The invisible metabolic world of fingermarks revealed by Magnetic Resonance Mass Spectrometry (MRMS)

Fingermarks leave behind a plethora of chemical information defined by metabolites, exogenous substances including pharmaceuticals, grooming products, and even drugs of abuse and explosives.

Abstract

The composition of this extremely complex mixture can be revealed by Magnetic Resonance Mass Spectromtry (MRMS) and the gathered information used for sex and age group identification. Moreover, specific metabolites were found to contribute the most for these differentiations, proving a biochemical foundation linking the metabolome to sex and age.

Keywords: Metabolomics, MRMS, fingerprints, chemical profile

Introduction

Fingermarks are unique to each individual and correspond to a complex pattern of ridges and valleys that are crucial in forensic science for subject identification [1]. Lophoscopic methods are based on the comparison of the epidermal fingerprint patterns with those contained in a database using AFIS (Automatic Fingerprint Identification System) or by direct comparison with the epidermal patterns of a given suspect. However, in the absence of a lophoscopic trace with an identifying value, the analysis of its chemical composition can reveal the sex and age group of the subject, among other informations of forensic value. Indeed, the fingermark contains endogenous metabolites, as well as exogenous substances, such as pharmaceuticals, drugs, personal care products, and food additives. Since latent fingermarks are invisible to the naked eye, different revealing agents must be used for their development that potentially interfere with subsequent compositional analysis [2]. To assess the interference of revealing powders on these profiles, we used the Instant White fingerprint powder, commonly used to develop latent fingerprints on non-porous and semi-porous surfaces.

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Considering the extreme complexity of the chemical composition of human fingermarks, as well as possible interference from revealing powder, the most suitable approach for its analysis is ultra high resolution mass spectrometry using MRMS. Untargeted metabolomics based on extreme mass resolution led to the identification of hundreds of metabolites, even in the presence of Instant White. The chemical composition of fingermarks was analyzed using MRMS, not only to reveal its composition but also to uncover hidden information related to sex and age group.

Sample preparation and analysis

Ethics

This study was approved by the Ethics Commission of the Faculty of Sciences, University of Lisbon, Portugal. Fingerprints were collected from male and female volunteers, with ages from 20 to 55 years.

Metabolite extraction from human fingermarks

Volunteers washed their hands with water and rubbed their indicator fingers on their forehead and behind their ears. Fingerprints were deposited in a clean glass slide and collected 60 minutes after deposition. To uncover the fingerprints, Instant White fingerprint powder (BVDA International BV, Haarlem, The Netherlands) was applied using a squirrel hair brush (BVDA). A water/methanol solvent system was used to collect the fingermark residue from revealed and non-revealed fingerprints. Twenty μL of methanol (LC-MS grade)/water (1:1) were applied in the center of the fingermark, washing dynamically for 20 seconds, and recovering the sample to a clean tube. This procedure was repeated five times to remove all the residue. 900 μL of solvent mixture was added to the microtube, the sample was centrifuged for 8 minutes at 4°C at 8000 rms and supernatant recovered for analysis. Three replicates were collected and analyzed per subject. Each sample was diluted 1:10 in methanol/water (1:1) containing 0.1% (v/v) formic acid and 0.1 μg/mL of human leucine enkephalin as internal standard ($[M+H]^+$ = 556.276575 *m/z*).

MRMS analysis

Samples were analyzed by direct infusion at 2 μL/min on a 7 T solariX XR MRMS (Bruker Daltonics GmbH & Co. KG), by electrospray in positive ionization mode (ESI^+). Mass spectra were acquired in magnitude mode, from 100 to 1500 *m/z*, with an accumulation of 200 transients at 4M. Spectra were acquired in magnitude mode at a resolution of 680,000 (*m/z* 200) enough to show fine isotopic patterns of individual compounds across the whole *m/z* range. Spectra were internally calibrated using two-point online calibration using the standard leucine enkephalin and later with the *m/z* signal at 273.267312 ($C_{16}H_{35}NO_2$, present in all samples at a very high signal intensity).

