A headspace collection chamber for whole body volatilomics

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Abstract

The human body secretes a complex blend of volatile organic compounds (VOCs) via the skin, breath and bodily fluids, the study of which can provide valuable insight into the physiological and metabolic state of an individual. Methods to profile human-derived volatiles typically source VOCs from bodily fluids, exhaled breath or skin of isolated body parts. To facilitate profiling the whole body volatilome, we have engineered a sampling chamber that enables the collection and analysis of headspace from the entire human body. Whole body VOCs were collected from a cohort of 20 humans and analyzed by thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) to characterize the compounds present in whole body headspace and evaluate chemical differences between individuals. A range of compounds were detected and identified in whole body headspace including ketones, carboxylic acids, aldehydes, alcohols, and aliphatic and aromatic hydrocarbons. Considerable heterogeneity in the chemical composition of whole body odor and the concentration of its constituent compounds was observed across individuals. Amongst the most common and abundant compounds detected in human whole body odor were sulcatone, acetoin, acetic acid and C_6-C_{10} aldehydes. This method facilitates standardized and quantitative analytical profiling of the human whole body volatilome.

Keywords: human body odor; volatilomics; scent; headspace; metabolomics

Introduction

Human scent is a complex blend composed of volatile organic compounds (VOCs) emitted via the skin, breath, and bodily fluids. Across studies profiling volatile compounds emitted by the healthy human body, 1488 and 623 volatiles have been identified in breath and skin emissions respectively.[1] Many additional human-derived compounds may also remain unidentified or undetected due to limitations in analytical methods. Profiling VOCs can offer a wealth of information on the physiological and metabolic state of an individual, providing insight into disease,[2,3] diet and lifestyle,[4,5] environmental exposure,[6,7] and even the chemical attraction of arthropod disease vectors such as mosquitoes to humans.[8] The volatile compounds emitted by the human body are influenced by a variety of factors, such as diet, hygiene habits, disease state and the constitution of the human microbiome. As such, human body odor can be considered specific to the individual. As a result of the growing interest in human-derived VOCs and the information that can be gleaned by their study, numerous analytical techniques have been developed for the collection and evaluation of volatile compounds from different biological matrices.

The analysis of exhaled breath is amongst the most prominent areas of study in human volatilomics, driven by the desire to develop rapid, non-invasive diagnostics for disease. Metabolic changes in breath have been studied for a variety of purposes, particularly the detection of biomarkers for cancer,[9,10] chronic obstructive pulmonary disease (COPD),[11] asthma,[12] malaria,[13,14] and COVID-19.[15–18] Exhaled breath is typically collected into an inert polymer bag (Tedlar), followed by collection onto tubes containing a sorbent material such as Tenax or a carbon-based material.[19] Alternatively, breath samples can also be collected using specialized breathing masks which enable the capture of breath volatiles directed onto sorbent tubes.[20] Collected VOCs are subsequently analyzed by thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS). Though less common, direct mass spectrometry methods have been used for real-time sampling of exhaled breath, particularly selected ion flow tube mass spectrometry (SIFT-MS), secondary electrospray ionization mass spectrometry (SESI), and proton transfer reaction mass spectrometry (PTR-MS).[21] Gas chromatography-ion mobility spectrometry (GC-IMS) has furthermore been applied to breath

analysis, particularly in the field of rapid disease diagnostics due to its portability and low cost in comparison to benchtop mass spectrometers.[22]

A major contributor to human scent is the multitude of volatile compounds released from the skin. Skin emanations are derived from secretions from the eccrine, sebaceous and apocrine glands, in addition to metabolites produced by the human skin microbiome.[23] As glands and microbes are distributed differently across the body, discrete areas of the body can produce distinct scent profiles. Although less commonly the subject of metabolomics investigations, skin VOCs can provide insight into the human metabolome and be utilized across numerous fields of research. The study of skin emanations has been conducted to develop analytical techniques for the detection of human scent during search and rescue operations,[24–28] explore individual differences in human odor[29–31], evaluate the attraction of mosquitoes to human odor,[32–34] and to fundamentally understand the biological basis of human scent.[35–38] Skin VOCs are typically collected using sorbent materials placed in direct contact with the skin. Glass beads have been rubbed on the hands or feet of participants to collect skin secretions, after which compounds transferred to the beads are desorbed for analysis.[33,39–41] This form of sample collection has similarly been achieved using SPME fibers,[42] and polydimethylsiloxane (PDMS), including coated stir bars,[43] patches,[44,45] and wearable wrist bands.[34,46] Aside from contact-based sampling methods, skin emanations have been explored using sampling devices and bags to collect headspace from isolated body parts, namely the hands and feet, onto SPME fibers or thermal desorption tubes.[37,38,42]

Most human volatilomics studies to date focus on the detection of volatiles in a particular medium, such as exhaled breath, a bodily fluid, or from an isolated part of the body. A small number of studies have made efforts to characterize human whole body emissions, however these have excluded head and breath VOC emissions,[24,47,48] used techniques unsuitable for compound identification (such as low resolution ion mobility spectrometry),[49] or have only been applied to single participants or small cohorts.[50,51] In order to comprehensively characterize the chemical composition of whole body odor headspace, improved methods for the collection of human-derived VOCs must be developed and applied to larger cohorts in order to understand heterogeneity in the human scent signature and emission rates of constituent VOCs.

To facilitate chemical analysis of the human whole body volatilome, here we describe the development of a chamber for the controlled sampling of whole body headspace from individual

humans. We engineered an acrylic sampling chamber that is flushable with purified air to provide a standardized atmosphere for the collection of human-derived VOCs from a seated human. The chamber is sealed during sampling mode to enable sufficient concentration of VOCs, has multiple ports for VOC collection and a sealable door for participant entry and exit. We applied this booth-style sampling chamber and TD-GC/MS to profile individual whole body VOC headspace of a diverse cohort of 20 human participants. We quantified emission rates of 43 select VOCs that we annotated in human whole body odor, yielding high content human scent signatures. This study lays the foundation for application of this sampling method in combination with a variety of gas chromatography and mass spectrometry techniques to comprehensively profile the human volatilome for fundamental and applied purposes.

