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*Abbreviations:* VOCs, volatile organic compounds; LFD low fat diet; PTR-MS, proton transfer reaction mass spectrometry; TOF, time of flight; pk, peak;

**Abstract**

Volatile organic compounds (VOCs) from breath can successfully be used to diagnose disease-specific pathological alterations in metabolism. However, the exact origin and underlying biochemical pathways that could be mapped to VOC signatures are mainly unknown. There is a knowledge gap regarding the contribution of tissues, organs, the gut microbiome, and exogenous factors to the "sum signal" from breath samples. Animal models for human disease such as mutant mice provide the possibility to reproduce genetic predisposition to disease, thereby allowing the in-depth analysis of metabolic and biochemical functions. We hypothesized that breath VOCs can be traced back to origins and organ-specific metabolic functions by combining breath concentrations with systemic levels detected in different organs and biological media (breath, blood, feces and urine). For this we fed C57Bl/6N mice a grain-based chow or a purified low-fat diet, thereby modifying the emission of methanol in breath whereas acetone levels were unaffected. We then measured headspace concentrations of both VOCs in ex-vivo samples of several biological media. Especially cecum content was identified as a likely source of systemic methanol, whereas liver showed highest acetone concentrations. Our findings are a first step to the systemic mapping of VOC patterns to metabolic functions in mice because differences between VOCs could be traced to different sources in the body. As a future aim, different levels of so-called omics technologies (genomics, proteomics, metabolomics, and breathomics) could be mapped to metabolic pathways in multiple tissues deepening our understanding of VOC metabolism and possibly leading to early non-invasive biomarkers for human pathologies.

## 1. Introduction

Volatile organic compounds (VOCs) measured in exhaled breath have been shown to provide information about metabolic state and disease in humans as well as in model organisms for human diseases. Breath analysis has the potential of being used as a reliable, low cost and easy-to-use method to classify healthy and diseased subjects in a number of pathologies(Baranska et al., 2016; Fernández del Río et al., 2015; Nakhleh et al., 2016; Obermeier et al., 2017; van Vliet et al., 2017). Closer investigations revealed that breath VOCs are derived from a combination of various exogenous and endogenous sources (Lindinger et al., 1997; Pleil et al., 2013). This is a major reason why the physiological link between VOC signatures and disease metabolism is poorly understood. While uptake from the environment, or food and beverages reflect the exposome, endogenous VOCs originate from either endogenous metabolic processes or the individual microbial metabolism. Both, changes in the metabolic status and modified composition of the microbiome may reflect or interact with disease. Thus, dissection of the origin of single VOCs from different organs, tissues and metabolic pathways is important to improve the robustness of the method and its usefulness to provide early biomarkers for disease.In the field of VOC analytics, several attempts were made to better understand which processes lead to altered VOC metabolism. For such approaches, stable isotope labelled substrates were administered in mice to follow up specific VOCs (Sinues et al., 2017). In humans, enzyme functions were monitored in vivo (Ruzsanyi et al., 2014). Other studies addressed the relation between blood and breath in humans (O'Hara et al., 2009), analysed VOC emissions from single cell lines (Brunner et al., 2010; Filipiak et al., 2016), from tissue samples (Filipiak et al., 2014) or (pathogenic) microbiota (Bean et al., 2016). A complete view on organ and tissue concentrations of external or endogenous VOCs is required to better understand the origin of VOCs. This can help to map VOCs to specific metabolic functions. Evidently mammalian model organisms such as laboratory rats or the C57BL/6N mouse facilitated pilot work in this field (Aprea et al., 2012; Hüppe et al., 2016; Kistler et al., 2016; Sinues et al., 2017; Szymczak et al., 2014).

We investigated systemic VOC distribution in organs, blood, feces, urine, and breath of male C57BL/6N mice that were fed a purified low fat experimental diet or standard laboratory chow. For this study, we selected methanol, a VOC modified by diet and found in breath after ingestion of fruits, vegetables, alcoholic or aspartame sweetened beverages (Lindinger et al., 1997; Španěl et al., 2015). We previously found it elevated in exhaled breath of mice in response to feeding a grain-based chow diet compared to a purified control diet (Kistler et al., 2014). Acetone, a better studied endogenous metabolite was selected as second compound. We hypothesized that breath signals of both compounds can be linked to headspace concentrations from several mouse tissues and media like urine, blood, feces, and cecum content. By using such a system-wide approach, the so far largely unexplored links

between several tissues and VOCs that finally contribute to the sum signal presented in breath can be investigated and mapped to metabolic functions.

## 2. Material and Methods

### *2.1. Animal housing and diet regimes*

Male mice (n = 34) were housed in type IIL polycarbonate cages with individual ventilation (Tecniplast, Italy) in specific pathogen-free conditions at the German Mouse Clinic (GMC) (Fuchs et al., 2009). Air humidity of 50-60% and a 12:12 light/dark cycle were maintained. Wood shavings were used for bedding (Altromin GmbH, Germany). All mice had *ad libitum* access to pelleted laboratory chow (no. 1314, Altromin, Lage, Germany) and drinking water from weaning on. From the age of 32 weeks on mice were randomly assigned to either continued chow diet feeding or a semi-purified low fat diet (low fat: E 15000-04, Ssniff, Soest, Germany) for a period of 3 weeks until the VOC analysis measurements were conducted. All experiments were performed following animal welfare regulations supervised by the district government of Upper Bavaria (Regierung von Oberbayern).

Analysis of VOCs from *ad libitum* fed mice was performed between 7 am and 1 pm. Mice were in a postprandial state as they typically feed in the early morning hours. Chow and LFD mice were measured in alternating order to remove potential systemic bias. Mice were weighed immediately before the VOC measurement to the nearest 0.1 g.

### *2.2. Proton-transfer reaction time-of-flight mass spectrometry*

A high-sensitivity Proton Transfer Reaction Mass Spectrometer (PTR-MS, Ionicon Analytic GmbH, Innsbruck, Austria) with a resolution of m/ $\Delta m \leq 2000$ was used. Settings and machine parameters were applied as described previously (Kistler et al., 2016). The following deviations were made: A mass range from m/z 0 to 356 was recorded and the sum spectra were stored with integration time of 1 s (TOF-DAQ, Tofwerk AG, Switzerland). Calibration was performed using known peaks $H_3{}^{18}O^+$ (m/z 21.0221), $NO^+$ (m/z 29.9971), and the 2 high mass peaks provided by the built-in PerMaSCal unit $C_6H_5I^+$ (m/z 203.9431) and $C_6H_5I_2{}^+$ (m/z 330.8481). A total of 306 peaks were selected manually from the spectra using PTR-MS Viewer (Version 3.2.1.2, Ionicon analytic GmbH, Innsbruck, Austria).

