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Metabolic interactions in asthma

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Key words

asthma; biomarkers; metabolic changes; metabolic pathways; obesity

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Doi
10.23822/EurAnnACI.1764-1489.101

Summary

Metabolomics can be used to explore altered metabolic pathways in asthma, giving insights into its pathophysiology. We aimed to review how metabolomics has been used to understand asthma by describing metabolic pathways under research and discussing clinical implications.

The search was performed in PubMed, and studies published since 2000 using a metabolomics approach, were included.

A total of 32 studies were analysed. Pathways related with cellular energy homeostasis, lipid metabolism and oxidative stress, immune and inflammatory processes and others were altered. Initial studies focused on biomarker discovery. But metabolomics can be used to evaluate drug effects on specific pathways, to highlight pathways that can further develop in new targeted treatments, and to identify differences according to asthma severity and phenotypes.

Abbreviations

BALF, bronchoalveolar lavage fluid; CS, corticosteroid; EBC, exhaled breath condensate; GCxGC-TOF/MS, two-dimensional gas chromatography with time-of-flight mass spectrometry; GC-MS: gas chromatography-mass spectrometry; GSH, glutathione; HETE, hydroxyeicosatetraenoic acids; HPETE: hydroperoxyeicosatetraenoic acids; LC-MS: liquid chromatography-mass spectrometry; LC-Q-TOF/MS: liquid chromatography quadrupole / time-of-flight-mass spectrometry; LT, leukotrienes; MS, mass spectrometry; NMR, nuclear magnetic resonance; NO, nitric oxide; PG, prostaglandin; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; VLDL, very low-density lipoprotein.

Introduction

Asthma is a heterogeneous condition characterized by variable respiratory symptoms and airflow limitation driven by underlying pathophysiology mechanisms, namely, airway inflammation and remodelling (1). As a complex disease, with genetic and environmental influences, the role of molecular determinants and related pathways are not fully elucidated yet. Additionally, regarding the management and burden of the disease, severe asthma remains a significant clinical problem, and search for biomarkers to improve the target of new treatments is believed to be crucial (2,3). Nowadays, the physician goal in chronic disorders is to offer the best personalized treatment and man-

agement. Precision medicine aims to identify which approaches will be effective for each patient according to genetic, environmental and lifestyle factors (4). However, the application of precision medicine in day-to-day healthcare is still limited, and current research provided by several approaches aims to discover and give insights into biomarkers and key pathophysiology determinants. Metabolomics is an important tool in medical research, being able to manage complex diseases by giving insights over metabolic changes and pathophysiology (5). Studies can be designed to provide metabolic signatures of asthma severity and corticosteroid (CS) resistance, and to help in defining phenotypes or to evaluate treatment effects.

Metabolomics is a comprehensive analysis of metabolites in biological specimens. Metabolites are small molecules, including peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins and others small molecules that drive cellular functions, such as energy production, representing the functional phenotype of a cell, tissue or organism (6). Since metabolomics aims to profile a large number of molecules than the standard clinical laboratory techniques, and to cover biological processes and metabolic pathways, it holds promise in biomarker discovery and precision medicine. The most used techniques are nuclear magnetic resonance (NMR) and mass spectrometry (MS) and the main methodologies used for identification can be targeted or untargeted. The untargeted methodology measures the wide range of metabolites extracted in a sample without a priori knowledge of the expected metabolome. The targeted analysis yields higher sensitivity and specificity, since metabolites are analysed based on a priori information, allowing to measure concentrations in the extracted sample. Moreover, targeted analysis is important to validate results from untargeted analysis. The major challenge related with metabolomics is the identification of meaningful metabolites and its validation (6).

This review focus on how metabolomics has been used to understand asthma. Metabolic pathways altered in asthma will be described, considering studies performed in humans. Research and clinical implications will be discussed, as well future perspectives.

Methods

The scientific literature used in this review covered studies published from 2000 to November 2018 in PubMed and was focused on metabolomics applied to asthma. Only full-text in English and trials performed in humans were assessed for eligibility, independently of the type of document (original article, review, comment, conference paper, letters and book chapters). The selected search keywords were “metabolomics” or “metabolic profile” and “asthma”. The adopted inclusion criteria were: a) diagnosis, monitoring or phenotyping of asthma using metabolomics; and b) clinical trials. The exclusion criteria consisted in: a) trials not related with asthma; b) trials not related with metabolomics; and c) trials not related with diagnosis and/or monitoring of asthma. Additionally, some studies were found by cross-referencing.