Materials and Methods

Whole Body Headspace Collection Chamber

A sampling chamber was constructed to enable the collection of VOCs from the human whole body. The 3.5’ L x 3.5’ W x 5.0’ H sampling chamber with a total volume of 1734.41 L was constructed within an aluminium frame (#9030, 80/20 Inc., Columbia City, USA) with four walls and a ceiling consisting of 0.6 mm thick polymethyl methacrylate sheets (ASTM D4802 CAT. B-1, Finish 1 Type UVA, Trident Plastics, USA) (Figure 1). The base of the chamber was a 6.4 mm thick acrylonitrile butadiene styrene white plastic sheet (Interstate Plastics, Sacramento, USA). The corners of the frame were fixed with nylon plastic 3-way corner connectors (#9150, 80/20 Inc., Columbia City, USA), and the chamber edges were sealed externally with black thermoplastic elastomer (#2117, 80/20 Inc., Columbia City, USA). A 2’ x 2’ door centered on the front wall of the chamber was fitted with magnetic discs (McMaster-Carr, Elmhurst, USA) to seal the door for participant entry and exit. On each side of the chamber were 4” duct flanges (#409004, DL Wholesale, Romulus USA), one of which was coupled to an inline fan and carbon filter (Model GLFANXINLINEEXPC4, iPower) using 4” aluminium ducting to enable rapid flushing of the chamber with filtered air prior to sampling. The alternate flange on the other side of the chamber was also connected to 4” aluminium ducting and served as the flushing vent. Sampling ports on each side of the chamber consisted of black plastic bulkheads and ¼” OD push-to-connect fittings (#MTC 1/4-N01, MettleAir, USA), which were coupled with low-power

pumps (Pocket Pump, SKC Inc., USA) to draw chamber air through ¼" PTFE tubing onto Tenax-TA thermal desorption tubes (Gerstel, USA), connected using ¼" Swagelok connectors. The sampling ports can be split with push-to-connect Y-unions to facilitate sampling onto additional TD tubes. During flushing mode, the sampling ports were plugged with push-to-connect plugs (#PP1/4, TechniFit). During sampling mode, the flushing port and flushing vent were plugged with steel duct end caps (#B08SBNNFJW, Europlast), and push-to-connect plugs removed from sampling ports to facilitate air sampling. A small fan (MiniFan, Comlife) was positioned internally above the chamber door to circulate air around the inside of the chamber to facilitate VOC uniformity. A monitor was positioned inside the chamber to record temperature, relative humidity and CO_2 levels (IAQ Mini, CO2Meter, USA). CO_2 was monitored throughout the sampling procedure both to ensure the safety of participants and to observe variation in carbon dioxide emissions between participants.

The chamber was situated in a temperature-controlled laboratory maintained at 21 ^{o}C. Prior to use, the interior surfaces of the chamber and accessories were wiped clean with 10% ethanol and the chamber flushed with filtered air at 20 L/s for 1 hr. Flushing of the chamber occurred immediately prior to background air sample collection and entry of participants into the chamber. Prior to sampling, Tenax-TA thermal desorption (TD) tubes (#020810-005-00, 6 x 60 mm, Gerstel, USA) were conditioned at 300 ^{o}C for 60 min in a stream of nitrogen at 50 mL/min^{-1} using the Gerstel tube conditioner (TC2, Gerstel, USA) and then spiked with 15 ng of 2-pentadecanone (Alfa Aesar, USA) as an internal standard. Background air samples from the chamber were collected simultaneously via sampling ports in triplicate onto TD tubes at 375 mL/min for 30 min immediately prior to participant sampling. Participants were instructed to change into clean scrubs (65/35 polyester/cotton, SmartScrubs, Phoenix, USA) washed only in water to reduce the introduction of exogenous VOCs from non-standardized clothing. Participants were then instructed to remove their socks and enter the chamber via the chamber door.

To maximise skin exposure for VOC collection, once inside the chamber participants were instructed to uncover the lower half of their legs by rolling the pant legs of the scrubs to be level with their knees. The provided scrub shirts were short sleeved, and therefore skin surfaces on the lower arms below the elbows were readily exposed. Participants were seated throughout the sampling procedure on a small high-density polyethylene and steel chair (#1173094, Lifetime,

Riverdale USA). Human whole body odor samples were collected as per background sample collection for 30 min. Three replicate human odor samples were collected from the chamber for each participant. The full experimental workflow is detailed in Figure S1.

During chamber development, a pilot human subject cohort of 10 participants was recruited to monitor chamber oxygen levels and participant blood oxygen levels during the 30 min sampling period. Chamber O_2 levels were monitored with a Coreel 4 portable gas monitor (CoreEL Technologies) and blood oxygen levels were monitored with a CMS-50D1 fingertip oximeter (AccuMed).

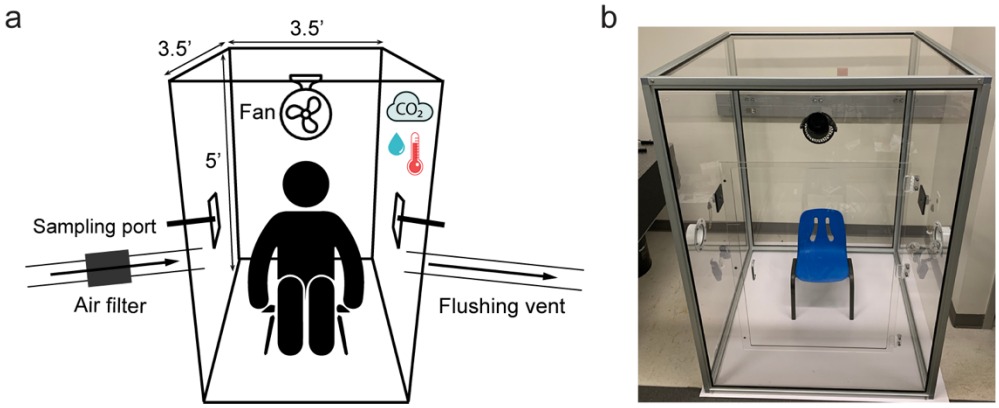

Figure 1. Whole body headspace collection chamber. (A) Schematic and (B) image of the booth-style headspace collection chamber for whole body volatilomics with a volume of ~1700L. Chamber dimensions are indicated. Prior to participant entry, the chamber is flushed with filtered air. VOC sampling occurs via symmetrical sampling ports on adjacent walls of the chamber. A fan positioned above the seated participant ensures homogeneity of VOCs across sampling ports. Temperature, humidity, and CO_2 levels inside the chamber are actively monitored during sampling. A sealed door in the front of the chamber facilitates participant entry and exit.