### 2.3. *Real-time VOC analysis in unrestrained and anaesthesized mice*

A setup and protocol for real-time measurement of VOCs in unrestrained mice using respiratory chambers was again applied as described previously (Kistler et al., 2016, 2014; Szymczak et al.,

2014). In brief, a polypropylene box (volume 600 mL) was connected to a proton transfer time-of-flight mass spectrometer (PTR-TOF-MS) and to a gas supply of synthetic air (20% oxygen,80% nitrogen, concentration of hydrocarbons 0.1 ppm,Linde AG, Germany). After flushing with synthetic air, blank samples were drawn from the empty chamber to control for leakage (5 min, flow 60 mL min−1 controlled by PTR). During a second flushing with synthetic air, the mouse was placed into the respirometry chamber and the accumulation of exhaled VOCs was monitored. Fushing (2 min, flow 3 L h−1) and accumulation of VOCs (5 min, flow 60 mL min−1 controlled by PTR) were repeated three times each. Air drawn from the chamber by the PTR was replenished from a Teflon bag reservoir filled with synthetic air (capacity of 10 L, Welch Fluorocarbon Inc., Dover, USA) connected to the chamber.

In addition to VOC measurements using mice in a chamber, we aimed to verify that methanol and acetone signals were mainly breath driven. Thus, we obtained breath data using a nose mask similar to previously published studies using rats (Aprea et al., 2012). In brief, a 15 ml falcon tube was shortened to a volume of 4 ml. The end of the tube was connected to the PTR using PTFE tubing. For the replacement of withdrawn gas, a 1 mm hole was placed at the side of the tube to allow steady airflow. A subgroup of anaesthetized mice (13 per group, i.p. injection of 100 mg/kg bodyweight ketamine and 10 mg/kg bodyweight xylazine) were measured twice for more than 20s. Surrounding room air was monitored both before and after breath measurements.

### *2.4. Organ sampling, pre-processing and head space measurement*

Blood from anesthetised mice was sampled from the orbital sinus. Coagulation was inhibited by sampling directly into EDTA-containing tubes, which were immediately shock-frozen in liquid nitrogen. After blood sampling, abdominal cavity was opened and the following organs and samples were taken: gastric, duodenal and cecum content; quadriceps femoris muscle, peri-renal white adipose tissue, kidney, liver, lung, heart, spleen, testis, whole brain. Urine and feces samples were collected from the respirometry box while breath samples were collected. Samples were shock-frozen in liquid nitrogen immediately after dissection. Samples were analysed within two weeks of storage at -80 °C.

For analysis of VOCs emitted, samples (except blood and urine) were homogenized (Tissue Lyzer MM400, Retsch GmbH, Haan, Germany). For this, pre-cooled steel balls were added to the sample tube and tubes were placed in a holder pre-frozen in liquid nitrogen. Homogenization was performed using a shaking frequency of 30 Hz for 2.5 minutes. A targeted mass of 250 mg homogenate was transferred to a glass vial, flushed with synthetic air and incubated for 3 minutes at 37 °C. Samples of organs with physiologically low mass (e.g. heart, lung, spleen, testes) or variable availability (e.g. urine, feces, gut content) were excluded if below 50 mg. In total, headspace of 516 samples was measured using a PTR-TOF-MS; extracted gas volume was replaced by synthetic air.

### *2.5. Data analysis and statistics*

#### 2.5.1. *Calculation of VOC source strengths and concentration*s

The source strength in ppb ml/min was derived by applying a compartment model on the recorded saturation curves (non-linear regression, described in **(Szymczak et al., 2014)**) and data pre-procession was performed as described previously if not stated otherwise **(Kistler et al., 2016, 2014)**. The concentration used for further analysis was determined using a mean of two maximal values. Outliers (defined as greater than four standard deviations from mean) were removed. Nose measurements were differentially corrected against room air background.

#### *2.5.2. Statistics and data visualization*

For the analysis of diet-induced effects on the VOC source strengths and concentrations, linear models were applied. Data were log-transformed to approximate a normal distribution (tested visually by qq-plotting). The variance between groups was controlled using both boxplots of source strength as well as residuals and residual versus fitted data plots. As a larger number of tests leads to summation of Type I – error, control of false discovery rate after Benjamini and Hochberg (Benjamini and Hochberg, 1995) was applied and all p-values were adjusted according to a 10% FDR.

Principal component analysis of scaled and centred data was performed using R. Boxplots were created using the R package *ggplot2* using means of maximal headspace concentrations and source strength data (Wickham, 2009). As a complete data-matrix is required to calculate the correlation plots, missing data was imputed using the *mice* R package **(Buuren and Groothuis-Oudshoorn, 2011)**, which accounted for 3.024% of data. Correlation plots were performed using the R package *corrplot* (pearson correlations).

## 3. Results

### *3.1. Elevation in systemic methanol levels might be driven by cecum methanol release*

Emission of methanol was increased in chow fed mice both in source strength in breath as well as in maximum headspace concentration of all organ and media samples during the measurement (Figure 1A). As a next step, we followed up on whether a difference in concentrations of the selected exhaled VOCs induced by a diet change can be recovered from the respective headspace concentrations of individual organ samples. A similar increase in the chow group was present in blood and, even more pronounced, in urine (Figure 1B). When following the digestive tract, methanol levels increased

tenfold from stomach to cecum (Figure 1C). In all internal organs, higher methanol headspace concentrations could be detected in chow fed mice (Figure 1D).

