ± 0.120	0.795 ± 0.264	<0.0001
Number of disease conditions* (mean \pm SD)	2.3 ± 1.5	1.8 ± 1.4	0.1448
Number of medications (mean \pm SD)	3.2 ± 1.8	2.8 ± 1.9	0.4115

*Includes hypertension, coronary artery disease, prior stroke, peripheral vascular disease, diabetes, chronic obstructive pulmonary disease, and osteoarthritis. BMI: body mass index; nonPF&S: non physically frail, non sarcopenic; PF&S: physical frailty & sarcopenia; SD: standard deviation; SPPB: Short Physical Performance Battery.

3.2. Participant classification according to PLS-DA

In order to verify the existence of a specific pattern of amino acids in participants with PF&S, a PLS-DA classification model was constructed and validated. The optimal PLS-DA model was built using 3 LVs that accounted for more than 44% of the variance originally present in the X block. As indicated by the double cross-validation procedure, the model allowed to correctly predict the presence of PF&S in $95.7 \pm 2.1\%$ of participants in the calibration phase ($94.7 \pm 3.8\%$ for PF&S and $96.7 \pm 4.6\%$ for controls), $84.1 \pm 2.7\%$ in the internal validation stage ($82.6 \pm 3.6\%$ for PF&S and $86.0 \pm 4.8\%$ for controls), and $76.6 \pm 3.9\%$ in external validation ($75.1 \pm 4.6\%$ for PF&S; $78.5 \pm 6.0\%$ for nonPF&S). The classification ability of the model is also evident by inspecting the projection of participants onto the space spanned by the first two LVs of the PLS-DA model (Figure 1), which shows a clear separation between participants with PF&S and controls.

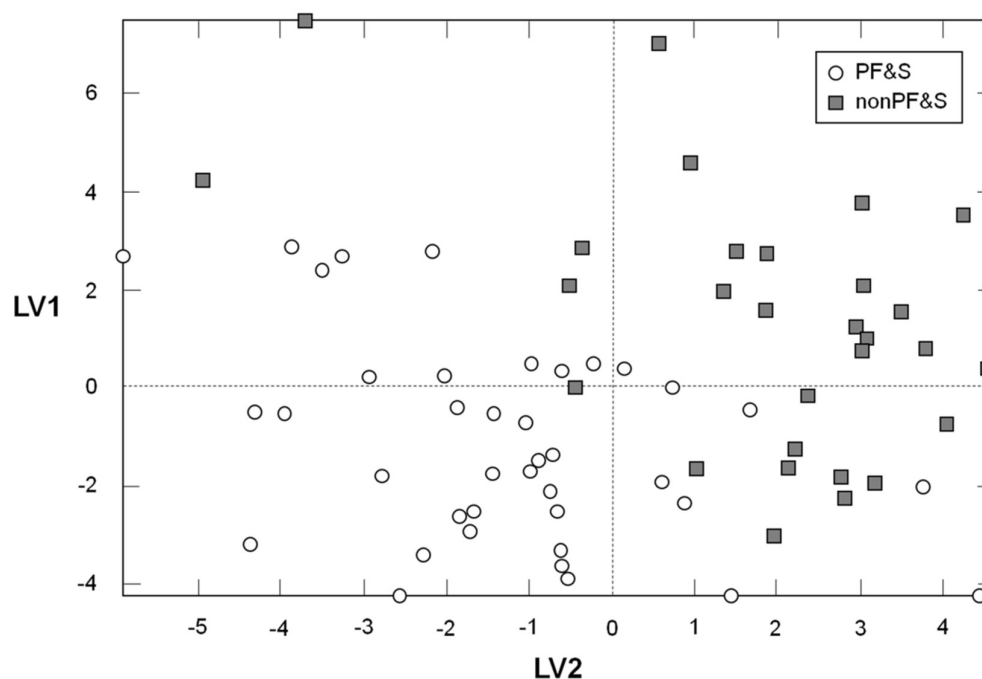


Figure 1. Scores plot showing the separation of participants according to the serum concentrations of amino acids and derivatives in the space spanned by the two latent variables (LV1 and LV2), as determined by PLS-DA.