Results

The systematic search using the aforementioned methodology yielded 89 studies. However, this number was increased to 102 after the inclusion of studies found by reference list searching. During the screening of titles and abstracts using the pre-specified inclusion criteria, 44 studies were rejected (studies not related with asthma n = 23; studies not related with metabolomics n = 14; and studies not related with diagnosis and/or monitoring of asthma n = 4), yielding 58 studies for full revision. After, each of these studies was entirely reviewed. In the end, 32 original articles were found to meet the inclusion criteria. Additionally, 26 reviews, comments, letters and book chapters met inclusion criteria and were used for reference list searching. **Figure 1** illustrates the flow diagram of search and selection process.

Urine (n = 9), serum (n = 6), plasma (n = 4), exhaled breath condensate (EBC) or exhaled breath (EB) (n = 13), and bronchoalveolar lavage fluid (BALF) (n = 1) were used to identify the metabolic profile of patients with asthma. Abnormal metabolic activity is primarily localized in the lung and respiratory tract; however, asthma can lead to systemic metabolic alterations as several circulating metabolites have been found to differ in asthmatics in regard to healthy individuals. MS and NMR were the main techniques used to achieve these discoveries.

Table I summarizes the main altered pathways in asthma found in studies using metabolomics - pathways related with 1) cellular energy homeostasis and hypoxia, 2) lipid metabolism and oxidative stress, 3) immune and inflammatory processes and 4) other pathways were described in several studies. Main metabolic changes reported are related with cellular energy homeostasis since inflammation, bronchoconstriction and airways hyperresponsiveness lead to a higher energetic burden. In response to these events, metabolites involved in tricarboxylic acid (TCA) cycle are increased, especially succinate, fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate. Poor oxygenation and hypoxic stress can also cause changes in TCA cycle, as well as in lactic fermentation, which is enhanced by inosine, to facilitate metabolism under these conditions. Finally, energetic demand obligates to lipids activation and mobilization. High levels of carnitine and acetyl-carnitine reinforce the oxidative burden, being essential to transport fatty acids into mitochondria for oxidation. Inflammatory status leads to oxidative stress, which triggers lipid peroxidation of polyunsaturated fatty acids (PUFAs) resulting in the release of hydrocarbons and other volatile compounds in the airways and urine. Additionally, some inflammatory markers were found increased, such as nicotinamide, adenosine mo-

nophosphate, arachidonate, arachidonic acid, leukotrienes and prostaglandins, contributing to pathophysiology. Furthermore, metabolites with anti-inflammatory properties were found decreased (urocanic acid). Amino acids metabolism was found deregulated, leading to changes in bile acids production and in urea cycle to eliminate end reaction products.

Most studies were designed to discover biomarkers able to differentiate asthmatics and healthy controls, although severity was also studied (n = 3), as well as corticosteroid resistance (n = 2), asthma control (n = 2) and treatment effects of inhaled therapy (n = 1).