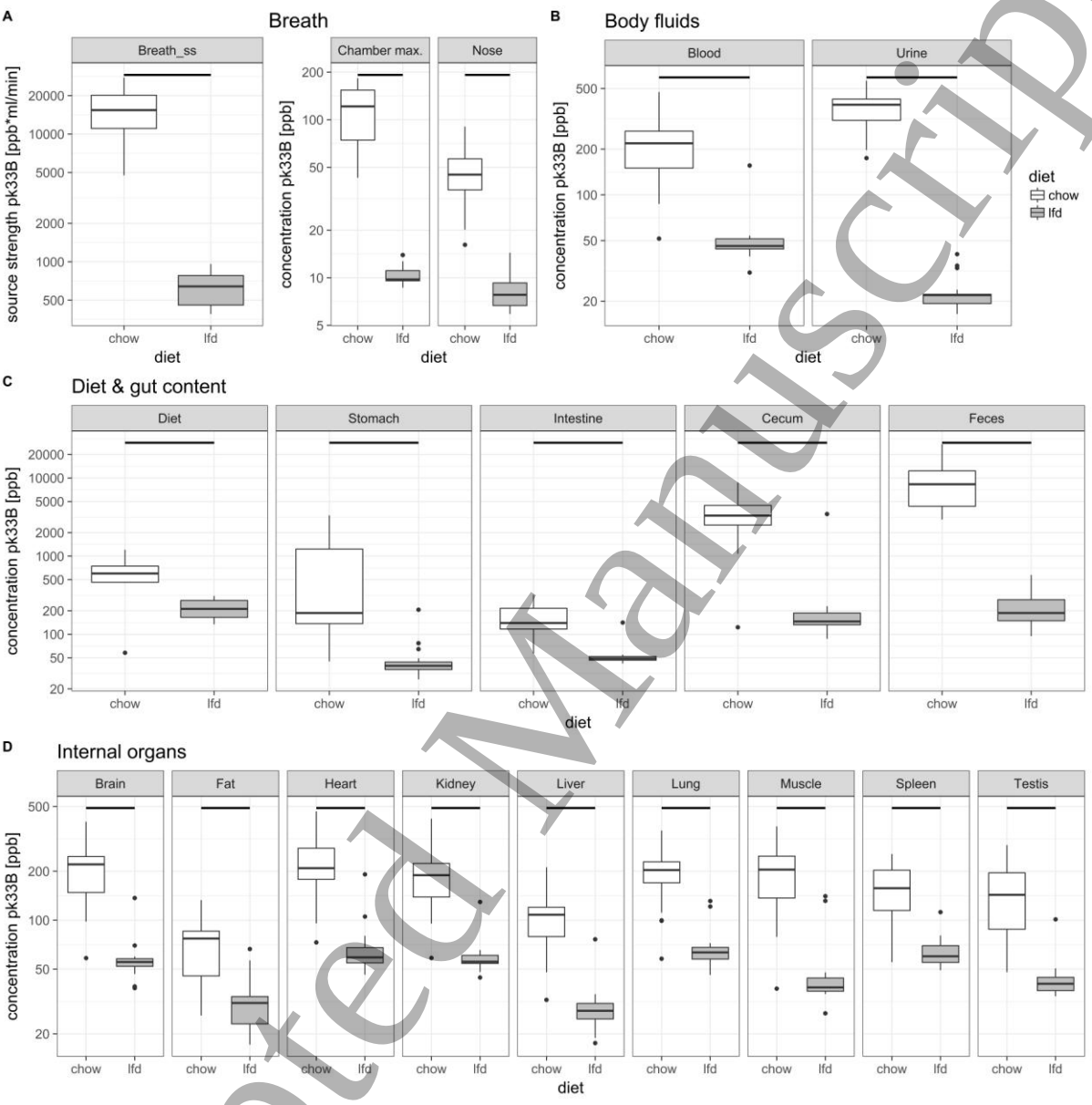



**Figure 1: Methanol concentrations from tissue homogenate headspaces.** Mice fed either chow (white, n = 17) or LFD (grey, n = 17). Methanol breath source strength, maximal concentration during mouse measurement and nose mask measurements for a subset (both n = 13) are shown in A. Headspace concentrations of blood and urine are shown in B. Diet, stomach, intestine and cecum content and feces head space concentrations are shown in C. Headspace concentrations of brain, fat, heart, kidney, liver, lung, muscle, spleen and testis are shown in D.

### *3.2. Liver is a major site of acetone release*

In contrast to methanol, acetone levels were comparable between groups after the diet change both in breath source strength as well as in maximum headspace concentration (Figure 2 A). No significant

difference could be detected in body fluids (Figure 2 B). When following the digestive tract, a slight increase could be observed in the content of the intestine (Figure 2 C). No major diet effect was found in the acetone concentration detected in any internal organs. Hepatic tissue homogenates showed highest acetone concentrations in headspace, brain, heart, kidney, lung, and spleen were intermediate. Lowest values were detected in white adipose tissue, muscle and testes (Figure 2 D).

