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Impact of exposure to atmospheric particulate matter in human skin-derived fibroblast cells: A metabolomics approach for the class of amino acids based on GC×GC-Q-TOFMS/MS

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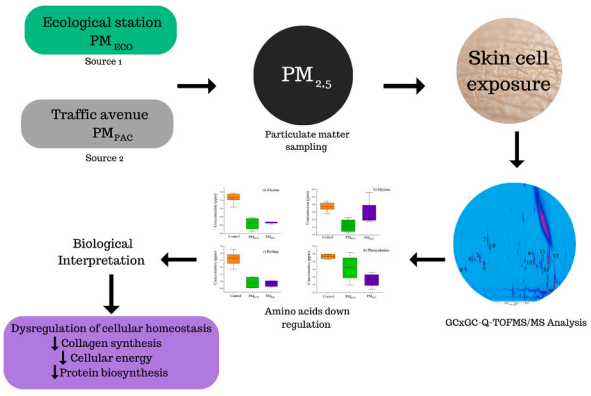
HIGHLIGHTS

- Exposure to particulate matter impairs skin health by decreasing cellular amino acid levels.
- Cellular energy and homeostasis, collagen synthesis, and protein biosynthesis are affected by exposure to particulate matter.
- This work correlates the metabolomic study of amino acids derived from fibroblast cells and analyzes by GCxGC-Q-TOFMS/MS.
- This study provides new insights into the risks of exposure to air pollution on the skin.

GRAPHICAL ABSTRACT



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ABSTRACT

The particulate matter (PM) in the air comprises particles containing a complex mixture of pollutants associated with various environmental and public health disturbances. However, studies related to the effects of PM on the skin are still incipient. In this work, the toxicity of particulate material to fibroblast cells derived from the human dermis was investigated using metabolomic analysis for the class of amino acids. For the analysis of amino acids, a new method with high selectivity and resolution based on comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-Q-TOFMS/MS) was developed and validated. The exposure impact of PM up to 2.5 μm ($PM_{2.5}$) on fibroblast cells was shown to be dose-dependent. Metabolomics results indicated that amino acid levels and metabolic pathways in fibroblasts were significantly affected by $PM_{2.5}$. Given the results, it was possible to correlate these effects to a series of responses, including decreased cellular energy, dysregulation of cellular homeostasis, decreased collagen synthesis, interference with wound healing, and suppression of protein biosynthesis.

Environmental implication: Although some progress has been made in air pollution control, the health risk related to $PM_{2.5}$ exposure remains important. The effects of air pollution on the skin have been extensively studied. However, few studies are related to the impact of $PM_{2.5}$ on the skin. This study determines the profile of amino

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acids from fibroblast cells exposed to $PM_{2.5}$, providing new insight into the damage to skin cells from atmospheric pollution.

1. Introduction

Particulate matter (PM) is associated with several problems that threaten global public health [7]. Chronic exposure to air pollutants affects the biological system of the skin, damaging its surface or more deeply, inducing dermatological diseases and accelerating its aging [23]. Particulate matter of up to 2.5 μm ($PM_{2.5}$) has already been reported as a possible cause of oxidative stress and inflammation in human skin [22]. Studies have also shown that polluting particles alter a series of mediators of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) family and positively regulate sensitive transcription factors within cell nuclei due to this high oxidative stress and inflammatory mechanisms [17].

The phenotypic changes in the cellular mechanisms of the skin due to air pollution have been extensively studied [1,24,8]. The consequences of skin degradation have already been reported both at genomic [10] and proteomic levels [29]; however, studies on biochemical changes and biomarker discoveries for clinical applications that result in changes in the skin metabolome are still in the early stages [5,30]. As the skin is a metabolically active organ, studying the changes in the metabolomic profile is essential to help understand the adverse effects, such as oxidative stress, and the positive effects, including the acceleration of repair mechanisms.

Metabolomics presents a comprehensive view of primary and intermediate metabolites and exogenous compounds, such as drugs and other chemical compounds, which constitute a cell, tissue, or organism [2,13]. Metabolomics analysis can effectively and sensitively separate and quantify many compounds from biological samples, such as sugars, acylcarnitines, amino acids, and lipids, and provide information on overall qualitative and quantitative changes in metabolism. It can also identify biochemical pathways and the general physiological status, including stress or system hyperactivity [36].

Strategies for analyzing metabolomic data are classified into targeted and untargeted [21]. The targeted analysis of the amino acid class can be an interesting path to discover various types of diseases or system modifications, as these compounds play crucial roles in both anabolic and catabolic metabolism. In addition to participating in protein building blocks and being a complementary tool for proteomic studies, amino acids provide metabolic energy and are precursors of many key metabolites for the functioning of the organism [39]. Another fact that must be considered is the well-established use of these compounds for the diagnosis and treatment of a wide variety of metabolic disorders, such as primary amino acid enzymopathies and amino acid transport disorders, as well as in assessments of nutritional status, adherence to diet, kidney function, and tissue damage [16]. Therefore, amino acids can offer an exciting route to study the damage caused by PM on the skin.

