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# A longitudinal study of ethanol and acetaldehyde in the exhaled breath of healthy volunteers using selected-ion flow-tube mass spectrometry

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**Selected-ion flow-tube mass spectrometry (SIFT-MS) has been used to monitor the volatile compounds in the exhaled breath of 30 volunteers (19 male, 11 female) over a 6-month period. Volunteers provided breath samples each week between 8:45 and 13:00 (before lunch), and the concentrations of several trace compounds were obtained. In this paper the focus is on ethanol and acetaldehyde, which were simultaneously quantified by SIFT-MS using $H_3O^+$ precursor ions. The mean ethanol level for all samples was 196 parts-per-billion (ppb) with a standard deviation of 244 ppb, and the range of values for breath samples analysed is 0 to 1663 ppb. The mean acetaldehyde level for all samples was 24 ppb with a standard deviation of 17 ppb, and the range of values for breath samples analysed is 0 to 104 ppb. Background (ambient air) levels of ethanol were around 50 ppb, whereas any background acetaldehyde was usually undetectable. Increased ethanol levels were observed if sweet drink/food had been consumed within the 2 h prior to providing the breath samples, but no increase was apparent when alcohol had been consumed the previous evening. The measured endogenous breath ethanol and acetaldehyde levels were not correlated. These data relating to healthy individuals are a prelude to using breath analysis for clinical diagnosis, for example, the recognition of bacterial overload in the gut (ethanol) or the possibly of detecting tumours in the body (acetaldehyde).** 

Ethanol is one of the few breath metabolites routinely measured, principally by law enforcement officers checking that it is below that permitted by law (in the UK 180 ppm, equivalent to a blood level of 0.8 g/L). However, the legal drink-drive limit is typically 1000 times that of the typical endogenously produced ethanol concentration measured in exhaled human breath. Endogenous ethanol in breath, i.e. that produced within the body and unrelated to ingestion of alcohol, has previously been measured over 30 days in five human volunteers using selected-ion flow-tube mass spectrometry (SIFT-MS), and found to be between 0 and 380 parts-per-billion (ppb),[1] with mean values for each of the five volunteers over the 30-day period ranging between 27 to 153 ppb. In the same study breath acetaldehyde was quantified simultaneously, resulting in a mean level for the five volunteers between 2 and 5 ppb.

The present work extends the earlier study[1] by studying the composition of exhaled breath in a larger cohort of 30 volunteers over an extended time period of 6 months (commonly termed a longitudinal study), and includes data from some volunteers who had ingested alcohol the previous day. The improved sensitivity of the SIFT-MS analytical instrument (see below) also allowed more accurate quantification of acetaldehyde in the breath. We also briefly examine the role of mouth production of breath ethanol, and demonstrate that its level can be influenced by mouth flora and the consumption of sweet food or drink prior to sampling, although this will be the topic of a later detailed study.

It has been known for decades that acetaldehyde is an intermediate in the metabolism of ethanol in the liver.[2] Ethanol is converted into acetaldehyde by the enzyme alcohol dehydrogenase, and the acetaldehyde is then converted into acetic acid by acetaldehyde dehydrogenase.[3] Acetaldehyde is a highly toxic compound, with a 50% lethal dose ($LD_{50}$) concentration in rats that is about ten times lower than that for ethanol.[4] Hence it needs to be removed from the

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body rapidly, and thus it is very difficult to measure in the body so that early observers noted that many observed blood acetaldehyde concentrations during normal ethanol oxidation were artefactual and arose during the analytical procedures.[5] It has also been suggested that acetaldehyde produced by microorganisms present in the throat may affect the breath analysis of acetaldehyde,[6] so one needs to be circumspect when analysing breath for acetaldehyde (and indeed other trace compounds).

