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In vitro profiling of volatile organic compounds released by Simpson-Golabi-Behmel syndrome adipocytes



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ABSTRACT

Breath analysis offers a non-invasive and rapid diagnostic method for detecting various volatile organic compounds that could be indicators for different diseases, particularly metabolic disorders including type 2 diabetes mellitus. The development of type 2 diabetes mellitus is closely linked to metabolic dysfunction of adipose tissue and adipocytes. However, the VOC profile of human adipocytes has not yet been investigated. Gas chromatography with mass spectrometric detection and head-space needle trap extraction (two-bed Carboxen X/Carboxen 1000 needle traps) were applied to profile VOCs produced and metabolised by human Simpson Golabi Behmel Syndrome adipocytes. In total, sixteen compounds were identified to be related to the metabolism of the cells. Four sulphur compounds (carbon disulphide, dimethyl sulphide, ethyl methyl sulphide and dimethyl disulphide), three heterocyclic compounds (2-ethylfuran, 2-methyl-5-(methyl-thio)-furan, and 2-pentylfuran), two ketones (acetone and 2-pentanone), two hydrocarbons (isoprene and *n*-heptane) and one ester (ethyl acetate) were produced, and four aldehydes (2-methyl-propanal, butanal, pentanal and hexanal) were found to be consumed by the cells of interest. This study presents the first profile of VOCs formed by human adipocytes, which may reflect the activity of the adipose tissue enzymes and provide evidence of their active role in metabolic regulation. Our data also suggest that a previously reported increase of isoprene and sulphur compounds in diabetic patients may be explained by their production by adipocytes. Moreover, the unique features of this profile, including a high emission of dimethyl sulphide and the production of furan-containing VOCs, increase our knowledge about metabolism in adipose tissue and provide diagnostic potential for future applications.

1. Introduction

Volatile organic compounds (VOCs) being produced by a human organism mirror normal and abnormal physiological processes and have, therefore, a great potential for use in medical diagnosis and therapy monitoring [1,2]. These compounds make up the human volatilome [3] and stem from various sources. While some of these VOCs are of endogenous origin, others can derive from exogenous sources

such as diet, environmental exposure, or microbiota activity. It has been demonstrated that abnormal processes, such as metabolic disorders, cancer, or other diseases can influence the volatilome profile by producing new VOCs, or by altering the concentrations of the VOCs that are produced normally by the human organism. The detection of these changes, via analysis of VOCs emitted by breath, skin, and other bodily fluids such as urine, saliva, or sweat, provides a unique opportunity to track microbiota activity, exposure to environmental toxins, or screen/

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monitor various diseases including cancer [1,4–6]. Despite this huge potential, the use of the human volatilome within a clinical setting is limited. A major unresolved issue is the poor understanding of the origin, behaviour, and metabolic fate of VOCs in human body.

VOC in vitro studies, involving cell cultures or pathogenic microorganisms (bacteria, fungi), could partially address this issue. In particular, this approach is of considerable importance for the exploration and understanding of blood-borne compounds observed in exhaled breath and other body emanations. For example, during the last few years a substantial effort has been made to identify volatiles released or consumed by cancer cells [7–10], bacteria [11–14], and fungi [15].

White adipose tissue is a major endocrine organ and secretes various bioactive compounds which are involved in different physiological and metabolic processes [16]. If production and release are dysregulated, as it is in the case of obesity, these compounds contribute to the development of metabolic disorders including diabetes, kidney and cardiovascular diseases, and thus can be used for diagnostic purposes [17]. Some recent studies were able to detect VOC changes in patient with chronic kidney disease and diabetes [18–21]. Diabetes, kidney disease, and cardiovascular disease are known to be interrelated and they are linked with obesity [17,22–25]. Thus, the mechanism that underlies their development takes place in adipocytes and the adipose tissue [17,26–29]. However, the VOC profile of human adipocytes has not been investigated yet.

Here, we report details on the VOCs produced and metabolised by human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes, which are well-established model cells for investigating metabolism in human fat tissue [30]. For this purpose, gas chromatography with mass spectrometric detection (GC–MS) and head-space needle trap extraction (two-bed Carboxen X/Carboxen 1000 NT needle traps) as the pre-concentration method have been applied.