The sampling, sample preparation, number of replicates, and analytical instrumentation employed are all critical for a successful metabolomics study. Common analytical techniques applied in metabolomics studies include Nuclear Magnetic Resonance (NMR-Nuclear Magnetic Resonance)[18], Fourier transform infrared spectroscopy (FTIR-Fourier-Transform Infrared) [31], and direct injection in the mass spectrometer (DIMS – Direct Injection Mass Spectrometry) [15]. However, hyphenated techniques such as gas chromatography coupled to mass spectrometry (GC/MS–Gas Chromatography- Mass Spectrometry) and liquid chromatography coupled to mass spectrometry (LC/MS – Liquid Chromatography-Mass Spectrometry) have become more attractive in this field of research as they combine the separation capacity of chromatography with the high selectivity of mass spectrometry [11,33,40].

This work aimed to evaluate metabolic changes in fibroblast cells derived from human skin exposed to atmospheric $PM_{2.5}$ collected at two different points, one with intense car traffic and the other with less traffic. Then, possible changes in amino acid levels and metabolic pathways of these cells were identified. The study investigated altered amino acids in cells through analysis performed by comprehensive two-dimensional gas chromatography coupled to high-resolution mass spectrometry with quadrupole-time-of-flight (GC×GC-Q-TOFMS/MS).

2. Material and methods

2.1. Standard amino acid solutions

Individual 1000.0 mg L^{-1} stock solutions of the 17 amino acids analytical standards purity ≥ 99% from Merck KGaA (Darmstadt, Germany) were prepared in ultrapure water. The amino acids used in this study were L-alanine (Ala), glycine (Gly), L-valine (Val), L-leucine (Leu), L-isoleucine (Ile), L-threonine (Thr), y-acid aminobutyric acid (GABA), L-proline (Pro), L-methionine (Met), L-aspartic acid (Asp), L-serine (Ser), L-phenylalanine (Phe), L-glutamic acid (Glu), L -lysine (Lys), L-histidine (His), L-tyrosine (Tyr), and L-tryptophan (Trp), as well as L-norvaline (Norv) as the internal standard. The solutions were prepared in calibrated flasks and stored in a refrigerator at 4 °C. Working solutions were prepared from the stock solution.

2.2. Collection of particulate matter

Field sampling occurred between March 10 and April 7, 2022. During that time, 17 collections were carried out with an average frequency of four collections per week, with a collection time of 24 h. The device and sampling parameters can be viewed in Supplementary Material 1. Two different locations were chosen for this study. The first was close to an ecological park (19°87′34.7″ S, 43°97′24.8″ W) with a high number of trees and little vehicle traffic (PM_{ECO}), and the other point was close to an avenue (19°51'47.3"S 43°57'30.2"W) with intense vehicle traffic (PM_{PAC}). Both places are located on the campus of the Federal University of Minas Gerais in Belo Horizonte, Brazil (Supplementary Material 1). A fraction of approximately 10 × 12 cm of the collected filters was randomly selected for the extraction of particulate matter. Subsequently, the selected parts were cut into small pieces, immersed in distilled water, and sonicated for 30 min to facilitate PM removal from the filter. The supernatant was then transferred to a clean flask, more distilled water added, and sonicated for another 30 min. This step was repeated three times until the complete cleaning of the filters and removal of all particulate matter. After the supernatant was transferred to a beaker, the suspension was allowed to stand overnight. After complete decantation of the material, the supernatant was removed, and the decanted fraction was dried under nitrogen flow. Subsequently, the particles were weighed and resuspended in a solution composed of L-glutamine, pyridoxine hydrochloride, and a high concentration of glucose in Dulbecco's Modified Eagle's Medium (DMEM) according to the test concentrations and stored at 4 °C. This session was based on [19] with some modifications.

In addition, scanning electron microscopy (SEM) analyses were carried out to observe the difference between the particles deposited on the filters collected in the two places, PM_{ECO} and PM_{PAC} (Supplementary Material 1).

2.3. Cell cultures

Fibroblasts derived from the adult human dermis (HDFa), acquired

from Thermo Fisher Scientific (CAS-C0135C, São Paulo, Brazil), were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% (v/v) fetal bovine serum (PBS) and 1% (v/v) penicillin-streptomycin solution in a humidified atmosphere of 5% CO_2 at 37 °C. The medium was replaced every two days. HDFa cells were seeded into 75 cm^2 flasks or 35 mm well plates for all assays.