Discussion

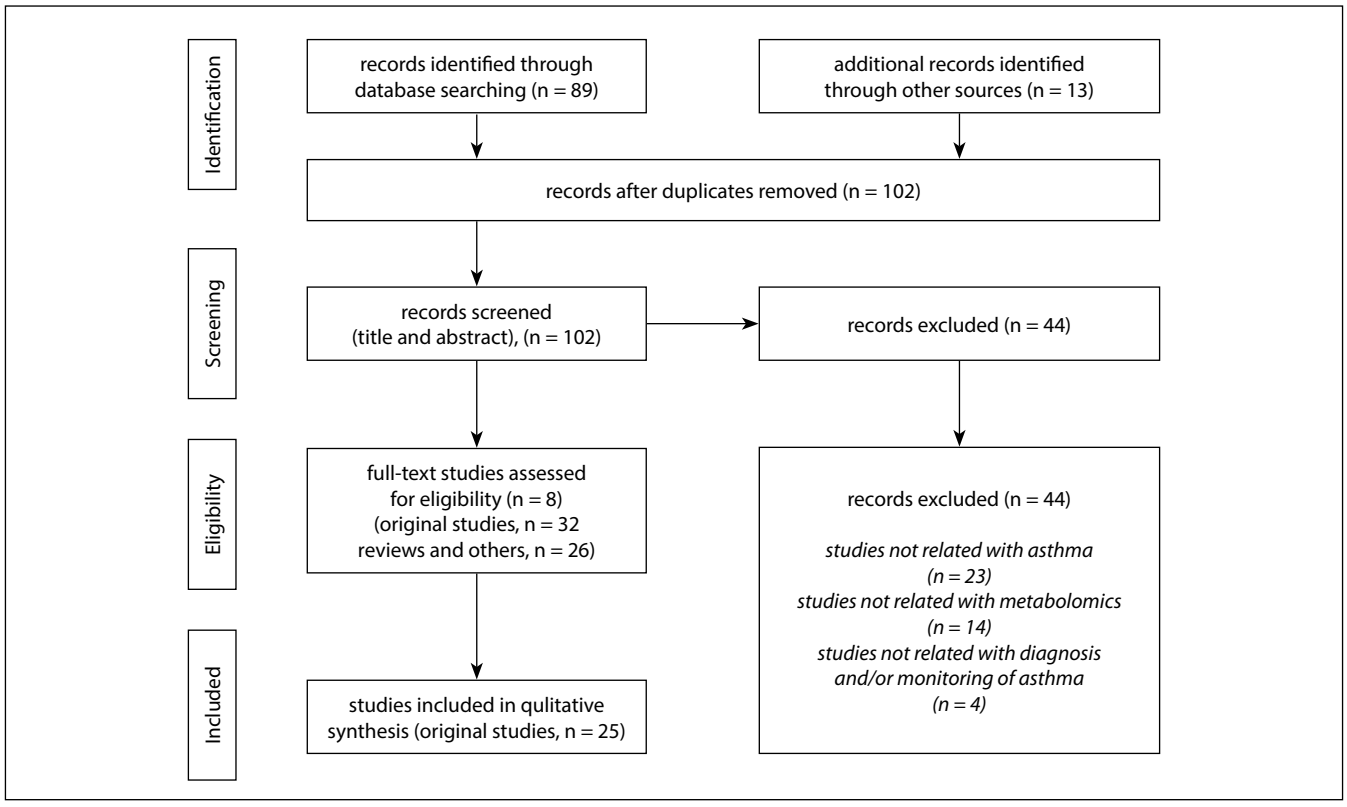
Metabolomics findings

Metabolomics studies revealed several altered pathways associated with asthma. The main findings in human studies, conducted in asthmatics and healthy controls, included not only changes in cellular energy and hypoxia, lipid metabolism and oxidative stress, immune / inflammatory pathways, but also amino acid, steroid, nitrogen and glutamate-glutamine metabolism, as well as bile acids production and vitamins metabolism. Most of these studies were targeted to identify diagnostic biomarkers for asthma or to improve its pathophysiology understanding.

Cellular energy homeostasis and hypoxia. Metabolites involved in tricarboxylic acid (TCA) cycle are increased in asthmatics, which possibly reflects the energetic burden due inflammation and bronchoconstriction. These metabolites were found in urine and serum of asthmatics, and succinate was the most consistent between studies (7-9). Fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate were also found higher in asthmatics who had recently suffered an exacerbation (9). TCA cycle changes can also be resultant of hypoxic stress due to reduced oxygenation, especially during an exacerbation (8). These changes are supported by the presence of high levels of lactate and low levels of glucose. Additionally, inosine, a breakdown product of adenosine, was increased in asthmatics and is capable of penetrating in cells and enhancing activity of pyruvate oxidase and other enzymes, facilitating cell metabolism under hypoxic stress during poor oxygenation (7).

Lipid metabolism and oxidative stress. Lipid metabolism is enriched in asthmatics since lipids drive inflammatory responses, promote release of histamine and are essential to cellular energy

Figure 1 *- Flow diagram of the search and selection process.*



metabolism (8,9,11-15,22,24). The presence of high levels of LDL, VLDL and its hydrolysis products have been found to activate the release of histamine, which promotes constriction of airways smooth muscle (8,12,26). The energetic demand causes a decrease in glucose levels and lipids can be activated to provide acetyl-CoA (8). Lipids breakdown, under insufficient glucose, leads to production of acetone which was found in high levels in serum of patients (8). However, low levels of acetone were found in a different study conducted in children with asthma which, until now, is a contradictory finding between studies (13). Also, increased levels of carnitine and acetyl-carnitine were found in urine and plasma of asthmatics during exacerbation, which highlights the oxidative burden, since these metabolites are essential to transport fatty acids into mitochondria for oxidation (9,11,12). The increased phosphocholine levels, an important component of the endothelial cell barrier, in the serum of patients, indicates a lack of airways protection (8,9). Moreover, the release of reactive oxygen species (ROS) by inflammatory cells and the decrease in glutathione levels, leads to oxidative stress which triggers lipid peroxidation of the polyunsaturated fatty acids (PUFAs) of cells, reducing the ability of the epithelium for damage repair (8,11,17). The resultant metabolites are compiled in a systematic review (27). End products of lipid peroxidation are mainly hydrocarbons including hexane, heptane, pentanal, heptanal, decanal, octane, nonadecane, 4-methylheptane, 2,4-dimethylheptane 2,4-dimethylpentane, 2-methylpentane and other alkanes and aldehydes (13-17,27-29). Interestingly, in elite swimmers, both with or without asthma, swimming was associated with a decrease in oxidative stress markers (30).

