Metabo-tip: a Metabolomics Platform for Lifestyle Monitoring

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Research Article

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Abstract

Exposure to bioactive compounds from nutrition, pharmaceuticals, environmental contaminants or other lifestyle habits may affect the human organism. To gain insight into the effects of these influences, as well as the fundamental biochemical mechanisms behind them, molecular profiling seems promising. To address this problem, we developed an assay for the analysis of sweat, collected from finger tips, using mass spectrometry – by far the most comprehensive and sensitive method for such analyses. To evaluate this assay, called metabo-tip, we exposed volunteers to various xenobiotics using standardized protocols and investigated their metabolic response. As early as 15 minutes after the consumption of a cup of coffee, 50g of dark chocolate or a serving of citrus fruits, significant changes in the sweat composition of the finger tips could be observed, providing relevant information in regards to the ingested substances. This included bioactive compounds such as natural flavonoids as well as potential hazardous substances such as pesticides. Furthermore, the identification of metabolites from nicotine after cigarette smoking and metabolites from orally ingested medications such as metamizole indicated the applicability of this assay to observe specific enzymatic processes. Remarkably, we found that the sweat composition fluctuated in a diurnal rhythm, supporting the hypothesis that the composition of sweat can be influenced by endogenous metabolic activities. This was further corroborated by the finding that histamine was significantly increased in the metabo-tip assay in individuals with allergic reactions. Metab-tip may thus enable direct access to individual metabolic profiles and may thus support predictive preventive personalised medicine (PPPM).

Introduction

It is now widely known, lifestyle choices are pivotal for a person's health and life quality, and it is estimated that at least 40% and up to 95% of chronic illnesses can be traced back to lifestyle risk factors (*e.g.* smoking, lack of physical activity and dietary habits) [1, 2]. Moreover, a positive correlation between the adoption of healthy lifestyle choices and a reduced risk of mortality as well as postponing or even avoiding many types of chronic illnesses such as cancer, cardiovascular diseases and metabolic syndrome, has been demonstrated [1, 3-8]. Insulin resistance, abnormal lipid metabolism, diabetes and hypertension are risk factors highly correlated with obesity [9-11], while smoking is known to cause several cancers, especially of the lung and upper airways [12, 13]. In contrast, regular physical activities as well as healthy eating habits such as the Mediterranean diet [14] have already shown to reduce the risk of several chronic conditions such as cardiovascular disease [15, 16], to play a protective role in cancer prevention [17], to promote longevity [18] and to decrease the risk of developing metabolic syndrome and type 2 diabetes [19]. Observational studies suggest that lifestyle changes, mainly dietary [20], can improve the immune system and reduce the risk of recurrence of certain types of cancers, such as ovarian cancer [21]. Therefore, fast and efficient monitoring of biomarkers reflecting individual lifestyle patterns could play a preventive role in the development of chronic disorders.

The investigation of metabolic response to environmental exposures is an emerging field of research in toxicology [22, 23]. The so-called exposome includes not only exogenous exposure to the environment,

diet or lifestyle factors but also to biological processes reflecting internal responses to exposure [24-26]. For example, a chronic low-dose exposure to mycotoxins, which are frequently detected as natural contaminants in foods, have already been associated with the onset of various diseases. The analysis of mycotoxins as biomarkers for exposure to contaminated food was successfully performed using plasma, serum, urine and milk samples [27, 28]. The origin of many metabolites, which are either endogenously produced, originate from the gut microbiome, or come from the environment *via* nutrition or smoking is already being investigated in great detail [29]. In particular, serum metabolomics has successfully revealed several biomarkers which have improved our understanding of disease mechanisms and which are being used in clinical settings for the diagnosis of diseases as well as in the monitoring of therapeutic outcomes [30].

A comprehensive analysis of endogenous processes related to the uptake and individual metabolism of xenobiotics requires sensitive analytical techniques in addition to non-invasive and fast sampling methods, allowing short interval sampling and therefore enabling kinetic time-course measurements in humans [31, 32]. The analysis of sweat from the fingertips fulfils these requirements and supports compliance of test subjects. Over decades, fingerprints have been used to identify individuals and more recently play an important role in lifestyle monitoring via imaging mass spectrometry [33]. Exogenous compounds found in bug sprays and sunscreens as well as food oils, alcohols and citrus fruits were detected in fingerprints, offering relevant chemical information about the tested person [34]. Moreover, fingerprints are used to detect illicit drugs and their metabolites [35, 36]. In contrast to the investigation of fingerprints, the analysis of sweat has been successfully proven in diagnostic medicine to enable the monitoring of individual metabolic and health states [37-39]. Sweat is mainly composed of water (99%), but includes also numerous substances such as electrolytes, lactate, pyruvate, urea, amino acids, proteins, peptides, fatty acids, hormones and xenobiotics (e.g. cosmetics, medications and drugs including ethanol) [40]. Antibodies and cytokines detected in sweat may serve as potential biomarkers for diseases [41] and, as already demonstrated, for disease states in cystic fibrosis [42] and active tuberculosis [43]. Moreover, cortisol has been successfully quantified in human eccrine sweat, demonstrating the potential of finger sweat analysis in regard to monitor endogenous processes related to stress [44].