Human Participants

A cohort of 20 healthy adults was recruited from the Baltimore metropolitan area (MD, USA) consisting of 10 males and 10 females with an age range of 19-39 years and a median age of 26.5 years. Participants were 55% white, 10% black or African American, 25% Asian, and 10% more than one race. The study was approved by the Johns Hopkins Bloomberg School of Public Health (JHSPH) Institutional Review Board (IRB no. 00014626) and all participants gave written informed consent prior to participation. Participants were provided with fragrance-free

shampoo and body wash (Vanicream, USA) to wash with prior to providing a whole body odor sample. All participants were requested to shower within 24 hr prior to sampling. After washing, participants did not use any other cleaning products, deodorants, cosmetics, skin creams or fragrances. For 12 hr prior to sampling participants were asked to refrain from the consumption of alcohol and odorous foods such as garlic, onions, and spicy foods. On the day of sampling, participants were required to provide details of their recent hygiene practices, diet, and occurrence of smoking or vaping. All individuals recruited complied with the study requirements and thus all were able to participate. It should be noted that whilst steps were taken to reduce the presence of VOCs (such as from hygiene products) traces of exogenous materials may still remain on the body without requiring participants to refrain from their use for substantially longer periods of time. Sampling for all participants occurred between the hours of 9:00 – 13:00. No adverse events to the study protocol were reported post-participation.

TD-GC/MS Analysis

Samples were analyzed by thermal desorption gas chromatography/mass spectrometry (7890B GC, 5977N MSD, Agilent, USA). Tenax-TA tubes were placed in a Gerstel Thermal Desorption Unit mounted onto a Gerstel Cooled Injector System (CIS4) PTV inlet (Gerstel, USA). Analytes were desorbed in splitless mode starting at 30 ºC followed by an increase of 720 ºC/min to 280 ºC and held for 3 min. Analytes were swept into the inlet which was held at -70 ºC and then heated at 720 ºC/min to desorb analytes onto a HP-INNOWAX capillary column (30 m length x 0.25 mm diameter x 0.25 µm film thickness). The GC oven was programmed with an initial temperature 40 ºC with a 2 min hold followed by an increase of 10 ºC/min to 250 ºC with a 5 min hold. A helium carrier gas with a flow rate of 1.2 mL/min^{-1} was used. The MS analyser was set to acquire over a range of *m/z* 30-300 and was operated in EI mode. The ion source and transfer line were set to 230 ºC and 250 ºC respectively.

Data were deconvoluted in Agilent Unknowns Analysis software (RT window size factor of 50 and 100, absolute area 1000 counts, peak sharpness threshold 25%, min match factor 50) and exported as .csv files. Identifiable analyte peak areas occurring above the limits of detection were normalized to the internal standard, background-subtracted, and mean values were taken from the three technical replicates. Compound identification was achieved by comparison of mass spectra with the NIST Mass Spectral Library version 2.2 and retention time matching with

analytical reference standards. MetaboAnalyst 5.0 was used for the production of heat maps and chemometric analysis. The heat map was produced using data normalized to the internal standard and on a logarithmic scale.

Results and Discussion

Sampling Chamber Development

To enable the sufficient accumulation of human body VOCs in the sampling chamber and prevent contamination with laboratory air, a sealed chamber is required. Air exchange within the chamber in sampling mode was evaluated using the CO_2 concentration decay test, a method used to determine air change in a room or container based on the decreasing concentration of a tracer gas.[52] A short pulse (~5 seconds) of compressed carbon dioxide (CD USP50, Airgas, Rosedale, MD) was introduced into the sealed chamber via one of the sampling ports and the CO_2 concentration monitored over time. Air exchange rate was calculated using equation (1): [53]

$$A_D = 1/\Delta t In\{(C_1 - C_R)/(C_0 - C_R)\} \qquad (1)$$

Where A_D is the air exchange rate, Δt is the period between measurements (hours), C_0 and C_1 are the measured CO_2 concentrations over the decay period (ppm), and C_R is the CO_2 concentration in the replacement air (i.e. the air outside the chamber). Using three replicate calculations across over five hours, the air change rate was calculated to be 0.072 air changes per hour, demonstrating a suitably low air exchange rate from the sealed chamber for controlled sampling.

During chamber development, we monitored chamber oxygen levels and participant blood oxygen concentrations to confirm safety during sampling mode within the chamber. Over the 30-minute duration of the sampling period, chamber oxygen levels decreased an average of 0.06% (mean starting O_2 concentration = 20.9%, mean ending O_2 concentration = 20.84%), with no measurable decrease detected for 90% of participants (n=10 total). At no point did chamber oxygen levels fall below the 19.5% concentration deemed to be considered an oxygen deficient environment by the Occupational Safety and Health Administration (OSHA).[54] Blood oxygen concentrations decreased an average of 0.6%, always remaining within the normal range of

peripheral oxygen saturation (SpO2) of 95-100% as measured by pulse oximetry,[55] indicating a minimal change in participants' blood oxygen levels throughout the sampling process (Table S1).

To ensure reproducible sampling from the collection ports, the chamber was fitted with a fan to circulate air and aid VOC uniformity throughout the chamber. In an initial test with a single participant, we measured 6 VOCs from varied structural classes and of known human origin in whole body headspace to evaluate sampling reproducibility across three collection ports. The average relative standard deviation (RSD) of pinene, hexanal, undecane, acetoin, sulcatone, and acetic acid across three sampling ports were 11.3, 10.6, 4.9, 7.1, 8.5, and 11.0 % RSD respectively, demonstrating low sampling variability across the sampling ports.

To ensure a clean chamber background prior to sample collection from humans, the interior surfaces of the chamber were wiped with 10% ethanol and the chamber flushed with filtered air. Background chamber air samples were collected in triplicate to confirm the removal of background contaminants and identify pre-existing components to disregard from human odor samples. Some artefacts from the plexiglass sampling chamber itself were present in all background samples, such as siloxanes ubiquitous in the laboratory, however no background contaminants interfered with peaks from human-derived analytes of interest. Headspace samples of the "scent-free" shampoo and body wash provided to participants were also collected and analyzed to determine the presence of any potential contaminants. The chemical profiles of these cleansing products were dominated by a large 1,2-hexanediol peak, the presence of which was disregarded from human odor samples.

We engineered our whole body headspace collection chamber to be transportable using materials that can be flat-packed and assembled within a tubular aluminium frame with corner connectors. This frame conveniently has flanges that allow the walls and roof of the chamber which are made from transparent plexiglass panels to slot into position, as well as a floor made of ABS plastic. These materials were chosen due to their inert and impact resistant nature. The use of transparent plexiglass panels also allows clear observation of each participant during sampling. Previously, whole body sampling chambers have been engineered using welded stainless steel and glass.[24,50,51] While these materials also provide excellent inert qualities and air tightness once sealed, we anticipate that the durability and relative light-weight nature of the materials used for construction of our whole body headspace collection chamber, will facilitate

ease of its assembly and portability in a wide-variety of laboratory, clinical and field-based contexts.

