Update on metabolomic findings in COPD patients

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Shareable abstract (@ERSpublications)
The clinical presentation of COPD is very heterogeneous. We need to improve diagnosis and classify each patient accurately (personalised medicine). The main metabolomic findings in COPD, its diverse phenotypes and its associated factors are reviewed. https://bit.ly/45tDLaO

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Abstract

COPD is a heterogeneous disorder that shows diverse clinical presentations (phenotypes and "treatable traits") and biological mechanisms (endotypes). This heterogeneity implies that to carry out a more personalised clinical management, it is necessary to classify each patient accurately. With this objective, and in addition to clinical features, it would be very useful to have well-defined biological markers. The search for these markers may either be done through more conventional laboratory and hypothesis-driven techniques or relatively blind high-throughput methods, with the omics approaches being suitable for the latter. Metabolomics is the science that studies biological processes through their metabolites, using various techniques such as gas and liquid chromatography, mass spectrometry and nuclear magnetic resonance. The most relevant metabolomics studies carried out in COPD highlight the importance of metabolites involved in pathways directly related to proteins (peptides and amino acids), nucleic acids (nitrogenous bases and nucleosides), and lipids and their derivatives (especially fatty acids, phospholipids, ceramides and eicosanoids). These findings indicate the relevance of inflammatory-immune processes, oxidative stress, increased catabolism and alterations in the energy production. However, some specific findings have also been reported for different COPD phenotypes, demographic characteristics of the patients, disease progression profiles, exacerbations, systemic manifestations and even diverse treatments. Unfortunately, the studies carried out to date have some limitations and shortcomings and there is still a need to define clear metabolomic profiles with clinical utility for the management of COPD and its implicit heterogeneity.

Introduction

COPD is a very prevalent disorder that has important consequences for social and healthcare systems [1, 2]. Although its clinical expression has certain common elements among patients, it is extremely heterogeneous. It can fundamentally vary in its progression profile, and the frequency and severity of acute episodes (exacerbations), characterised by an increase in symptoms. For this reason, many attempts have been made to define differentiated clinical profiles in COPD, which have been named phenotypes or, more recently and specifically, "treatable traits" [3]. The most classical are pulmonary emphysema and chronic bronchitis, although in the last decade those groups of patients with frequent exacerbations, or those with eosinophilia and/or the asthma interposition syndrome (ACO), have also been included. In addition, it is clear that the disease expresses differently depending on the age and sex of the patients, and that some of them show an accelerated loss of lung function, or associated loss of body weight and lean mass, muscle dysfunction, cardiovascular involvement or even lung cancer. Undoubtedly, the majority of COPD patients share general pathophysiological mechanisms, but the clinical heterogeneity of this entity suggests that they differ in others, with specific biological profiles (endotypes) involved in each case [1, 2]. Both the time of the initiation of changes leading to the onset of the disease, as well as the dynamic interaction of the various noxae with the individual's immune system, and other intercurrent circumstances also advocate for a relative specificity in some mechanisms. This has two very important clinical consequences. On the one

hand, the potential existence of differentiated markers between patients with diverse clinical presentations. Secondly, the possibility of identifying precise therapeutic targets for each case. However, we are still far from having an accurate classification for each COPD patient in order to provide a more precise and personalised care [4]. This is especially worrisome when new treatments, aimed precisely to modify the above-mentioned pathophysiological mechanisms, are constantly being investigated and translated to clinics [5]. This is a therapeutic approach that has already become common in other respiratory diseases, such as bronchial asthma and pulmonary fibrosis [6, 7]. Therefore, now it is very important to be able to identify the mechanisms present in each group of patients and discover what type of determinations can help to better classify each case. In this sense, there are already some blood biomarkers that correspond to specific clinical outcomes and have promoted precise therapeutic recommendations, for instance, the number of eosinophils in peripheral blood [1, 2]. However, there is still a need to search deeper for markers and, therefore, for specific endotype profiles that would allow patients to become properly segregated into homogeneous subgroups. The search for these biomarkers can be performed in two different general ways. The most traditional is to direct the research approach by a specific hypothesis, derived from previous knowledge and generally employing conventional laboratory techniques. The alternative way is the "scanning search" (more or less blind) that uses massive prospecting techniques, without any or a fully defined hypothesis. For this approach, high-throughput techniques, such as those linked to omic sciences, stand out because of their usefulness. They are not totally blind since the search is always based on the previous experience of the group on a particular subject and the related library. Nevertheless, it is possible to perform an initial search for candidates to be good biomarkers, which must be further validated with the same or other methodologies in other patient cohorts. Furthermore, the analysis of these potential candidates would suggest the biological mechanisms involved in each case ("enrichment" approaches), with potential consequences in diagnosis, management and even therapy [8].

Omic sciences include different conceptual or molecular substrata. This is the case of the exposome or the microbiome amongst the former, and transcriptomics, proteomics or metabolomics amongst the latter. Metabolomics in particular is the science that studies metabolites present in a sample (generally from a living being), inferring the underlying metabolic processes from which these metabolites derive. Such metabolites include carbohydrates, lipid, amino acids and peptides, as well as components of nucleic acids, among others.

Respiratory diseases, including COPD, involve metabolic changes in their pathophysiology (lung and systemic inflammation, oxidative stress, *etc.*) that can be analysed through metabolomic tools. These are aimed at detecting and eventually quantifying certain signals (called "analytes"), which in turn allow for the identification of their molecular origin. From there, the so-called enrichment techniques (profiles based on bioinformatic tools) will suggest the metabolic pathways that are most likely to be involved in a given situation. Analyte separation techniques include capillary electrophoresis, gas and liquid chromatographies (GC and LC, respectively), and especially the high-pressure LC (HPLC) and "ultra-high performance" LC (UHPLC) [9, 10]. Analytes can also be directly separated by mass spectrometry (MS). However, this latter technique is mostly employed to identify (and eventually quantify) the analytes once they have become separated by any of the aforementioned methods [11]. Electrospray ionisation (ESI), which transfers ions from macromolecules to a gas phase, can be used for further MS. This combination is known as ES-MS and can be optimised by coupling it with tandem mass spectrometry (MS/MS, defined by the consecutive use of two or more spectrometers). MS/MS is especially useful for detecting peptides and proteins, and can also be coupled to GC or LC systems, such as HPLC. The quadrupole time of flight (Q-TOF or QTOF) is a modality of MS that combines the use of four parallel rods (quadrupole) and a collision cell, with the calculation of the time of flight (TOF) for each ion, gaining in sensitivity and speed. Finally, nuclear magnetic resonance spectroscopy allows for the identification of metabolites without the need of separating them in advance [12]. Although the results of metabolomic studies are usually expressed as relative within a specific study, some normalisation methods have been proposed to approximate comparisons between the results of different authors [13].

The other important element of metabolomic studies applied to disease status is the type of samples that have been analysed. Those which are organ-specific are generally more difficult to obtain but very valuable due to their proximity to the main events that take place in a disorder [14]. By contrast, blood, urine and even saliva or sweat samples, which are much easier to collect, will only indirectly reflect what is happening in a particular organ or system. In the case of primary lung diseases, samples from the respiratory system include lung tissue or bronchial epithelium, exhaled air condensate and airway secretions (bronchial aspirate, bronchoalveolar lavage (BAL), induced sputum, *etc.*). However, in the particular case of COPD it is important to keep in mind that this is a disorder with significant systemic manifestations and associated comorbidities. This enhances the significance of findings obtained in blood samples.

Finally, the research groups that have participated in metabolomic studies have their own field and technical specialisation, so their results may have limitations or contain involuntary omissions and shortcuts when interpreting the overall metabolic picture. Only an integrated approach including the most relevant findings provided by different groups and techniques can give us a clear picture of the present knowledge on metabolomics in COPD.

In the following sections, the exposome most related to the potential future development of COPD and its effects on the metabolism will be reviewed first. This will be followed by a section with the most relevant findings reported for COPD as a single entity, with no segregation for phenotypes or other characteristics that may also influence the results. Finally, this review will focus on those studies that have specified their analyses by considering the presence of exacerbations, COPD phenotypes or other factors that may also influence metabolomic profiles.

The "risky-exposome"

Exposome is the set of external and internal factors that act on an individual from conception (and even before) to death. In the case of COPD, tobacco smoking is the external factor traditionally considered the most important, with the development and profile of the immune system probably being the most relevant internal counterpoint. Both factors dynamically interact throughout the entire life of the patient. There are obviously many other elements to be considered: environmental pollution, exposure to wood or charcoal smoke, lifestyle, early stages of the lung development and microbiome, among others. The combined impact of all these elements and their mutual interactions will cause specific physiological and pathophysiological changes in each patient or group of patients. These will be likely reflected in their metabolites at each stage of the disease [15, 16].