Only recently, we successfully demonstrated kinetic time-course measurements of metabolic activities in humans, indicating that finger sweat analyses may become a valuable tool for precision medicine [32]. In contrast to other minimally invasive approaches [45, 46], the presented non-invasive metabolomics assay works quickly and easily while offering tremendous investigative power. We have termed it “metabo-tip”. Indeed, the ingestion of many bioactive compounds contained in food may become detectable via metabo-tip within minutes after consumption. Applying mathematical modelling strategies, it was possible to overcome critical normalization challenges and to obtain quantitative measures for individual metabolic properties [32]. In this project, we investigated metabo-tip in regards to monitoring lifestyle parameters such as the presence of endogenous and exogenous bioactive compounds or exposure to toxins contained in foods or beverages. These parameters could be described by time-dependent metabolic patterns detected in sweat from the fingertip. The observation of metabolic diurnal rhythms

and distinct individual responses to potentially adverse exposure promises successful future applications of metabo-tip analysis not only in the general assessment of individual lifestyle-parameters but also in predictive preventive personalised medicine (PPPM).

Results

Both food and beverage consumption leave characteristic imprints on finger sweat composition and enables conclusions to be drawn about the general lifestyle of individuals.

Figure 1A shows extracted ion chromatograms identifying caffeine, 7-, 3- and 1-methylxanthine as well as catechin and epicatechin in a finger sweat sample of an individual 15 min after consumption of 50g dark chocolate. None of these substances were detectable in the finger sweat of individuals before consumption; the significant increase of these compounds in finger sweat after chocolate consumption could be reproduced in a controlled study with ten different individuals (Figure 1B). Furthermore, the levels of different caffeine metabolites after chocolate intake were compared to those after drinking a cup of coffee. Although both caffeine and theobromine are contained in chocolate as well as coffee, different levels of these two compounds found in finger sweat enabled the identification of the respective consumption groups (Figure 1C). Paraxanthine is the catabolic metabolite of caffeine and apparently accumulates in regular coffee consumers [47, 48]. In our experiment, we could confirm this finding in individuals consuming coffee regularly through elevated baseline levels of paraxanthine in finger sweat (Figure 1D). Thus, a metabolic property detected in finger sweat may be related to individual lifestyle preferences, such as coffee consumption.

Cigarette smoke, pharmaceuticals and exposure to environmental toxins leave characteristic imprints on finger sweat composition

Not only a specific diet, but also personal habits such as smoking and regular use of pharmacological drugs will result in characteristic changes in finger sweat. Moreover, metabo-tip has the potential to detect even trace amounts of pesticides as demonstrated in Figure 2. The consumption of an orange allowed for the identification of natural bioactive compounds such as flavonoids (nobiletin, hesperidin and tangeritin) together with pesticides such as enilconazole (Figure 2A). In addition, metabo-tip was applied to detect smoking of tobacco (cigarettes) in finger sweat via characteristic compounds such as nicotine and anatabine (Figure 2B). The simultaneous detection of precursor molecules and their metabolites such as nicotine, cotinine and 3-hydroxycotinine facilitate individual metabolic profiling (Figure 2B). In the case that xenobiotics are not directly detectable, evidence can be found by the detection of specific metabolites. For example, the pain killer metamizole was not detectable in sweat, but after consumption of metamizole, a group of metamizole-derived metabolites could be detected in finger sweat, as demonstrated in Figure 2C.

Sweat composition provides a wide spectrum of information regarding individual lifestyle

Metabo-tip not only allows the detection of specific compounds after consumption of a certain food or medication, it also enables comprehensive, untargeted screening of exogenous bioactive compounds, for instance from cosmetics or environmental pollutants. In addition, it detects specific markers for general lifestyle habits (Figure 3, Supplementary Table). Based on previously published studies [49, 50], it could be assumed that basic compounds are more likely to get transported into sweat ducts; however our results do not seem to discriminate between analytes based on their pK_A values, therefore allowing for a comprehensive metabolic profile (Supplementary Table with listed pK_A values). The combined detection of exogenous compounds as well as endogenously produced metabolites results in a specific molecular signature in the finger sweat of each individual. Moreover, the wide array of information obtained from finger sweat analysis may reveal information about the general health status of an individual. Exposure to bioactive compounds as well as to various medications and drugs could be detected. Moreover, environmental pollutants such as fungicides, mycotoxins and compounds derived from plastic containers such as bisphenol S and melamine were detectable and may be of great relevance concerning individual health.

Metabo-tip analysis reveals diurnal fluctuations in metabolism and individual endogenous responses

Principal Component Analysis (PCA) of finger sweat samples collected from three donors in the morning and evening over a time period of 30 days revealed differences in finger sweat composition with the possibility to distinguish between these individuals based on their general lifestyle (Figure 4A-B). Donor A and donor B could not be completely separated by PCA which might be contributed to a similar lifestyle. Intriguingly, a regular shift of metabolites in a diurnal rhythm was observed (Figure 4C). Analysis of the PCA loadings plot suggested coffee or chocolate consumption (caffeine, theobromine, theophylline and paraxanthine) and amino acids as most influential compounds (Figure 4D).