Data analysis

Raw data was analyzed with Bruker MetaboScape® 5.0 using the T-ReX® 2D (MRMS single spectrum) algorithm. All samples' peak lists were aligned in a single bucket table and the intensities normalized with the standard leucine enkephalin. Molecular formulas for each mass were assigned using the SmartFormula function and putative annotation of metabolites performed using the analyte lists from the Human Metabolome Database (HMDB [3]), from 17 Nov 2021, uploaded to MetaboScape 5.0. After spectra alignment, a total of 4050 buckets were obtained. The number of identified features in MetaboScape by HMDB database were 465 (all with formulas assigned) and 3479 features had formulas assigned using SmartFormula.

Multivariate statistical analysis was performed in Bruker MetaboScape 5.0. Principal Components Analysis (PCA) models were built using Pareto scaled data, retaining a minimum number of principal components necessary to explain 98% of variance. Sample Hierarchical Clustering (agglomerative) was performed considering a Euclidean distance and using the Ward distances method. A Partial Least Squares Discriminant Analysis (PLS-DA) was performed to identify the compounds that better discriminate between male and female donors. Variable Influence on Projection (VIP) scores were calculated from the PLS-DA model and the specific metabolites that contributed the most to the differentiation between both groups (male and female).

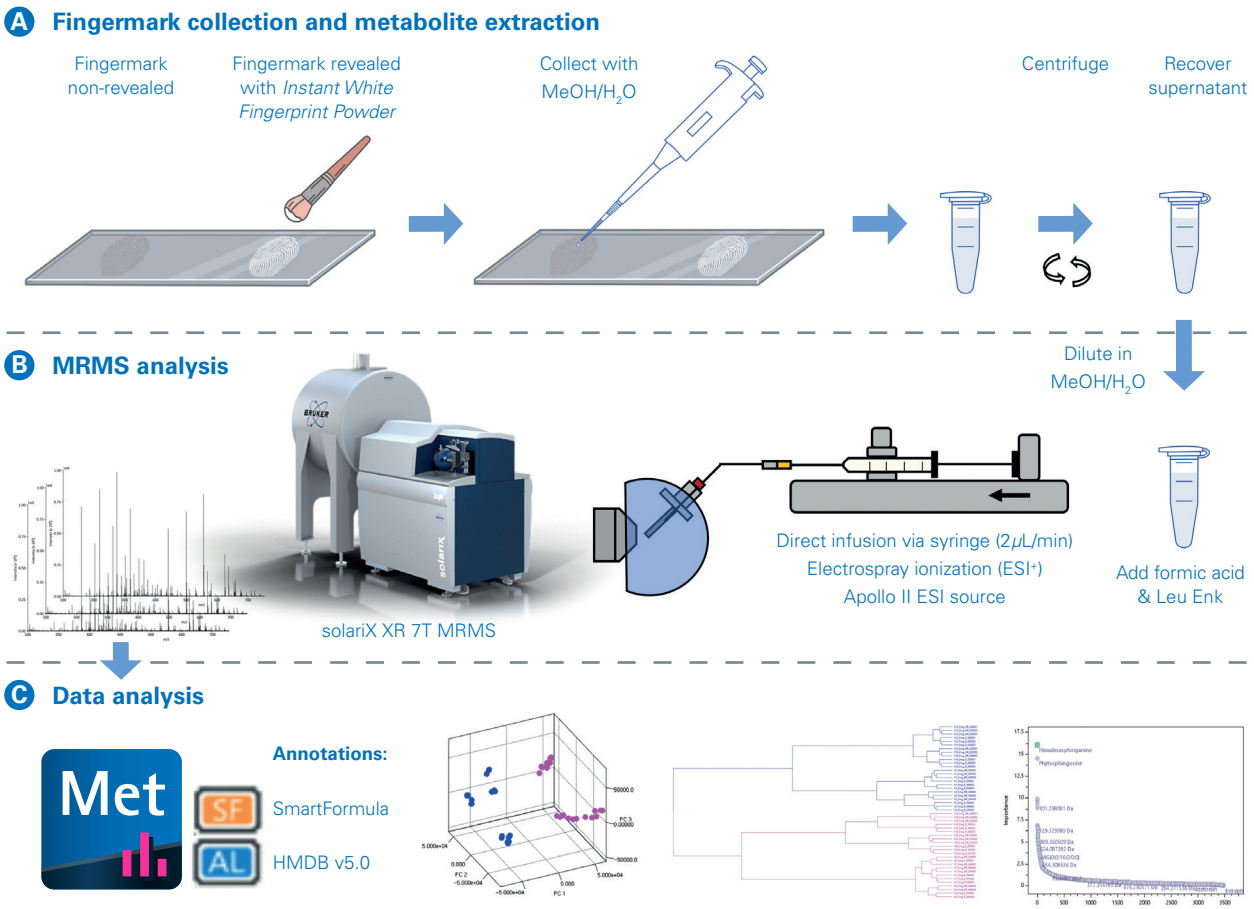

Figure 1
Schematic workflow of the experimental procedure, including (A) fingermark collection and metabolite extraction, (B) MRMS analysis and (C) data analysis.

Results

An untargeted metabolomics analysis using direct infusion MRMS was performed to analyze the chemical profiles of human fingerprints.

PCA and hierarchical clustering analysis (HCA) were applied to validate data reproducibility and to detect inter-group metabolic similarities among the different samples. A clear separation between male and female donors, and between the two defined age groups was observed in the PCA score plots, with low variability between replicates from the same sample (Figure 2A). Hierarchical clustering further confirmed both this separation and the high reproducibility of the data. (Figure 2B). This separation trend indicates that multivariate statistical analysis of human fingermarks' based on MRMS spectra is powerful enough for sex and age group discrimination.