Tobacco

The effects of tobacco smoking on metabolism are numerous, so only some of the most significant findings are mentioned here. In one of the most relevant studies, carried out with blood samples of smokers, significant associations were found between lung function and various phospholipids (both glycerophospholipids and sphingo phospholipids) [17]. Other authors have reported similar associations, extending them to branched-chain amino acids (leucine, isoleucine and valine), eicosanoids [18–20], fibrinogen [21] or even the metabolism of certain ions [22]. Pregnant smokers, in turn, show a significant alteration in multiple amino acids and the urea cycle [23]. Moreover, some other abnormalities such as methylation of various molecules (including DNA) have also recently been reported in users of new smoking alternatives such as tobacco consumption with vaporisers [24]. Even passive exposure to tobacco smoke appears to alter the metabolism of phospholipids, amino acids, peptides and purine components, with effects on inflammation, oxidative stress and cell signalling pathways [25, 26]. However, recent studies seem to indicate that quitting smoking can partially restore the metabolic profile in ex-smokers [27].

Products of biomass combustion and air pollution

The combustion smoke from materials such as firewood and coal is an important factor in the development of COPD in many countries, and not only in the developing ones [28–30], as it is also able to enhance the effects of tobacco smoking [30]. Although specific metabolomic studies are lacking, there are some on exposure to particles that can be derived from biomass combustion. In this regard, exposure to small and even ultrafine particles can induce changes in phospholipids and glutathione, with a probable impact on the level of inflammation and oxidative stress, and subsequent damage to tissues and key molecules [31].

Metabolomic studies of environmental pollution have reported changes in various lipids, carbohydrates and amino acids, as well as in nucleotides, vitamins and hormones [31–35], even following relatively short exposures. Again, the most intensely affected pathways seem to be those related to inflammation, oxidative stress and, in this case, also the metabolism of steroids [15, 32]. Moreover, most of them are changes that seem to precede the onset of the pulmonary disease.

Summary

The exposome is an essential element in the pathophysiology of COPD. The effects of tobacco smoking mainly involve the metabolism of phospholipids and branched-chain amino acids with the additional methylation of different molecules, which together would condition the activation of inflammatory pathways and oxidative stress. However, these effects appear to be relatively reversible after smoking cessation. Environmental pollution and the inhalation of biomass combustion products, however, have been less studied but seem to have similar effects, with the addition of changes in the synthesis of some hormones.

COPD "as a single disorder"

Significant metabolomic changes have also been found in subjects with an already established COPD (figure 1), although their meaning still needs to be clarified to transfer this knowledge to clinical practice. Some of these studies have been performed on samples from the respiratory system, but most results have been obtained in blood (plasma or serum), due to the ease of obtaining samples. Fewer studies carried out on urine or even faecal samples are also available. Most of the authors compare their findings obtained in COPD patients with those from control populations of healthy subjects, although one aspect that always needs to be taken into consideration is whether the latter were smokers or not, since as mentioned above tobacco "*per se*" can induce changes in the metabolism.

Findings in samples from the respiratory system

Lung and airway tissue

Very few metabolomic studies have been carried out on patients' lung tissue, and in them, some authors have found changes in various phospholipids (specifically, phosphatidylinositol and phosphatidylserine), mostly in relation to the presence and degree of emphysema [36]. Other studies have tried to define specific lipid profiles according to the severity of the disease, and it appears that patients with mild or moderate COPD have higher levels of ceramides in their lungs than healthy subjects, which probably reflects an increased level of parenchymal destruction [37]. In contrast, patients with more severe COPD show higher levels of the phospholipid sphingosine-1-phosphate but lower levels of the aforementioned ceramides [37], perhaps indicating that tissue destruction is no longer as active.

Bronchoalveolar lavage

As in the other samples from the respiratory system, an intrinsic problem is not knowing the exact degree of dilution of a particular sample when analysing the results of BAL. This may hinder comparisons, not only among individuals or groups belonging to the same study, but also with results from different studies. In any case, the already mentioned associations of COPD with changes in not only some lipids (mainly fatty acids and phospholipids in this case, as well as in those mediators derived from cytochrome P450), but also in amino acids (such as threonine, glycine, cysteine and homocysteine) and peptides (for instance those containing valine and alanine), have been reported (figure 1), being also proportional to the degree of functional impairment [14, 38, 39].

Induced sputum

This is the easiest sample to be obtained directly from the respiratory system and, therefore, many studies have used it. In an interesting study carried out by Esther *et al*. [40] these authors observed that COPD patients showed increases in sialic acid and in various metabolites involved in the synthesis or modifications in nucleic acids, as is the case of nitrogenous bases and purine derivatives (adenine and methylthioadenosine, hypoxanthine and xanthines), as well as in the level of glutathione, a compound with an important role in the control of oxidative stress. In another interesting study published by Van der Does *et al*. [41] stable COPD patients exhibited increases in ω-3 DPA (a docosapentaenoic acid) and arachidonic acids, as well as reductions in other fatty acids and their derivatives (linoleic, linolenic, ω-3 EPA (eicosapentaenoic), and ω-3 DHA (another docosapentaenoic acid)). Moreover, the increase in arachidonic acid observed in these patients appeared to be secondary to changes in the metabolism of sphingolipids [42]. Other molecules of lipid origin, such as eicosanoids, have also shown specific changes in the sputum of COPD patients, who showed increases in some leukotrienes (LTD4 and LTE4), prostaglandins (PGD2, PGE2, PGF2a and their isoforms) and thromboxanes (TBX2), as well as in some eicosatetranoic acids, simultaneously with decreases in some metabolites of the same prostaglandins (PGEM from PGE1 and PGE2, and PGDM from PGD2) (figure 1) [43]. Regarding the changes in amino acids, another study has reported an impairment in tryptophan metabolism, fundamentally due to a reduction of hydroxyl indoleacetic acid, secondary in turn to the activity of interleukin-22 (IL-22). This chain of events is possibly related to apoptotic pathways, and perhaps even to the microbiota of such patients [44].

Other studies have also reported important differences in the sputum of patients with mild-to-moderate COPD compared to those with a more severe disease. In one of them, the difference reached 500 different metabolites [45], highlighting the lower levels observed in severely ill patients that are related to glycerophospholipids, with parallel increases in the powerful antioxidant enzyme superoxide dismutase (SOD), myeloperoxidase (MPO) and an isoform of the prostaglandin PGF2α. All these changes are considered as probably being related to the level of oxidative stress within the lungs [45].

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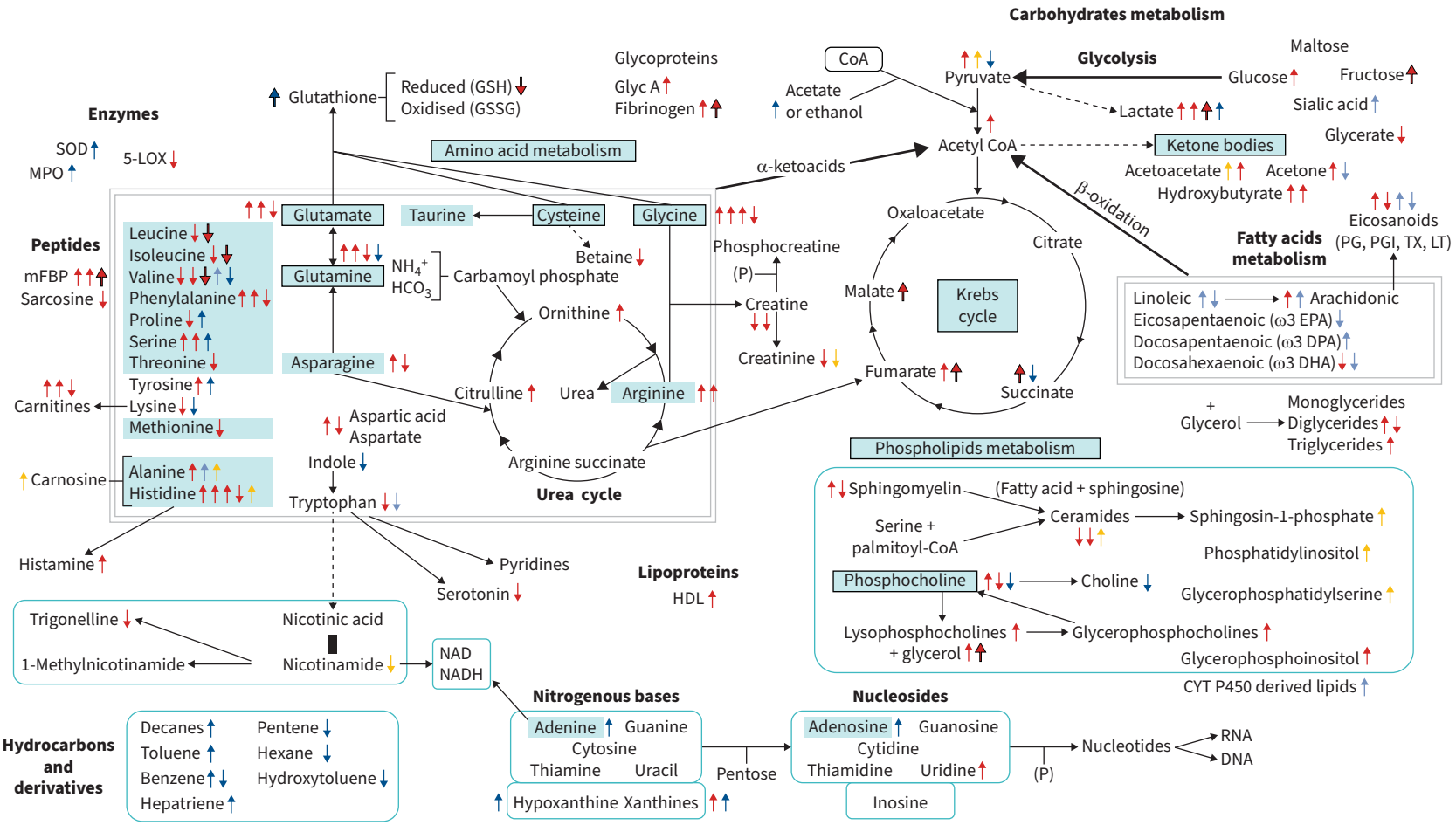