2.4. Cell viability assay

Cell viability was measured using MTT (3- (4,5-dimethylthiazol-2-yl)− 2,5-diphenyl tetrazolium bromide). For the MTT assay, HDFa cells were seeded (1.0×10^4 cells/well) on 96 well plates. After 24 h incubation (37 °C, 95% v/v humidity, and 5% v/v CO_2), cells were treated with 100.0 μL of different concentrations of PM_{ECO} (0.312; 0.625; 1.250; 2.500; 5.000; and 10.000 mg L^{-1}) and PM collected near the metropolitan avenue -PM_{PAC} (0.312; 0.625; 1.250; 2.500; 5.000; and 10.000 mg L^{-1}). Experiments were performed in triplicate, including 3 blank and 3 non-treated control wells. After another 24 h incubation, the medium was removed and washed with phosphate-buffered saline (PBS). Next, 10.0 μL of MTT solution (5 mg ml^{-1}) and 90.0 μL of DMEM were added per well. The plates were incubated for 3 h until the formazan crystallization and the formazan salts were dissolved with sodium dodecyl sulfate-10% HCl (v/v) solution. The absorbance was measured at 570 nm using a Multiskan GO microplate reader Thermo Scientific (Waltham, USA). The absorbance from blank wells was subtracted from each measurement, and the cell viability was calculated as a function of the percentage of the control group absorbance. Data were processed using La Jolla Prism 6 software from La Jolla (CA, USA).

2.5. PM exposure

HDFa cells were divided into a control group without PM treatment and a treated group exposed to PM. Cells were cultured in 35 mm plates (1×10^5 cells/well) in DMEM at 37 °C in an atmosphere of 5% CO_2 for 48 h, followed by exposure of 100.0 μL of particulate matter solution to predetermined doses according to the IC50 (0.900 mg L^{-1} for PM_{ECO} and 0.400 mg L^{-1} for PM_{PAC}) for another 24 h. Five parallel treatments for the control group and four parallel treatments were performed for each PM_{ECO} and PM_{PAC} exposure.

2.6. Cell harvest

After cells grew to 95% confluency, the medium was removed, and the culture was rinsed twice with PBS for 30 s. Then, 450.0 μL of 80% (v/v) methanol (pre-cooled) was added, and the plate was shaken for about 20 s. Cells were separated in methanol using a scraper for about 1 min. Subsequently, the suspension was poured into an Eppendorf tube and stored at − 80 °C until amino acid analysis. Based on [37].

2.7. Extraction of metabolites

Before metabolomic analysis, samples collected in methanolic solution were dried in a speed vacuum at 30 °C for about 4 h. Subsequently, the cell pellets were sonicated in 100.0 μL of PBS and incubated in an ice bath for 10 min. The proteins were removed by adding 450.0 μL of cold methanol/acetonitrile solution (1:1 v/v) with subsequent centrifugation for 10 min at 14.000 g. The supernatant was transferred to a test tube and dried under vacuum at 45 °C for approximately 30 min. The extraction of metabolites was based on previous work by [37] with some modifications.

2.8. Analysis of amino acids

The amino acids were derivatized and then analyzed on an Agilent system two-dimensional gas chromatograph, model 7890B GC coupled to a mass spectrometer with hybrid analyzer quadrupole – time-of-flight Q-TOFMS/MS, model 7250 from Agilent (Wilmington, USA). The derivatization steps and analysis conditions, such as the set of columns used, modulation period, oven temperature, and injector temperature, together with the method validation process for quantitative determination are described in detail in Supplementary Material 2.

2.9. Data processing

Metabolomic data were processed using GC Image software version 2.9r2 GCxGC-HRMS with a research system equipped with a NIST 107 library version 2.3 (2017). The metabolites (amino acids) concentrations in each sample were measured by calibration curves (shown in Supplementary Material 2). Statistical significance was calculated by analysis of variance (ANOVA) with Tukey post hoc test with p-value< 0.05 using OriginPro® 9 SR0, version 2019 (Northampton, USA).

3. Results

3.1. Cytotoxicity after exposure to PM

The cytotoxicity of the samples collected in two different areas was determined through the cells exposed to particulate matter ($PM_{2.5}$) ranging from 0.312 to 10.000 mg L^{-1} after incubation for 24 h with MTT (Fig. 1). Cell viability in both treatments significantly decreased, depending on concentration compared to control cells. In this assay, the IC50 for PM_{ECO} was 0.9498 mg L^{-1} and an R^2 of 0.9500, indicating the high reliability of the results. The IC50 for MP_{PAC} was 0.4116 mg L^{-1} with an R^2 of 0.9700. Viability showed a pronounced dependence on the concentration gradient (p-value < 0.05). In addition, the viability curve of the particulate material collected near an urban avenue (PM_{PAC}) was more pronounced than for the material collected near an ecological area (PM_{ECO}), an indication that the constituents of PM_{PAC} have more toxicity than PM_{ECO} as expected. Given these results, the concentrations selected for subsequent tests were 0.900 mg L^{-1} for PM_{ECO} and 0.400 mg L^{-1} for PM_{PAC}.