Instant White fingerprint contains 80-90% (w/w) titanium dioxide and 10-20% (w/w) zinc stearate. No differences were observed between samples revealed with this fingerprint powder and non-revealed ones (Figure 2B, C), showing that the revealing agent did not interfere with the fingermark recovery and chemical composition identification by MRMS.

To identify the metabolites that contributed the most for sex and age separation, PLS-DA models were fitted to the MS intensity data, building a system of components that maximized covariance between the groups (male / female and age 20-30 / 45-55).

The sphingolipid hexadecasphinganine ($C_{16}H_{35}NO_2$), with an *m/z* value of 273.267312 ($M+H^+$), showed the highest VIP score (Figure 2D, E), most contributing for the gender and age separation. Another sphingolipid that also greatly contributes to gender and age discrimination is phytosphingosine ($C_{18}H_{39}NO_3$, *m/z* 318.30027 ($M+H^+$)), identified both by name using HMBD and by formula assignment (Figure 2D, E). The ultra high resolution, mass accuracy and dynamic range achieved at 7T MRMS, allowed the identification of hexadecasphinganine and phytosphingosine isotopologues, unequivocally assigning their molecular formula (Figures 3C, D, E and 4C, D, E). The molecular formula of both sphingosines were additionally confirmed by their isotopic fine structure (Figure 3D and 4D).

Both hexadecasphinganine and phytosphingosine were detected at a higher concentration in female fingermarks (Figures 3A and 4A) and in younger people with the same sex, although the difference in females is more evident (Figures 3B and 4B). Hexadecasphinganine is a component of sphingomyelins (ceramide phosphocholines), present in the human epidermis. Phytosphingosine, also present in ceramides, stimulates the differentiation of human keratinocytes [4] and enhances moisture level in human skin barrier [5], being therefore essential to prevent dehydration and prevent microbes and allergens from entering tissues from the outside [6].