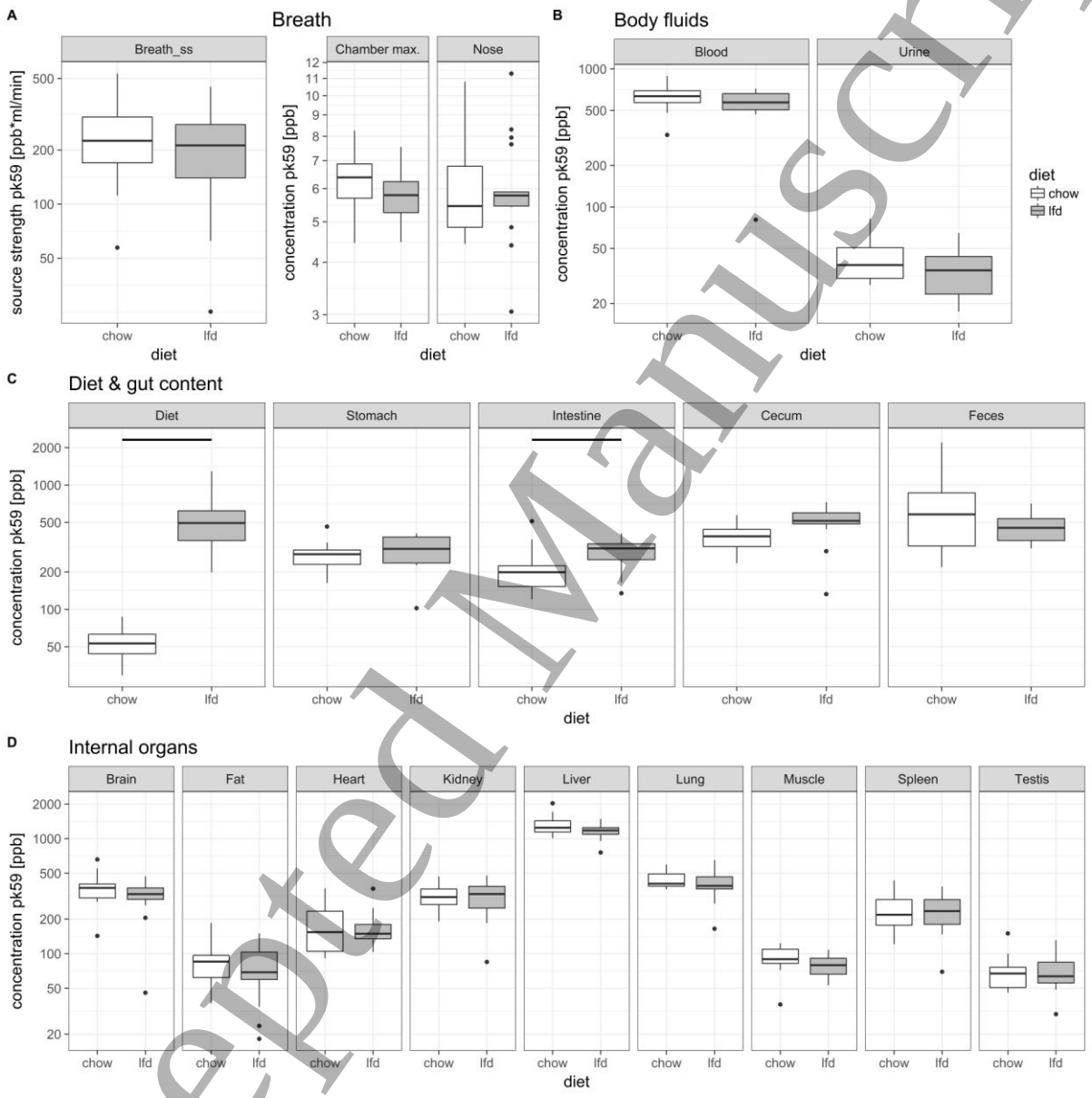
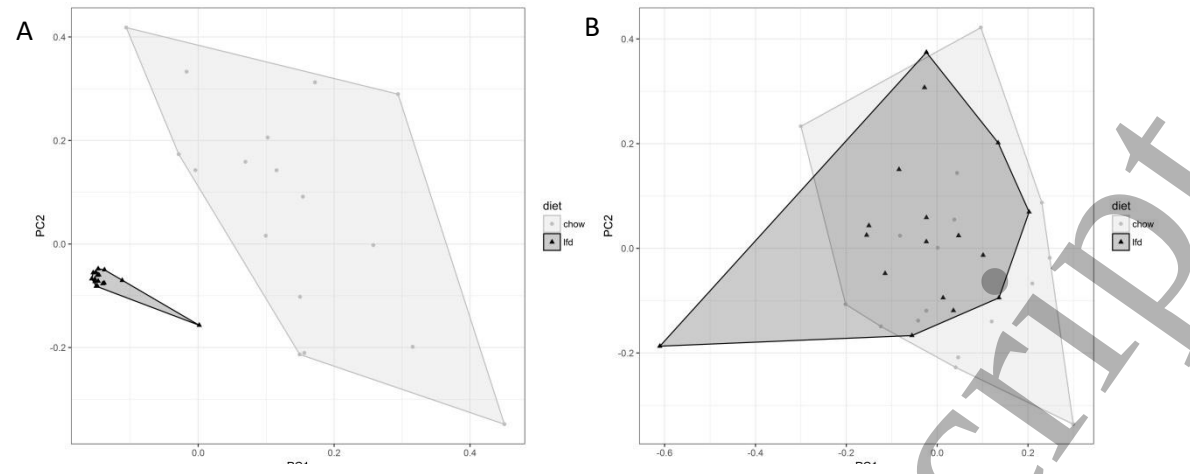
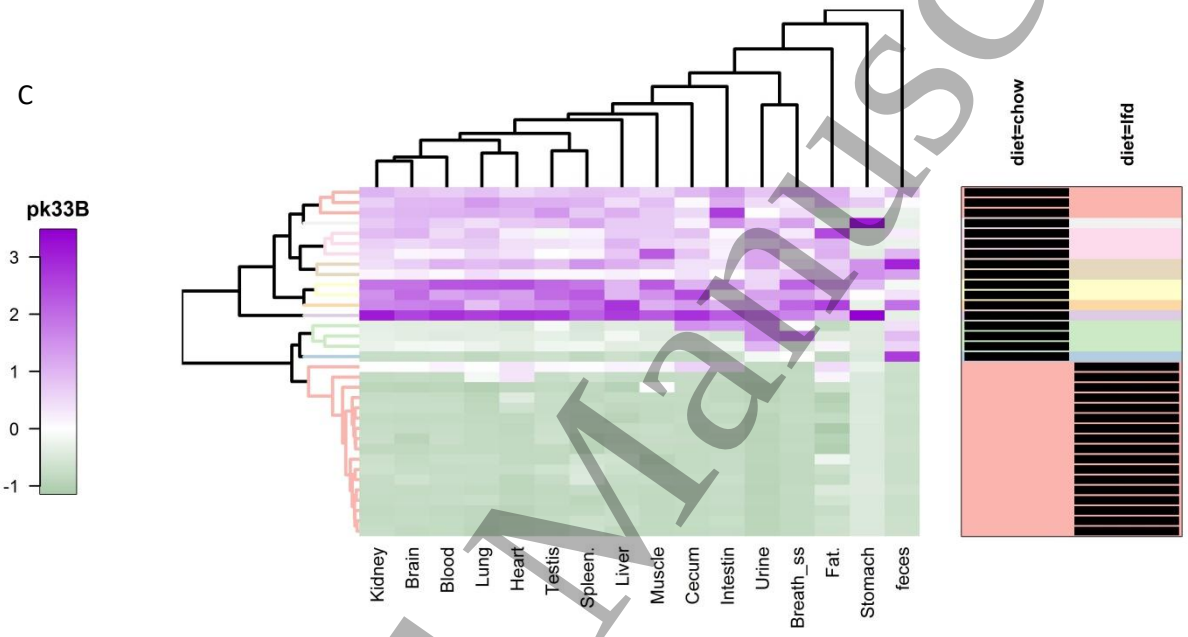



**Figure 2: Acetone concentrations from various tissue homogenate headspaces.** Mice fed either chow (white, n = 17) or LFD (grey, n= 17). Acetone breath source strength, maximal concentration during mouse measurement and nose mask measurements for a subset (both n = 13) are shown in A. Headspace concentrations of blood and urine are shown in B. Diet, stomach, intestine and cecum content and feces head space concentrations are shown in C. Headspace concentrations of brain, fat, heart, kidney, liver, lung, muscle, spleen and testis are shown in D.

### *3.3. Methanol levels of tissue samples and media cluster by diet-interventions*

Next we used unsupervised clustering methods to determine whether groups clustered by diet. Methanol levels in all samples were clearly separated by diet as we could show by principle component analysis (Figure 3A) and hierarchical clustering (Figure 3C). In contrast, acetone levels in different media were not separated by diet both in PCA and hierarchical clustering (Figure 3B and D).