3.2. Identification of differential metabolites by GC×GC-Q-TOFMS/MS

A new method of analysis based on two-dimensional gas chromatography was proposed for the metabolomic study of cells exposed to particulate matter collected at two different points (PM_{ECO} and PM_{PAC}). It was possible to identify, with high selectivity and sensitivity, the 17 investigated amino acids. The determination of analytes concentration in the control and exposed groups was used to evaluate the behavior of amino acids. Some criteria were adopted for selecting differentiated amino acids, such as statistical treatment using ANOVA (p-value < 0.05) and the Turkey test to discover significant differences between the groups. It was possible to statistically identify 4 different amino acids between the control and treated groups, all showing a decreased concentration when exposed to particulate matter. Details of the analysis method development process can be viewed in Supplementary Material 2. Fig. 2 illustrates the amino acids differentiated between the control, PM_{ECO} and PM_{PAC} exposed groups.

Data previously treated statistically in the concentration format were imported into metaboanalyst 5.0 to visualize the separation between groups. Fig. 3 shows the PCA results for three types of samples: control, treated with $PM_{ECO,}$ and treated with PM_{PAC}. A clear separation can be observed between the control samples and those treated with PM_{PAC}. The data shows less differentiation when the cells are treated with the particulate matter collected in a more ecological area. This analysis will likely predict a clear correlation between pollution and metabolic changes in fibroblast cells, the more polluted the site, the more significant the difference between the groups.

MetaboAnalyst 5.0 was also used for metabolic pathway analysis. For this, the amino acids differentiated between the groups were imported separately for each group, and the analysis was performed. A total of 8

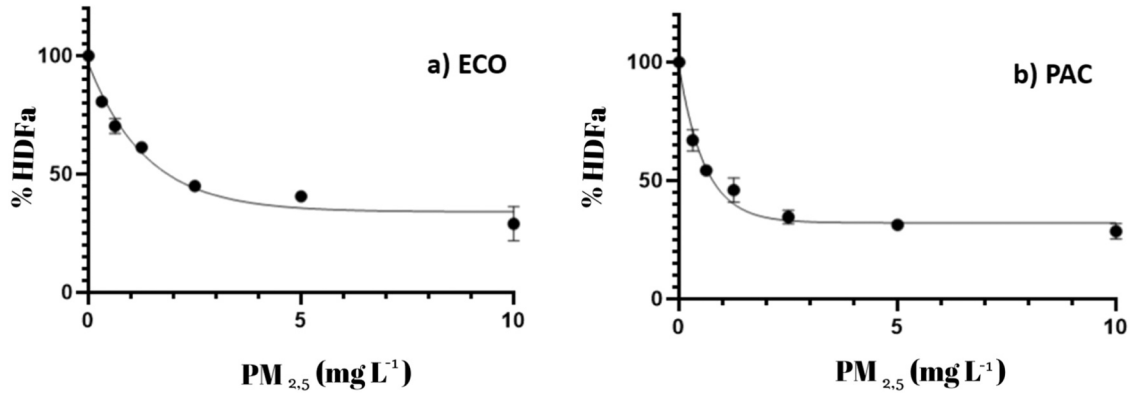

Fig. 1. Cytotoxicity of HDFa cells exposed to different PM concentrations. a) Cytotoxicity of HDFa exposed to near an ecological (PM_{ECO}) b) Cytotoxicity of HDFa exposed to near a major avenue (PM_{PAC}).