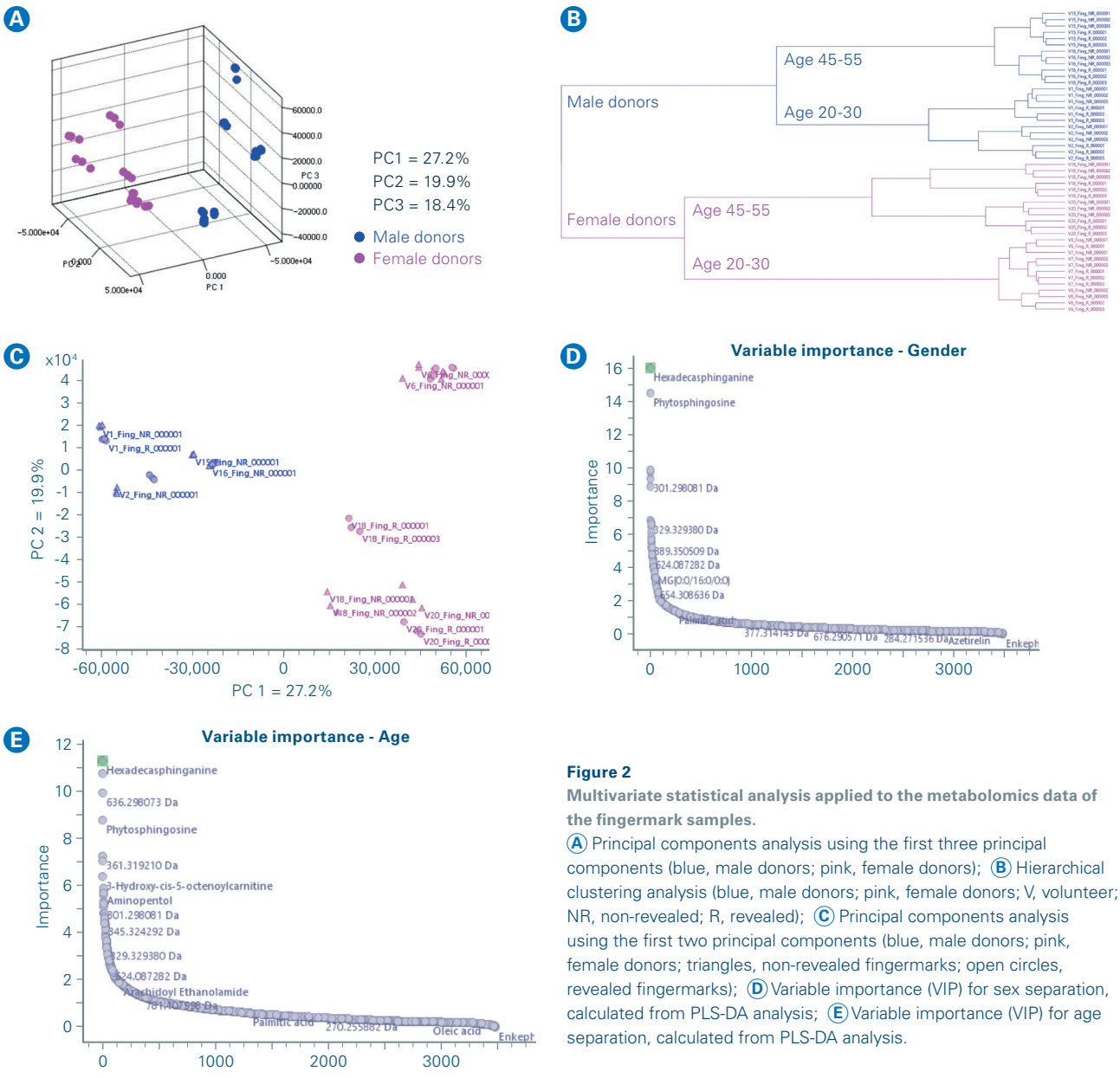

Figure 2

Multivariate statistical analysis applied to the metabolomics data of the fingermark samples.