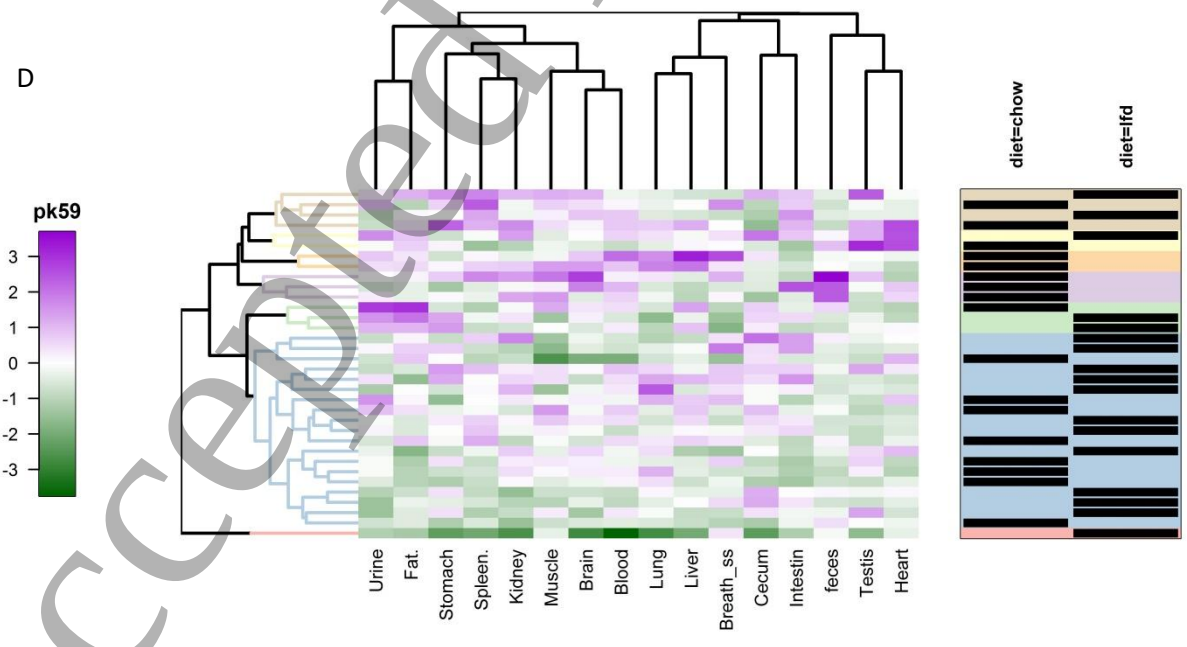



**Figure 3: Principal component analysis (PCA) and heatmaps with hierarchical clustering of emissions from various tissues and samples.** Components one and two of a PCA of main methanol signal at 33.05 (A, pk33B) or main acetone signal at 59.05 (B, pk59) are shown for stomach, intestine and cecum content, feces, liver, heart, lung, brain, muscle, spleen, testis, fat, blood, urine and source strength in breath from mice fed either chow (white, n = 17) or LFD (grey, n = 17). Heatmaps of methanol (C) and acetone (D) data are shown with hierarchical clustering of individual mice (mean data, rows, sub-clusters colored) and tissue and media samples (columns). Data is scaled and centered. Color-coding legend shown on the left. Classification of individual mice is annotated on the right (diet = chow or low fat (lfd)).

### 3.4. Methanol concentrations are highly correlated in internal organs and blood

Methanol concentrations in the headspace of different media were highly positively correlated especially within the internal organs and blood (Figure 4 A). In contrast, acetone concentrations in the same media samples showed fewer significant and generally weaker correlations.

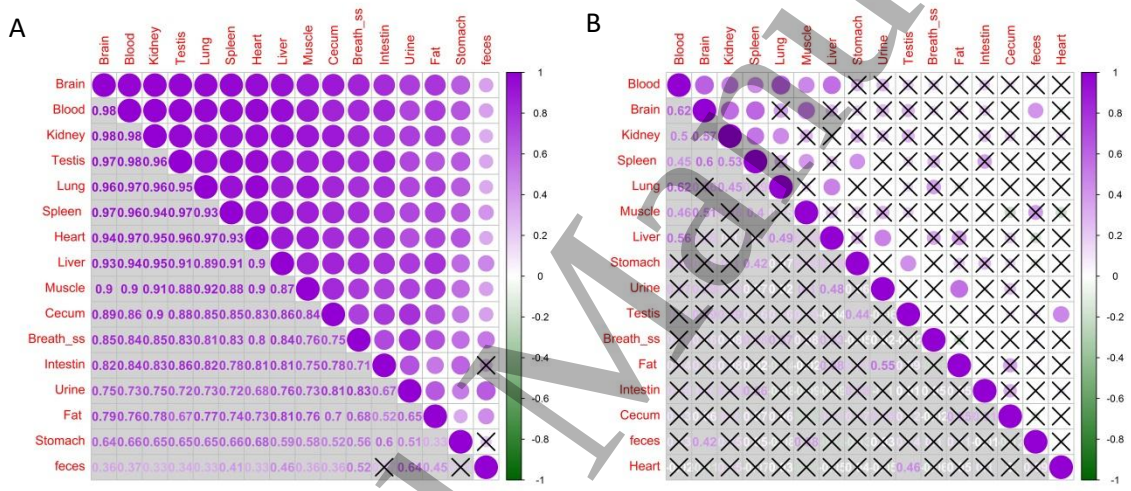



**Figure 4: Correlation matrix ordered after first principal component.** Pearson correlations between headspace concentrations of main methanol signal at 33.05 (A, pk33B) or main acetone signal at 59.05 (B, pk59) are shown for stomach, intestine and cecum content, faeces, liver, heart, lung, brain, muscle, spleen, testis, fat, blood, urine and source strength in breath from mice fed either chow (white, n = 17) or LFD (grey, n = 17). Correlation strength is color-coded and shown in circle size (upper half) as well as absolute coefficients (lower half). Non-significant correlations are marked with a cross.

## 4. Discussion

In this study we compared the distributions of mostly dietary modified methanol with endogenously produced acetone through a series of gastro-intestinal contents, organ lysates and body fluids. As shown earlier, feeding mice a grain-based chow diet in comparison to a purified diet that only contains synthetic ingredients increases emitted levels of methanol (Kistler et al., 2014). Interestingly,

this did not only hold true for breath methanol but the difference can be replicated in every tissue, fluid and biomaterial investigated in this study. In addition, principal component analysis and hierarchical clustering clearly separated samples from mice fed a chow diet from LFD mice. Exemplarily, we selected acetone, a well-studied endogenous VOC where breath emission is not influenced by dietary matrix change. As expected from breath data, no diet related differences could be detected in organs selected for headspace analysis as well. Therefore, we show that systemic changes of VOCs can be detected with this approach.