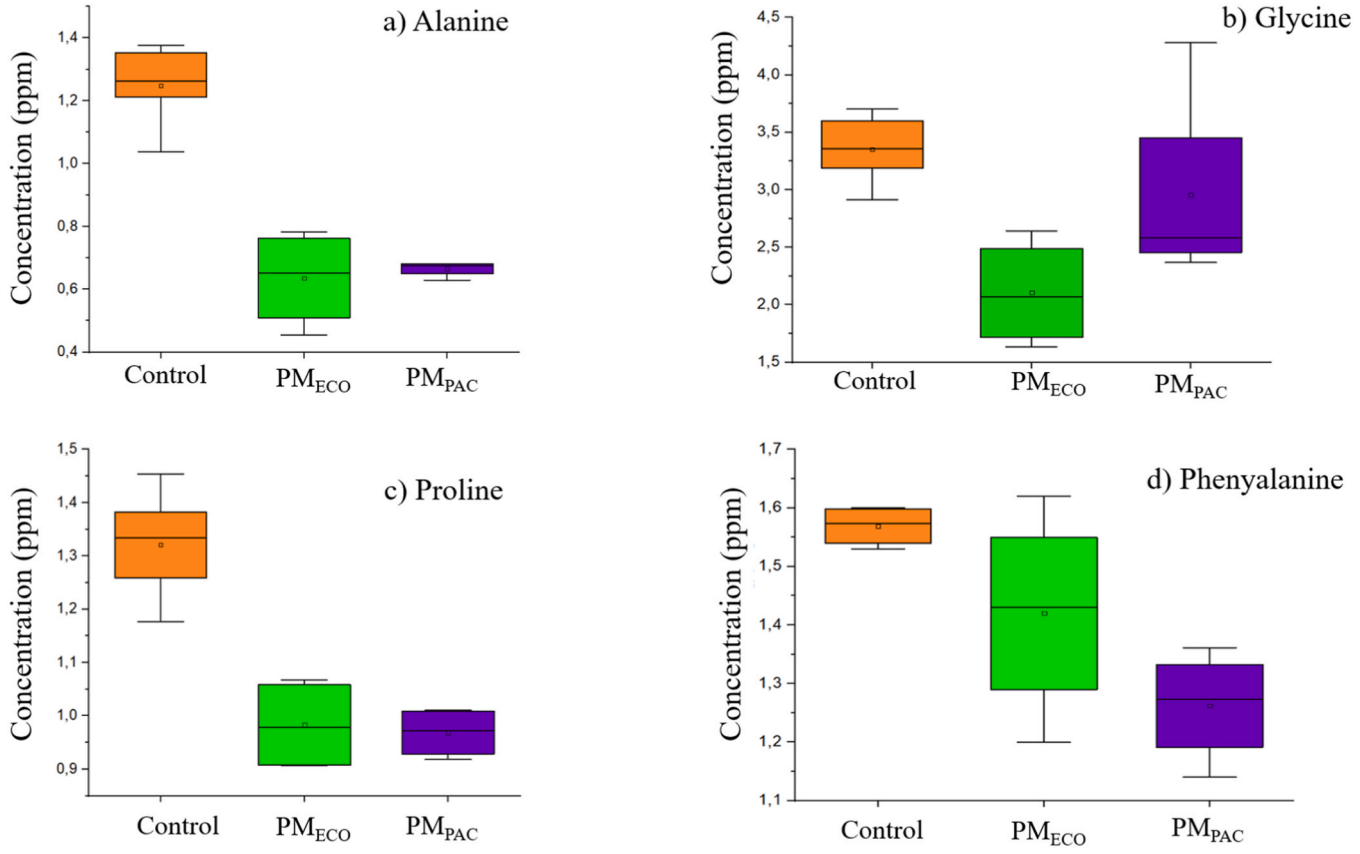

Fig. 2. Differentiated amino acids between control, PM_{ECO}, and PM_{PAC} groups. a) and c) represent the differentiated amino acids between the Control and PM_{ECO} groups and the Control and PM_{PAC} groups; while b) represents the amino acid differentiated only between the Control and PM_{ECO} groups; and d) represents the amino acid differentiated only between the Control and PM_{PAC} groups (Control n = 5, PM_{ECO} n = 4 and PM_{PAC} n = 4).

metabolic pathways were disrupted for the PM_{ECO}-treated group, and a total of 6 metabolic pathways were disrupted for the PM_{PAC}-treated group. In both groups, the aminoacyl-tRNA biosynthesis was the most significantly altered pathway, followed by the phenylalanine, tyrosine, and tryptophan biosynthesis pathway altered only in the PM_{PAC} group. The summary of the pathway analysis is illustrated in Fig. 4, with the detailed results of the route analysis in Table 1.

4. Discussion

In the present study, GC×GC/Q-TOFMS/MS was applied to identify metabolic alterations directed to the class of amino acids associated with exposures of atmospheric pollutants collected at two different points, PM_{ECO} and PM_{PAC}, in fibroblast cells. The results suggested that short-term exposure to PM_{ECO} and PM_{PAC} induced changes in metabolic pathways and caused significant changes in some amino acids derived from fibroblast cells. We identified several biological pathways associated with these environmental particles. Among them, some changes were common to both PM_{ECO} and PM_{PAC}, such as the metabolism of alanine, aspartate, and glutamate, which is associated with acute inflammation [20].