To further validate the classification model and rule out the possibility of chance correlations, the results of the double cross-validation procedure were compared with the distributions of specific figures of merit under the null hypothesis. The distribution of NMC, AUROC and DQ2 under their respective null hypothesis, as estimated by permutation tests, is reported in Figure 2. The corresponding values obtained by the PLS-DA model on unpermuted data, as evaluated by the double cross-validation procedure, are also shown. The results of the PLS-DA classification model were statistically significant, as for all of the three figures of merit, value obtained on the real dataset fell outside of the corresponding null hypothesis distribution (corresponding to a $p < 0.05$). In order to identify the metabolites that were mostly involved in discriminating between cases and controls, the values of the VIP indices were inspected.

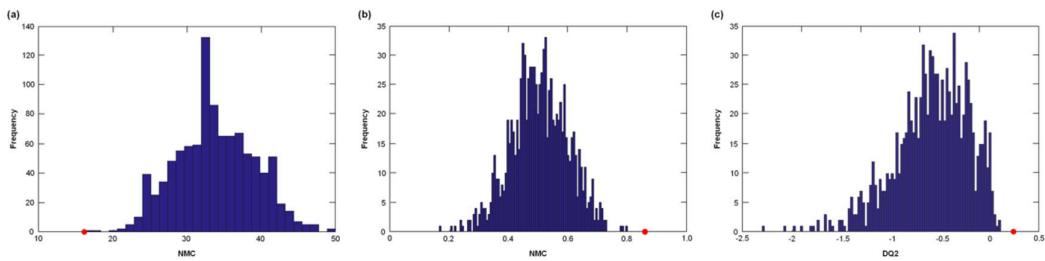


Figure 2. Distribution of (a) number of misclassifications (NMC), (b) area under the receiver operating characteristic (ROC) curve (AUROC), and (c) discriminant Q2 (DQ2) values under their respective null hypothesis as estimated by permutation tests (blue histograms) and the corresponding values obtained by the PLS-DA model on unpermuted data (red circles).

The variables corresponding to a VIP greater than 1 are reported in Table 2. Nine amino acids were found to contribute significantly to the discrimination model. The sign of the regression coefficients of the PLS-DA model is reported to indicate how the concentration of each analyte varied between PF&S and controls. Since the former was coded as +1 in the dummy y vector, all biomarkers with positive regression coefficient had a higher concentration in the PF&S group relative to controls, and vice versa. Accordingly, people with PF&S were characterized by higher levels of asparagine, aspartic acid, ethanolamine, glutamic acid, citrulline, sarcosine, and taurine. Conversely, the profile of non-PF&S individuals was defined by higher levels of α -aminobutyric acid (AABA) and methionine.

Table 2. Serum concentrations of discriminant analytes, variable importance in projection (VIP) values, and rank product (RP) values in BIOSPHERE participants with and without of physical frailty & sarcopenia (PF&S).

	PF&S (n=38)	nonPF&S (n=30)	VIP	RP
α -aminobutyric acid ($\mu\text{mol/l}$)	20.0 \pm 4.9	22.3 \pm 5.7	2.2	8.0
Asparagine ($\mu\text{mol/l}$)	91.0 \pm 12.6	77.8 \pm 13.4	3.4	2.0
Aspartic Acid ($\mu\text{mol/l}$)	24.6 \pm 5.4	17.0 \pm 4.0	5.8	2.6
Citrulline ($\mu\text{mol/l}$)	44.8 \pm 12.1	36.8 \pm 11.5	2.1	2.8

Ethanolamine (μmol/l)	10.3 ± 1.7	9.0 ± 2.2	1.7	9.9
Glutamic acid (μmol/l)	71.7 ± 16.6	54.3 ± 21.2	2.3	8.5
Methionine (μmol/l)	22.6 ± 2.8	23.4 ± 5.7	1.3	6.3
Sarcosine (μmol/l)	1.9 ± 0.6	1.5 ± 0.5	1.4	8.0
Taurine (μmol/l)	220.1 ± 36.5	189.5 ± 47.2	1.8	6.7

4. Discussion

In the present study, we report the first preliminary results from the BIOSPHERE study. This project was designed to identify a set of biomarkers associated with PF&S and develop an analytical strategy to integrate biomarkers into the clinical assessment of the condition. In this exploratory study, an innovative analytical approach based on amino acid profiling coupled with PLS-DA was adopted to unveil possible associations between alterations in protein-amino acids networks and the PF&S condition.