2. Materials and methods

2.1. Chemicals and calibration mixtures

Multi-compound calibration mixtures were prepared from high-purity liquid substances. The reference substances with purities ranging from 95 to 99.9% were purchased from Sigma-Aldrich (Austria) and Fluka (Switzerland). A detailed description of the production of gaseous calibration mixtures is provided elsewhere [31,32] and, therefore, only a short outline of the procedure is provided here. Gaseous mixtures of water soluble species were produced by means of a GasLab calibration mixtures generator (Breitfuss Messtechnik, Germany). The device generates gas mixtures at different humidity levels via evaporation of liquid species. To achieve desired concentrations, pure substances were additionally diluted with water at the ratios of 1:2000–1:3000. Effectively, gas mixtures exhibiting VOCs volume fractions ranging from 10 ppt (parts-per-trillion) to 500 ppb (parts-per-billion) were used for calibration. For more volatile compounds, multi-compound gaseous standards were prepared by injecting and evaporating a few microliters of liquid or gaseous analyte into evacuated glass bulbs (Supelco, Canada). The desired calibration levels were achieved by transferring adequate volumes of the primary standard into Tedlar bags (SKC Inc., USA) filled with predefined amounts of humidified zero air, the latter being produced by the GasLab generator. Calibration curves were obtained on the basis of 2-fold analyses of five distinct and independent concentration levels.

2.2. Adipose cells cultivation

Human Simpson Golabi Behmel Syndrome (SGBS) preadipocyte cells [30] were maintained in 15 mL DMEM/Ham's F12 (1:1) medium (Invitrogen, Paisley, UK) containing 10% fetal calf serum (FCS; Invitrogen), 100 U/mL penicillin (Invitrogen), 100 µg/mL streptomycin (Invitrogen), 33 µM biotin, and 17 µM pantothenate. When SGBS

preadipocyte cells were near confluent, differentiation into mature adipocytes was started. They were washed with phosphate buffered saline (PBS) and cultured in FCS-free differentiation medium containing DMEM/Ham's F12 (1:1) medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 33 µM biotin, 17 µM pantothenate, 10 µg/mL human transferrin, 10 nM insulin, 100 nM hydrocortisone, 0.2 nM triiodothyronine, 25 nM dexamethasone, 500 µM 3-isobutyl-1-methylxanthine (IBMX), and 2 µM rosiglitazone. After four days, this medium was replaced by differentiation medium excluding dexamethasone, IBMX, and rosiglitazone and cells were cultured for up to 10 days.

The glass cultivation and measurement flasks (Ruprecht, Austria) occupy a volume of $21 \times 5.5 \times 11.5 \text{ cm}^3$ (1 L nominal volume, bottom area of approximately 240 cm²). Their detailed description is given elsewhere [7]. In total 12 pairs of samples (cell culture and medium without cells) were prepared.

2.3. Sample preparation and head-space needle trap extraction procedure

Volatiles released by cells were pre-concentrated using head-space needle trap extraction. Two-bed 23-gauge Silcosteel-treated stainless steel needle trap devices (NTD) (2 cm of Carboxen X and 1 cm of Carboxen 1000, both 60/80 mesh, PAS Technology, Germany) were employed for trapping the VOCs. Prior to their use all NTDs were pre-conditioned at 290 °C by flushing them with high-purity nitrogen (6.0–99.9999%) for 15 min. The NTD trapping of the head space constituents was performed dynamically by inserting the NTD via a septum into a cultivation flask and drawing 100 mL of the head space gas at a steady flow rate of 2 mL/min. To maintain a constant pressure in the flask during sampling high purity zero air was continuously introduced into the flask at a flow equal to the sampling flow. Following extraction, the NTD was introduced into the inlet of the gas chromatograph where the volatiles were thermally desorbed at 290 °C in a splitless mode (1 min). In parallel, one blank sample containing nitrogen was analyzed using the same protocol to identify possible contaminants stemming from sources other than cells/medium. The resulting concentration levels were subtracted (if applicable) from the respective values in the associated HS samples.