We finally assessed the presence of the biogenic monoamine histamine, which serves both as a neurotransmitter and modulator in inflammatory responses. Figure 4E exemplifies the levels of histamine determined by metabo-tip analysis on consecutive days. Histamine peaks correlated with symptoms associated with food intolerance and allergy reported on the same day. This observation indicates that specific individual responses to food consumption can be detected by metabo-tip analysis.

Discussion

Diagnosis and treatment of chronic disease is often difficult, because these diseases often have multiple causes – as evidenced in case of arteriosclerosis, type II diabetes, cancer or neurodegenerative diseases. Actually they may be caused or promoted by an imbalance of metabolic homoeostasis due to long-lasting stress. The investigation of metabolic imbalances in humans has been difficult though such investigation is crucial to predictive preventive personalised medicine (PPPM) improvement [51]. Due to its dynamic nature, metabolomic measurements call for repeated analyses in a narrow timeframe. Blood sampling is therefore not feasible and leads to compliance issues. Non-invasive methods would be preferable but the analysis of urine, for instance, does not allow repeated time-series analyses in a narrow

timeframe because urine is stored in the bladder prior to sampling. Saliva may suffer from contaminants such as bacteria and food as well as from mucous glycoproteins which may interfere with metabolite extraction and which may impair sensitivity of measurement. Sweat is only available in limited amounts, and the risk of potential contamination from the skin also causes quantification challenges. To overcome some of these obstacles we investigated sweat collected from clean fingertips via metabo-tip. This procedure is fast, non-invasive and easy to use, thereby supporting multiple measurements within a short timeframe, and ensuring good compliance by the test subjects [31]. The determination of individual enzymatic activities and the management of normalization issues, related to the total amount of sweat, has already been demonstrated [32]. Here, we evaluated whether this analytical approach could detect specific substances that would allow lifestyle habits which may be relevant to health to be determined.

We demonstrated that the uptake of as little as 0.2 mg of a chemical compound may be sufficient to be detected by metabo-tip analysis, and investigated if this approach was limited to basic compounds showing higher solubility in the slightly acidic sweat pH [49, 50]. Plotting the identified substances versus pK_A values did not show any relation, suggesting no discrimination of substances based on their pK_A (Supplementary Table). Thus, it seems that metabo-tip analysis may provide a representative overview of substances we are exposed to. Present data therefore suggest a broad applicability of metabo-tip. The detection of bioactive compounds such as flavonoids and even pesticides after the consumption of oranges demonstrated the practicability of the assay.

Furthermore, the metabolism of xenobiotics could be observed using metabo-tip analysis as demonstrated in the case of xanthines, nicotine and metamizole (Figure 1 and 2). Individual differences in the expression of P450 isoenzymes [52] account for individual variations in drug effects and toxicity [53]. Metabo-tip analysis allows for the detection of substances and their metabolites, supporting the systematic study of different individuals.

We have observed an apparent correlation between allergic symptoms and histamine detected by metabo-tip analysis (Figure 4), which might also make it possible to obtain biochemical signatures of the adverse effects. Such an experimental approach would allow us to investigate apparent detrimental effects with scientific rigour, enabling reasonable conclusions to be made regarding risk assessment. Furthermore, the presented analytical workflow offers the opportunity to monitor individual responses to plant-derived bioactive compounds, especially flavonoids, which are intensely discussed in the context of PPPM because of their beneficial impact on carcinogenesis [54, 55].

The opportunities offered by metabo-tip analysis are raising many questions. Only large-scale studies will allow us to decide e.g. whether the apparent diurnal rhythm observed by metabo-tip in this study (Figure 4) was related to endogenous factors (e.g. through circadian regulation), or rather exogenous factors (such as regular nutrient patterns or repeated exposure to xenobiotics). Furthermore, technical improvements regarding robustness, sensitivity and throughput may also support the development of stress monitoring devices as well as therapy monitoring strategies, bringing about crucial progress in the

field of PPPM. In conclusion, the non-invasive and timely accessibility to individualised metabolomics data provided by metabo-tip analysis may have a large number of practical applications.

Methods

Reagents and Chemicals

LC-MS grade formic acid, methanol and water used for chromatographic separation as well as for preparation of internal standards and samples were purchased from VWR (Germany). Xenobiotic and metabolic standards were obtained either from Sanova Pharma GmbH (Austria), Sigma Aldrich (Austria), or from Honeywell Fluka (GER). Filter papers (standardized to 1 cm^2) used for sampling were stamped out of fuzz-free paper from Kimtech Science (U.S.A).

Cohort Design

Several volunteers were recruited after giving written, informed consent for the different studies as outlined in Table 1. These experiments were approved by the ethical committee of the University of Vienna (no. 00337). Volunteers may be part of more than one study (A-E). Gender distribution of participants was equally balanced between male and female and their ages ranged from 20-50 years. Some studies (C-E) required a fasting period of 12 hours for caffeinated foods (chocolate, energy bars) and beverages (coffee, tea, energy drinks) before beginning with the experiment in order to avoid interferences with the investigation of xenobiotic metabolism. Finger sweat samples were collected just before donors consumed a 50g chocolate bar or a double espresso (0 min). Finger sweat samples were thereafter collected in short intervals as outlined in Table 1. In study A and B, the general lifestyle of volunteers was monitored. There were no dietary restrictions placed on the test subjects.