We hypothesized in our previous study that methanol production depends on microbial activity in the gastro-intestinal tract. This was further substantiated by the fact that upon overnight food restriction (and thus microbial substrate restriction), methanol levels are reduced (Kistler et al., 2016). When following the diet and gastro-intestinal content through the digestive system, a massive ~10 fold increase in methanol concentrations can be found beginning in cecum. It is well known that microbial density increases towards the lower digestive tract to up to $10^{12}$ organisms per gram (Hooper and Gordon, 2001). Methanol is a ubiquitous compound present in the breath of humans and other mammals (Eriksen *et al*, 1963). For a long time, it was considered to be exclusively due to exogenous production after ingestion of fruits, vegetables, alcoholic or aspartame sweetened beverages (Lindinger *et al*, 1997). Therefore, it is very likely that methanol release is linked to the metabolism of the gut microbiome. Interestingly, there is also literature about genuine endogenous methanol release, for example from carboxy methylated proteins, which can be freed by carboxymethylase (Diliberto and Axelrod, 1976, 1974) or under neutral or basic conditions, or from S-adenosyl methionine (SAM) (Axelrod and Daly, 1965). However, our data suggested that exogenous sources seem to dominate systemic levels. Interestingly, acetone concentrations, which are not affected by diet, tissue headspaces detected in both groups were remarkably comparable and reproducible. We assume that the tissue with the highest headspace concentration is a likely candidate for the endogenous production of acetone. Highest levels could be found in liver tissue. This is well in accordance with published work about acetone metabolism, as liver is the major site of ketogenesis. Acetone can be derived from the other ketone bodies aceto-acetate and indirectly from beta-hydroxybutyrate (Puchalska and Crawford, 2017). Thus, according to these two examples information about the origin of certain targeted VOCs can be gained by this methodology.

Remarkably, using this method of sampling from various parts of the organism, interesting distributions in the two selected VOCs can be detected. In methanol, we found a high correlation of levels in the internal organs, which is reduced but still relatively high in other samples. Internal organs are linked by blood as a transfer compartment, and after gastro-intestinal uptake of methanol the blood circulation is the major route of distribution through the body. Interestingly, the solubility of methanol in blood and lean tissues is similar (Fiserova-Bergerova, 1985), which is in accordance with similar headspace concentrations in blood and lean tissues. In addition a reduction in methanol levels

could be found in fat and liver tissue. Existing pharmacokinetic models of methanol exposure via inhalation typically differentiate between liver as the metabolic clearance compartment, kidney for excretion, and richly or slowly perfused organs (Fiserova-Bergerova, 1985; Fiserova-Bergerova and Diaz, 1986; Horton et al., 1992; Paterson and Mackay, 1989; Ward et al., 1997). The perfusion of adipose tissue is significantly less compared to other organs and depends on the physical activity of the exanimated models (Fiserova-Bergerova, 1985). Moreover, the fat-gas partition coefficient and the large difference in the methanol solubility of the slowly perfused fat compared to lean tissues explain the corresponding reduced level (Paterson and Mackay, 1989).

Similar to fat, reduced methanol levels were observed in the liver as well. The hepatic tissue is not only a richly blood-perfused tissue, but it is one of the most metabolically active tissues in the body. It is known that the degradation of methanol primarily occurs in the hepatic tissue (96.9 % vs. 0.6% via urine and 2.5% via breath) which might explain lower observed ex-vivo levels (Skrzydlewska, 2003). Furthermore the clearance of methanol via urine might be indicated by the increase of methanol from blood to urine samples in chow feed mice. In LFD fed mice, absolute levels in urine are reduced by tendency, which could be explained if methanol is reabsorbed (actively) from primary urine. This leads to the hypothesis that there exists a physiological set point, pointing out potential physiological roles for methanol (Dorokhov et al., 2015). In contrast to methanol, acetone urine levels are reduced compared to blood levels in both groups. As acetone contains metabolizable energy, it is reabsorbed from primary urine. This is especially true in ad libitum fed conditions, when ketone body concentrations are low in comparison to fasted or other ketogenic states where the reabsorption capacity is overcome (Puchalska and Crawford, 2017). Regarding acetone, concentrations in different samples were generally weaker correlated (Figure 4b). Since there is no dedicated intervention, *ad libitum* fed acetone levels vary less than methanol levels (due to the intervention), and physiological situations like fasting might be an interesting future experiment to perform in combination with this methodology. Despite the high concentrations in liver headspace there seems to be a typical pattern very similar in both feeding groups, indicating that something like an organ specific VOC-signature might exist.

When studying methanol metabolism, differences in methanol degradation between rodents and primates need to be taken into account. Degradation of methanol to formaldehyde and further metabolites in rodents is primarily performed by the enzyme catalase, whereas in contrast primates use alcohol dehydrogenase and cytochrome P2E1 (Sweeting et al., 2010). Furthermore, formate detoxification in primates is limited by folate availability, leading to accumulation and intoxication to which rodents are not prone. As a consequence, methanol is cleared much faster than in humans. Still, in this study, it can be seen in mouse tissue samples after mice consumed chow food presumably hours before. Another interesting question is to what extent remaining blood in the organs could contribute to the observed levels. For other biological questions, protocols for e.g. saline perfusion to

remove blood from tissues are established. To our knowledge, no study has shown or systematically evaluated the effect of such procedures to the remaining VOC content, the effects and usefulness of perfusion need to be addressed in further studies. In addition to the question of potential saline perfusion discussed above, for several aspects of the methodology other options can be discussed. In the performed protocol, we aimed to optimize the procedure for speed, thus minimizing the time for VOC losses due to emission from organs or (bio-) chemical spontaneous or enzyme-mediated processes. As such, no perfusion was performed, since time from killing the mouse to shock freezing in liquid nitrogen would have been doubled at least. In addition, we decided to homogenize the tissue in a frozen state. By destroying tissue organisation, VOC release can be increased with reduced gradients through the sample. However, other groups have used complete tissue or complete and "chewed" food samples for headspace analysis of VOCs (Farneti et al., 2017; Filipiak et al., 2014). It might depend on the question of interest which sample preparation is feasible.

## 5. Conclusion and perspective

In the presented work we showed that by using dietary modification of a VOC and measuring breath in combination with an ex-vivo headspace of organs approach, information about volatile distribution and physiology can be gained as shown in two well-studied proof of principle VOCs. By applying this method, the origin and metabolism of unknown breath VOCs can be studied. Furthermore, understanding the contribution of single organ systems to breath levels can be instrumental for alternative diagnosis of organ pathologies in the clinics. In combination with different levels of so-called omics technologies (genomics, proteomics, metabolomics, and breathomics), a multi-organ view could contribute to map metabolic pathways and origins of VOCs.