Chemical Constitution of Human Whole Body Headspace

The human headspace collection chamber enabled the profiling of human whole body volatile organic compounds, in addition to facilitating quantification of exhaled CO_2 and measurement of water vapor from 20 healthy human participants. Carbon dioxide is one of the primary VOCs in exhaled breath, present at approximately 4-5% by volume and produced as a by-product of cellular metabolism. The measurement of respiratory CO_2 can provide clinical insight into the severity of respiratory conditions such as asthma and COPD,[56] how effectively CO_2 is being eliminated from the body,[57] and response to medical interventions such as tracheal intubation and anaesthesia.[58] Carbon dioxide is also a crucial VOC in the attraction of arthropod disease vectors, such as mosquitoes, black flies, triatomine bugs and bed bugs to humans,[59–62] and variation in exhaled CO_2 may play a role in the differential attractiveness of individuals to mosquitoes. For instance, Brady *et al* assessed the attraction of mosquitoes to different human hosts over a 10 week period, demonstrating that variation of CO_2 production between different human participants may be a major chemical factor driving differential attractiveness.[63] Other areas of interest for the study of exhaled CO_2 include exercise monitoring,[64–66] the development of chemical sensors to detect trapped humans in search and rescue operations,[25,67] and the monitoring of human contributions to indoor air contaminants.[68]

In this study, the concentration of CO_2 in the sampling chamber was recorded at 5-minute intervals throughout the 30-minute sampling period. Chamber CO_2 was monitored both to ensure CO_2 levels did not exceed permissible exposure limits as established by OSHA (5000 ppm as an 8 hour time-weighted average)[69] and to evaluate individual differences in CO_2 emissions. Across the 20 participants, CO_2 emissions over the 30-minute sampling period ranged from 89 to 197 ppm/min, with a mean emission of 135 ppm/min. The total CO2 concentration in the sampling chamber at the end of the 30 min sampling period ranged from 3130 ppm to 6407 ppm with a mean total of 4482 ppm. There was a moderate correlation between body weight and CO_2 emission rates (Figure 2), although variation in CO_2 emissions were observed between participants of similar weights.

Temperature and humidity within the sampling chamber were also recorded at the beginning of the experiment and at 5-minute intervals throughout the sampling process. The laboratory in which the human headspace collection chamber was situated was maintained at a constant temperature and the mean starting temperature inside the chamber was 21.1 ºC. By the end of the 30-minute sampling period, the interior temperature of the chamber increased by an average of 2.5 ºC (range of 1.7 to 3.2 ºC increase). Average starting relative humidity in the sampling chamber was 56.9% with an average increase of 19.3% (range of 12 to 30%) by the end of the 30-minute sampling period. In this study, the sorbent tubes used for VOC sampling contained only hydrophobic Tenax-TA which does not retain water and thus the increase in relative humidity was not deemed problematic for downstream VOC analysis.

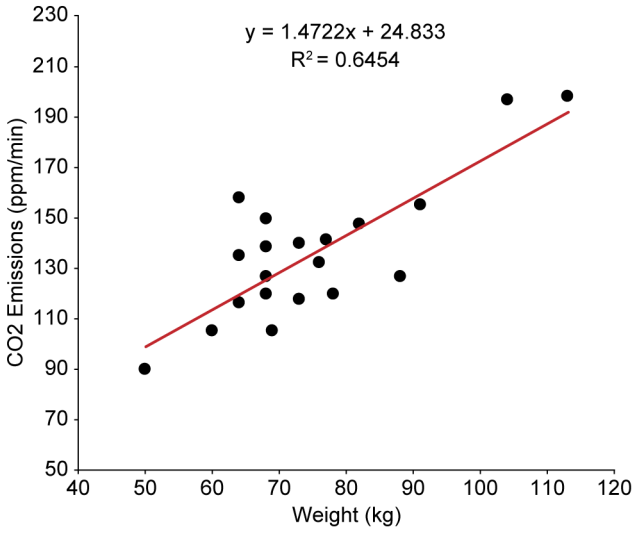

Figure 2. Whole body carbon dioxide emission rates are moderately correlated with human body size. Whole body carbon dioxide emissions (ppm/min) and body weights (kg) of each human sampled are plotted. n = 20 participants.

A large number of VOCs were detected in human whole body headspace belonging to a range of compounds classes, including aldehydes, ketones, carboxylic acids, alcohols, and aliphatic and aromatic hydrocarbons. Across the 20 participants, we detected a minimum of 797 and maximum of 1140 features, with a mean of 983 features detected in human headspace. On average, 326 of these features had a possible match in NIST library. In this study, the identity of approximately 13% of features with a NIST match have been confirmed. See Table S2 for a breakdown of the number of features detected in the headspace of each participant.

Whole body headspace exhibited heterogeneous chemistry across the 20 participants, as detailed in Figure 3, which shows the relative abundance of 43 confirmed VOCs annotated in headspace samples. These compounds were selected based on their frequency of detection and availability of analytical standards to validate their identity. The majority of these 43 detected compounds were conserved in human whole body headspace, present in all or many of the participants (Table 1), though distinct differences were observed in the relative abundance of the compounds detected. Furthermore, the quantity of individual compounds detected varied considerably across participants (Figure 4).