2.4. Chromatographic analysis

Chromatographic analyses were carried out using an Agilent 7890A/5975C GC–MS instrument (Agilent, USA). During desorption the GC inlet operated in the splitless mode (1 min), followed by a split mode using a ratio of 1 to 50. VOCs were separated using an Rt-Q-BOND column (30 m × 0.25 mm, film thickness 8 µm, 100% divinylbenzene phase, Restek, USA) working in a constant flow of helium at 1 mL/min. The column temperature program was as follows: 40 °C for 1 min, increased at a rate of 5 °C min⁻¹ to 210 °C, a constant temperature of 210 °C for 4 min, increased at a rate of 3 °C × min⁻¹ to 260 °C, and a constant temperature of 260 °C for 14 min. The mass spectrometer worked in a combined SCAN/SIM mode. The SCAN mode with *m/z* ranging from 20 to 200 was used for the identification of compounds as well as for the quantification of more abundant species. In case of the latter, peak integration was based on extracted ion chromatograms. The substance specific *m/z* ratios allowed in majority of cases for a separation of the peaks of interest from its neighbors, even when the latter were overlapping in the total ion count chromatogram. Selected less abundant VOCs were quantified using SIM (selective ion monitoring mode). The applied SCAN quantifier ions and SIM *m/z* values, and dwell times are given in Table 1. The quadrupole, ion source and transfer line temperatures were kept at 150 °C, 230 °C and 280 °C, respectively.

The identification of compounds was performed in two steps. Firstly, the peak spectrum was checked against the NIST mass spectral library. Next, the NIST identification was confirmed by comparing the

Table 1

Retention times (R_t) [min], quantifier ions, SIM dwell times [μ s], LODs [ppb], RSDs (%), coefficients of variation (R^2) and linear ranges [ppb] for VOC compounds of interest. The compound in italics was not quantified for reasons mentioned in the text. Compounds are ordered with respect to increasing retention time.

VOC	CAS	R_t [min]	Quantifier ion (SIM dwell time [μ s])	LOD		RSD [%]	R^2	Linear range	
				[ppb]	[pmol L^{-1}]			[ppb]	[pmol L^{-1}]
Acetone	67-64-1	19.42	43	1.0	40	6	0.979	3.3–100	129–3900
Carbon disulphide (CS_2)	75-15-0	19.72	76 (80)	0.13	5.1	7	0.969	0.43–8	17–310
Dimethyl sulphide (DMS)	75-18-3	19.97	62	4.0	156	10	0.998	13–500	510–19,500
Isoprene	78-79-5	22.21	67 (80)	0.16	6.3	7	0.970	0.53–5	21–200
Propanal, 2-methyl	78-84-2	24.09	72	5	200	7	0.993	16.5–180	640–7000
Butanal	123-72-8	25.36	72	5	200	12	0.996	16.5–40	640–1600
Ethyl methyl sulphide (EMS)	624-89-5	26.09	61 (80)	0.04	1.6	9	0.998	0.13–4.5	5.2–180
Ethyl acetate	141-78-6	26.44	43	0.7	27	8	0.998	2.3–17	90–660
2-Ethylfuran	3208-16-0	30.58	81 (80)	0.03	1.2	11	0.999	0.1–4	3.9–160
2-Pentanone	107-87-9	30.76	43	0.06	2.4	10	0.999	0.2–10	7.7–390
Pentanal	110-62-3	31.05	58	1.8	70	9	0.999	6.0–38	230–1500
Dimethyl disulphide (DMDS)	624-92-0	31.78	94	0.05	2.0	8	0.987	0.17–4.3	6.4–170
<i>n</i> -Heptane	142-82-5	33.31	71 (80)	0.03	1.2	5	0.974	0.1–2.5	3.9–98
Hexanal	66-25-1	36.03	56	1.2	47	7	0.999	3.9–250	150–9800
<i>2-Methyl-5-(methyl thio) furan</i>	<i>2371-70-2</i>	<i>42.80</i>	<i>128 (80)</i>	–	–	–	–	–	–
2-Pentylfuran	3777-69-3	48.00	81 (80)	0.03	1.2	12	0.998	0.1–5	3.9–200

respective retention times with retention times obtained for reference mixtures prepared as outlined above.