Air pollutants penetrate the skin through nanoparticles and generate quinones, redox-cycle chemicals that produce reactive oxygen species (ROS). ROS stimulates the release of pro-inflammatory mediators,

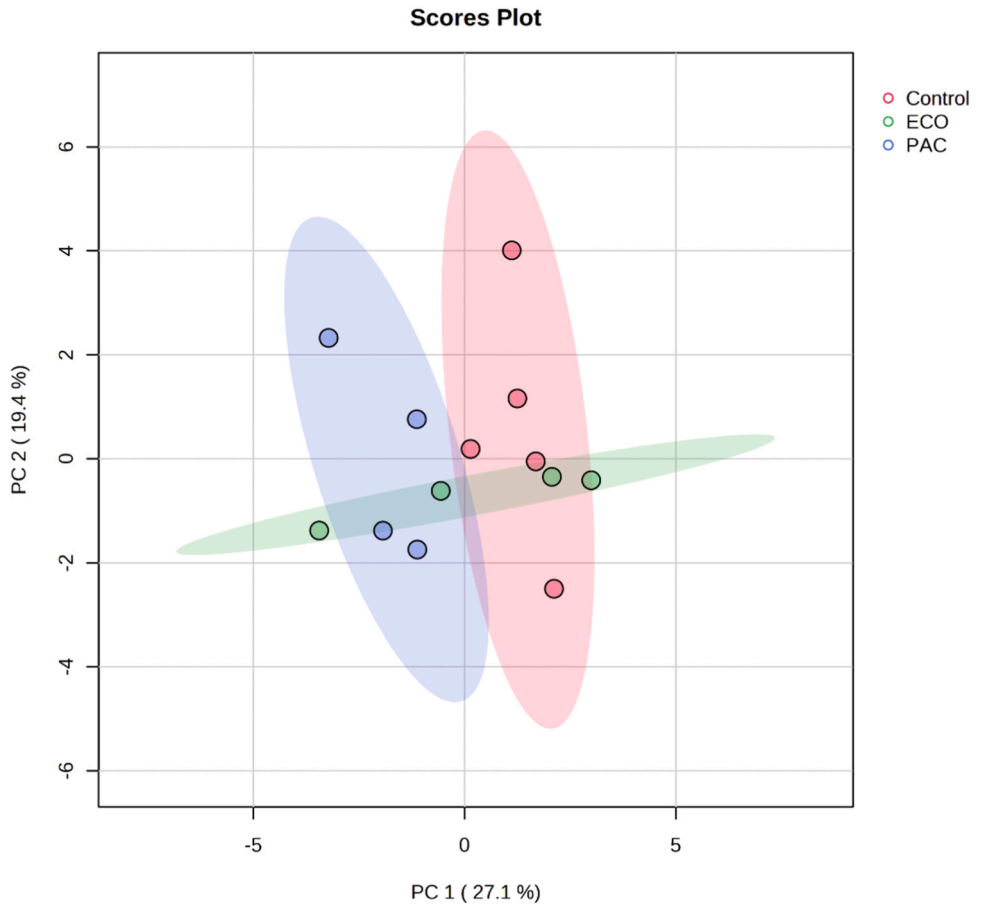

Fig. 3. PC1 ×PC2 score plot of control, MP_{ECO}, and MP_{PAC} exposed fibroblast cells.