## 6. Author contributions

M. K. conceived and designed the experiments, reviewed and analysed data and wrote, reviewed and edited the manuscript.

A.M. researched data, reviewed data and wrote, reviewed and edited the manuscript.

G.M., V.H., C.H., R.Z. and M.H.A. contributed to discussion, reviewed and edited the manuscript.

J.R. conceived and designed the experiments, reviewed data, wrote, reviewed and edited the manuscript.

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## 7. References


Aprea, E., Morisco, F., Biasioli, F., Vitaglione, P., Cappellin, L., Soukoulis, C., Lembo, V., Gasperi, F., D'Argenio, G., Fogliano, V., Caporaso, N., 2012. Analysis of breath by proton transfer reaction time of flight mass spectrometry in rats with steatohepatitis induced by high-fat diet. J. Mass Spectrom. JMS 47, 1098–1103. doi:10.1002/jms.3009

Axelrod, J., Daly, J., 1965. Pituitary gland: enzymic formation of methanol from S-adenosylmethionine. Science 150, 892–893.

Baranska, A., Mujagic, Z., Smolinska, A., Dallinga, J.W., Jonkers, D.M. a. E., Tigchelaar, E.F., Dekens, J., Zhernakova, A., Ludwig, T., Masclee, A. a. M., Wijmenga, C., van Schooten, F.J., 2016. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. Aliment. Pharmacol. Ther. 44, 45–56. doi:10.1111/apt.13654

Bean, H.D., Rees, C.A., Hill, J.E., 2016. Comparative analysis of the volatile metabolomes of Pseudomonas aeruginosa clinical isolates. J. Breath Res. 10, 047102. doi:10.1088/1752-7155/10/4/047102

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Brunner, C., Szymczak, W., Höllriegl, V., Mörtl, S., Oelmez, H., Bergner, A., Huber, R.M., Hoeschen, C., Oeh, U., 2010. Discrimination of cancerous and non-cancerous cell lines by headspace-analysis with PTR-MS. Anal. Bioanal. Chem. 397, 2315–2324. doi:10.1007/s00216-010-3838-x

Buuren, S. van, Groothuis-Oudshoorn, K., 2011. mice: Multivariate Imputation by Chained Equations in R. J. Stat. Softw. 45, 1–67.

Diliberto, E.J., Axelrod, J., 1976. Regional and Subcellular Distribution of Protein Carboxymethylase in Brain and Other Tissues. J. Neurochem. 26, 1159–1165. doi:10.1111/j.1471-4159.1976.tb07001.x

Diliberto, E.J., Axelrod, J., 1974. Characterization and substrate specificity of a protein carboxymethylase in the pituitary gland. Proc. Natl. Acad. Sci. 71, 1701–1704.

Dorokhov, Y.L., Shindyapina, A.V., Sheshukova, E.V., Komarova, T.V., 2015. Metabolic Methanol: Molecular Pathways and Physiological Roles. Physiol. Rev. 95, 603–644. doi:10.1152/physrev.00034.2014

Farneti, B., Di Guardo, M., Khomenko, I., Cappellin, L., Biasioli, F., Velasco, R., Costa, F., 2017. Genome-wide association study unravels the genetic control of the apple volatilome and its interplay with fruit texture. J. Exp. Bot. doi:10.1093/jxb/erx018

Fernández del Río, R., O'Hara, M.E., Holt, A., Pemberton, P., Shah, T., Whitehouse, T., Mayhew, C.A., 2015. Volatile Biomarkers in Breath Associated With Liver Cirrhosis — Comparisons of Pre- and Post-liver Transplant Breath Samples. EBioMedicine 2, 1243–1250. doi:10.1016/j.ebiom.2015.07.027

Filipiak, W., Filipiak, A., Sponring, A., Schmid, T., Zelger, B., Ager, C., Ewa Klodzinska, Denz, H., Pizzini, A., Lucciarini, P., Jamnig, H., Troppmair, J., Amann, A., 2014. Comparative analyses of volatile organic compounds (VOCs) from patients, tumors and transformed cell lines for the validation of lung cancer-derived breath markers. J. Breath Res. 8, 027111. doi:10.1088/1752-7155/8/2/027111

Filipiak, W., Mochalski, P., Troppmair, J., Unterkofler, K., Agapiou, A., Davis, C., Cumeras, R., Ager, C., Filipiak, A., 2016. A compendium of volatile organic compounds (VOCs) released by human cell lines. Curr. Med. Chem. 23, 1–1. doi:10.2174/0929867323666160510122913

Fiserova-Bergerova, V., 1985. Toxicokinetics of organic solvents. Scand. J. Work. Environ. Health 11 Suppl 1, 7–21.

Fiserova-Bergerova, V., Diaz, M.L., 1986. Determination and prediction of tissue-gas partition coefficients. Int. Arch. Occup. Environ. Health 58, 75–87.

Fuchs, H., Gailus-Durner, V., Adler, T., Pimentel, J.A.A., Becker, L., Bolle, I., Brielmeier, M., Calzada-Wack, J., Dalke, C., Ehrhardt, N., Fasnacht, N., Ferwagner, B., Frischmann, U., Hans, W., Hölter, S.M., Hölzlwimmer, G., Horsch, M., Javaheri, A., Kallnik, M., Kling, E., Lengger, C., Maier, H., Mossbrugger, I., Mörth, C., Naton, B., Nöth, U., Pasche, B., Prehn,

C., Przemeck, G., Puk, O., Racz, I., Rathkolb, B., Rozman, J., Schäble, K., Schreiner, R., Schrewe, A., Sina, C., Steinkamp, R., Thiele, F., Willershäuser, M., Zeh, R., Adamski, J., Busch, D.H., Beckers, J., Behrendt, H., Daniel, H., Esposito, I., Favor, J., Graw, J., Heldmaier, G., Höfler, H., Ivandic, B., Katus, H., Klingenspor, M., Klopstock, T., Lengeling, A., Mempel, M., Müller, W., Neschen, S., Ollert, M., Quintanilla-Martinez, L., Rosenstiel, P., Schmidt, J., Schreiber, S., Schughart, K., Schulz, H., Wolf, E., Wurst, W., Zimmer, A., Hrabé de Angelis, M., 2009. The German Mouse Clinic: a platform for systemic phenotype analysis of mouse models. Curr. Pharm. Biotechnol. 10, 236–243.