Table 1: Overview of all studies discussed in this publication.

Study	Participants	Design	Sampling time points	Restrictions
A	6 participants	30 consecutive days of sampling	2x per day (morning and evening)	no restrictions
B	10 participants	10 consecutive days of sampling	3x per day (morning, lunch, evening)	no restrictions, 4 smokers
C	10 participants	50 g chocolate bar	0, 15, 30, 45, 60, 90 and 120 min after consumption	fasting caffeinated foods and drinks 12 hours previous to the start of the experiment
D	6 participants	50g chocolate bar or double espresso or neither (control)	0, 20, 40, 60, 90, 120 and 120 min after consumption	fasting caffeinated foods and drinks 12 hours previous to the start of the experiment
E	20 Participants	regular *versus* rare coffee drinkers	sampled one time to check baseline levels	fasting caffeinated foods and drinks 12 hours previous to the start of the experiment

Collection of Sweat from the Fingertip

Finger sweat samples were collected as previously published [32]. In short, filter papers were stamped out of fuzz free paper to get a circular area of 1 cm^2, filter papers were pre-wetted with 3 µl of LC-MS grade water and stored in 0.5 ml Eppendorf Tubes. For each sweat collection, donors cleaned their hands with warm tap water and then dried their hands with disposable paper towels. Donors kept their hands open in the air at room temperature for 1 min. Then, the sampling unit was placed between thumb and index finger with clean tweezers and held for 1 min. Filters were transferred back to labelled 0.5 ml Eppendorf tubes using clean tweezers and stored at 4 °C until sample preparation.

Sample Preparation

Each finger sweat sample collected on filter papers was extracted with 120 µl of extraction solution (an aqueous solution of 1 pg $µl^{-1}$ caffeine-trimethyl-D9 with 0.2% formic acid). Metabolites were extracted *via* repeated pipetting of the extraction solvent 15 times. The filter paper was pelleted on the bottom of the Eppendorf Tube and the supernatant was transferred into HPLC glass vials equipped with a 200 µl V-Shape glass insert (Macherey-Nagel GmbH & Co.KG) and analysed by LC-MS/MS. To determine background metabolites or potential contaminants blank filter papers were additionally extracted in the same manner. Standard solutions were prepared in methanol in a concentration of 1 mg ml^{-1}. After that they were diluted to concentrations of 100 pg $µl^{-1}$ and 10 pg $µl^{-1}$.

LC-MS/MS Analysis

Chromatographic separation was performed on a Vanquish UHPLC system (Thermo Fisher Scientific) and analysed in an untargeted fashion with a hybrid instrument consisting of a quadrupole mass filter and an orbitrap mass analyser (Q Exactive HF, Thermo Fisher Scientific). A reversed phase Kinetex XB-C18 column (100 Å, 2.6 µm, 100 × 2.1 mm, Phenomenex Inc.) was used to separate analytes present in finger sweat samples. Mobile phase A consisted of water with 0.2% formic acid, mobile phase B of methanol with 0.2% formic acid. The following gradient was applied: 1–5% B in 0.3 min and then 5–40% B from 0.3–4.5 min, followed by a column washing phase of 1.4 min at 80% B and a re-equilibration phase of 1.6 min at 1% B resulting in a total runtime of 7.5 min. Flow rate was set to 500 µl min^{-1} and the injection volume was 10 µl. Electrospray ionisation was performed in positive as well as negative ionisation mode. MS scan range was m/z 100-1000 and the resolution was set to 60000 (at *m/z* 200) for MS1 and 15000 (at *m/z* 200) for MS2. A top 4 method was applied and dynamic exclusion was set to 6 seconds. Collision energy was 30 eV. Instrument control was performed with Xcalibur software (Thermo Fisher Scientific).

Data Analysis

Raw files generated by the Q Exactive HF were searched in the Compound Discoverer Software 3.1 (Thermo Fisher Scientific) applying a user-defined work-flow. All identified compounds with a match factor ≥80 were manually reviewed using Xcalibur 4.0 Qual browser (Thermo Fisher Scientific) and the obtained MS2 spectra were compared to reference spectra taken from *mzcloud* (Copyright © 2013–2020 HighChem LLC, Slovakia). A maximum retention time shift of 0.1 min was allowed and the mass tolerance was restricted to 10 ppm for MS1 and MS2. Identified compounds were also verified using purchased analytical standards applying the same LC-MS method. The Tracefinder Software 4.1 (Thermo Fisher Scientific) was used for peak integration and calculation of peak areas. Generated batch tables were exported and further process with Microsoft Excel, GraphPad Prism (for two-tailed, paired t-tests) and the Perseus [56] (for principal component analysis) software. ACD/Labs' ChemSketch (Freeware) 2020.1.1 was used to draw structures.

Abbreviations

CV – Coefficient of Variation, **LC-MS** – liquid chromatography mass spectrometry, ***m/z*** – mass over charge, **nAUC** – normalised area under the curve, **ns** – not significant, **PCA** – principle component analysis, **pK_A** – acid dissociation constant, **PPPM** – predictive preventive personalised medicine, **RT** – Retention Time

Declarations

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

Raw data is secured and available on request.