Immune and inflammatory processes. Urocanic acid, which is an intermediate of histidine catabolism and a potent immune-suppressor, was decreased in asthmatics urine and EBC, contributing to a poor resolution of the inflammatory process. Nicotinamide, adenosine monophosphate and arachidonate are inflammatory markers, and were increased in plasma of asthmatics (19). In addition, leukotrienes B4, D4 and E4 were found higher in EBC. Leukotrienes are potent inflammatory lipid mediators and chemoattractant of granulocytes, contributing to the pathophysiology of asthma and being synthetized from arachidonic acid via 5-lipoxygenase. Seventeen PUFAs were found in high levels in the urine of asthmatics, specifically hydroxyeicosatetraenoic acids (HETE), hydroperoxyeicosatetraenoic acids (HPETE), prostaglandins and arachidonic acid. These compounds are biological mediators linked to inflammatory and immune responses. Arachidonate, an inflammatory biomarker and precursor of leukotrienes, was found high in plasma and was positively correlated with taurine levels, highlighting the relation between its oxidation and the release of taurine.

Other metabolic pathways. Amino acid metabolism is also altered in asthmatics. Some amino acids appeared to be found in higher levels and others in lower levels. Glutamine, asparagine, leucine, valine, alanine and arginine were found in low levels in serum and phenylalanine, methionine, histidine and taurine were upregulated (7,8,19). Histamine and its downstream product, 1-methylhistamine, were higher in asthmatics urine, being involved in inflammation and bronchoconstriction (8). The precursor of histamine, histidine, was increased in plasma (8). High taurine levels were found in plasma and its release by cells is associated to taurine-releasing pathways that are activated by arachidonic acid oxidation via 5-lipoxygenase to leukotrienes (19). Taurine levels were also associated with bile acids production (cholate, glycocholate, glycodeoxycholate, taurocholate and lathosterol, an intermediate) which is the major pathway for its elimination (19). Nitrogen metabolism was also changed in the serum of asthmatics, showing low levels of ornithine, citrulline, arginine and formate, suggesting alterations in the urea cycle (important pathway in the excretion of ammonia resultant from amino acids catabolism) (7,8). Sinha et al. detected low levels of ammonia in the EBC and connected the finding to low levels of glutaminase activity, possibly indicating alterations in the glutamate-glutamine cycle (25). Glutaminase is responsible for the generation of glutamate and ammonia from glutamine. This hypothesis is reinforced by Jung et al., that found increased levels of glutamine and glutamate in the serum of asthmatics (8).