FIGURE 1 The most relevant metabolites and pathways involved in changes in the metabolome of COPD are shown. Arrows indicate the direction of changes reported for each molecule, their colour being indicative of the type of sample: dark red (blood); light blue (sputum or BAL); dark blue (condensate of exhaled breath); and dark gold (urine). Any arrow colour outlined in black means predictive of bad prognosis or even death. Green boxes around metabolites, their groups or even pathways highlight those considered key by occasional (without outline) or multiple (outlined boxes) authors. SOD: superoxide dismutase; MPO: myeloperoxidase; 5-LOX: 5-lipooxygenase; CoA: coenzyme A; mFBP: membrane-associated folate binding protein; PG: prostaglandin; PGI: prostacyclin; TX: thromboxane; LT: leukotriene; (P): phosphate; HDL: high-density lipoprotein; NAD: nicotinamide adenine dinucleotide; NADH: reduced NAD; CYT: cytochrome.

Condensate of exhaled breath

Although the exhaled air is another sample of great potential value, its collection and analysis also present the previously mentioned methodological problem (the degree of sample dilution), as well as the possibility of contaminating the concentrate with substances external to the subject [46]. However, it also has some advantages since it allows for the collection of some metabolites rarely identifiable in other biological samples (*e.g.* volatile organic compounds and some inorganic molecules) (figure 1) [46–48]. In one of the first studies published on exhaled air in COPD patients, De Lurentis *et al*. [49] showed that they exhibited reductions in exhaled pyruvate compared to controls, with absence of succinate; a finding that could indicate a serious alteration of the energy production in the Krebs cycle. There were no traces of the amino acid glutamine, nor of choline (a compound necessary for the synthesis of various phospholipids or alternatively, a product of their degradation) or any of its intermediate metabolites (such as phosphorylcholine, trimethylamine oxide), which in contrast were present in controls [49]. In a later study, also performed in exhaled air, the same authors observed differences between both groups for ethanol, lactate, the amino acid threonine, acetoin (involved in the synthesis of branched-chain amino acids), some fatty acids and propionate [50]. These results were partially confirmed 2 years later but extended with the addition of increased levels of acetate and more amino acids (such as proline, serine and tyrosine) in COPD, along with lower quantities of acetone and other amino acids (lysine and valine) [51]. In another interesting study, Bregy *et al*. [52] observed significant differences between patients and controls, especially with regard to fatty acids, amino acids and aldehydes. Some studies focusing especially on volatile compounds have reported that COPD patients show higher values of some decanes, with decreases in benzene, pentene, hexane, propanol, the ketone cyclohexanone, limonin, fluorophosphate (a cholinesterase inhibitor) and the antioxidant butylated hydroxytoluene than healthy controls [53, 54]. However, not all the authors agree with these results, since some others such as Gaida *et al*. [55] have reported higher levels of benzene, toluene, vinylacetate and 1,6-dimethyl-1,3,5-heptatriene in COPD patients than in controls, with lower values for indole.

Finally, it is worth mentioning that it is also possible to detect metabolites in exhaled air using a device called “electronic nose” (eNOSE). The article published by Rodríguez-Aguilar *et al*. [56] clearly stands out among different studies since they reported higher levels of acetaldehyde, cyclopentanone, octane, methylisobutarate, 2-propanol and 3-hexanone in COPD patients when compared to controls, with lower concentrations for tetradecane, δ-dodecalactone, 2-methylbutanoic acid, vinylpyrazine (a diazine such as pyrimidines) and 2-acetylpyridine.

Findings in systemic samples

Blood

There are several factors that must always be considered when analysing blood samples. The first is whether the extraction has been performed while the subject is fasting, since food intake can modify various metabolites, generating misinterpretations. The second factor is to clarify if the sample is serum or plasma, since fibrinogen and other coagulation factors will be absent in the first. This issue, that will be mentioned later in the present review, could be especially relevant in the case of COPD. Finally, the studies published to date have been carried out using either very large but relatively few cohorts of patients (*i.e.* COPDGene, ECLIPSE, SPIROMICS, *etc.*) [57–60] or with much smaller populations. At the end of the present review some of the advantages and disadvantages of each one of these complementary approaches are briefly discussed.

In most published studies, the role played by various lipids clearly stands out among other findings. In this regard, a dysregulation in some diglycerides and phospholipids has been repeatedly evidenced in the serum of COPD patients [61–69]. If referring specifically to the latter, lower levels of phosphatidylcholines and sphingomyelin have been specially highlighted by different authors [62, 64, 65, 67]. Both are phospholipids but while the former is the main lipid component in the lung surfactant (around 85% if considering dipalmitoyl phosphatidylcholine plus phosphatidylcholine) [70, 71], the latter (phosphocholine or phosphoethanolamine plus a ceramide, *i.e.* a combination of sphingosine and fat acid(s)) is especially abundant in cell membranes and the nervous system. It should be noted that phosphatidylcholines seem to be diminished in the surfactant of COPD patients, a finding that has been associated with their functional impairment [72]. However, other phospholipids, their precursors or even their metabolites (such as glycerophosphocholine, phosphocholine, lysophosphocholine and lysophosphatidylcholine), as well as some triglycerides, seem to be increased in COPD patients compared to controls (figure 1) [60, 62, 65–69]. In fact, when globally analysed as an interaction network, all these lipids showed a strong association with the pulmonary disease [64]. Some of these same investigators have also reported low levels of polyunsaturated fatty acids in COPD patients, although monounsaturated fatty acids in turn seem to be elevated, with increased values also being observed in various eicosanoids (such as those derived from CYP

oxidases, and platelet 12-HETE (12-hydroxyeicosatetranoic) and 12-HHTrE (12-hydroxyheptadecatrienoic) acids), accompanied by low levels of 5-lipooxygenase (5-LOX, involved in the transformation of fatty acids into leukotrienes) [39, 61, 62, 68]. Another interesting finding is that of high levels of ketone bodies observed in COPD patients [69, 73], which would express a greater level of acetyl-CoA due to oxidation of both pyruvate and fatty acids. Moreover, another interesting study showed increases in acetyl-CoA degradation products (specifically acetoacetate and 3-hydroxybutyrate) in COPD patients when compared to controls [74]. Such increases have also been reported in lipoproteins, especially those of high-density (HDL) [74], although paradoxically other studies have shown a decrease in distinct lipoproteins [69, 75]. Also in relation to lipid metabolism, additional changes have been found in the level of acylcarnitines, which are responsible for their transport to mitochondria to become degradated [64, 66, 68].

Many authors have also emphasised the relevant role played by changes in protein metabolism in COPD, which is, above all, expressed by modifications in their amino acidic and peptide components, especially branched-chain amino acids, with high levels shown by COPD patients for histidine and 3-methylhistidine (and secondarily for histamine), arginine, glutamine and phenylalanine [66, 68, 69, 75–80]. Other amino acids or their derivatives, such as creatine (from glycine and arginine) and its derivative creatinine, threonine, leucine, valine, glycine and dimethylglycine, as well as glutamine-glutamate, show lower values in patients than those observed in healthy individuals [66, 68, 69, 75, 76, 79–81]. There are also some isolated reports on changes observed in other amino acids such as cysteine, lysine, methionine, proline, taurine and dimethylarginine [40, 65, 82]. All the above-mentioned findings have been related to systemic inflammation [75, 76, 82], and particularly in the case of branched-chain amino acids, they appear to be more evident in severe COPD. Moreover, increases in the glycoprotein GlycA (that derives from acute phase proteins, being therefore a marker of low-grade inflammation) and the fibrinogen peptide mFBP have been reported in COPD patients [21, 74]. Other authors, such as Huang *et al*. [79] and Carpenter *et al*. [80], have found strong associations of COPD, not only with the metabolism of amino acids and lipids, but also with that of nucleic acids. Seen as a whole, an alteration in the pathways of protein metabolism has been repeatedly observed, with simultaneous evidence of oxidative phenomena, overall suggesting an increased catabolism [40, 65].