(A) Principal components analysis using the first three principal components (blue, male donors; pink, female donors); (B) Hierarchical clustering analysis (blue, male donors; pink, female donors; V, volunteer; NR, non-revealed; R, revealed); (C) Principal components analysis using the first two principal components (blue, male donors; pink, female donors; triangles, non-revealed fingermarks; open circles, revealed fingermarks); (D) Variable importance (VIP) for sex separation, calculated from PLS-DA analysis; (E) Variable importance (VIP) for age separation, calculated from PLS-DA analysis.

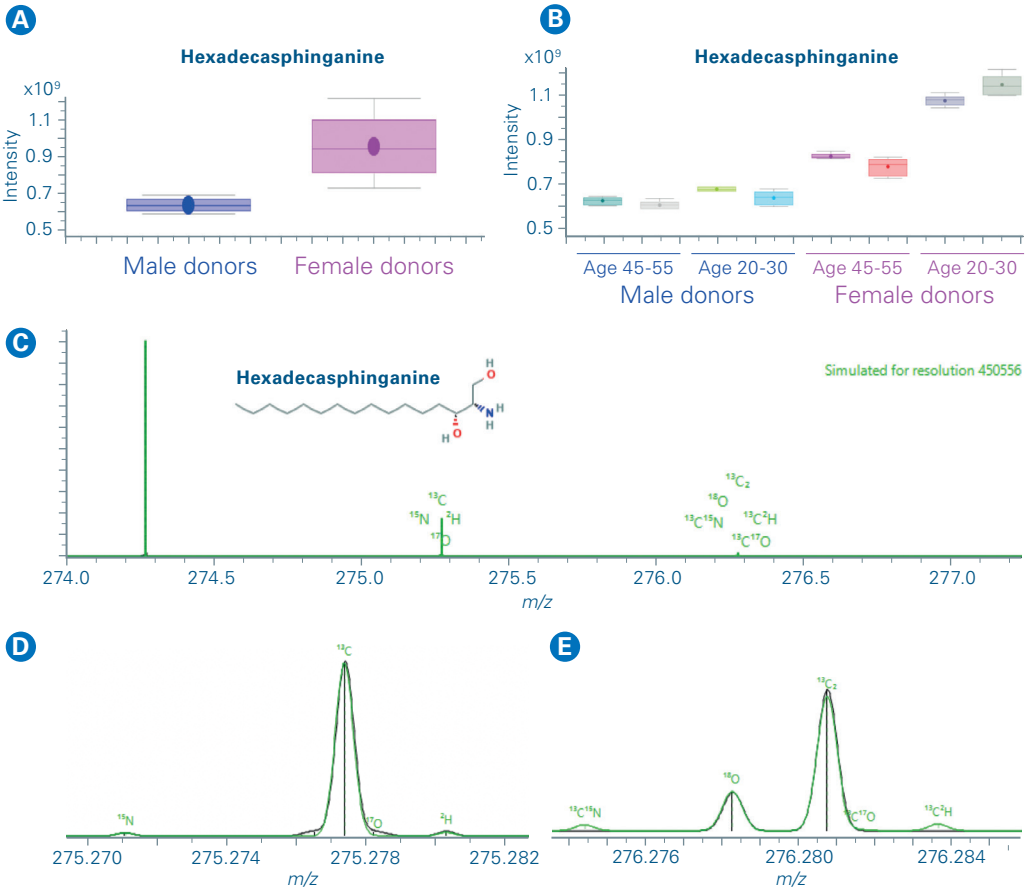

Figure 3
Hexadecasphinganine box plot and fine isotopic distribution. Hexadecasphinganine box plot (A) by sex and (B) for all samples; (C) Hexadecasphinganine structure and fine isotopic distribution, highlighting the (D) second and (E) third peaks. Black line, experimental; green line, theoretical isotopic distribution.