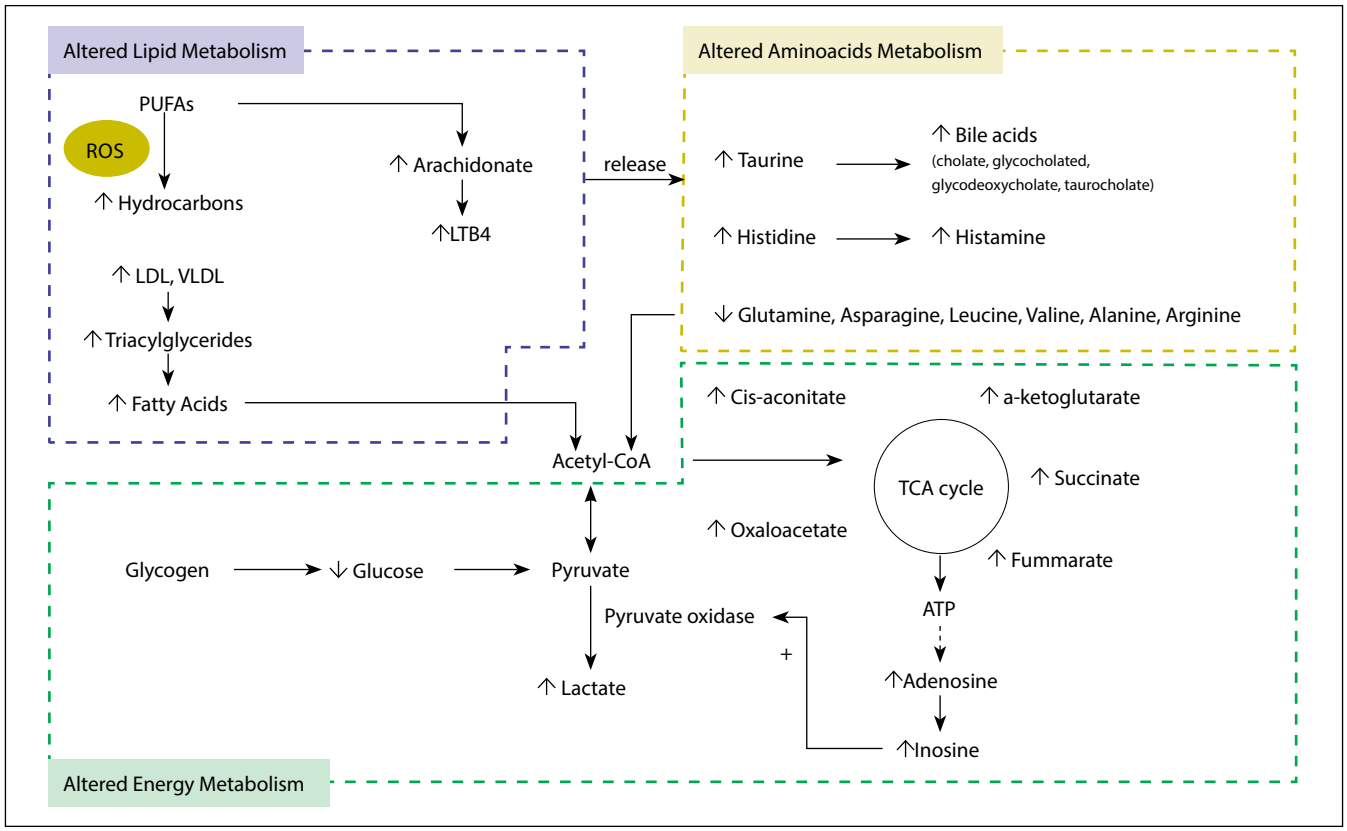
In summary, asthma is associated with abnormalities in energy metabolism such as TCA cycle, lipid and amino acids metabolism, possibly relating to increased respiratory muscles activity and to reduced oxygenation leading to hypoxic stress. Immune and inflammatory markers are also amplified in asthmatics. Some of these altered pathways are schematized in **figure 2**.

Research and Clinical value

Metabolomics studies comparing asthmatics and healthy controls are useful to identify altered metabolic pathways and to expand our knowledge about the disease pathophysiology. Metabolomics can be also useful for clinical practice, such as evaluating consequences and the effect of a specific treatment or giving insights about altered pathways in treatment-resistant subjects and encourage the development of new target therapies (18,19,22,31-34). Metabolomics can also be used to differentiate asthma among other airway diseases, such as chronic obstructive pulmonary disease (35).

Severe asthmatics, usually including those taking high doses of corticosteroid (CS), exhibited pronounced metabolic effects on steroid metabolism when compared to other asthmatics not taking CS therapy or under low doses. This group of patients is characterized by low levels of steroids in plasma (1-stearoylglycerol, dehydroisoandrosterone sulphate, androsterone sulphate and epi-androsterone sulphate) and in urine (dehydroepiandrosterone, cortisone, cortisol, urocortisol and urocortisone)

Figure 2 *- Representation of some of the altered metabolic pathways in asthma linked to cellular energy, lipid and amino acid metabolism. ATP, adenosine triphosphate; LDL, low-density lipoprotein; LTB4, leukotriene B4; PUFA's, polyunsaturated fatty acids; ROS, reactive oxygen species; TCA cycle, tricarboxylic acid cycle; VLDL, very-low-density lipoprotein.*



(18,19,31). Metabolomics revealed a hypothalamus-pituitary-adrenal axis suppression, which is now a well-documented consequence of this treatment. Additionally, decreased levels of prolyl-hydroxyproline (ProHyp) and pipecolic acid were found in urine and were associated to an increased risk of osteoporosis and bone injury due to CS treatment (31). Metabolomics was also used to evaluate the effect of a combined treatment of budesonide and salbutamol in children during asthma acute exacerbation (36). Arginine and proline metabolism, as well TCA cycle, were the most impacted pathways. This combined treatment, improving asthmatic symptoms, interacts also with arginine metabolism, since arginine and its downstream products, such as proline, are involved in collagen synthesis and cell proliferation during tissue remodelling. These findings suggest a potential metabolic reprogramming due to this combined treatment, and contribute to understand metabolic regulation of budesonide and salbutamol in asthmatic children at the molecular level.