On the other hand, in a very large study published by Yu *et al*. [83] multiple metabolites from both lipid and amino acid lineages were found to be associated with COPD. These authors reported that patients showed higher levels than controls for metabolites linked to tyrosine (3-methoxytyrosine), citrulline (homocitrulline) and ornithine, as well as to carnitines (succinylcarnitine and oleoylcarnitine), 5-dodecenoate (basis of the lauric acid), 3–4-hydroxyphenyl-lactate, 7-α-hydroxy-3-oxo-4-cholesthenoate (derived from cholesterol), glycerol, an isomer of the nucleoside uridine (pseudouridine), and some xanthines and their metabolites (such as l-methylurate), being lower for glycerate, serotonin, ω-3 DHA and androsterone sulfate. In another large study, a model composed of >20 metabolites with high predictive capacity for the disease was generated [21]. This model included sugars (such as myo-inositol), lipid derivatives (glycerophospho-inositol), various peptides (again the mFBP), derivatives of protein oxidation (cysteine sulfonic acid) and elements of the Krebs cycle (such as fumarate). Regarding this same key cycle, different authors have reported elevated serum levels of maleate, pyruvate and lactate in COPD [84]. All these results strongly suggest problems in oxidative metabolism, with predominance of anaerobic glycolysis and negative impact on energy production [84, 85]. More recently, and based on findings from various large studies, Godbole *et al*. [86] have also attempted to generate a predictive model of the functional severity of COPD patients. With >100 metabolites, which included not only amino acids and lipids but also nucleosides and even vanillylmandelic acid (catecholamine metabolism), the authors were able to predict the variability of forced expiratory volume in 1 s. However, unfortunately, and similarly to other attempts, these encouraging results could not be further validated.

Urine

Altered levels of various metabolites have also been described in urine. Elevations of pyruvate, acetates and ketone bodies, as well as α-ketoglutarate and phenylacetyl glycine (all of them indicative of anaerobic metabolism) have been reported [74]. Moreover, increased levels of carnosine (dipeptide with antioxidant properties) and the amino acids β-alanine and histidine have also been observed in COPD patients, while other derivatives of protein metabolism, such as creatinine or even nicotinamide, showed reduced values. In another study carried out on urine, it was also observed that hippurate, formate (involved in the metabolism of various amino acids) and trigonelline (alkaloid), all of them related to diet, but which can also be produced by the microbiota, were associated with the level of functional impairment in COPD patients [87].

Summary

If we consider COPD as a single-homogeneous entity, it could be said that it conditions very relevant metabolic changes, which can be detected from samples from the lung or its secretions, but also through their systemic repercussions. Samples of pulmonary origin basically indicate modifications in the metabolism of phospholipids, fatty acids and ceramides, especially in the less severe phases of the disease, probably indicating the active destruction of the lung parenchyma in these stages, among other phenomena. Moreover, we could add to these findings changes in some eicosanoids, and signs of increased protein catabolism, with changes in the levels of various peptides and amino acids, as well as oxidative stress and difficulties in energy production. All these phenomena are reflected at the systemic level (*i.e.* blood) but coexist with additional alterations in the metabolism of some blood lipids and coagulation factors, which could have relevant cardiovascular implications for COPD patients.

Some factors modulating metabolites in COPD

There are a number of factors with potential relevance in the clinical presentation of COPD, and therefore in its differential pathophysiology and metabolic expression. Age, sex, exacerbations, the severity of the functional impairment, microbiome and associated comorbidities, among others, appear to be of significant importance (table 1).

Influence of age and sex

The influence of ageing on the metabolomic expression of COPD is also a subject of great interest, as it compares the earliest phases of the disease with the more advanced; two concepts that do not always overlap. In fact, other factors such as the development profile of the lungs, physical activity, diet or obesity appear to influence the early *versus* late onset of the disease [88, 89].

It is well known that ageing *per se* is associated with structural and functional changes in the lung that are similar to those observed in COPD. Indeed, various authors have pointed out that COPD may be associated with accelerated ageing of the lung [90]. At the metabolic level, ageing causes significant disturbances in proteostasis (*i.e.* dynamic homeostasis of proteins) and in the extracellular matrix, with higher degrees of low-grade inflammation and stress in mitochondria functioning [91]. As already mentioned in the present review, many of these changes are also present in COPD, but few studies have specifically analysed the interaction between COPD and ageing using metabolomics, with that of Xue *et al*. [84] worth mentioning. These authors reported greater dysfunctions in components of the citric acid cycle in older patients compared to younger ones. Mangoti *et al*. [92] similarly showed that COPD patients display changes derived from methylation in the metabolism of some amino acids such as arginine in advanced age. Another potential biomarker for both physiological and accelerated ageing would be hydrogen sulfide, a gaseous molecule with anti-inflammatory and antioxidant properties, also implicated in the development of the lung [93]. It has been reported that patients with either eosinophilic or frequent exacerbator phenotypes, as well as those with a more severe disease, show lower levels of this metabolite in their exhaled air when compared to those not presenting these characteristics [94]. Moreover, its serum levels are also lower in patients suffering from an exacerbation than in those who are in a stable phase [95]. Dyhydroandrosterone (DHEA) also shows clear decreased serum levels with ageing, a change that seems to be more accentuated in COPD patients [96]. Interestingly, this finding (as those changes occurring in other androgenic hormones) may be involved in the loss of muscle mass suffered by many patients [97]. Something similar happens with β-hydroxy-β-methylbutyrate (HMB), an α-keto acid derived from leucine, that is low in elderly patients with loss of muscle mass, and more especially in those suffering from chronic diseases such as COPD [98].

Regarding the earliest stages of the disease, and regardless of the age of the patients, there is currently a study underway (BIOEARLY-COPD), one of whose objectives is to define the metabolomic profile of these patients, but its main results are still pending [99].

Serum carnitine levels are lower in female COPD patients than in healthy women. Moreover, these levels are also lower than those observed in male COPD patients [64, 85]. In addition, while the pathways of nitric oxide and the metabolism of some amino acids (such as arginine) seem to be more altered in female COPD than in male patients (perhaps in relation to greater presence of nitrosative stress) [85], the latter show a greater involvement in their lipid metabolism (sphingolipids, ceramides and fatty acids) [64]. On the other hand, various lipid metabolites found in BAL, such as octadecenoic, heptadecatrienoic, octadecadienoic and eicosatetraenoic acids or thromboxane TXB2, seem capable of differentiating between female smokers with and without COPD [39]; a metabolic profile with potential diagnostic utility that, however, lacks any usefulness in men. Considering all these, and other complementary findings, some modules of interaction that seem to be very sex-specific have been described. In this regard, those formed

TABLE 1 Main metabolite changes associated with some relevant characteristics of the patients and those associated with some of the most prominent COPD phenotypes