Studies on the quantification and decay of ethanol and acetaldehyde in breath following the ingestion of ethanol have been performed using SIFT-MS.[7] In that study the ingestion of ethanol led to a rapid increase in ethanol and acetaldehyde in breath, and there was a clear correlation between the levels of ethanol and acetaldehyde. However, it was previously indicated[6] that acetaldehyde generated during ethanol metabolism may become tightly bound to endogenous proteins and phospholipids before passing into blood, which would suggest that the correlation between these two compounds in breath may not be obvious, contrary to other findings.[7] The data obtained in the present study, that did not involve ethanol ingestion, allows any correlation between endogenous ethanol and acetaldehyde to be checked in a significant cohort of healthy volunteers.

Measurement of base levels of ethanol and acetaldehyde in breath in healthy people has significance in that these compounds, when elevated above the normal, may be important indicators of disease. It is known that ethanol is greatly elevated in the breath of some patients in end-stage renal disease,[8] and this has been tentatively attributed to bacterial overgrowth in the gut of these patients. Concerning acetaldehyde, it has been shown by SIFT-MS measurements that this compound is produced in easily measurable quantities by the lung cancer cell lines SK-MES and CALU-1 *in vitro*.[9] The rate of production of acetaldehyde per cell was calculated (typically $10^6$ molecules/cell/min) and, although cells *in vivo* may act differently, it is an important finding that indicates that volatile compounds produced by abnormal cells may transfer into blood and hence into breath, thus becoming potential disease markers.

## EXPERIMENTAL

### SIFT-MS analysis

SIFT-MS has been described in detail elsewhere,[10–13] so only a brief outline is provided here. Precursor ions generated in a microwave discharge are selected by a quadrupole mass filter and injected into a fast flowing helium carrier gas (Fig. 1). These ions are thermalised to the helium carrier gas temperature (300 K) by multiple collisions with helium atoms before reacting with the trace gases, e.g. ethanol and acetaldehyde, in a breath sample introduced at a known flow rate via a heated calibrated capillary. An appropriate precursor ion species can be selected from $H_3O^+$, $NO^+$ and $O_2^+$. The choice of precursor depends on the trace gas compounds to be analysed; each produces characteristic product ions from reactions with each particular trace gas compound. The precursor and product ions in the carrier gas are sampled by a downstream orifice, and pass into a differentially pumped quadrupole mass spectrometer and ion-counting system for analysis.

There are two distinct analytical modes of operation for SIFT-MS. The 'full-scan mode', in which a conventional mass spectrum is obtained over a chosen $m/z$ range, is used to identify the precursor and product ions and their respective count rates. The on-line computer then immediately calculates the partial pressures of those trace gas compounds present in the breath sample for which identifiable product