Regarding CS resistance, metabolomics can also provide some valuable results to further developing new therapy targets. CS-resistant asthmatic children presented statistically significant differences for some metabolites, such as γ-glutamylcysteine and cysteine-glycine, suggesting a decrease in glutathione (GSH) synthesis (32). GSH, as an antioxidant, plays an important role to prevent oxidative stress and its pathway can be a target for some cases of CS resistance. Moreover, lower levels of ascorbic acid were reported in the serum of children with asthma (17). Ascorbic acid has an important role in protecting lung tissues, especially the alveoli, against oxidative stress, and decreased levels were associated with pulmonary dysfunction (37). On the other hand, retinoic acid was found to be increased in asthmatic children, especially in severe cases, and its levels have been associated to inflammation and airway remodelling in asthma (22). Controlled and uncontrolled asthmatics were also studied, and differences were found in their exhaled breath and plasma metabolomics (33,34). Loss of asthma control was evaluated in a longitudinal study using two breath analysis methods, mass spectrometry and electronic nose technology (33). Participants enrolled in this study had a previous history of medical diagnosis of

mild to moderate persistent asthma, and presented a good control of the disease according to the parameters established in the study. The samples were collected in three phases in time: baseline, loss of control after cessation of inhaled CS, and recovery. GC-MS distinguished the samples with an accuracy of 68-77% and electronic nose achieved an accuracy of 86-95%. Previously, McGeachie et al. studied biochemical predictors of asthma control in the plasma of children with controlled and uncontrolled asthma, using liquid chromatography tandem mass spectrometry (LC-MS) (34). Metabolites related with linoleic acids metabolism (linoleic acid and γ-linoleic acid) and arachidonic acid metabolism (arachidonic acid, 5-HETE, PGE2, 12(S)-HPETE, 15(S)-HETE and LTB4) were different between the two groups, showing a high pathway impact score despite no statistical significant differences, probably due to the small sample size.

These studies provide useful clues that can lead to improvements in diagnosis and inspire further studies to discover new pathological pathways and possible therapeutic targets and biomarkers. Metabolic changes related with asthma severity and control of the disease may be suitable as specific biomarkers for diagnosis and management or to identify targets to develop new specific treatments.

The obesity-related asthma phenotype

There is evidence that obesity increases the risk of developing asthma (38-41). Generally, obese-asthma patients present decreased levels of airway eosinophilic inflammation, increased symptoms, risk of hospitalization, healthcare-associated costs and poor response to CS (39,40). Several proposed mechanisms suggest the pro-inflammatory role of adipocytes, that can lead to the development of airway inflammation and asthma (38). Differences in obese and non-obese asthmatics were detected for some metabolites, methane (energy metabolism) and pyruvate, glyoxylate and dicarboxylate (carbohydrate metabolism) in the EBC (42). Additionally, other mechanisms independent of the inflammatory status can be present, such as hyperglycaemia, hyperinsulinemia and dyslipidaemia in the context of metabolic syndrome. Metabolic syndrome was associated with asthma in a prospective study and an odds ratio of 1.57 (95% CI 1.31-1.87) was achieved after adjustments, being considered a risk factor to develop asthma (43). Other studies also support a relationship between metabolic syndrome and asthma (44-46).

The arginine-NO pathway is altered in asthmatics and in patients with metabolic syndrome, being a potential involved pathway in obese-asthma patients (47,48). Arginine is a substrate for enzymes, such as NO synthases (NOS) and arginases, which are induced by inflammation and arginine availability (reduced in these patients). Arginine can be converted to citrulline in a reaction catalysed by endothelial NOS in the airways, releasing NO. In the aforementioned studies (**table I**), citrulline and arginine were found decreased in asthmatics serum (7,8). Additionally, supplementation with arginine in experimental asthma resulted in a proper arginine balance and a decrease of inflammation and airway hyperreactivity (49). Nevertheless, a clinical trial (NCT00280683) evaluated arginine supplementation in moderate to severe persistent asthmatics, and no significant differences were found in the number of exacerbations, exhaled nitric oxide levels or lung function (50). The effect of arginine supplementation in severe asthmatics, grouped by nitric oxide levels, and citrulline supplementation in overweight late onset asthmatics, are also being studied, but results are not available yet (NCT01841281 and NCT01715844, respectively). Supplementation in subjects with metabolic syndrome also achieved good results on glucose levels, insulin sensitivity, endothelial function and oxidative stress (51).