Ageing
Condensate of exhaled breath: hydrogen sulfide ↓; fatty acids, derived and associated metabolites (EpOMEs (epoxides of linoleic acid) ↓, DiHOMEs (dihydroxylation of octadecadienoic acid) ↓)
Blood: amino acid methylations ↑; hormones (dihydroandrosterone ↓)
Sex
Bronchoalveolar lavage:
Female: fatty acids, derived and associated metabolites (octadecenoic (oleic) ↑, heptadecatrienoic (norlinolenic) ↑, octadecadienoic (linoleic) ↑ and eicosatetraenoic (arachidonic) ↑ acids); prostaglandins and thromboxanes (TXB2 ↑)
Blood:
Female: amino acids, derived and associated metabolites (arginine ↓, homocitrulline ↓, α-ketoglutaramate ↓, carnitine ↑, acylcarnitines ↑, dityrosine ↑); fatty acids, derived and associated metabolites (hydroxydocosahexaenoic (HdoHE) ↑, hydroxy-eicosatetraenoic (HETE) ↑); phospholipids (phosphatidylethanolamine ↑, phosphatidylcholine ↑); sphingoid base (behenoyl-sphingadienine ↓); other carboxylic acids (dihydroxy-methylbutirate ↓); erythroniacid ↑
Male: amino acids, derived and associated metabolites (5-hydroxylysine ↓, γ-glutamylmethionine ↓, nervonoylcarnitine ↑); fatty acids, derived and associated metabolites (octadecenedioic ↑); ceramides ↑; phospholipids (N-stearoyl-sphingosine ↑)
Exacerbations
Sputum: carbohydrates (sialic acid ↑); fatty acids, derived and associated metabolites (arachidonic ↑, docosapentaneoic ↑); hypoxanthines ↑
Blood: carbohydrate degradation metabolites ↑; amino acids, derived and associated metabolites (glycine ↓/↑, leucine ↓, isoleucine ↓, lysine ↓, tryptophan ↓, taurine ↓, valine ↓, glutamyl phenylalanine ↓, phosphocreatine ↓ (if associated with pneumonia), carnitines ↑, acetylcarnitine ↑); ceramides (trihexosylceramides ↑); phospholipids (sphingosine-1-phosphate ↓); nucleoside degradation metabolites ↑; other carboxylic acids (formate ↓); ketone bodies ((hydroxybutyrate ↓), hydrogen sulfide ↓
Severity of functional impairment (FEV_1)
Lung tissue, severe COPD: ceramides ↓ (although ↑ in mild-moderate COPD); phospholipids (sphingosine-1-phosphate ↑)
Bronchoalveolar lavage, severe COPD: amino acids, derived and associated metabolites (cysteine ↑, homocysteine ↑, glycine ↓, threonine ↑/↓); phospholipids (phosphatidyletanolamine ↓, phosphatidylserine ↓); ceramides ↑; nicotine metabolites (P-cresol ↓)
Surfactant, severe COPD: phospholipids (phosphatidylcholines ↓)
Sputum, severe COPD: fatty acids, derived and associated metabolites (5- and 12-oxoeicosatetranoic acids ↓); glycerophospholipids ↓; enzymes (SOD ↑, MPO ↑); prostaglandins-thromoboxanes (prostaglandins F2α (PGF2α) ↑ and D2 (PGD2) ↑/↓, thromboxane B2 (TXB2) ↑)
Exhaled breath condensate: amino acids, derived and associated metabolites (aspartic acid ↑, hydroxy-L-homoarginine ↓, oxoglutaric acid ↑); fatty acids, derived and associated metabolites (acetohydroxybutanoic ↑, octadecadienoic ↑, hydroxyeicosapentanoic ↑, 5-hydroxyeicosatetranoic ↑, 12-hydroxyeicosatetranoic ↓, trihydroxyoctadecenoic acid ↑ (TriHOME), EPOMEs ↑, DiHOME ↑, hydroxyheptadecatrienoic acid (HHTrE) ↑,); other carboxylic acids (hydroxyundecanoic ↓, oxotetradecenoic ↓, hexadecatrienoic ↓, oxoheptadecanoic ↓); ketone body (hydroxybutyrate ↑); prostaglandins-thromoboxanes (PGF2α ↓, TXB2 ↑),
Blood, severe COPD: carbohydrates and derived and associated metabolites (glucosaminic acid ↑); amino acids, derived and associated metabolites (arginine ↑/↓, asparagine ↑/↓, glutamylcysteine ↑, glutamine ↑/↓, glycine ↓, glutamylglycine ↑, dimethylglycine ↓, acetylglycine ↓, glutamylglutamate ↑, histidine ↓, methylhistidine ↑, isoleucine ↑/↓, leucine ↓, carboxymethylysine ↑, phenylalanine ↑, proline ↓, serine ↓, threonine ↓, tryptophan ↓, glycosyltryptophan ↑, tyrosine ↑, 3-methoxytyrosine ↑, valine ↑/↓, creatine ↓, octanoyl-l-carinitine ↑, decanoylcarnitine ↑, dedecenoylcarnitine ↑, laurylcarnitine ↑, myristoleoylcarnitine ↑, methylbutyrylcarnitine ↑/↓, propionylcarnitine ↓, palmitoleoylcarnitine ↑, serotonin ↓, aminoadipate ↓, ergothioneine ↓, γ-glutamyl-aminobutyrate (GABA)) ↓); fatty acids, derived and associated metabolites (palmitoleic ↑); glycerol ↑/↓ and diglycerides ↓; latosterol ↓; phospholipids, derived and associated metabolites (sphingomyelins ↑, sphinganine-1-phosphate ↓, lysophosphatidic acid ↓, phosphocholine ↓); ceramides ↑; nucleotides (dimethylguanosine ↑); other carboxylic acids – their salts and Krebs cycle (citrate ↓, isocitrate ↑, malate ↑, pyruvate ↑, succinate ↓, Lactate ↑/↓, hydroxyphenyllactate ↑, hydroxyisovalerate ↑, hydrocinnamate ↓, ippurate ↓, fumarate ↓); ketonic bodies (hydroxybutyrate ↑/↓, α-ketoglutarate ↓); amines-polyamines (trimethylamine ↑, acetylspermidine ↑); xanthines ↑; xenobiotics (ergothioneine ↓); metabolism of vitamin B3 (trigonelline ↓); hormones (androsterone sulfate ↓, dehydroisoandrosterone sulfate ↓, dehydroepiandrosterone sulfate ↓)
Short-term mortality
Blood: carbohydrates (fructose ↑, ribose ↑, fucose ↑); branched amino acids (leucine ↓, isoleucine ↓, valine ↓); peptides (from degradation of bradykinin ↑, Factor XII ↑ and fibrinogen ↑); other carboxylic acids – their salts and Krebs cycle (caproate ↓, succinate ↑, fumarate ↑, α-ketoglutarate ↑, malate ↑, glycerol and glycerate ↑/↓, lactate ↑); glycerophospholipids ↓; hormones (dehydroandrosterone ↓)
Low BMI – cachexia
Blood: carbohydrates (glucose ↓); amino acids, derived and associated metabolites (arginine ↑, asparagine ↑, aspartate ↑, citrulline ↑, glutamine ↑/↓, glycine ↑, histidine ↑, methylhistidine ↓, isoleucine ↓, hydroxylisine ↓, methionine ↑, phenylalanine ↑, proline ↑, thiaproline ↓, cystathionine ↓, serine ↑, valine ↑/↓, aminoadipate ↓, sarcosine ↓); lipoproteins (HDL ↑); triglycerides ↓; ketonic bodies and derived metabolites (acetate ↑/↓, hydroxybutyrate ↑, β-hydroxy-β-methylbutyrate ↓); nitrogenous bases and derived metabolites (β-aminoisobutyric ↓); other carboxylic acids – their salts and Krebs cycle (piruvate ↓); vitamin metabolism (vitamin C/ascorbic acid ↑/↓, vitamin B3/nicotinic acid ↑)
Microbiota (possible/probable relationships)
Sputum: carboxylic acids – their salts and derived metabolites (acetate ↑, butyrate ↓, propionate ↓)
Faeces: amino acids, derived and associated metabolites (acetyltaurine ↑/↓, acetylglutamate ↓, γ-glutamylglutamate ↓, acetylproline ↓; acetylcadaverine ↑); other carboxylic acids and derived metabolites (suberate ↓, undecanedioic acid ↓)
Urine: carboxylic acids, their salts and derived metabolites (formate ↑, hippurate ↑)

Continued

TABLE 1 Continued

Drugs
Inhaled β-agonists:
Urine: amino acids, derived and associated metabolites (glycine ↑); amines-polyamines (trimethylamine ↑)
Sputum: ketonic bodies and derived metabolites (acetate ↓); other carboxylic acids – their salts and derived metabolites (propionate ↓); acetoin ↑
Inhaled β-agonists+steroids:
Blood: amino acids, derived and associated metabolites (glutamine ↓); ketonic bodies and derived metabolites (acetoacetate ↑)
Urine: nicotinamide metabolism (methyl-dicotinamide ↑)
Condensate of exhaled breath: ketonic bodies and derived metabolites (acetate ↓)
Association COPD+lung cancer *versus* lung cancer
Non-tumoral lung tissue: carbohydrates (turanose ↑); amino acids, derived and associated metabolites (phosphoserine ↑); fatty acids, derived and associated metabolites (anandamide ↓); ketones (hydroxyacetone ↑); other carboxylic acids (azelaic ↓; 3-methylglutaric ↓)
Condensate of exhaled breath: carboxylic acids (propanoic ↑)
Phenotypes
Emphysema
Lung tissue: phospholipids (phosphatidylinositol ↑, phosphatidylserine ↑)
Blood: amino acids, peptides, derived and associated metabolites (alanine ↑, arginine ↑, asparagine ↑, acetylcarnitine ↑/↓, citrulline ↑, phenylalanine ↑, glutamine ↑, glycine ↑, dimethylglycine ↓, histidine ↑, 1- and 3-methylhistidine ↑, isoleucine-serine ↑, leucine ↑, lysine ↑, hydroxylysine ↓, proline ↑/↓, serine ↑, tryptophan ↓, tyrosine ↓, valine ↓; aminoadipate↓, betaine ↑/↓, carnitine ↑/↓, hydroxylhexanocarnitine ↑, creatine ↑/↓, sarcosine ↓, pyroglutamate ↓, aminoadipic acid ↓); fatty acids, derived and associated metabolites (myristoleic ↓, isovaleric ↓); ceramides ↓; diglycerides (palmitoyl-linoleoyl-glycerol ↓); phospholipids (glycerophospholipids ↑, sphingomyelins ↑/↓); nitrogenous bases, derived and associated metabolites (adenine ↑, β-aminoisobutyric ↓); nucleosides (ribosyl-imidazolacetate ↑/↓); other carboxylic acids and Krebs cycle (citric acid ↑); ketone bodies (3-hydroxybutyrate ↑); hormones (glucagon ↓, adiponectin ↓, androsterone ↓)
Chronic bronchitis
Sputum: carbohydrates (sialic acid ↑),
Blood: amino acids, peptides, derived and associated metabolites (phenylalanine ↓, tyrosine ↑, acetylcarnitine ↑, hydroxylhexanocarnitine ↓, pyroglutamate ↑); fatty acids (myristoleic ↑); phospholipids (sphingomyelins ↑); nitrogenous bases, derived and associated metabolites (adenine ↓)
Frequent exacerbators
Condensate of exhaled breath: hydrogen sulfide ↓
Sputum: carbohydrates (sialic acid ↑); amino acids, derived and associated metabolites (carnitines ↑, glutathione ↑); hypoxanthines and xanthines ↑; nitrogenous bases, derived and associated metabolites (adenine ↑, methylthioadenosine ↑),
Blood: amino acids, derived and associated metabolites (alanine ↑, arginine ↑, glutamine ↓, leucine ↓, isoleucine ↓, proline ↑, serine ↓, threonine ↑/↓, tryptophan ↓, tyrosine ↓, valine ↓; betaine ↑, carnitines ↑, trimethyl-l-alanine-l-proline-betaine ↑/↓); phospholipids (glycerosphingolipids ↑); amides (oleamide ↓)
Eosinophilia and/or ACO *versus* "pure COPD"
Condensate of exhaled breath: hydrogen sulfide ↓, amino acids, derived and associated metabolites (valine ↓); fatty acids ↑/↓; ketone bodies (acetone ↑); other carboxylic acids, their salts and Krebs cycle (lactic acid-lactate ↑; propionate ↑); alcohols (isopropyl alcohol ↑, methanol ↑)
Blood: carbohydrates (D-mannose ↓, glucose ↓); amino acids, derived and associated metabolites (asparagine ↓, glutamine ↓, histidine ↓, isoleucine ↓, leucine ↓, lysine ↓, phenylalanine ↓, serine ↓, threonine ↓, valine ↓; N-acetyl-glycoproteins ↓); fatty acids (linoleic ↑, other octadecadienoic ↑, stearic ↑), phospholipids, derived and associated metabolites (ethanolamine ↓); cholesterol ↓, triglycerides (palmitoylglycerol ↓); eicosanoids (5- and 13-hydroperoxyeicosatetraenoic (5- and 12- HpETE) ↑, 9- and 13-hydroperoxyoctadeca-9 dienoic (HPODE) ↑, 5-, 8-, 11- 12- and 15-hydroperoxyeicosatetraenoic (HETE) ↑ and 12-hydroxyeicosapentaenoic (HEPE) ↑ acids; thromboxane D2 (TXD2) ↑); other carboxylic acids – their salts and Krebs cycle (citrate ↓, succinate ↓, lactate ↑)
Urine: L-histidine ↑