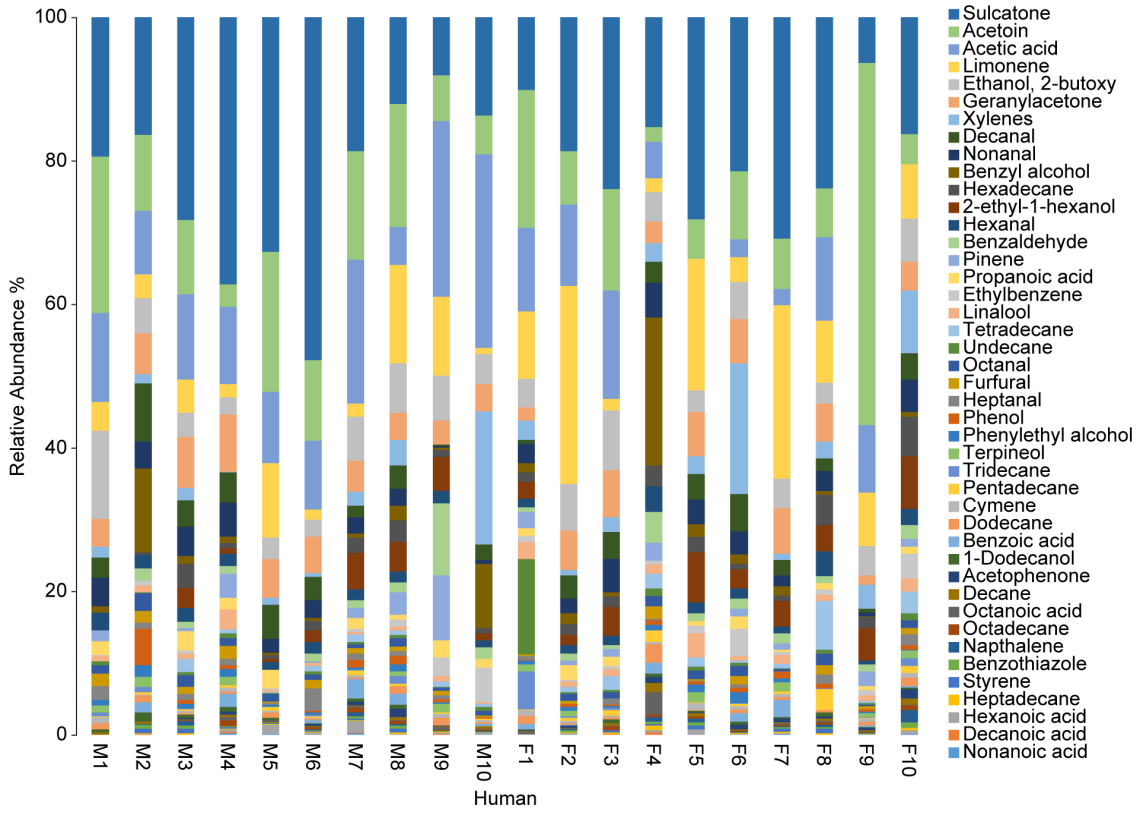

Figure 3. Relative abundances of 43 volatile organic compounds detected in whole body headspace from humans. Columns represent relative VOC signatures from individual humans, with compounds organized approximately from most to least abundant. n = 20 participants. M = male, F = female.

The compounds we detected in whole body headspace represent a broad range of VOCs of endogenous and microbial origin from skin emissions and exhaled breath. Many of these are of known interest in various areas of metabolomics. Squalene is an abundant component of human sebum, a lipid-rich substance secreted by the sebaceous glands.[70] Although not a volatile

compound, squalene is a major contributor to human odor due to the number of volatiles that have been demonstrated to originate from the reaction of squalene with ozone present in the atmosphere. Numerous studies have demonstrated that ozone oxidation of squalene results in the production of various VOCs including 6-methyl-5-hepten-2-one (sulcatone), 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), acetone, hydroxyacetone and 4-oxopentanal.[71–73] Sulcatone and geranylacetone were amongst the most abundant compounds detected in whole body headspace, present in 100% of participants. Both compounds are of particular interest in the study of mosquito host-seeking and are potentially important human odor cues in the differential attraction of mosquitoes to different individuals.[74,75]

Similarly, long-chain aldehydes are known products of the reaction between numerous unsaturated fatty acids found on the skin and ozone.[73] In this study, several aldehydes were detected, in particular hexanal, heptanal, octanal, nonanal and decanal, which were found in the whole body headspace of almost all participants. Aldehydes are of particular interest in clinical metabolomics, with numerous studies detecting changes in the aforementioned aldehydes in breath and skin emissions with various diseases including lung cancer,[76,77] breast cancer,[78] colorectal cancer,[79] COPD,[80] and malaria.[81–83] The whole body headspace of all participants contained substantial amounts of 3-hydroxy-2-butanone (acetoin), a known bacterial metabolite produced during the catabolism of pyruvate. Acetoin has previously been detected in the headspace of several types of bacteria, including *Staphylococcus epidermidis*[84,85] and *Staphylococcus aureus*[85,86] both of which are present on human skin. The high abundance of acetoin in human headspace may therefore primarily be a bacterial metabolite, though acetoin has also been detected in exhaled breath and has been highlighted as a potential breath biomarker for lung cancer.[87–89]

Short chain fatty acids (SCFAs) have been previously reported to be abundant components of human odor, produced via the catabolism of skin lipids into long-chain fatty acids and subsequently highly volatile SCFAs by *Staphylococcus, Propionibacteria* and *Corynebacteria*.[90] Furthermore, VFAs can also be produced from the conversion of branched aliphatic amino acids by *Staphylococcus* bacteria.[91] In this study, acetic acid was amongst the most abundant compounds present and was detected across 90% of the participants. Other volatile fatty acids were also detected, including propanoic and hexanoic acid, though these were present in considerably lower concentrations than acetic acid.

In addition to SCFAs, longer chain saturated fatty acids were detected at relatively low abundance, including octanoic, nonanoic and decanoic acid. Although readily produced by skin bacteria, volatile fatty acids are also present in breath and are linked to oral malodor.[92] Finally, the presence of fatty acids in human odor may also be a product of oxidation. Pleik *et al* conducted a study on degradation products in fingerprint residues, demonstrating decanoic acid was produced from the oxidation of decanal, which was amongst the most abundant aldehydes detected in whole body headspace using the sampling chamber.[93] Organic acids in exhaled breath have gained some clinical interest in recent years, with changes in the levels of certain carboxylic acids being associated with lung cancer[94] and gastrointestinal cancer.[95,96] Furthermore, carboxylic acids are known attractants to anthropophilic mosquitoes, and the further study of these in human odor could provide important insight into mosquito host-seeking.[97,98] Benzoic acid, an aromatic carboxylic acid detected in the whole body headspace of 55% of participants in this study, is a breath- and skin-derived compound that has been linked to both stress response and respiratory disease. Martin *et al* demonstrated the upregulation of benzoic acid in skin VOCs following psychological stress,[45] whereas Dallinga *et al* identified benzoic acid in exhaled breath is a biomarker for distinguishing asthma patients from healthy controls.[99]

The majority of compounds profiled using this method can be attributed to endogenous and microbial volatiles released from the skin, though some VOCs detected in whole body headspace are likely derived from exhaled breath (Table 1). Numerous aromatic hydrocarbons and terpenes were identified, including xylene, ethylbenzene, styrene, limonene, pinene, and cymene, all of which have been frequently detected in exhaled breath in previous studies.[1] Aliphatic hydrocarbons were also detected in this study, which have been previously detected in both exhaled breath and skin emissions.[1] Although volatile hydrocarbons are found in the exhaled breath of healthy individuals, several of the compounds, including styrene, xylene and ethylbenzene, are known biomarkers of environmental exposure. The elevated presence of some of these aromatic compounds has been found in the breath and biological fluids of chemical plant workers,[100] petrochemical industry workers,[101] and smokers.[102,103] Similarly, elevated levels of certain alkanes in exhaled breath have been described as biomarkers of oxidative stress.[104]

In this study only thermal desorption tubes with Tenax-TA sorbent were utilized, which is not suitable for the trapping of highly volatile species.[105] For instance, acetone and isoprene, which are well known to be two of the most abundant VOCs in human breath, were not well

captured using this sorbent. In future studies, the use of additional sorbents such as Carboxen and Carbopack may enable the collection and detection of a broader range of volatile compounds. Furthermore, the use of alternative techniques could be employed to improve analyte identification and quantification. The use of two-dimensional GC (GCxGC) would provide an additional degree of separation, enabling the improved separation and detection of co-eluting analytes and reducing the need for deconvolution for analyte separation and detection. Furthermore direct mass spectrometry, such as proton-transfer reaction MS (PTR-MS), could be utilized for the real-time analysis of compounds difficult to detect by traditional GC/MS, such as highly volatile compounds. The use of such instrumentation can furthermore enable VOC analysis without the need to collect samples onto sorbent tubes, reducing the potential for loss or reaction of analytes. In addition to PTR-MS, gas-specific monitors could be used to measured highly volatile compounds such as ammonia.