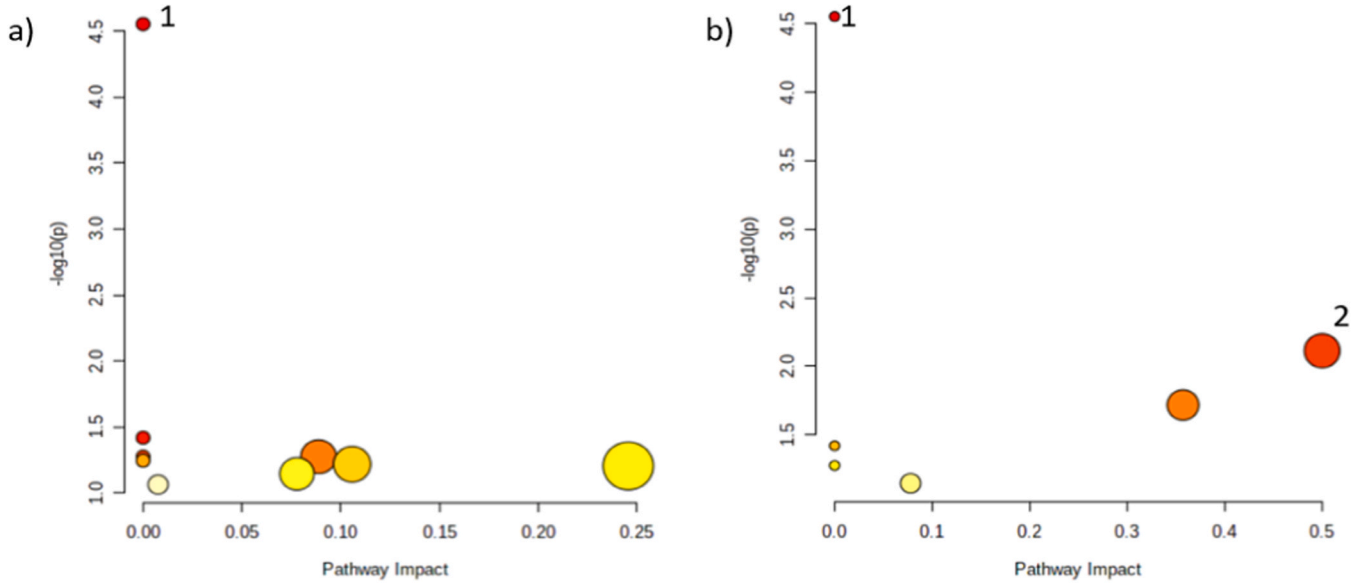

Fig. 4. Summary of metabolic pathways analyzed by MetaboAnalyst software: a) PM_{ECO} group b) PM_{PAC} group. Numbers 1 and 2 signify the aminoacyl-tRNA biosynthesis pathway and the Biosynthesis pathway of phenylalanine, tyrosine, and tryptophan, respectively.

accumulating neutrophils and other phagocytic cells that generate even more free radicals, resulting in a vicious cycle of inflammation and metabolic deficiencies. In addition, these species interact with the lipid-rich plasma membrane, initiating a lipid peroxidation reaction that triggers proteolytic activity, causing further tissue damage. The deleterious effects of this on the skin can be premature aging, lentigines, Melasma, atopic dermatitis, cancer, acne, among others [12,3,32].

Fig. 4 shows that aminoacyl-tRNA (b-tRNA) biosynthesis was significantly affected by PM_{ECO} and PM_{PAC} exposures. Along with the aminoacyl-tRNA synthetase (aaRS) enzymes, this pathway is a key factor

Table 1
Pathway analysis of differential metabolites of damaged cell.

	Total	Hits	Raw p	Holm adjust	FDR	Impact	Group
Aminoacyl-tRNA biosynthesis	48	3	2.79E-5	0.00234	0.00234	0	**ECO**
Selenocompound metabolism	20	1	0.0382	1	0.750	0	
Alanine, aspartate, and glutamate metabolism	28	1	0.0532	1	0.750	0	
Glutathione metabolism	28	1	0.0532	1	0.750	0.08873	
Porphyrin and chlorophyll metabolism	30	1	0.0569	1	0.750	0	
Glyoxylate and dicarboxylate metabolism	32	1	0.0607	1	0.750	0.10582	
Glycine, serine, and threonine metabolism	33	1	0.0625	1	0.750	0.2457	
Arginine and Proline metabolism	38	1	0.0718	1	0.753	0.0778	
Aminoacyl-tRNA biosynthesis	48	3	2.79E-5	0.00234	0.00234	0	**PAC**
Phenylalanine, tyrosine, and tryptophan biosynthesis	4	1	0.00772	0.64134	0.324	0.5	
Phenylalanine metabolism	10	1	0.0192	1	0.53879	0.357	
Selenocompound metabolism	20	1	0.0382	1	0.80297	0	
Alanine, aspartate, and glutamate metabolism	28	1	0.0532	1	0.89467	0	
Arginine and proline metabolism	38	1	0.0718	1	1	0.0778	

necessary for protein biosynthesis and cell viability. In addition, the expression of these enzymes can be pathologically associated with several human diseases, including neuronal diseases, cancer, autoimmune diseases, and diabetes that may also result in skin diseases [27]. Kalkhof et al. [9] identified that both proteins and amino acids were down-regulated in response to benzo[*a*]pyrene (B[*a*]P) exposure in murine hepatocytes in a time-dependent manner. The authors concluded that protein synthesis, lipid metabolism, and membrane dysfunction were identified as specific effects of B[*a*]P. Therefore, in this work, the reduction in metabolic activity associated with PAHs and the reduction in amino acid levels suggest that translation was suppressed after exposure to particulate matter.

The fibroblast exposure to PM_{PAC} also identified the biosynthesis of phenylalanine, tyrosine, and tryptophan. Phenylalanine is an essential amino acid and the precursor to tyrosine. It has been reported that tyrosine metabolism plays an important role in synthesizing thyroid hormone, catecholamine, and melanin [4].

The particulate matter can carry metallic ions and/or organic compounds such as polycyclic aromatic hydrocarbons-PHAs, which are highly lipophilic and can penetrate in the deeper layers of the skin. PAHs are often attached to the surface of combustion-derived PM, where they can bind to aryl hydrocarbon receptors and induce melanocyte proliferation [14]. Vierkötter et al. [35] associated skin aging symptoms with the formation of pigment spots caused by exposure to traffic-related PM. These particles develop skin damage not only directly, as has been shown, but also indirectly triggering a series of signaling pathways, such as oxidative stress and changes in melanin levels [22].