Code availability

Not applicable.

Author Contributions

J.B. performed research, interpreted data, analysed data and wrote the manuscript, A.B. interpreted data and wrote the manuscript, T.S. interpreted data and wrote the manuscript, F.R. performed research and wrote the manuscript, G.F. performed research, S.M.M. performed research, analysed and interpreted data and wrote the manuscript, C.G. conceptualized the project, interpreted data and wrote the manuscript.

Ethics approval

This study was approved by the ethical committee of the University of Vienna (no. 00337).

Consent to participate

Volunteers have given their written, informed consent to participate in this study.

Consent for publication

Volunteers have given their written, informed consent for publishing the data.

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Figures

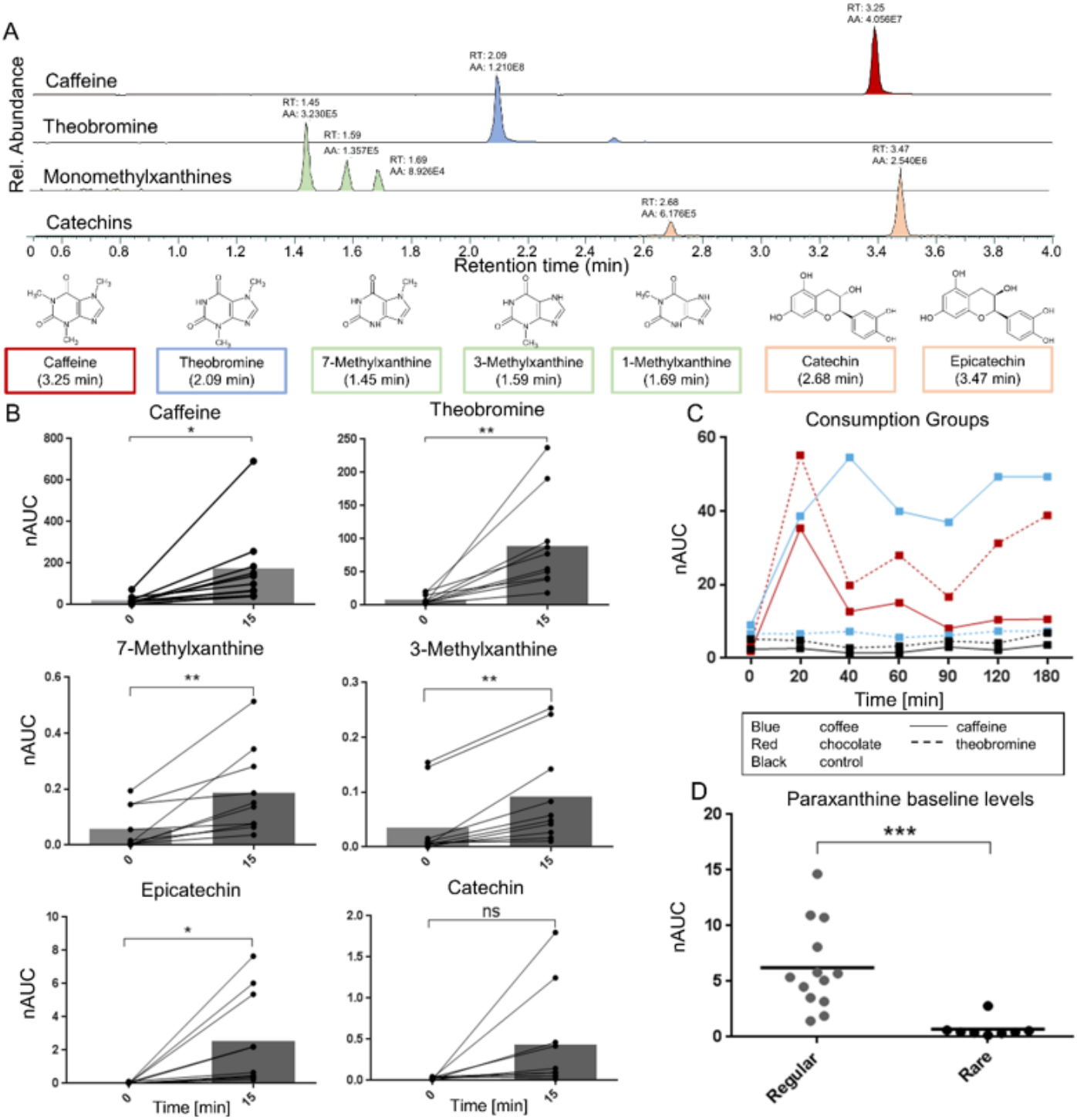

Figure 1

Imprints of food and beverages on finger sweat composition via high-resolution LC-MS/MS. (A) Characteristic metabolic profile after the consumption of chocolate exemplified by extracted ion chromatograms of caffeine (retention time (RT) = 3.25 min, m/z 195.0877), theobromine (RT = 2.09 min, m/z 181.0720), 7-, 3- and 1-methylxanthine (RT = 1.45, 1.59 and 1.69 min, m/z 167.0564) as well as catechin and epicatechin (RT = 2.68 and 3.47 min, m/z 291.0863) for a single donor. (B) Controlled study (study C) of 10 donors eating a 50 g chocolate bar. Normalised areas under the curve (nAUC) before (0