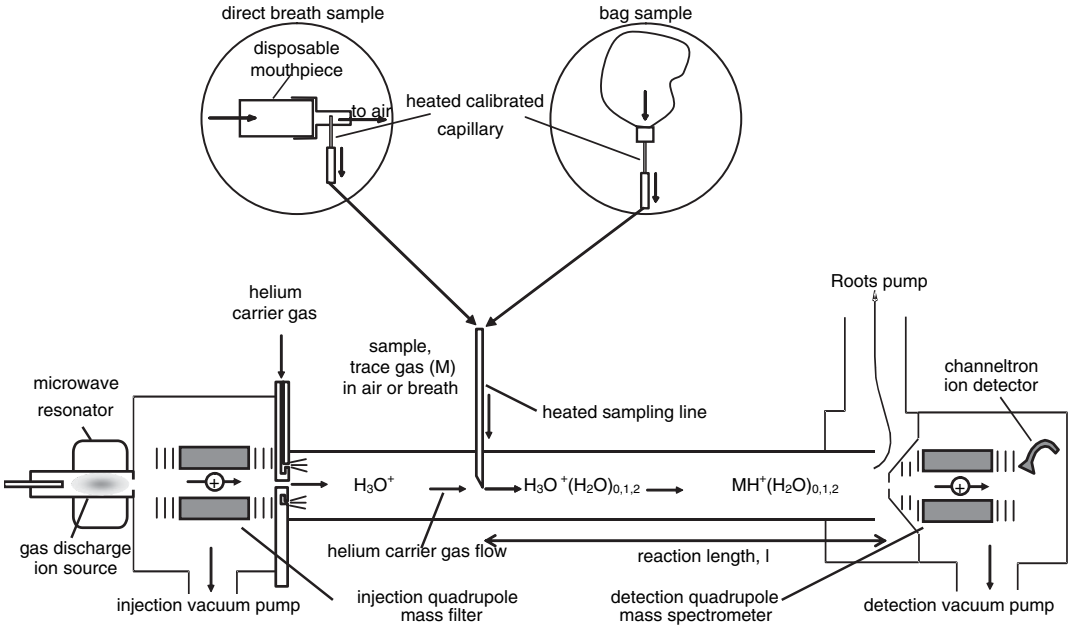


**Figure 1.** A schematic diagram of the SIFT-MS instrument indicating the main components. Direct breath samples, or indirect breath samples collected into a bag, may be analysed, as illustrated, although the present study used only direct breath measurements.

ions are present and which are included in the kinetics database required for the analysis. The kinetics database comprises the rate coefficients and the product ions of the particular precursor ion/trace gas compound reactions. The database has been constructed from numerous detailed SIFT studies of various classes of compounds (alcohols, aldehydes, ketones, hydrocarbons, etc.) with the three precursor ions.[10–12] The 'multiple ion monitoring mode', in which the downstream analytical mass spectrometer is rapidly switched between selected *m/z* values for both the precursor and chosen product ions, is used to quantify both water vapour and the targeted trace compounds. This mode of operation provides more accurate quantification of the chosen trace compounds than does the broad sweep full-scan mode. An example of the data obtained using the multiple ion monitoring mode is shown in Fig. 2, which shows the time profiles of ethanol and methanol concentrations in three exhalations, analysed by SIFT-MS using $H_3O^+$ precursor ions. This mode was used here to obtain the concentrations of ethanol and acetaldehyde in alveolar air. Note also the important point that, during the breath inhalation cycles, the levels of the targeted compounds in the ambient air are determined to check whether their observed levels are influenced by high ambient levels of these compounds.

Ethanol reacts rapidly with all three available precursor ions, $H_3O^+$, $NO^+$ and $O_2^+$. With $H_3O^+$, the reaction process is direct, non-dissociative proton transfer to give the protonated ethanol species at *m/z* 47. Reaction (1) is exothermic and occurs at the collision rate:[14]

$$H_3O^+ + C_2H_5OH \rightarrow C_2H_5OH_2^+ + H_2O \qquad (1)$$

Hydrates of the product ions readily form when humid breath is introduced into the carrier gas. Both the monohydrate and the dihydrate of protonated ethanol will be present at *m/z* 65 ($C_2H_5OH_2^+ \cdot H_2O$) and 83 ($C_2H_5OH_2^+ (H_2O)_2$), and these must be included in the analysis of the ethanol. The ion chemistry of acetaldehyde is similar in that $CH_3CHOH^+$ (*m/z* 45), $CH_3CHOH^+ \cdot H_2O$ (*m/z* 63) and $CH_3CHOH^+(H_2O)_2$ (*m/z* 81) are formed, all of which must be included in the analysis. Further details of the ion chemistry involved in the analysis of particular trace gases in breath are given in a recent review article[12] and a recently published book.[13]

## Breath monitoring

In the previous study by Diskin *et al.*[1] the concentrations of five breath metabolites, including ethanol and acetaldehyde, were monitored in the breath of just five volunteers over a 30-day period. To obtain a more reliable picture of the range of normal values for ethanol and acetaldehyde in healthy individuals under a variety of circumstances, in the present study 30 apparently healthy adult volunteers were recruited with ages over the range from 24 to 59 years; the body mass index (BMI) ranged from 18.4 to 30.6. Eleven of the volunteers were female and 19 male. Volunteers were asked to provide breath samples at approximately weekly intervals for about 6 months; some volunteers provided samples more frequently than others. In previous studies, diurnal and postprandial variations in the concentrations of some breath volatile compounds have been noted;[15,16] hence, to minimise the influence of variations caused by diet, sleep, activity, etc., the breath samples were always taken between 8:45 and 13:00 (prior to lunch), although not necessarily at the same time or on the same day each week for individual volunteers.