Such integrative approaches may further be used to comprehensively characterize the whole human body volatilome and understand the temporal stability of individual scent signatures over time. A comprehensive understanding of the qualitative and quantitative composition of whole body VOC signatures may also assist to demonstrate what body parts and associated gut, oral and skin microbiome communities contribute most to human body headspace. Additional participant monitoring could also be implemented, including skin conductivity, skin hydration, and heart rate. Future studies could also benefit from increased control of participant conditions prior to sampling including defined dietary regimes and standardized hygiene practices. Restricted use of hygiene products and intake of certain foods for longer time periods prior to sampling may help to reduce exogenous volatiles. Furthermore, slight differences in the time between washing and sampling could introduce variability in the type and abundance of VOCs detected, thus stricter control of these activities could be implemented. It should also be noted that the cotton scrubs and the seated position of participants that we implemented for scent collection, may have excluded or altered the release rates of VOCs from certain areas of the body covered by these substrates, thus influencing compounds profiled in this study protocol. We employed standardized cotton scrubs to minimize potential introduction of exogenous volatiles during sampling and provided participants with seating to maximize their comfort and minimize potential for venous pooling, commonly associated with standing, throughout the 30 minute

sampling period. However, if of interest, future studies may choose to evaluate the effects of clothing and body position on the chemical composition of the whole body volatilome.

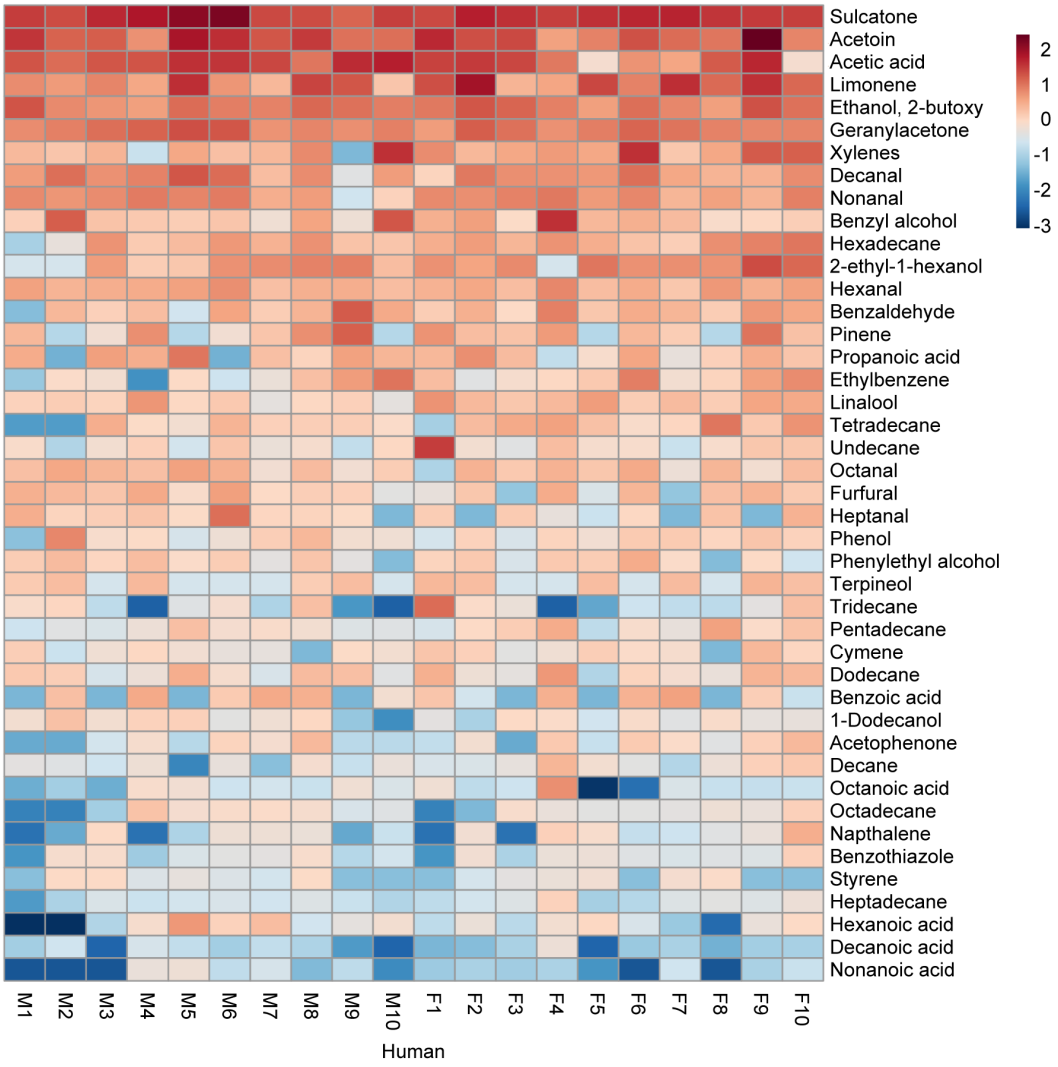

Figure 4. Heatmap of 43 volatile organic compounds detected in whole body headspace, ordered from highest to lowest mean absolute abundance across humans. VOC signatures from individual male (M) and female (F) participants are arranged across columns, n = 20 total. Scale bar represents concentration of analytes detected normalized to 15 ng of a 2-pentadecanone internal standard, with red indicating a higher concentration and blue indicating lower concentration. Heatmap constructed using MetaboAnalyst 5.0 using logarithmic scaling and data obtained from three replicate samples.

This study demonstrates the utility of our headspace collection chamber design for standardized sampling and quantitative analysis of human whole body volatilome. The high-content scent signatures derived using this method highlight the most frequent and abundant

components of whole body VOC emissions, as well as the complexity and heterogeneity of human body odor. This method could be a powerful addition to several fields of research in human volatilomics, including clinical metabolomics to detect changes in the chemistry of human scent in response to disease and stress, exposomics to study evaluated human exposure to environmental contaminants, and chemical ecology to identify VOCs that attract arthropod disease vectors to human hosts.