Hooper, L.V., Gordon, J.I., 2001. Commensal Host-Bacterial Relationships in the Gut. Science 292, 1115–1118. doi:10.1126/science.1058709

Horton, V.L., Higuchi, M.A., Rickert, D.E., 1992. Physiologically based pharmacokinetic model for methanol in rats, monkeys, and humans. Toxicol. Appl. Pharmacol. 117, 26–36.

Hüppe, T., Lorenz, D., Maurer, F., Albrecht, F.W., Schnauber, K., Wolf, B., Sessler, D.I., Volk, T., Fink, T., Kreuer, S., 2016. Exhalation of volatile organic compounds during hemorrhagic shock and reperfusion in rats: an exploratory trial. J. Breath Res. 10, 016016. doi:10.1088/1752-7155/10/1/016016

Kistler, M., Muntean, A., Szymczak, W., Rink, N., Fuchs, H., Valerie Gailus-Durner, Wurst, W., Hoeschen, C., Klingenspor, M., Angelis, M.H. de, Jan Rozman, 2016. Diet-induced and mono-genetic obesity alter volatile organic compound signature in mice. J. Breath Res. 10, 016009. doi:10.1088/1752-7155/10/1/016009

Kistler, M., Szymczak, W., Fedrigo, M., Fiamoncini, J., Höllriegl, V., Hoeschen, C., Klingenspor, M., Hrabě de Angelis, M., Rozman, J., 2014. Effects of diet-matrix on volatile organic compounds in breath in diet-induced obese mice. J. Breath Res. 8, 016004. doi:10.1088/1752-7155/8/1/016004

Lindinger, W., Taucher, J., Jordan, A., Hansel, A., Vogel, W., 1997. Endogenous Production of Methanol after the Consumption of Fruit. Alcohol. Clin. Exp. Res. 21, 939–943. doi:10.1111/j.1530-0277.1997.tb03862.x

Nakhleh, M.K., Amal, H., Jeries, R., Broza, Y.Y., Aboud, M., Gharra, A., Ivgi, H., Khatib, S., Badarneh, S., Har-Shai, L., Glass-Marmor, L., Lejbkowicz, I., Miller, A., Badarny, S., Winer, R., Finberg, J., Cohen-Kaminsky, S., Perros, F., Montani, D., Girerd, B., Garcia, G., Simonneau, G., Nakhoul, F., Baram, S., Salim, R., Hakim, M., Gruber, M., Ronen, O., Marshak, T., Doweck, I., Nativ, O., Bahouth, Z., Shi, D., Zhang, W., Hua, Q., Pan, Y., Tao, L., Liu, H., Karban, A., Koifman, E., Rainis, T., Skapars, R., Sivins, A., Ancans, G., Liepniece-Karele, I., Kikuste, I., Lasina, I., Tolmanis, I., Johnson, D., Millstone, S.Z., Fulton, J., Wells, J.W., Wilf, L.H., Humbert, M., Leja, M., Peled, N., Haick, H., 2016. Diagnosis and Classification of 17 Diseases from 1404 Subjects via Pattern Analysis of Exhaled Molecules. ACS Nano. doi:10.1021/acsnano.6b04930

Obermeier, J., Trefz, P., Happ, J., Schubert, J.K., Staude, H., Fischer, D.-C., Miekisch, W., 2017. Exhaled volatile substances mirror clinical conditions in pediatric chronic kidney disease. PloS One 12, e0178745. doi:10.1371/journal.pone.0178745

O'Hara, M.E., Clutton-Brock, T.H., Green, S., Mayhew, C.A., 2009. Endogenous volatile organic compounds in breath and blood of healthy volunteers: examining breath analysis as a surrogate for blood measurements. J. Breath Res. 3, 027005. doi:10.1088/1752-7155/3/2/027005

Paterson, S., Mackay, D., 1989. Correlation of tissue, blood, and air partition coefficients of volatile organic chemicals. Br. J. Ind. Med. 46, 321–328.

Pleil, J.D., Stiegel, M.A., Risby, T.H., 2013. Clinical breath analysis: discriminating between human endogenous compounds and exogenous (environmental) chemical confounders. J. Breath Res. 7, 017107. doi:10.1088/1752-7155/7/1/017107

Puchalska, P., Crawford, P.A., 2017. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. Cell Metab. 25, 262–284. doi:10.1016/j.cmet.2016.12.022

Ruzsanyi, V., Lederer, W., Seger, C., Calenic, B., Liedl, K.R., Amann, A., 2014. Non-13CO2 targeted breath tests: a feasibility study. J. Breath Res. 8, 046005. doi:10.1088/1752-7155/8/4/046005

Sinues, P.M.-L., Kohler, M., Brown, S.A., Zenobi, R., Dallmann, R., 2017. Gauging circadian variation in ketamine metabolism by real-time breath analysis. Chem. Commun. 53, 2264–2267. doi:10.1039/C6CC09061C

Skrzydlewska, E., 2003. Toxicological and Metabolic Consequences of Methanol Poisoning. Toxicol. Mech. Methods 13, 277–293. doi:10.1080/713857189

Španěl, P., Dryahina, K., Vicherková, P., Smith, D., 2015. Increase of methanol in exhaled breath quantified by SIFT-MS following aspartame ingestion. J. Breath Res. 9, 047104. doi:10.1088/1752-7155/9/4/047104

Sweeting, J.N., Siu, M., McCallum, G.P., Miller, L., Wells, P.G., 2010. Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates. Toxicol. Appl. Pharmacol. 247, 28–35. doi:10.1016/j.taap.2010.05.009

Szymczak, W., Rozman, J., Höllriegl, V., Kistler, M., Keller, S., Peters, D., Kneipp, M., Schulz, H., Hoeschen, C., Klingenspor, M., de Angelis, M.H., 2014. Online breath gas analysis in unrestrained mice by hs-PTR-MS. Mamm. Genome Off. J. Int. Mamm. Genome Soc. 25, 129–140. doi:10.1007/s00335-013-9493-8

van Vliet, D., Smolinska, A., Jöbsis, Q., Rosias, P., Muris, J., Dallinga, J., Dompeling, E., van Schooten, F.-J., 2017. Can exhaled volatile organic compounds predict asthma exacerbations in children? J. Breath Res. 11, 016016. doi:10.1088/1752-7163/aa5a8b

Ward, K.W., Blumenthal, G.M., Welsch, F., Pollack, G.M., 1997. Development of a physiologically based pharmacokinetic model to describe the disposition of methanol in pregnant rats and mice. Toxicol. Appl. Pharmacol. 145, 311–322. doi:10.1006/taap.1997.8170

Wickham, H., 2009. ggplot2: elegant graphics for data analysis. http://had.co.nz/ggplot2/book. Springer New York.