min) and 15 minutes after consumption are shown for caffeine, theobromine, 7-methylxanthine, 3-methylxanthine, epicatechin and catechin, demonstrating an increase in all individuals after 15 min. * p-value ≤ 0.05, ** p-value ≤ 0.01, ns not significant. (C) Time-course measurements of caffeine and theobromine shown for three donors consuming either a double espresso (blue), a 50 g chocolate bar (red) or neither (control, black) demonstrates an increase of those metabolites in a highly characteristic fashion. nAUC, normalised area under curve. (D) Comparison of paraxanthine baseline levels of regular (at least 1 cup of coffee per day) with rare coffee consumers. *** p-value ≤ 0.001; nAUC, normalised area under the curve.

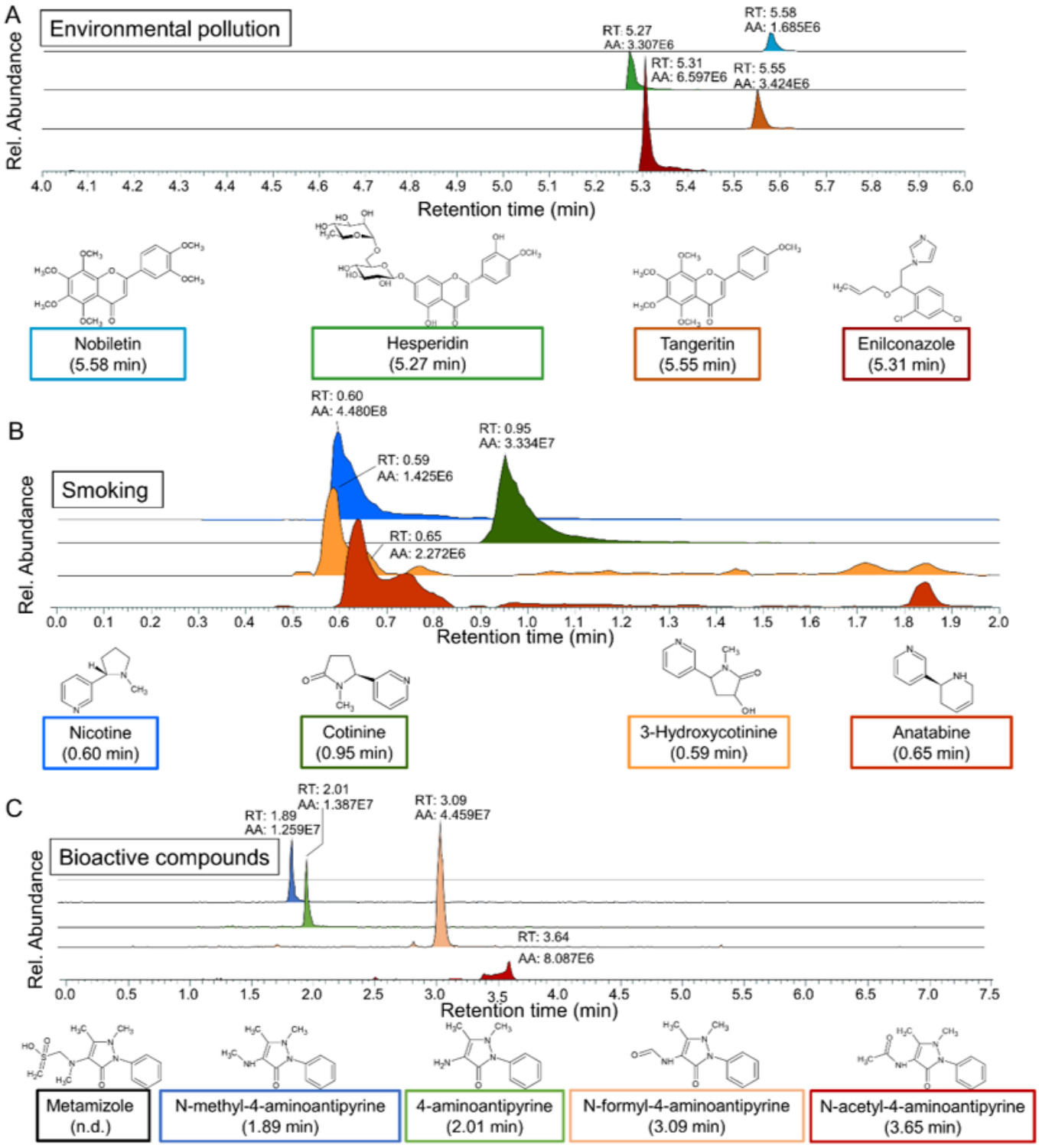

Figure 2

Metabo-tip reveals environmental pollution in nutrition, smoking habits and medication (A) The intake of an orange (donor from study A) results in the detection of distinct metabolites in finger sweat. Extracted ion chromatograms are shown for nobiletin (RT = 5.58 min, m/z 403.1387), hesperidin (RT = 5.27 min, m/z 611.1970), tangeritin (RT = 5.55 min, m/z 373.1282) and enilconazole (RT = 5.31 min, m/z 297.0556), which is a fungicide predominantly used in the agriculture of citrus fruits. (B) The imprint of