In this study, the two types of particulate matter were collected on the same day and time and under the same temperature conditions. The difference between them was that the PM_{PAC} was collected near an avenue with intense traffic, and the PM_{ECO} near an ecological park with little vehicle traffic. Even with this difference between the two types of collection, the results were similar. Although PM_{ECO} presented more altered pathways, PM_{PAC} had more significant alterations (Fig. 4), which was expected, as the environmental pollutants in this location were more concentrated. These results suggest that the altered pathways in the metabolism of cells exposed to PM_{ECO} occur because even with little car traffic in the area, the small concentration of particles still poses some toxicity.

Exposure to PM_{ECO} and PM_{PAC} caused a significant decrease (p-value < 0.05) in the levels of some cellular amino acids. Alanine is a non-essential amino acid produced by the body by converting pyruvate. It is highly concentrated and released by the muscles, functioning as an important energy source [6]. Decreased levels of alanine may indicate that the level of pyruvate is negatively regulated. As pyruvate is the main carrier that transports NH_4^+ from the tricarboxylic acid (TCA) cycle to the ornithine cycle, the decrease in pyruvate leads to a weakening of the TCA cycle and consequently a decrease in cellular energy. Many diseases in the human body are accompanied by metabolic disorders of energy metabolism in cells [34]. Intracellular energy transfer is via ATP, and ATP production is related to the energy metabolism of cells, mainly in the (TCA) cycle and glycolysis [38].

Similar results can be found in the work of Shi et al. [34], in which reduced levels of alanine and other amino acids were found in liver tissues exposed to fine particulate matter. The authors concluded that exposure to $PM_{2.5}$ disturbed amino acid metabolism, the TCA cycle, the urea cycle, and purine metabolism, which caused a certain degree of liver damage and oxidative stress. These results suggest that reduced alanine levels can affect the metabolism of skin cells and lead to several types of injuries that are still unknown and deserve investigation.

Proline, another amino acid down-regulated in both types of exposure, is a unique secondary AA required for protein biosynthesis. In addition, it plays a critical role in cellular bioenergetics, osmoregulation, stress protection, cell cycle blockade, autophagy, and apoptosis [26,28].

A metabolomic and transcriptomic study showed reduced changes in proline in aged skin compared to young skin. The authors correlated this result with an imbalance in osmoregulation in aging skin. Organic osmolytes regulate cell volume, act as protectors of epidermal water loss, and play several roles in protecting cells against external stressors [15]. According to these data, both PM_{ECO} and PM_{PAC} may indicate aspects that lead to cell aging due to these significant changes in their amino acids. Furthermore, we can infer that negatively regulated levels of proline affect collagen synthesis and promote a series of metabolic cascades that interfere with the structure and the wound-healing process of the skin [25].

Our results indicated that the particulate matter affects the metabolism of fibroblast cells, which in turn can present results both at internal levels, such as metabolism itself, and at external levels, such as the appearance of spots, wrinkles, and diseases in the skin. As far as we know, this work is the pioneer in evaluating amino acid changes in fibroblast cells exposed to atmospheric pollution. Although the results are promising, it is still too early to indicate a biomarker for this type of exposure, as more tests, replications, and different kinds of samples will be needed.

5. Conclusions

In this work, a new method based on GC×GC-Q-TOFMS/MS was developed to analyze amino acids derived from fibroblast cells. Due to the high sensitivity and selectivity of the technique, it was possible to identify some negatively regulated amino acids in cells exposed to particulate matter, such as alanine and proline. These might be correlated with various disorders caused in the skin, such as decreased cellular energy, dysregulation of cellular homeostasis, decreased collagen synthesis, interference with wound healing, and suppression of protein biosynthesis. In addition, the analysis of the pathways identified altered metabolic pathways, including aminoacyl-tRNA biosynthesis, which may be associated with the suppression of metabolic activity.

In summary, this study demonstrated that metabolomics based on targeted analysis of amino acids could provide information on metabolic levels and indicated that fibroblast cells are significantly affected by exposure to particulate matter. Furthermore, comparing the two types of particulate matter collected, PM_{ECO} and PM_{PAC}, elucidated that even with a small amount of traffic, the collected particles still showed some toxicity to skin-derived cells.

Finally, the new insights gained from metabolomics based on target amino acid analysis will clarify the effect of PM exposure, providing a new perspective on the damage of air pollution to skin cells.

CRediT authorship contribution statement

Samantha C. H. Rodrigues: Writing - original draft, Methodology, Validation, Investigation. **Helvécio C. Menezes**: Conceptualization, Resources. **Dawidson A. Gomes:** Conceptualization, Resources. **Zenilda L. Cardeal**: Conceptualization, Resources, Supervision. Conceituação, Resources, Supervisão.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zenilda L Cardeal and Samantha C H Rodrigues reports financial support was provided by National Council for Scientific and Technological Development. Zenilda L Cardeal reports financial support was provided by Fundação de Amparo á Pesquisa do Estado de Minas Gerais - FAPEMIG.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132606.

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