3. Results and discussion

3.1. Method validation

The validation parameters for VOCs under study are given in Table 1. Limits of detection (LOD) were estimated using the procedure proposed by Huber [33]. More specifically, the standard deviation of 5 consecutive blank signals and 1% probability ($1-\alpha$) for the type 1 error resulting in the coverage factor of 4.1 were used for these purposes. The LODs ranged from 0.03 to 5 ppb. The limit of quantification (LOQ) was defined as $3.3 \times \text{LOD}$. Relative standard deviations (RSDs), calculated on the basis of the consecutive analyses of 5 medium samples, varied from 5 to 12% and were considered appropriate for the purpose of this study. The GC–MS instrument response was found to be linear within the investigated concentration ranges (Table 1), with coefficients of determination ranging from 0.969 to 0.999.

3.2. Adipose cell cultures

The total number of adipose cells in particular experiments and their viability at the time of measurement are shown in Table 2. The total number of cells fell within the range of 1.25×10^6 and 4.12×10^6 (mean 2.6×10^6), whereas the viability varied from 85.4% to 97.1%

Table 2

Total number of cells, number of living cells and viability at the time of the measurement.

Culture	Total number of cells $\times 10^6$	Number of living cells $\times 10^6$	Viability [%]
1	1.28	1.15	90.2
2	2.63	2.55	97.1
3	2.07	1.88	90.4
4	2.45	2.38	96.9
5	2.90	2.78	95.7
6	1.85	1.75	94.6
7	2.56	2.28	89.1
8	2.32	2.20	94.8
9	3.04	2.60	85.5
10	2.84	2.72	95.8
11	3.12	2.92	93.6
12	4.12	3.52	85.4
Mean	2.60	2.39	92.4

(mean 92.4%). Consequently, the applied experimental procedure did not affect the cells' viability and it can be assumed that the release and uptake of the head-space VOCs mirror their metabolism.

3.3. Volatiles released and metabolised by adipose cells

A Wilcoxon signed rank test was used to evaluate the production and consumption of VOCs by the cells under study, and $p < 0.05$ was taken as being significant. For the purpose of the Wilcoxon test the left-censored data were estimated by a value $\text{LOQ}/\sqrt{2}$, or $\text{LOD}/\sqrt{2}$ [34]. Levels of 16 compounds were found to be significantly dependent on the presence of the adipose cells in the cultivation flask. Their detection and quantification incidences, as well as their concentrations in the cultures head-space (ppb), are given in Table 3. Twelve VOCs were produced and a further four were metabolised by the adipose cells under study. Amongst the liberated compounds there were four volatile sulphur compounds (carbon disulphide (CS_2), dimethyl sulphide (DMS), ethyl methyl sulphide (EMS) and dimethyl disulphide (DMDS)), three heterocyclic compounds (2-ethylfuran, 2-methyl-5-(methyl-thio)-furan and 2-pentylfuran), two ketones (acetone and 2-pentanone), two hydrocarbons (isoprene and *n*-heptane) and one ester (ethyl acetate). All metabolised species belong to aldehydes. All compounds but one, (2-methyl-5-(methylthio)-furan), were quantified using the aforementioned procedures. 2-methyl-5-(methylthio)-furan was not quantified due to problems related to the preparation of reliable reference mixtures and its levels were assessed only on the basis of peak areas. The highest concentration levels were noted for DMS (median of 317 ppb in the cell cultures) and acetone (57 ppb). The majority of the remaining species exhibited median concentration values below 5 ppb.

A total of four aldehydes (2-methyl-propanal, butanal, pentanal, and hexanal) were metabolised by SGBS adipocytes. This finding agrees with results from other human cell cultures, in which consumption of aldehydes is commonly observed [7–10,35,36] and ascribed to the expression of aldehyde dehydrogenases (ALDHs) irreversibly oxidizing compounds from this chemical family into their corresponding carboxylic acids [37,38]. Indeed, fatty aldehyde dehydrogenase (FALDH) has been identified in rat adipocytes as a key component of the detoxification pathway of aldehydes arising from lipid peroxidation events [39,40]. Alternatively, aldehydes could also be reduced to alcohols by alcohol dehydrogenases (ADHs). However, low levels of ADHs in adipocytes render the oxidation of aldehydes by ALDHs a more plausible reason for aldehydes uptake [40].

A number of metabolic pathways could be involved in the production of acetone and 2-pentanone by cells under scrutiny. These

Table 3

Detection (n_d) and quantification (n_q) incidences, concentration ranges, and medians of VOCs under study in the headspace of media and cell cultures. The compound in italics was not quantified for reasons mentioned in the text (the peak area is provided). Compounds are ordered with respect to increasing retention time.