Conclusion

Human odor is a complex blend of volatile compounds released via the skin, breath and bodily fluids, but characterizing whole body odor in its entirety has been challenging due to limitations in available sampling configurations. This study aimed to develop a controlled and standardized method for headspace collection from seated humans to facilitate chemical analysis of the human whole body volatilome. A booth-style sampling chamber was engineered and used to profile whole body volatiles from a pilot cohort of 20 human participants. Human headspace samples were collected onto Tenax-TA thermal desorption tubes and analyzed by TD-GC/MS to identify and quantify human odor components. This approach enabled the characterization of a broad range of endogenous and microbial skin and breath-derived volatiles, including ketones, aldehydes, hydrocarbons, carboxylic acids and alcohols. Many compounds were identified across all participants, whereas some were only present in the headspace of select participants at varying levels. Such inter-individual variation in VOC frequency and abundance highlights both common and heterogeneous features of human scent chemistry. This new analytical approach to profile the human whole body volatilome could be readily used to characterize the contribution of the human microbiome to VOCs detected in whole body headspace, and for varied applications in clinical metabolomics, exposomics, chemical ecology, security and forensics to yield high-content human scent signatures.

Conflicts of interest

There are no conflicts of interest to declare.

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Table 1. Volatile organic compounds detected in human whole body headspace. Identified compounds ordered by detection frequency and mean emission rate.

Compound	CAS No.	Chemical Class	Detection Frequency	Mean Emission Rate (µg/hr)	Possible Origin
6-methyl-5-hepten-2-one (Sulcatone)	110-93-0	Ketone	100%	14.56	Skin (squalene oxidation, breath)[73]
3-hydroxy-2-butanone (Acetoin)	513-86-0	Ketone	100%	10.48	Breath, skin (microbial metabolite)[85]
Limonene	138-86-3	Terpene	100%	5.65	Breath, skin (diet, cleaning products)[57]
Ethanol, 2-butoxy-	111-76-2	Alcohol	100%	3.19	Skin (microbial metabolite)[106]
6,10-dimethyl-5,9-undecadien-2-one (Geranylacetone)	3796-70-1	Ketone	100%	3.07	Skin (squalene oxidation)[73]
Decanal	112-31-2	Aldehyde	100%	1.98	Breath, skin (fatty acid degradation, microbial metabolite)[73,85]
Nonanal	124-19-6	Aldehyde	100%	1.72	Breath, skin (fatty acid degradation)[31,73]
Benzyl alcohol	100-51-6	Alcohol	100%	1.63	Breath, skin (toluene metabolism, microbial metabolite)[5,85]
Hexanal	66-25-1	Aldehyde	100%	1.04	Breath, skin (fatty acid degradation)[31,73]
Undecane	1120-21-4	Hydrocarbon	100%	0.74	Breath, skin (lipid peroxidation, microbial metabolite)[104,106]
Linalool*	78-70-6	Terpenoid	100%	0.67	Breath, skin (unknown)
Dodecane	112-40-3	Hydrocarbon	100%	0.44	Breath, skin (microbial metabolite)[85,106]

Pentadecane	629-62-9	Hydrocarbon	100%	0.32	Breath, skin (lipid peroxidation, microbial metabolite)[85,104]
Xylenes	1330-20-7	Aromatic	95%	2.45	Breath (exogenous)[45]
Hexadecane	544-76-3	Hydrocarbon	95%	1.11	Breath, skin (lipid peroxidation)[104]
Benzaldehyde	100-52-7	Aldehyde	95%	1.01	Breath, skin (benzyl alcohol oxidation, microbial metabolite)[85,106,107]
Ethylbenzene	100-41-4	Aromatic	95%	0.72	Breath, skin (exogenous)
Octanal	124-13-0	Aldehyde	95%	0.61	Breath, skin (fatty acid oxidation)[73]
Phenol	108-95-2	Aromatic	95%	0.39	Breath, skin (microbial metabolite)[85]
1-Dodecanol	112-53-8	Alcohol	95%	0.21	Breath, skin (microbial metabolite)[85]
Octanoic acid	124-07-2	Acid	95%	0.18	Breath, skin (sebaceous gland secretions)[108]
Decane	124-18-5	Hydrocarbon	95%	0.18	Breath, skin (lipid peroxidation, microbial metabolite)[85, 104,106]
Heptadecane	629-78-7	Hydrocarbon	95%	0.09	Breath, skin (lipid peroxidation)[104]
Acetic acid	64-19-7	Acid	90%	6.95	Breath, skin (human metabolism, microbial metabolite)[49, 57,106]
Propanoic acid	79-09-4	Acid	90%	0.81	Breath, skin (microbial metabolite)[85]
Tetradecane	629-59-4	Hydrocarbon	90%	0.65	Breath, skin (lipid peroxidation, microbial metabolite)[85,104]
Furfural	98-01-1	Aldehyde	90%	0.51	Breath, skin (unknown)
Phenylethyl Alcohol	60-12-8	Alcohol	90%	0.35	Skin (microbial metabolite)[85]

Cymene	527-84-4	Aromatic	90%	0.26	Breath (diet)[109]
Hexanoic acid	142-62-1	Acid	90%	0.23	Breath, skin (microbial metabolite)[110]
Benzothiazole	95-16-9	Thiazole	90%	0.13	Breath, skin (unknown)
2-ethyl-1-hexanol	104-76-7	Alcohol	85%	1.82	Breath, skin (exogenous)[111]
Tridecane	629-50-5	Hydrocarbon	85%	0.34	Breath, skin (lipid peroxidation, microbial metabolite)[85,104]
Acetophenone	98-86-2	Ketone	85%	0.22	Breath, skin (unknown)
Octadecane	593-45-3	Hydrocarbon	85%	0.17	Breath, skin (lipid peroxidation)[104]
Decanoic acid	334-48-5	Acid	85%	0.04	Breath and skin (sebaceous gland secretions)[108]
Heptanal	111-71-7	Aldehyde	80%	0.49	Breath, skin (fatty acid degradation)[31]
Naphthalene	91-20-3	Aromatic	80%	0.16	Skin (microbial metabolite, exogenous)[112]
Pinene	80-56-8	Terpene	75%	0.98	Breath, skin (exogenous)[37]
Nonanoic acid	112-05-0	Acid	75%	0.04	Breath, skin (sebaceous gland secretions)[108]
Benzoic acid	65-85-0	Acid	65%	0.41	Breath, skin (benzyl alcohol oxidation)[107]
Styrene	100-42-5	Aromatic	65%	0.12	Breath, skin (microbial metabolite)[85]
Terpineol	8000-41-7	Terpenoid	55%	0.35	Breath, skin (exogenous)[57]

* Tentative identification by mass spectral library matching only.