smoking on the finger sweat (donor from study B). Not only nicotine but also its metabolites were detected in sweat as well as anatabine, which is found in tobacco. Extracted ion chromatograms of nicotine (RT = 0.60 min, m/z 163.1230), cotinine (RT = 0.95 min, m/z 177.1022), 3-hydroxycotinine (RT = 0.59 min, m/z 193.0972), and anatabine (RT = 0.65 min, m/z 161.1073) are depicted. (C) Evidence for the intake of a pain killer by the successful detection of its metabolites in finger sweat. Extracted ion chromatograms of metamizole (m/z 312.1013) and its metabolites N-methyl-4-aminoantipyrine (RT = 1.89 min, m/z 218.1288), 4-aminoantipyrine (RT = 2.01 min, m/z 204.1131), N-formyl-4-aminoantipyrine (RT = 3.09 min, m/z 232.1086) and N-acetyl-4-aminoantipyrine (RT = 3.65 min, m/z 246.1237).

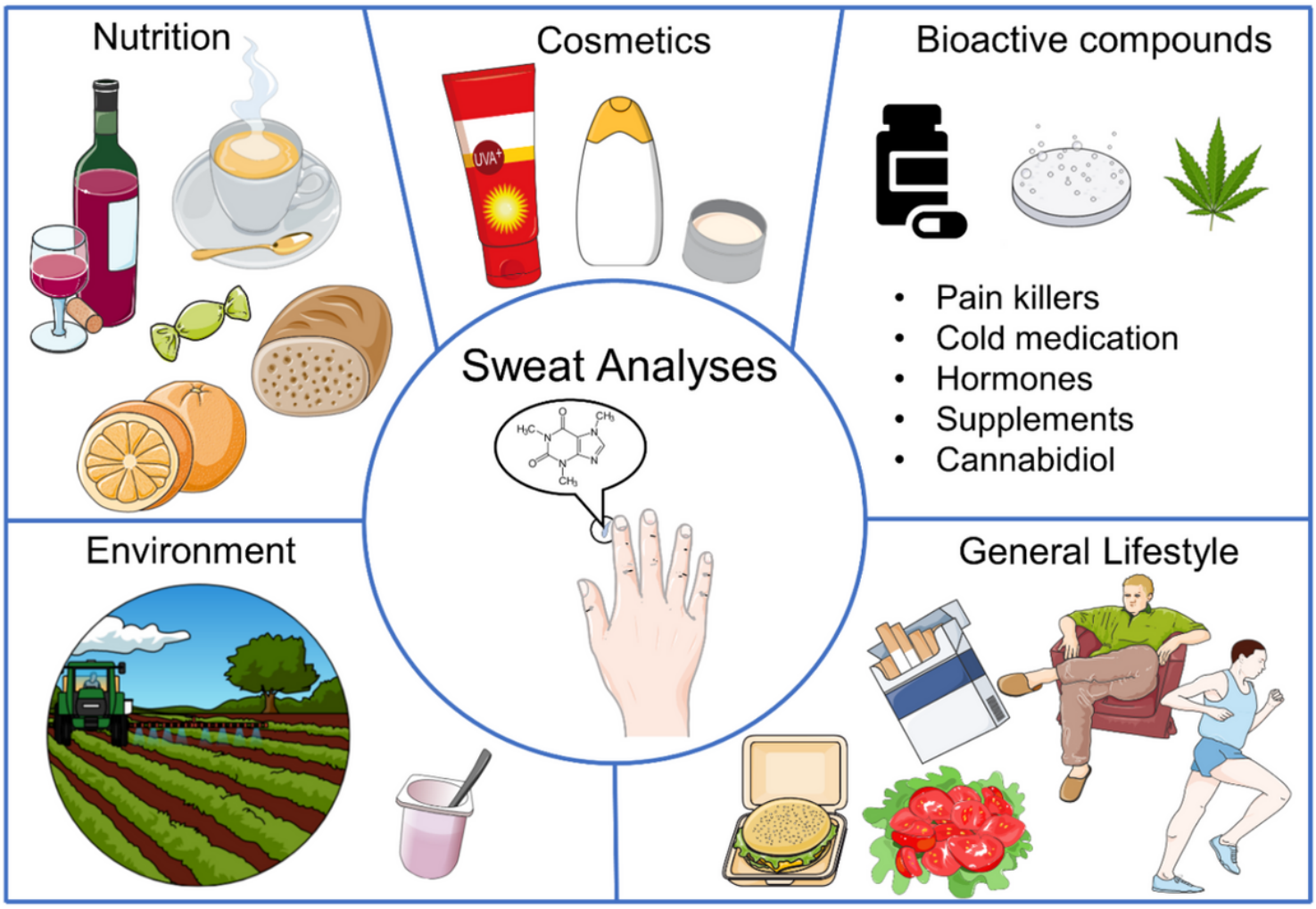

Figure 3

Monitoring lifestyle parameters via finger sweat analysis. Sweat composition is highly dynamic and changes when specific foods and beverages are consumed, when supplements are taken or when certain types of hygiene products have been used. Moreover, the general health status of a person can be assessed by revealing individual medication (e.g. for pain and fever, for colds, hormones, or cannabidiol). Next to other lifestyle habits like smoking, exposure to environmental toxins derived from consumed products like herbicides, pesticides and fungicides as well as toxic compounds in plastics found in the packaging of foods and beverages can be detected in the sweat of test subjects.