	VOC	CAS	Cell cultures		Medium			Wilcoxon signed-rank test p value	
			Incidence $n_d(n_q)$	Range (median) [ppb] [pmol L ⁻¹]	Incidence $n_d(n_q)$	Range (median) [ppb] [pmol L ⁻¹]			
Uptake	Propanal, 2-methyl	78-84-2	12(12)	21–56 (30)	820–2200 (1200)	12(12)	51–235 (83)	2000–9200 (3200)	4.88×10^{-4}
	Butanal	123-72-8	6(1)	20	780	12(8)	19–36 (29)	740–1400 (1100)	4.88×10^{-4}
	Pentanal	110-62-3	12(3)	6.7–7.7 (7.4)	260–300 (290)	12(12)	7.2–49 (8.6)	280–1900 (340)	4.88×10^{-4}
	Hexanal	66-25-1	12(10)	3.6–8.5 (6.5)	140–330 (250)	12(12)	7.5–424 (14)	290–16,500 (550)	1.47×10^{-3}
Release	Acetone	67-64-1	12(12)	25–94 (57)	980–3700 (2200)	12(12)	6.9–8.0 (7.6)	270–310 (300)	4.88×10^{-4}
	Carbon disulphide	75-15-0	12(12)	2.1–8.0 (2.5)	80–310 (100)	12(12)	0.7–1.6 (1.1)	27–62 (43)	4.88×10^{-4}
	Dimethyl sulphide	75-18-3	12(12)	171–480 (317)	6700–18,700 (12,300)	9(0)	< LOQ	< LOQ	4.88×10^{-4}
	Isoprene	78-79-5	12(9)	0.5–2.9 (1.3)	20–110 (50)	12(7)	0.5–1.1 (0.9)	20–43 (35)	1.17×10^{-3}
	Ethyl methyl sulphide	624-89-5	12(6)	0.15–1.14 (0.34)	6–44 (13)	0(0)	< LOD	< LOD	4.88×10^{-4}
	Ethyl acetate	141-78-6	12(12)	4.5–11.4 (7.4)	180–440 (290)	12(10)	2.2–4.0 (3.2)	86–160 (130)	4.88×10^{-4}
	2-Ethyl furan	3208-16-0	10(6)	0.24–1.0 (0.58)	9–39 (23)	10(0)	< LOQ	< LOQ	3.9×10^{-2}
	2-Pentanone	107-87-9	12(12)	0.48–1.35 (0.72)	19–53 (28)	12(7)	0.21–0.27 (0.22)	8.2–11 (9)	4.88×10^{-4}
	Dimethyl disulphide	624-92-0	12(12)	0.24–1.7 (0.46)	9–66 (18)	12(6)	0.25–0.37 (0.3)	9.8–14 (12)	4.88×10^{-4}
	<i>n</i> -Heptane	142-82-5	12(12)	0.09–0.95 (0.18)	3.5–37 (7)	12(12)	0.09–0.27 (0.16)	3.5–10.5 (6)	6.84×10^{-3}
	2-methyl 5-(methylthio) furan	2371-70-2	12(12)	48,000–328,000 (83,000)	–	12(12)	700–1400 (1000)	–	4.88×10^{-4}
	2-Pentylfuran	3777-69-3	12(10)	0.08–1.4 (0.25)	3.2–55 (10)	12(10)	0.09–0.1 (0.1)	3.5–3.9 (4)	5.0×10^{-2}

embrace: (i) ADHs-related oxidation of secondary alcohols, (ii) β -oxidation of branched-chain fatty acids, and (iii) decarboxylation of Acetyl-CoA. Although ADHs are mainly responsible for ethanol metabolism; they are also capable of oxidizing long-chain, cyclic and secondary alcohols [37,41]. Thus, 2-pentanone may be the product of 2-pentanol oxidation; whereas, acetone can derive from 2-propanol. Even though 2-pentanol was not detected in the cultures/media head-space, huge amounts of 2-propanol were present in the applied medium. Interestingly, 2-pentanone liberation has been previously seen for hepatocyte carcinoma cells (HepG2), lung adenocarcinoma cells (A549), human bronchial epithelial primary cells (HBEpC) and human fibroblasts (hFB) [7,8]. β -Oxidation of fatty acids is another potential source of ketones in humans. For example, 2-heptanone and 4-heptanone are the products of the 2-ethylhexanoic acid oxidation [42], and 2-propyl pentanoic acid is metabolised to 3-heptanone [43]. Walker et al. [44] provided some evidence that 2-pentanone could be produced in an analogous way in the peroxisomal pathway through β -oxidation of hexanoic acid. Acetone is a major volatile in the human body found in breath, blood, and urine [32,45–47]. It is mainly formed in the liver during fatty acids oxidation from acetoacetate undergoing spontaneous decarboxylation [47,48].