Supplementary Information

A headspace collection chamber for whole body volatilomics

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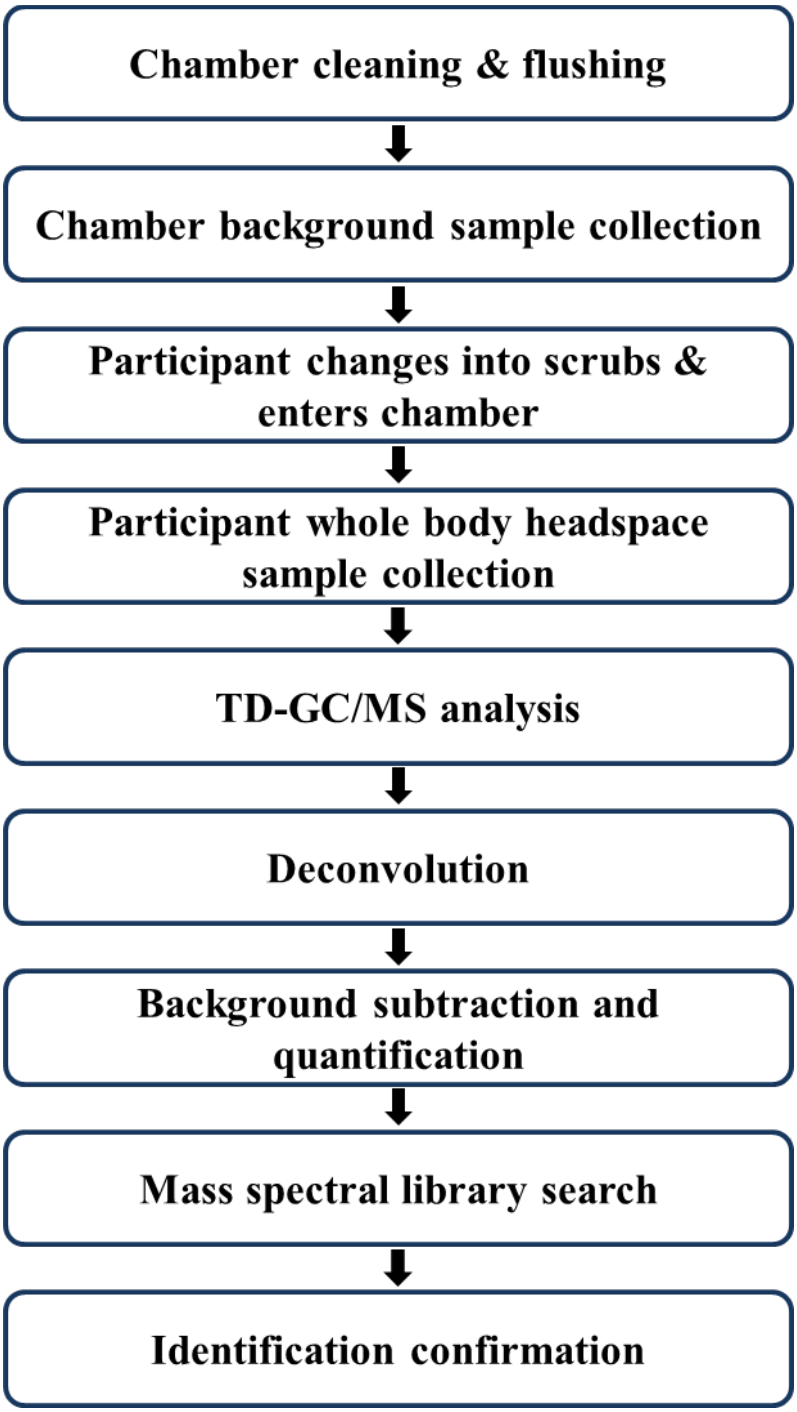

Figure S1. Schematic of the experimental workflow.

Table S1. Sampling chamber and blood oxygen levels across 10 participants at the start (0 mins) and end (30 mins) of whole body headspace sampling.

Participant	Sex	Weight (kg)	Starting Chamber O2 (%)	Ending Chamber O2 (%)	Chamber O2 Change (%)	Starting SpO2 (%)	Ending SpO2 (%)	SpO2 Change (%)
1	Female	48	20.9	20.9	0	99	98	-1
2	Male	111	20.9	20.3	0.6	95	97	+2
3	Male	79	20.9	20.9	0	98	97	-1
4	Female	68	20.9	20.9	0	97	97	0
5	Female	68	20.9	20.9	0	99	97	-2
6	Female	45	20.9	20.9	0	97	98	+1
7	Female	61	20.9	20.9	0	99	98	-1
8	Female	59	20.9	20.9	0	97	98	+1
9	Female	61	20.9	20.9	0	99	98	-1
10	Male	67	20.9	20.9	0	98	98	0

Table S2. Cohort metadata including sex, height, weight, CO_2 emission rate, total CO2 concentration in the headspace collection chamber at the end of 30 min sampling period, and the mean number of MS features detected in whole body headspace of each participant.

Participant	Sex	Height (cm)	Weight (kg)	Sampling Time	CO2 Emissions (ppm/min)	CO2 Total Concentration (ppm)	Mean MS Features	Mean MS Features with NIST Hits
M1	Male	192	113	10:00	197	6407	1140	371
M2	Male	185	82	10:00	147	4834	1110	358
M3	Male	180	73	10:00	139	4601	1079	352
M4	Male	186	76	10:00	132	4349	1030	333
M5	Male	173	64	13:00	157	5096	827	274
M6	Male	183	68	10:00	119	3986	797	269
M7	Male	183	73	12:00	117	3913	882	293
M8	Male	185	104	9:00	196	6317	945	319
M9	Male	178	91	10:00	154	5057	945	322
M10	Male	173	77	9:00	140	4648	907	306
F1	Female	168	64	10:00	116	3934	1093	355
F2	Female	176	64	10:00	134	4409	1110	373
F3	Female	163	68	10:00	126	4186	1046	341
F4	Female	178	78	9:00	119	3984	1030	339
F5	Female	158	50	9:00	89	3130	965	324
F6	Female	169	68	10:00	137	4567	1017	334
F7	Female	178	68	10:00	149	4888	933	315
F8	Female	157	60	13:00	104	3548	873	295
F9	Female	165	69	10:00	104	3581	965	322
F10	Female	163	88	10:00	126	4207	960	319