FEV_1: forced expiratory volume in 1 s; BMI: body mass index; ACO: asthma interposition syndrome.

by amino acids–lysophospholipids–bile acids–acylcholines, and amino acids–Krebs cycle–xenobiotics would be characteristic of male COPD, while a module basically formed by steroids would be more specific of women with the disease [64]. The most common explanation for the clinical and biological differences observed between COPD in males and females is that they are probably related to a differentiated exposome linked to the cultural environment and/or hormonal-related differences [100, 101].

COPD exacerbations

Relatively few studies have analysed metabolomic data during exacerbations. Among them, it is worth noting the one published by Gulcev *et al.* [102], who reported reductions in the tryptophan level at the onset of these acute episodes, an outcome that was probably related to the associated increase in its catabolic enzyme indoleamine 2,3-dioxygenase. Zhou *et al.* [65] in turn observed lower levels of peptides and amino acids such as glutamyl phenylalanine and taurine, respectively, during COPD exacerbations compared to periods of stability. Van der Does *et al.* [41] showed increases in DPA and arachidonic acids,

accompanied by high levels of cyclooxygenase-2 mediators (synthesis of prostaglandins) in the sputum of exacerbated patients.

Other works also aimed to search for metabolomic markers of severity in these acute episodes. This is the case for those published by Cruickshank-Quinn *et al*. [17] and Paris *et al*. [48], who observed that the pathways most affected in severe exacerbations were again those related to catabolism of peptides and amino acids, lipids (mostly fatty acids and sphingolipids), carbohydrates and nucleosides, with a highly probable negative impact on energy metabolism. In turn, Fortis *et al*. [103] reported low levels of formate and glycine in those patients with such severe exacerbations that hospital admission and noninvasive mechanical ventilation were required. Furthermore, if the acute event is due to pneumonia, which would not always be considered an exacerbation by many authors and guidelines, low blood levels seem to include phosphocreatine (energy reserve) as well as different amino acids [103]. Moreover, and regarding predictability, in the already mentioned work by Esther *et al*. [40] certain markers, such as some hypoxanthines and sialic acid, were increased in the sputum of patients who were close to presenting a new acute episode. However, and despite all these findings, there is still a lack of precise and already validated metabolic markers of either exacerbations or the severity of these acute episodes.

Severity of the functional impairment

Some of the existing associations reported between the severity of respiratory function impairment and certain metabolites (such as various amino acids and proteins, as well as glycerophospholipids) have already been mentioned in previous sections [14, 17–19, 21, 104]. Different groups have also described direct or inverse associations between pulmonary function variables and additional metabolites, including various carbohydrates, glycerol, diglycerides, more phospholipids (sphingomyelins), amino acids (including creatine, histidine, proline, threonine, serine and asparagine) and γ-glutamyl amino acids (those linked to glutathione to facilitate its cellular transport, as is the case for glycine, cysteine and glutamate), aminoacyl-tRNA (a transfer RNA linked to an amino acid), some amines derived from amino acids (2-methylbutyrylcarnitine and propionylcarnitine), a nucleotide (N2-N2-dimethylguanosine), trigonelline (derived from Vitamin B3), some carboxylic acids (3-phenylpropionate and 3-(4-hydroxyphenyl)lactate), the xenobiotic ergothioneine and ATP-dependent transmembrane transporters (ABC) [17, 19, 62, 76, 83]. More recently, and using machine-learning techniques, Carpenter *et al*. [80] confirmed the relevance of the networks related to the metabolism of various amino acids (more specifically arginine and proline, cysteine and methionine, glycine, serine and threonine), as well as those including the ligand-neuroactive receptor, pyrimidines and the aforementioned ABC transporters.

To summarise, the pathways that appear to be more closely associated with lung function deterioration include those related to the metabolism of nitrogen, lipids, proteins and some nucleic acids, as well as to the transportation through cell membranes and the absorption of certain minerals. Some of these associations showed more strength if the sample analysed comes directly from the respiratory system rather than blood [14]. From these results it may be inferred that some of the aforementioned metabolites and/or pathways could allow for the biological monitoring of COPD patients in parallel to those variables classically based on lung function, perhaps being able to help find a better definition of evolutionary or prognostic profiles of the disease.

Short-term mortality

Some patients show a rapidly unfavourable clinical course that leads to early death. This patient profile, with reduced life expectancy, has shown some special metabolic characteristics (figure 1), with a more marked reduction in branched-chain amino acids but higher levels of sugars such as fructose, metabolites of bradykinin (vasodilator), the coagulation factor XII and fibrinogen (the latter described as being associated with the spontaneous formation of thrombi), glycerate, various components of the Krebs cycle (such as succinate, fumarate and malate), ketones (α-ketoglutarate) and lactate than those with a better life course [105, 106]. Furthermore, patients with shorter life expectancies have also shown a well-defined alteration in their glycerophospholipid and pentose-phosphate pathways, as well as in the metabolism of glycoxylate (produced in a variant of the Krebs cycle) and dicarboxylate (related to the metabolism of fatty acids) [105]. All this suggests important problems in the generation of energy, in clear agreement with the networks described by other authors [62, 64].

COPD and microbiota

Although intestinal flora is the main contributor to human microbiota, respiratory microbiota as well as its interactions with the former one (the “gut-lung axis”) have also been proposed as relevant for the genesis and development of different lung diseases, including COPD [107]. Moreover, there is increasing evidence of the role played by the microbiota, not only in the development and maturation of the immune system,

but in different organs such as the lung [108]. Interestingly, the microbiota of an individual varies throughout his/her life in relation to multiple factors (medications, infections, lifestyle) [109], and therefore it would also be possible to modulate its composition by potentially therapeutic actions [110–112].

Although studies with faecal samples from COPD patients are scarce, various catabolites have been found to be differentially expressed when compared to healthy controls. This is the case, for instance, for acetylcadaverine, acetylglutamate, suberate and undecanedioic acids [113], as a probable consequence of their different composition of intestinal microbiota. The latter appears to be characterised in COPD patients by an increased presence of *Streptococcus*, Rothia and members of the *Escherichia* genus [111]. Interestingly, other biological samples can also express metabolomic changes derived from gut microbiota. In this regard, some metabolites with this probable origin (*e.g.* hippurate and formate) have been detected in the urine of COPD patients in different proportions than in controls [104]. Something similar occurs with serum markers, such as some carboxylic acids (*e.g.* acetic, butyric and propionic), which also have a probable origin in the intestinal microbiota [114]. It has also been suggested that some of these metabolites would be related to different disease phenotypes or stages [114], perhaps due to differences in their gut flora. For example, those patients with more severe disease show clear differences from those with milder forms (amongst other changes, the former having more presence of fusobacterial and aerococci than the latter), as is also the case between those with the eosinophilic phenotype *versus* those without [115]. With regard to respiratory microbiota and its derived metabolites, it also seems to show differences between diverse phenotypes and different severity levels. Patients with an eosinophilic phenotype, for instance, show greater germ sex diversity than non-eosinophilic patients, with a greater abundance of some of them such as the thermophilic *Streptococcus* and diverse fungi [116, 117]. Similarly, those patients with more severe functional disease have also shown differences compared with those with milder forms, with the former having a higher number of Pseudomonas spp. and fewer treponemes than the latter [118, 119].