CS₂, DMS, EMS and DMDS, were produced by adipocytes, with DMS having the highest concentration of all liberated compounds. The presence of some volatile sulphur compounds in human organism is being linked to the metabolism of two sulphur-containing amino acids; methionine and cysteine. DMS was suggested to be produced by thiol S-methyltransferase via the methylation of methanethiol [49]. The latter in turn can be formed from (i) methionine by L-methionine γ -lyase, or (ii) hydrogen sulphide (H₂S) by S-methyltransferase. In the latter case H₂S can stem from desulphydration of cysteine performed by cystathionine- β -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulphurtransferase [49]. Analogously, S-methyltransferase could form other methyl thioethers, such as e.g. EMS, via the methylation of respective thiols. Recently, a small population study with diabetic and non-diabetic human subjects found differences in the concentrations of sulphur compounds in breath, with higher concentrations of methanethiol being found in diabetic patients and a trend for higher DMS with increasing H₂S [21]. We therefore suggest that the increased production of sulphur compounds in diabetic patients might stem from adipocytes in adipose tissue. CS₂ is presumably an environmental or occupational intoxicant absorbed by inhalation, oral, and

dermal routes [50]. In humans it is metabolised to carbonyl sulphide, atomic sulphur and monothiocarbonate by cytochrome P450 monooxygenase [50]. Although at present the metabolic pathways leading to the CS₂ production are unknown, it has been recently suggested to play a bioregulatory or therapeutic roles in mammals [50].

Isoprene is a terpenoid of unclear origin and function in the human organism. In mammals it is postulated to be produced from isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) in the mevalonic acid (MVA) pathway [51]. More specifically, it is suggested to be produced in the cytosol of hepatocytes during acid-catalyzed formation from DMAPP [52]. However, this reaction is too slow to explain high isoprene levels in human organism [53]. Moreover, a number of recent studies suggest that other metabolic pathways may contribute to isoprene production in humans [35,54,55]. Interestingly, in breath gas analysis this species is considered to be of use as a sensitive non-invasive biomarker for several metabolic effects in humans [51,56,57]. It is also postulated to offer some protection against oxidative stress, analogously to that hypothesized in plant physiology, and act as a reactive oxygen species sweeper reacting with radicals through the double bond system [55,58]. More recently, Neupane et al [18] have found that isoprene rises during hypoglycaemia in diabetic subjects. However, the mechanism behind this increase of isoprene is unclear. Thus, again, our data are indicating that this rise of isoprene is, at least in part, based on its production in adipocytes. The metabolic pathways leading to the formation of *n*-heptane by the adipocytes under scrutiny remain unclear. It could be assumed that it is produced during lipid peroxidation processes, but this needs to be addressed in future studies. It should also be mentioned here that *n*-heptane was found to be consumed by lung cancer cells (A549) [59].

Ethyl acetate seems to derive from the esterification of ethanol with acetic acid. Although, this reaction in the absence of a catalyst is expected to be very slow, ethyl acetate could be formed from acetic acid, which is a common volatile found in the human organism originating from normal human biochemistry (e.g. Krebs cycle, pyruvate metabolism, or ethanol metabolism). Indeed, ethanol was observed to be present the cell culture and media head-space. The production of ethyl acetate, similar as in the case of SGBS adipocytes, has been reported in cultures of human umbilical vein endothelial cells (HUVEC) and lung adenocarcinoma cells (A549) [36,59].