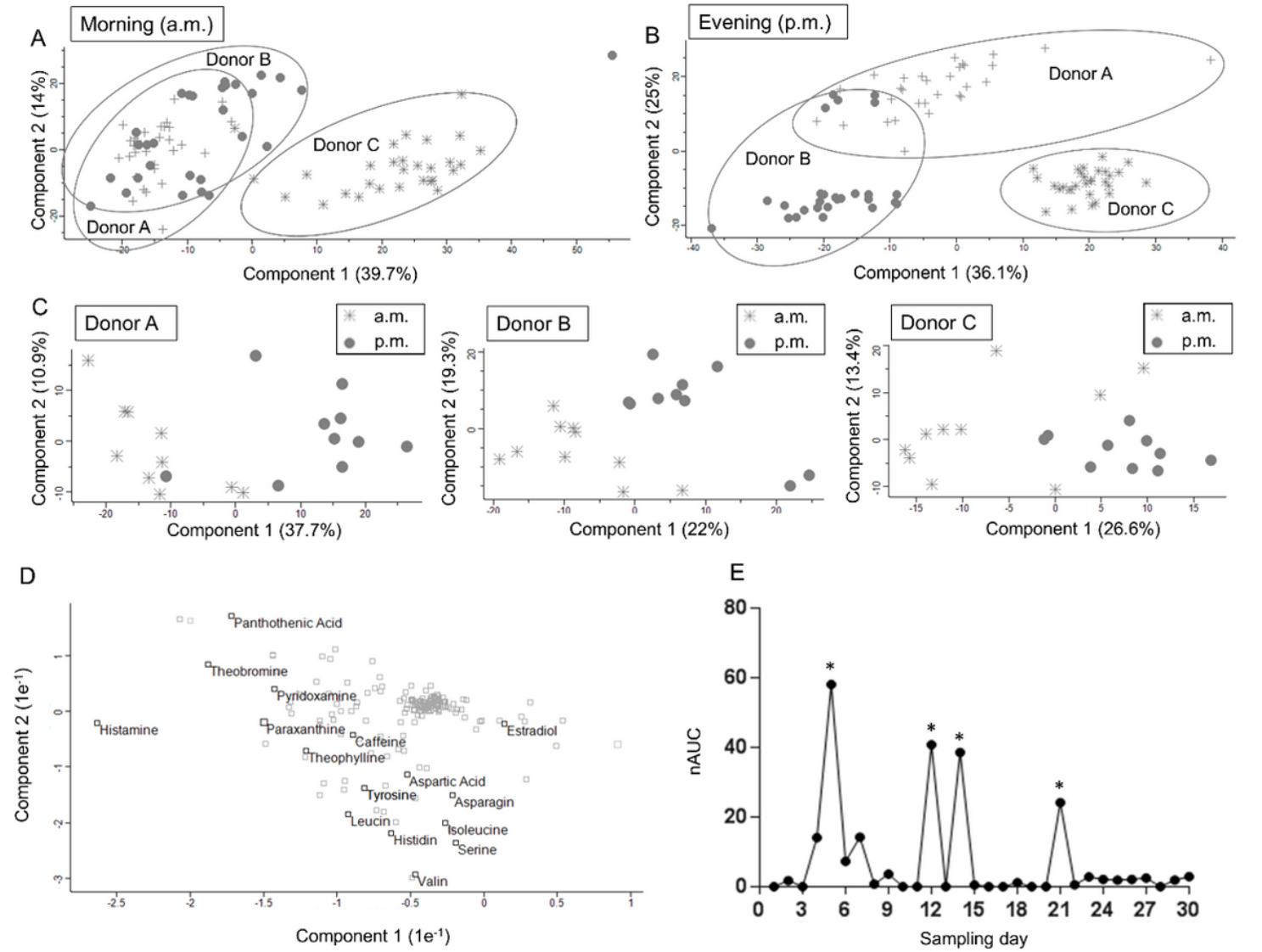

Figure 4

Diurnal metabolic fluctuations and individual endogenous responses. Principle component analyses (PCA) of finger sweat samples collected from three donors (study A) in the morning (A) and evening (B) over 30 days are depicted. (C) PCA of the finger sweat samples of three donors reveals diurnal metabolic fluctuations demonstrated by a consistent shift of component 1 to the right from morning to evening in each individual. (D) Loadings plot for the PCA depicted in Figure 4B, showing the strong influence of histamine, coffee or chocolate consumption (caffeine, theobromine, theophylline and paraxanthine), and general diet (amino acids) on component 1 and 2. (E) Time-course analysis of histamine over a time period of 30 days.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.xlsx