It is known that frequent exacerbators show not only a reduced bacterial diversity, but also a greater presence of *Pseudomonas* spp. [116, 120]. Moreover, it is obvious that the microbial load also influences the probability of suffering these acute events; not only as a direct effect of the infective capacity of some germs, but also due to their ability to damage lung tissues, with the release of white cells and proinflammatory and/or oxidative stress-inducing molecules, as well as qualitative and quantitative changes in mucins [121, 122]. Furthermore, the impoverishment of the respiratory microbiome has been associated with an increased risk of death in COPD patients, especially if accompanied by a *Haemophilus* predominance [123]. Unfortunately, and in terms of predictive capacity, there are no metabolic markers available yet linked to the underlying microbiota that can indicate the risk of a new episode of exacerbation.

Drugs and non-pharmacological treatments in COPD

Some drugs used in COPD can induce changes in body metabolism and, therefore, in markers detectable in biological samples. This should be considered when analysing the results of metabolomics studies. β-agonist bronchodilators for instance can alter the metabolism of nicotinic acid (and therefore of nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH)) and some fatty acids, such as linoleic and arachidonic (with consequences also for eicosanoid derivatives) [124, 125]. Inhaled steroids have also shown the possibility of inducing changes in diverse metabolites. For instance, adding budesonide to a β-agonist has been shown to be able to modify the levels of serum markers related to proteins, formate and components of the citric acid cycle [125, 126]. Combinations of phenotypic traits with treatments should also be considered. Tan *et al*. [127] reported that patients with the emphysematous phenotype showed a different metabolic response to treatment with tiotropium than those with predominant chronic bronchitis. Regarding non-pharmacological treatments, Maniscalco *et al*. [128] have published that clinical improvements after a pulmonary rehabilitation programme are accompanied by changes in various metabolites in the exhaled breath condensate. The decrease in methanol stands out among them; a finding that is probably indicative of a lower degree of lung inflammation.

A frequent comorbidity, COPD and lung cancer

The association between lung cancer and COPD, and more specifically with the presence of pulmonary emphysema, is very well established and has been considered as partially independent of the direct effect of tobacco smoking. To date there have been some attempts to identify specific metabolic profiles that could help in the early diagnosis of lung cancer in high-risk patients such as those with COPD. Li *et al*. [129] observed that cancer patients showed relevant differences in their non-tumour lung tissue depending on whether or not they associate COPD. These changes involved ABC transporters, and some steps in the synthesis pathways of pantothenate and CoA, as well as on the metabolism of amino acids (especially alanine) and purines. In another work worth mentioning, Muñoz-Lucas *et al*. [130] showed relevant

differences for propanoic acid (derived from fatty acids, and probably a product of gut microbiota) in exhaled breath condensate between these two populations. A complementary issue would be to be able to identify differences between the metabolic profiles of COPD alone or lung cancer alone. In this regard, a study published by the Callejón-Leblic group [66] reported differences in around 30 serum metabolites; several amino acids and derivatives (*e.g.* ornithine, glutamine-glutamate, threonine and creatine) and phospholipids (*e.g.* phosphatidylcholine) standing out among them.

Summary

Different factors, such as age and sex, can influence the metabolic response of patients with COPD. Advanced age seems to condition difficulties in the energy production pathways, which is added to a deterioration in protein metabolism, derived partially from various methylations and alterations in the production of some anabolic hormones, and loss of antioxidant capacity. Some of these phenomena may be related to the coexistence of an early metabolic senescence. In turn, the profiles of men and women with COPD differ in various metabolomic aspects, since while the former show predominant alterations in various steroids and amino acids, the latter present abnormalities in different lipids and the citric acid cycle. Other factors that can influence the metabolism of patients are exacerbations, where there are signs of an even more increased protein and lipid catabolism than in stable phases, with a parallel impairment on pathways of energy production. The severity of the disease, considered through the level of functional impairment or the patient's life expectancy, also has an impact on the metabolism. Thus, patients with more severe disease show a greater impact on pathways related to protein-amino acid and phospholipid metabolism, in this case added to abnormalities in those of glycerol, diglycerides, ABC transporters and nucleic acids. On the other hand, the presence of reductions in branched-chain amino acids and increases in various coagulation factors and in components of the Krebs cycle and other energy pathways seem to be associated with an early mortality of patients. Their microbiota, both intestinal and respiratory, can also have an impact on the metabolomic expression of the disease. In this regard, it can condition changes in both the host metabolites detected and, above all, the coexistence with molecules directly or indirectly related to microorganisms. Interestingly, microbiota seem to differ qualitatively and quantitatively between patients with severe *versus* milder disease, and between different phenotypes. Moreover, even the drugs usually used for the treatment of COPD patients can also affect their metabolism. β-agonists, for example, can modify some pathways related to nicotinic acid (with consequences in the production of NAD and NADH) and various fatty acids, while combinations of these drugs with inhaled steroids may improve protein metabolism and energy production. Finally, comorbidities can also intervene in the metabolomic profile of patients with COPD. Lung cancer, for example, is an entity that associates with COPD with relative frequency. Patients with both processes seem to have a specific metabolic profile, with differences in the metabolism of various amino acids, ABC transporters and CoA synthesis compared to patients with only one of these two entities.

Phenotypes and treatable traits

As discussed in previous sections, various attempts have been carried out to achieve a greater personalisation in the clinical management of COPD patients, grouping them based on certain criteria of homogeneity in their clinical expression. These groups, unfortunately still too big, are called phenotypes or even better, "treatable traits", since one of the main objectives of these classifications is the ability to offer them particularisations in the clinical management, including treatment [3]. Each of them also seems to show somewhat more specific metabolomic characteristics (table 1).

Classical phenotypes: pulmonary emphysema versus *chronic bronchitis*

Pulmonary emphysema was one of the first phenotypes described in COPD. These patients are classically known as "pink puffers", and they suffer from severe dyspnoea and frequently associate body weight loss. However, metabolic studies in emphysema are sometimes hampered by the different criteria used in its definition. Probably the most reasonable one is to use computed tomography (CT) images, which can also allow for the quantification of parenchymal loss. Taking all this into consideration, metabolomic studies have shown that patients with classic emphysema show higher levels of various amino acids or related molecules (specifically glutamine, arginine, serine, asparagine/aspartate, and the derived acetylcarnitine) and ketone bodies (such as 3-hydroxybutyrate); with lower levels of other amino acids and associated metabolites (such as tryptophan, histidine, 3-methylhistidine, proline and valine, as well as betaine (trimethyl-glycine or TMG, a regulator of cell division and involved in osmotic homeostasis), creatine, carnitine and aminoadipate (a lysine precursor)), peptides (such as sarcosine), lipids (such as ceramides, diglycerides and various phospholipids), molecules derived from nitrogenous bases (such as the β-aminoisobutyric acid (BAIBA)), ribonucleosides (1-ribosyl-imidazolacetate) and hormones (such as glucagon, adiponectin or androsterone) [18, 73, 75, 76, 131, 132]. In contrast, contradictory results have been reported for some other amino acids (such as phenylalanine). Furthermore, the Krebs cycle and

oxidative phosphorylation stand out among those metabolic pathways that appear to be closely associated with emphysema [17, 19], perhaps due to the simultaneous presence of oxidative and/or nitrosative stress. In the networks recently obtained by Gillenwater *et al.* [64] three modules were found to be involved in this pulmonary abnormality: steroids, amino acids–lysophospholipids–bile acids–acylcholines, and amino acids–Krebs cycle–xenobiotics. However, these promising results obtained using patients' samples from the COPDGene study, were not fully confirmed in a subsequent attempt carried out in another wide cohort (SPIROMICS). Even more recently, Godbole *et al.* [86] have described a predictive model of emphysema and its intensity using around 130 metabolites obtained from patients belonging to the above-mentioned two cohorts, but again failing the subsequent confirmation attempt. Finally, it is also worth mentioning that in the study carried out by Halper-Stromberg *et al.* [14] in BAL samples, they found a high number of metabolites associated with emphysema, including those related to diverse amino acids, fatty acids and phospholipids. Carpenter *et al.* [80] in turn, using machine-learning analysis, obtained strong associations between the degree of emphysema and amino acid metabolism (specifically those concerning alanine, histidine, glycine, serine, and threonine) on the one hand, as well as the already mentioned ligand-neuroactive receptor and ABC transporter networks on the other.

The loss of body weight, with or without associated muscle dysfunction, is relatively common in emphysematous patients. Analysing this distinctive aspect, Ubhi *et al.* [73] observed that nutritional status was negatively associated with the levels of glutamine, and directly with those of HDL lipoproteins and ascorbic (*i.e.* Vit. C) and nicotinic (*i.e.* Vit. B3, and a precursor in turn of NAD and NADH) acids. Focusing on the presence of an already established cachexia, this extreme situation seems to be associated with low levels of different branched-chain amino acids (such as valine and isoleucine), ascorbic acid-ascorbate, pyruvate and glucose, with increases in methionine, glutamine and glycine, as well as in 3-hydroxybutyrate, BAIBA and acetate [73]. Moreover, muscle dysfunction is another frequent manifestation in many COPD patients, being also especially prevalent in those with emphysema [133]. To the best of our knowledge, there is only one study, published by Rodríguez *et al.* [134] >10 years ago, in which the plasma metabolomic profile was investigated in COPD patients with skeletal muscle dysfunction. These authors reported significant reductions in some amino acids such as isoleucine, valine and alanine in this subgroup of patients. Surprisingly, and despite the fact that muscle dysfunction appears to be inversely associated with life expectancy in COPD patients, no subsequent work has been published until now on this subject.