Three heterocyclic compounds belonging to the furan family (2-ethylfuran, 2-methyl-5-(methylthio)-furan and 2-pentylfuran) were

also found to be released by the SGBS adipocytes. These species are not known to be produced by human metabolism. However, the latter was found in the breath of patients with *Aspergillus fumigatus* infections [60,61] and human skin emanation [62]. They are also present in many foods and beverages and used as flavoring agents. To the best of our knowledge, the production of VOCs from the furan family by human cells in vitro has not been reported before [10]. Therefore, this feature seems to be unique for the adipocyte tissue metabolism. Interestingly, furan has been found to be released from the prostate and has been suggested to be of use as a biomarker for prostate cancer – even though its origin has remained unclear [63]. Its production by natural dehydration of monosaccharides and the oxidation of some fatty acids catalyzed by lipoxygenases [63] could take place in adipocytes in the context of lipid peroxidation. Whether these compounds might have a value for use as biomarkers for metabolic diseases, including diabetes, remains to be clarified.

3.4. Potential of adipocyte-specific volatiles as metabolic biomarkers

Type 2 diabetes mellitus is an inflammatory disease and the mechanism that underlies its development take place in the adipose tissue. It is also the leading cause of kidney failure and it increases the risk of heart attacks and strokes by the factor of 2–3 [64]. Obesity is the main cause for type 2 diabetes mellitus and cardiovascular disease. It is also linked to kidney disease [65–69]. Accumulating evidence indicates that obesity causes chronic low-grade inflammation and that this contributes to systemic metabolic dysfunction that is associated with obesity-linked disorders [17]. Thus, the early and easy detection of a metabolic switch or a dysregulated metabolism in adipose tissue via VOC profiling could speed-up and enhance sensitivity of clinical diagnosis.

Similar to the link between diabetes, obesity, and adipose tissue metabolism, a change in breath VOCs in diabetic patients [21] might mirror a change in release of VOCs by adipocytes. It has been hypothesized previously that a metabolic switch, which enables adipocytes to survive and sustain stress episodes, e.g. hypoxia (as it is the case in the obese state) may provide energy abundance and trigger lipogenesis [70]. In the long term, this could explain the vicious circle of exacerbating obesity, shifted metabolism and metabolic disease manifestation. In agreement with this, comprehensive microarray analysis has found striking correlations between the gene expression pattern of human adipocytes and matched biomarkers for metabolic diseases including diabetes and insulin resistance [70].

4. Conclusions

The present study is the first study to provide a VOC profile of human adipocytes. It underlines the potential of VOC analysis as a potential non-invasive tool for monitoring metabolic changes in adipose tissue as well as the whole body. Sixteen VOCs were found to be related to the metabolism of the cells under study. Twelve VOCs were found to be produced and a further four metabolised. There are two unique features of the VOC fingerprint formed by the SGBS adipocytes: (i) the presence of VOCs from the furan family and (ii) a very high production of dimethyl sulphide. Although the exact pathway of the DMS production remains unclear, the presence of this species might mirror the activity of enzymes detoxifying reactive volatile sulphur compounds converting them into less toxic forms. Moreover, the release of some ketones and ethyl acetate by the cultures under scrutiny seem to reflect ADHs and ALDHs expression and further confirm the detoxification role of the adipocytes in human organism. If so, the adipose tissue could play a more active role in metabolic regulation in general and detoxification mechanisms in particular, as is currently assumed. Isoprene and sulphur compounds have also been reported to be increased in diabetic patients' breath. The results of this study imply that, at least partially, these VOCs originate from the adipose tissue. Although, the origin of the observed furan-containing compounds remains unclear,

they might be related to some unique metabolic functions of the adipose tissue and therefore have some diagnostic potential. As the fuel reservoir, adipose tissue controls the lipid mobilization. Therefore, the adipose tissue dysfunction can be reflected by changes in the human volatilome, which could in turn be detected non-invasively via breath, or urine analysis. The occurrence of species stemming from lipid peroxidation could, in this context, be considered as valuable markers of this metabolic state.

Several limitations of the study should be raised. First, this study has been conducted with a human adipocytes cell culture model, using cells derived from a patients suffering from Simpson-Golabi-Behmel syndrome. Thus, our data may not necessarily reflect the in vivo situation of adipocytes in healthy subjects. Next, only C3–C11 species were targeted. Consequently, heavier volatile compounds may be produced but are not reported in the present study.

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