Dyslipidaemia is also a characteristic of obese subjects, and asthma patients also experienced changes in cholesterol levels. LDL and VLDL were increased in asthmatics plasma and HDL was diminished (8,52). Thus, the use of statins in obese-asthma patients can be convenient. The use of statins added to inhaled CS and bronchodilators in severe obese asthmatics resulted in better asthma control, through ACQ questionnaire evaluation, and improvement of lung function, when compared to non-statin users (53,54). However, a systematic review showed statins seem not to have additional benefits in asthma control, regardless the decrease of airway inflammation and slight improvement of lung function in individuals with mild allergic asthma (55). Still, more research is needed to verify the benefits of statins in certain subpopulations, such as the obese-asthma patient.

Mitochondrial dysfunction in various organs is known in metabolic syndrome and was recently discovered in airway epithelial injury and asthma (56). Mabalirajan et al. showed that 13-S-hydroxyoctadecadienoic acid (13-S-HODE), a lipid metabolite derived from linoleic acid, induces mitochondrial dysfunction in airway epithelia to drive severe asthma in experimental asthma, and demonstrated increased 13-S-HODE levels in human asthmatic airways (57). Moreover, the imbalance between oxidant and antioxidant species may also lead to mitochondrial changes (56). Many mitochondrial-targeted antioxidants have shown beneficial effects in metabolic syndrome and asthma in independent studies, such as coenzyme-Q10 and α-tocopherol (58-61). Coenzyme-Q10 showed beneficial effects by reducing CS dosage in asthmatics and, in experimental metabolic syndrome, prevented hyperinsulinemia, improved endothelial dysfunction and reduced hypertension and oxidative markers (59,60). α-tocopherol also demonstrated promising results in reducing mitochondrial dysfunction in experimental asthma and in individuals with metabolic syndrome (58,61). Mitochondrial dysfunction seems to be shared by both conditions.

Obesity-related asthma phenotype is characterized by a variable and non-eosinophilic inflammation and CS resistance. There-

Table I *- Summary of metabolomics analysis and identification of altered pathways in asthma found in studies conducted in humans (asthmatics vs healthy controls).*

Cellular pathway	Altered metabolites		Biofluid	Method
	high levels	low levels		
1. Cellular energy homeostasis and hypoxia				
cellular energy homeostasis and hypoxia	succinate (7,10), inosine (10), lactate (7)	glucose (7)	serum	GC-MS (10), NMR (7)
	fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate (11)	-	urine	NMR (11)
2. Lipid metabolism and oxidative stress				
lipid metabolism	VLDL and hydrolysis products, acetone (8)(7), phosphatidylcholines (10)(8)	phosphocholine, choline (8)	serum	NMR (8), MS (10)
	carnitine, acetyl-carnitine (9,11)	-	urine	NMR (9,11)
	carnitine (12), VLDL (12)	-	plasma	NMR (12)
lipid peroxidation and oxidative stress	2,4-dimethylpentane (13), 2,4-dimethylheptane (13), 2-undecenal, octane (13), 2-methylpentane (13), 2-methylhexane (13), 1-(methylsulfanyl)propane (14), ethylbenzene (14), 2-octenal (14), butanoic acid (15), benzoic acid (15), tridecane (15) and other VOC	acetone (13), 2,2,4-trimethylheptane (13), 2,3,6-trimethyloctane (13), 1-pent-2-one (15), undecane (15), p-xylene (15)	exhaled breath	GC-MS (13-15)
	hexane, heptane, pentanal, heptanal, decanal, octane, nonadecane, 4-methylheptane, 2,4-dimethylheptane and other alkanes and aldehydes (16)	-	urine	GCxGC-ToFMS (16)
	hypoxanthine (17)	glutathione (17)	serum	LC-MS (17)
3. Immune and inflammatory processes				
immune and inflammatory processes	histamine, 1-methylhistamine, nicotinamide (9)	urocanic acid (18)	urine	NMR (9), LC-MS (18)
	nicotinamide, adenosine monophosphate, arachidonate (19)	-	plasma	GC-MS (19)
	LTB4 (20,21), LTD4 (21), LTE4 (21), deoxyadenosine (22), thromboxane B2 (22)	urocanic acid, adenosine (23)	EBC	LC-MS (20,22), NMR (23), GC-MS (21)
arachidonic acid pathway	hydroxyeicosatetraenoic acids (15-HETE, 8-HETE, 11-HETE, 5-HETE, 12-HETE), hydroperoxyeicosatetraenoic acids (15-HPETE, 5-HPETE), prostaglandins (PGE1, PGF1a, PGJ2, PGF2a, PGA2, PGB2, 15-keto-PGF2a), arachidonic acid (24)	-	urine	LC-Q-TOF/MS (24)
	20-hydroxy-PGF2a, 6-keto-PGF1a (22)	-	EBC	LC-MS (22)
4. Other pathways				
amino acid metabolism	phenylalanine (7), histidine (8), methionine (8), glycine (8)	asparagine (7), arginine (8), leucine (8), valine (8), alanine (8), isoleucine (8)	serum	GC-MS (7), NMR (8)
	alanine, threonine (11)	-	urine	NMR (11)
	taurine (19)	tyrosine (12), isoleucine (12), leucine (12), valine (12), alanine (12)	plasma	GC-MS (19), NMR (12)
	alanine, proline, phenylalanine, arginine, isoleucine (23)	valine, tyrosine (23)	EBC	NMR (23)
nitrogen metabolism and urea cycle	-	ornithine (7), citrulline (7), formate (8), arginine (8)	serum	GC-MS (7), NMR (8)
	-	creatine (12), creatinine (12)	plasma	NMR (12)
glutamate-glutamine pathway	-	ammonia (25)	EBC	NMR (25)
	glutamate, glutamine (8)	-	serum	NMR (8)
bile acids production pathway	taurine, lathosterol, cholate, glycocholate, glycodeoxycholate, taurocholate (19)	-	plasma	GC-MS (19)
	ursodeoxycholic acid, isodeoxycholic acid (24)	-	urine	LC-Q-TOF/MS (24)
vitamins metabolism	retinoic acid (22)	ercalcitriol (22)	EBC	LC-MS (22)
	-	ascorbic acid (17)	serum	LC-MS (17)