Regarding the other classical phenotype, chronic bronchitis, also known as the "blue bloater" profile, being characterised by abundant cough and expectoration, some studies have investigated its metabolomics profile. This is the case of the work published by Esther *et al.*, who found elevations of the α-keto sialic acid in the sputum of such patients [40], although this change does not have a parallel expression in blood [19, 66, 131]. A discrepancy that may be related to the fact that chronic bronchitis mostly involves the airways, with relative absence of systemic manifestations. On the other hand, a mild increase in sphingomyelins has been occasionally reported in the blood of patients with a relatively pure chronic bronchitis phenotype, although this finding remains controversial [131].

Regarding differences among these two classical phenotypes (*i.e.* pulmonary emphysema *versus* chronic bronchitis), Callejón-Leblkic *et al.* [66] reported that the former show higher serum levels of glucose, adenine, and some amino acids and derivatives (such as phenylalanine or hydroxylhexanocarnitine) than the latter, but lower levels of pyroglutamate, tyrosine, acetylcarnitine and the myristoleic fat acid. Moreover, in an interesting article Tan *et al.* [127] reported the differences between a mixed emphysema-bronchitis (defined by both loss of lung parenchyma and structural alterations in bronchial walls observed in the CT scan) and pure emphysema phenotypes, observing that the former showed lower levels of glutamine and alanine than the latter.

Frequent exacerbators

Those patients with frequent or severe exacerbations have also shown distinct metabolite profiles. In sputum samples from the SPIROMICS cohort for instance, frequent exacerbators showed elevated levels of the already mentioned sialic acid, but also of xanthines, adenine and its S-methylated form methylthioadenosine (aerobic metabolism), and glutathione. A footprint profile that also allows for the prediction of future exacerbations [40]. In another study, carried out in the same cohort, patients with previous exacerbations showed low levels of numerous metabolites in their sera, with tryptophan and branched-chain amino acids standing out among them [135]. Relatively similar results were published by Cruickshank *et al.* [17], who using plasma from the COPDGene cohort, observed that frequent exacerbators associated changes in some sugars, various amino acids (serine, threonine, and arginine) and derivatives (carnitine and aminoacyl-tRNA). The pathways most involved in such changes were those

related to the metabolism of proteins and nucleosides, as well as to ABC transporters. Gillenwater *et al*. [19], also using blood samples from the COPDGene cohort, found that another amino acid derivative denominated trimethyl-L-alanine-L-proline betaine (TMAP) was closely associated with the number of exacerbations. Finally, Bowler *et al*. [131] found that sphingolipid metabolism was altered in plasma of COPD patients from the same multicenter cohort, but glycerosphingolipids specifically predominated in frequent exacerbators.

The eosinophilic phenotype and the asthma-COPD interposition syndrome

One frequent (from a third to a quarter of all COPD patients) and well-defined phenotype is the "eosinophilic" one, characterised for an increased number of these cells in peripheral blood (being >300 per μL the most widely accepted diagnosis criteria) [136, 137]. Although they are not equivalent concepts, the already published metabolomic studies frequently overlap this eosinophilic phenotype with that of the Asthma-COPD interposition syndrome (ACO, which includes up to 10 additional criteria), therefore both concepts are discussed together in the following paragraphs.

Those studies that have studied the metabolomics alterations present in ACO patients have found interesting relationships between their lung function and serum levels of the amino acids valine, serine and threonine, the carbohydrates glucose and manose, cholesterol, glutamate, citrate and succinic acid [138]. Similar relationships have been reported with the urinary amount of histidine [139], or the exhaled air levels of some other amino acids (such as valine), fatty acids, alcohols (isopropanol and methanol), lactate and formate, ketone bodies and propionate [140]. In addition to these metabolites, other molecules including more amino acids and carbohydrates, ethanolamine and different lipids and derivatives (cholesterol, triglycerides, and few fatty acids such as linoleic and stearic) also appear to differentiate patients with ACO from other COPD phenotypes [141]. Furthermore, ACO patients also seem to associate a marked dysregulation of the metabolism of many eicosanoids [142] and cytokines [141], a profile that has been proposed as related to their hypermetabolic status [143, 144] and worse evolution [48, 145].

COPD versus *asthma*

Another complementary and interesting subject to be explored is that of metabolomic differences between the purer forms of either COPD or bronchial asthma. In two noteworthy papers, De Laurentis *et al*. [146] and Maniscalco *et al*. [147] reported that COPD patients showed higher levels of alcohols (such as ethanol and methanol) in their exhaled breath than asthmatics, while the amounts of acetone/acetoin and formate were lower. Liang *et al*. [148], in turn, observed that patients with COPD showed plasmatic levels of some amino acids (such as valine, norleucine, leucine and phenylalanine), fatty acids and related molecules (arachidonic and pyroglutamic acids as well as indoxyl sulfate), succinate (Krebs cycle) and some xanthines that were higher than in people with asthma, with lower quantities of other xanthines, the nucleoside inosine, palmitic acid and bilirubin (metabolism of the haem group). From their viewpoint, Adamko *et al*. [149] reported that COPD patients showed higher urinary levels than subjects with asthma in arginine and dimethylamine, 3-hydroxyisovalerate (CoA metabolism), betaine, choline (component of cell membranes and precursor of acetylcholine) and methylnicotinamide, but lower in glutamine (glutathione and protein metabolism), succinate, pantothenate (CoA synthesis) and uracil.

Summary

The different phenotypes and/or treatable traits described in COPD also show some specific metabolomic characteristics that to some extent differ from those already mentioned for the disease in general. Pulmonary emphysema is amongst the most classic phenotypes and is characterised by altered levels of various molecules involved in the metabolism of proteins, lipids and nucleic acids, as well as components of the Krebs cycle, and hormones with anabolic properties. Systemic findings are less evident in patients with chronic bronchitis, who appear to exhibit changes in some carbohydrates present in their respiratory secretions. COPD frequent exacerbators show changes in the blood levels of sugars, amino acids and glycerophospholipids, with consequences, above all, for protein and nucleic acid metabolism, as well as for membrane transporters. Finally, patients who are associated with blood eosinophilia and/or having ACO are characterised not only by changes in lipid and protein metabolism in general, but also by dysregulation of pathways related to eicosanoids and cytokines.

Limitations of previous studies and present questions

Most of the studies published to date refer to the general metabolic expression of COPD, or that of the already better-defined phenotypes, but almost always using hypothesis-driven approaches. Moreover, many of these metabolomics studies have been carried out with samples from a limited number of cohorts, which not only include wide populations but also very specific inclusion and exclusion criteria for both patients and controls. Furthermore, the vast majority of models described for the disease or its different

circumstances have shown limited reproducibility in the validation steps. We agree with most authors of other recent reviews that there are still important deficits regarding the specific importance of factors such as age, sex, the temporal phases of the disease, its severity, exacerbations and progression trajectories to define more personalised metabolic profiles potentially susceptible of different future therapeutic approaches [48, 150–152]. Moreover, the complementary use of relatively blind approaches such as those provided by wide metabolic screenings plus further cluster analyses will help to find still unknown endotypes and new pathophysiological mechanisms potentially involved in COPD.

Conclusions

As a conclusion it is evident, from the most relevant findings published in the available literature, that COPD mostly impacts on the metabolism of proteins (evidenced through findings observed in amino acids and peptides) and lipids (with special involvement of fatty acids and phospholipids), with a minor role for changes in the metabolism of carbohydrates. These changes result in impaired proteostasis and abnormalities in those pathways related to obtaining and using energy, coagulation and the immune-inflammatory response. Some of these findings may be, directly or indirectly, related to the semiology usually present in COPD patients, as is the case of weight and fat-free mass loss, fatigue, thromboembolic and cardiovascular complications, the proclivity to suffer exacerbations and the characteristics of their clinical trajectories. Moreover, some of the different phenotypes already described for the disease (emphysema/chronic bronchitis, eosinophilia–ACO, frequent exacerbators) also appear to have relatively specific metabolomic profiles. Emphysema, for instance, seems to be associated with relevant alterations in protein and phospholipid metabolism, with a potential impact on nutritional status, muscle function and the homeostasis of pulmonary surfactant, while the chronic bronchitis phenotype shows changes that are more localised in the airways. Patients with ACO in turn are characterised by changes in eicosanoids and a highly specific cytokine profile, while frequent exacerbators appear to show overstimulation of both inflammatory pathways and oxidative stress, increased catabolism and probable epigenetic changes at various levels. In addition, some other factors such as the modality of risk-exposures, age, sex, comorbidities and even microbiota can also have an impact on patient metabolism.

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