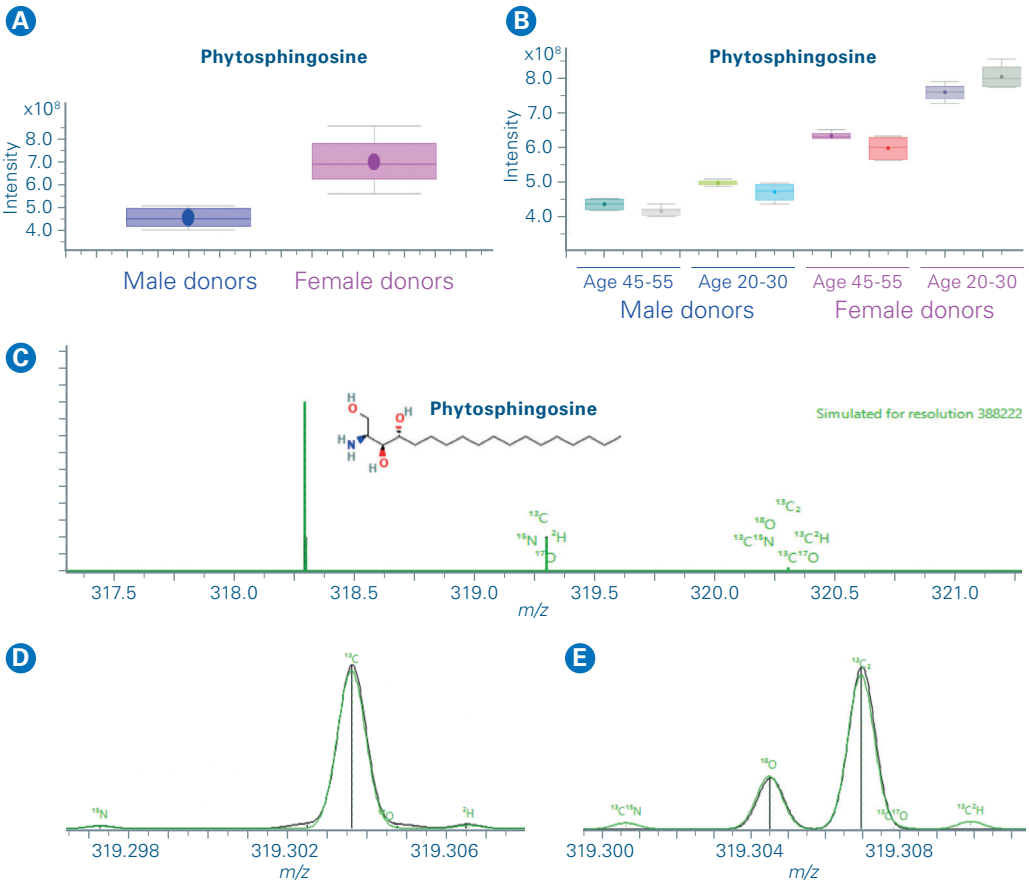

Figure 4
Phytosphingosine box plot and fine isotopic distribution. Phytosphingosine box plot (A) by sex and (B) for all samples; (C) Phytosphingosine structure and fine isotopic distribution, highlighting the (D) second and (E) third peaks. Black line, experimental; green line, theoretical isotopic distribution.

Conclusion

- An untargeted metabolomics characterization of human fingermarks with extremely high-resolution magnetic resonance mass spectrometry can accurately discriminate subjects by sex and age.
- Sphingolipids play a central role in sex and age discrimination.
- The revealing agent White fingerprint powder did not interfere with the analysis of dermopapillary residue analysis, as the chemical composition of revealed and non-revealed samples was very similar.
- MRMS analysis of human fingermarks metabolome can provide an important contribution to forensic science.

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