The volunteers were colleagues of one of us (at SRI), and the distance travelled to provide a breath sample was a short walk to the laboratory, requiring minimal effort or exercise. For each breath sample obtained, volunteers were asked to

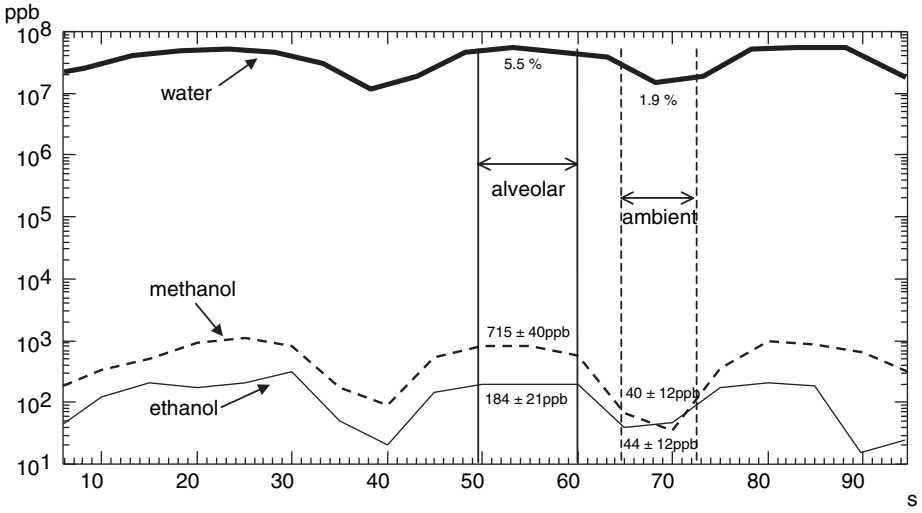


**Figure 2.** Quantitative SIFT-MS analyses of the ethanol and methanol levels in exhaled breath calculated using the ion signal ratios of the precursor ions ($H_3O^+$) and their hydrates and the characteristic product ions of ethanol ($C_2H_5OH_2^+$, *m/z* 47) and methanol ($CH_3OH_2^+$, *m/z* 31) and their hydrates, together with the known reaction time and the known sample and carrier gas flow rates. Both the alveolar portions of the exhalation and the ambient air portion, that are used to calculate the mean exhalation levels and the levels in the laboratory air, are indicated.

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**Table 1.** Summary of ethanol concentrations in all the breath samples from 30 volunteers over a 6-month period