EBC, exhaled breath condensate; GCxGC-TOF/MS, two-dimensional gas chromatography with time-of- flight mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HETE, hydroxyeicosatetraenoic acids; HPETE, hydroperoxyeicosatetraenoic acids; LC-MS, liquid chromatography-mass spectrometry; LC-Q-TOF/MS, liquid chromatography quadrupole / time-of-flight-mass spectrometry; LT, leukotrienes; NMR, nuclear magnetic resonance; PG, prostaglandin; VLDL, very low-density lipoprotein.

fore, it is relevant to studying altered metabolic pathways in this population, and understanding the possible overlapping mechanisms between metabolic syndrome and asthma. Arginine-NO pathway, mitochondrial dysfunction and altered cholesterol levels seem to be common pathophysiological features in both conditions. Thus, exploring metabolic overlapping mechanisms between obesity and asthma could open new therapeutic hypothesis for the obese-asthma phenotype, such as supplementation with arginine, citrulline, statins and mitochondrial target antioxidants.

Conclusions and future perspective

The metabolome is highly dependent of several variables and confounders, such as sample type, sample collection, age, sex, circadian rhythm, exercise, diet, microbiome, medication and other xenobiotics. Other major limitations concern procedure standardization, from data collection to data processing and interpretation, and external validation of the results. However, the pathophysiology understandings described in this review and the recent nature of most studies encourage the design of new ones in this field. Initial studies were focused on biomarker discovery for asthma and performed in asthmatics and healthy controls. Several altered pathways were described and replicated in more than one study, such as cellular energy homeostasis and hypoxia by TCA cycle alterations, lipid metabolism, including induction of lipid peroxidation due to oxidative stress, and increased levels of carnitine and lipids breakdown metabolites; metabolites of immune and inflammatory processes with significant alterations in the arachidonic acid pathway, as well as in other pathways such as amino acid metabolism (up regulation of urea cycle and bile acids production), steroid metabolism, and vitamins metabolism. Still, metabolomics can be used with other purposes, such as to evaluate drug effects on different pathways, and even its adverse consequences, to highlight pathways that can be better studied to achieve new drug targets, and to identify differences according to severity or even phenotypes (obesity-related asthma phenotype). Additionally, these results, despite still limited to date, emphasize the need of longitudinal studies to evaluate predictive biomarkers or to monitor specific treatment approaches.

Conflict of interests

The authors declare that they have no conflict of interest.

Funding

The authors acknowledge funding support by FCT - Fundação para Ciência e Tecnologia, for the project ExPOSE - Establishing protocols to assess occupational exposure to microbiota in clinical settings (02/SAICT/2016, Project no. 23222).

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