The most noticeable finding was that a distinct profile of circulating amino acids characterized older individuals with PF&S. In particular, elderly persons with PF&S displayed higher serum levels of asparagine, aspartic acid, citrulline, ethanolamine, glutamic acid, sarcosine and taurine. Conversely, the profile of non-PF&S individuals was defined by higher levels of AABA and methionine. Although the study was not designed to address mechanistic hypotheses, the present findings add some interesting cues into the physiopathology of PF&S. Indeed, serum amino acids are part of a pool of free amino acids that results from several processes, including food ingestion and nutrient absorption, metabolic pathways, hormonal stimulation, endogenous synthesis, and gut microbiota activities [36].

PF&S was associated with lower circulating levels of the essential amino acids EAAs methionine and histidine. EAA are defined as those amino acids that must be provided with the diet to meet optimal requirements [36]. The reduction of serum concentrations of a number of EAAs (including methionine) with age was reported in both genders and was purportedly associated with decreases in total energy and protein intake [37]. In addition, low plasma levels of EAA were found in severely frail older people [25]. These findings may be linked to malnutrition (both quantitative and qualitative), a common causative factor of frailty and sarcopenia [38,39]. The concomitant low serum concentration of the non-essential non-proteinogenic amino acid AABA seems to corroborate the previous finding since AABA may derive from the catabolism of methionine [40]. Further to this, plasma levels of AABA were found to be associated with both the quality and amount of dietary protein [41,42]. Although these findings seem to point towards a poor-quality protein diet or (selective) malabsorption, further studies are needed to clarify the correlation between diet and circulating EAA levels in the context of PF&S.

Methionine is also involved in one-carbon metabolism, a crucial pathway that modulates multiple physiological processes, including nucleotide biosynthesis, amino acid homeostasis, epigenetic maintenance, and redox balance [43]. Not surprisingly, alterations in one-carbon metabolism were observed in aging and age-related diseases, including as cancer, cardiovascular disease, and neurodegeneration [43,44]. Sarcosine, the N-methyl-derivative of glycine, is another relevant intermediate of one-carbon metabolism [43]. Sarcosine is formed from dietary choline and the metabolism of methionine [45,46] and can be found in muscles and other body tissues. A recent metabolomic study showed that circulating sarcosine levels were reduced with aging both in rodents and humans, while dietary restriction was able to prevent this decline in both species [47]. Counterintuitively, sarcosine levels were higher in persons with PF&S relative to controls. However, it is important to note that circulating sarcosine may increase in case of folate deficiency, because folate mediates the conversion of sarcosine to glycine [46]. Thus, this finding may be linked to insufficient folate ingestion and/or perturbation in folate/one-carbon metabolism.

Sarcosine also activates autophagy in mouse fibroblasts in a dose-dependent fashion [47] and alterations in myocyte quality control mechanisms (including autophagy) may contribute to

sarcopenia [48,49]. In this context, the presence of ethanolamine among the most discriminant metabolites for PF&S classification is of particular interest. Ethanolamine is a naturally occurring amino alcohol that exerts a pivotal role in the synthesis of phosphatidylethanolamine, a central intermediate of lipid metabolism and a major component of biological membranes [50]. Importantly, phosphatidylethanolamine is also directly involved in the regulation of autophagy [51] and it is postulated that ethanolamine treatment or the consumption of ethanolamine-rich foods may increase cellular phosphatidylethanolamine levels, induce autophagy and exert beneficial anti-aging effects across species [51]. While serum ethanolamine levels were different between PF&S and controls, this did not result in a corresponding difference in serum phosphatidylethanolamine concentrations, suggesting alterations in CDP-ethanolamine pathway, the major route of phosphatidylethanolamine production [52]. Interestingly, the disruption of CDP-ethanolamine pathway in muscle was associated with alterations in mitochondrial biogenesis and the reduction in muscle mass in mice [53].

Taurine is a ubiquitous non-proteinogenic sulfur-containing amino acid that represents the most abundant free amino acid in the heart, retina, skeletal muscle, brain, and leukocytes, accounting for approximately 0.1% of total body weight [54]. In skeletal muscle, which contains 70% of total body taurine, this amino acid is involved in the regulation of ion channel function, membrane stability, mitochondrial quality control, and calcium homeostasis [55–58]. In muscle, taurine also serves osmoregulatory, anti-oxidant, and anti-inflammatory functions [55–58]. Given these multiple actions, taurine has recently been proposed as a candidate therapeutic molecule against sarcopenia [59]. While it is reported that serum taurine concentrations decline with age in males [37], increased levels of serum taurine have been retrieved in the metabolic profiles of old wild-type mice from different genetic backgrounds [60]. Circulating levels of taurine are regulated by the balance among different factors, including dietary intake, intestinal absorption, bile acid conjugation, urinary excretion, and endogenous synthesis from methionine and cysteine [54]. Taurine may be released from cells following osmotic perturbations, oxidative stress and (chronic) inflammatory stimulation [57]. Further studies are needed to unveil the mechanisms responsible for the high circulating taurine levels observed in older adults with PF&S.

Citrulline is a non-essential non-protein amino acid with a key role in nitrogen homeostasis [61]. Citrulline is an end product of glutamine metabolism and an endogenous precursor of arginine [62]. For its capacity of promoting endothelial nitric oxide availability and vasodilation, “sparing” arginine and glutamine from hepatic catabolism and the supposed ability to activate mTORC1 signaling [63], citrulline was proposed as a pharmaconutrient to counteract sarcopenia [64]. Several reports have shown that serum citrulline increases with age [37,65,66]. In addition, in a metabolomic study assessing the individual variability in human blood metabolites [67], citrulline was among the circulating molecules that exhibit a remarkable age-related increase. The authors attributed this finding to impairment in urea cycle efficiency due to the progressive decay of liver and renal function with age [67]. However, no differences in kidney or liver function were observed between participants belonging to the two BIOSPHERE study groups. Further investigation on interorgan nitrogen homeostasis pathways are needed to explain the higher circulating values of citrulline found in people with PF&S.

Asparagine, aspartic acid and glutamic acid are among the six amino acids that are metabolized in resting muscles [68]. These amino acids provide the amino groups and the ammonia required for the synthesis of glutamine and alanine, which are released following protein meals and in the post-absorptive state [68]. The carbon skeletons of these metabolites may be used solely for de novo synthesis of TCA-cycle intermediates and glutamine [69]. The higher levels of asparagine, aspartic and glutamic acid observed in persons with PF&Ss may be suggestive of perturbations in muscle energy metabolism associated with muscle wasting. Interestingly, a pattern of metabolic changes accompany muscle remodeling after disuse, including energy substrate accumulation (e.g., asparagine) in the atrophied muscles [70,71].

In the present study, no significant changes were observed in the serum levels of branched-chain amino acids. This finding is not consistent with previous investigations that

reported changes in branched-chain amino acids concentration in relation to sarcopenia, low muscle mass, and functional limitation [21–23]. These discrepancies may be due to the different operational definitions adopted in the studies and the different study designs. In addition, heterogeneity in ethnic groups and eating habits among participants of the different studies may contribute of the contrasting results.

Although reporting novel findings, our study presents some limitations that need to be acknowledged. First, analyses were conducted in a relatively small group of persons and involved a vast array of experimental variables. However, as reported previously [30], the PLS–DA approach is particularly suited for such an experimental design. Moreover, the very stringent double cross–validation procedure confirmed the reliability of the PLS–DA model. The study sample was exclusively comprised of Caucasian individuals, which impedes generalizing the findings to other ethnic groups. Other factors that might affect circulating amino acid levels include lifestyle and eating habits [72,73]. Although only people not engaged in regular physical exercise were enrolled, the amount of physical activity of participants was not quantified. Hence, the possible influence of physical activity on amino acid profiles in the context of PF&S could not be established. The same applies to the possible influence of different nutritional patterns. However, as highlighted by recent evidence, the differences in circulating amino acid are less marked than the differences between amino acid intakes [72]. The cross-sectional design of the study does not allow inference to be drawn on the time course of changes of the variables considered and on cause–effect relationship. Finally, although a fairly large number of amino acids and derivatives was assayed, it cannot be excluded that more powerful biomarkers of PF&S might be obtained through the analysis of a larger range of biomediators.

5. Conclusions

In the present study, a PLS-DA-based approach allowed identifying distinct patterns of circulating amino acids and derivatives in older persons with PF&S. The different pathways identified could be used to generate new mechanistic hypotheses on the pathophysiology of PF&S. The longitudinal implementation of such an innovative strategy could allow for the tracking of PF&S condition over time and the monitoring of response to treatments. This may represent a first relevant step towards the integration of specific biochemical measurements into the assessment of PF&S both in clinical and research settings.

Supplementary Materials: Table S1: Eligibility criteria in BIOSPHERE.

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