| Volunteer no. | Age | BMI | Gender | Mean ppb | SD | Low ppb | High ppb | No. of samples |
|---|---|---|---|---|---|---|---|---|
| 21 | 24 | 22.2 | F | 277 | 351 | 12 | 1109 | 16 |
| 22 | 29 | 29.1 | F | 329 | 155 | 106 | 503 | 6 |
| 1 | 39 | 22.9 | F | 43 | 38 | 7 | 146 | 23 |
| 4 | 39 | 24.3 | F | 336 | 195 | 81 | 868 | 18 |
| 23 | 42 | 23 | F | 106 | 85 | 32 | 345 | 18 |
| 27 | 43 | 18.4 | F | 132 | 88 | 47 | 374 | 17 |
| 3 | 44 | 30.6 | F | 111 | 40 | 64 | 171 | 11 |
| 13 | 44 | 28.7 | F | 95 | 74 | 10 | 315 | 20 |
| 16 | 46 | 23.6 | F | 166 | 98 | 4 | 364 | 15 |
| 19 | 47 | 30.1 | F | 113 | 109 | 11 | 427 | 18 |
| 15 | 56 | 28 | F | 205 | 253 | 60 | 1007 | 15 |
| 29 | 27 | 20 | M | 155 | 131 | 34 | 459 | 11 |
| 8 | 33 | 22.6 | M | 138 | 126 | 21 | 432 | 16 |
| 17 | 39 | 20.7 | M | 333 | 164 | 88 | 702 | 19 |
| 26 | 41 | 23.6 | M | 99 | 53 | 23 | 204 | 18 |
| 7 | 41 | 27.5 | M | 406 | 317 | 97 | 1157 | 16 |
| 30 | 45 | 24.2 | M | 625 | 446 | 53 | 1508 | 12 |
| 25 | 48 | 25.4 | M | 1091 | 316 | 467 | 1663 | 10 |
| 28 | 49 | 25.7 | M | 151 | 62 | 38 | 267 | 16 |
| 12 | 49 | 26.5 | M | 164 | 117 | 0 | 468 | 16 |
| 5 | 50 | 22.8 | M | 249 | 197 | 0 | 725 | 19 |
| 2 | 52 | 24.6 | M | 157 | 104 | 0 | 376 | 16 |
| 14 | 52 | 25.3 | M | 54 | 49 | 13 | 230 | 18 |
| 20 | 52 | 24.2 | M | 114 | 103 | 4 | 410 | 17 |
| 6 | 55 | 27.6 | M | 359 | 306 | 36 | 1078 | 17 |
| 10 | 55 | 23 | M | 76 | 79 | 3 | 333 | 17 |
| 24 | 56 | 27.6 | M | 59 | 67 | 23 | 269 | 13 |
| 11 | 58 | 22.6 | M | 79 | 48 | 38 | 217 | 17 |
| 9 | 59 | 25.3 | M | 113 | 91 | 28 | 321 | 15 |
| 18 | 59 | 25.7 | M | 109 | 109 | 0 | 367 | 18 |

complete a questionnaire detailing information about their diet, sleep, exercise, medication, alcohol intake, cigarette smoking, general feeling of health and state of stress, medication taken, etc. This information was then collated and subjected to various statistical analyses to identify relevant correlations with breath components. A total of 478 mean ethanol concentration values were recorded during the course of the study; each ethanol concentration (Table 1) was the mean of three values measured in three separate consecutive breath exhalations. Similarly, 391 breath samples were taken to quantify acetaldehyde; this is fewer measurements than for ethanol, because unfortunately acetaldehyde was not measured from the very beginning of the study.

Volunteers were asked to exhale by mouth through a cardboard tube positioned at the entrance to the sample inlet system of the SIFT-MS instrument, and breath was sampled directly via a capillary that provided an accurately known flow rate of sample into the carrier gas. Volunteers were not required to rinse their mouths out prior to sampling, or depart in any way from their normal daily routine.

## RESULTS

### Ethanol

As noted above, the ethanol concentration recorded for each volunteer for each day was the mean value for three consecutive breaths, as exemplified in Fig. 2. A total of 478 mean breath values, obtained from 1434 single breath exhalations, are considered here. The mean breath ethanol concentrations for each volunteer over the 6-month observational period, together with their standard deviations (SD) and the spread in these values over the number of breath samples shown, are given in Table 1 (in ppb). The data are listed by increasing age of the volunteers, and separated according to gender; the BMI of each volunteer is also recorded. The spread in breath ethanol concentrations is very great among individuals, but generally not so widely spread for each individual. The values for all volunteers range from 0 to 1663 ppb with a mean of 196 ppb and a SD of 244 ppb; the median value is 112 ppb. The distribution of breath ethanol levels for all 30 volunteers is depicted by the histogram in Fig. 3(a), representing all 478 measurements of concentrations in alveolar breath; the distribution is close to log-normal, as expected for variable (and by their nature necessarily positive) breath metabolite levels. Because our very recent preliminary investigations demonstrated that the consumption of sweet drinks or food enhances breath ethanol levels, either through the action of mouth flora or gut flora on sugars (data not yet thoroughly assessed), we questioned whether this phenomenon could be significantly influencing the breath ethanol levels determined in this study. Hence, the total data (Table 1) were divided into those breath samples given when the volunteer had not consumed sweet drinks or food within 2 h of the sample, and those that had. Figure 3(b) is a histogram of 292 breath ethanol samples for which no sugary food or drink had been consumed within the previous 2 h, and Fig. 3(c) is a histogram

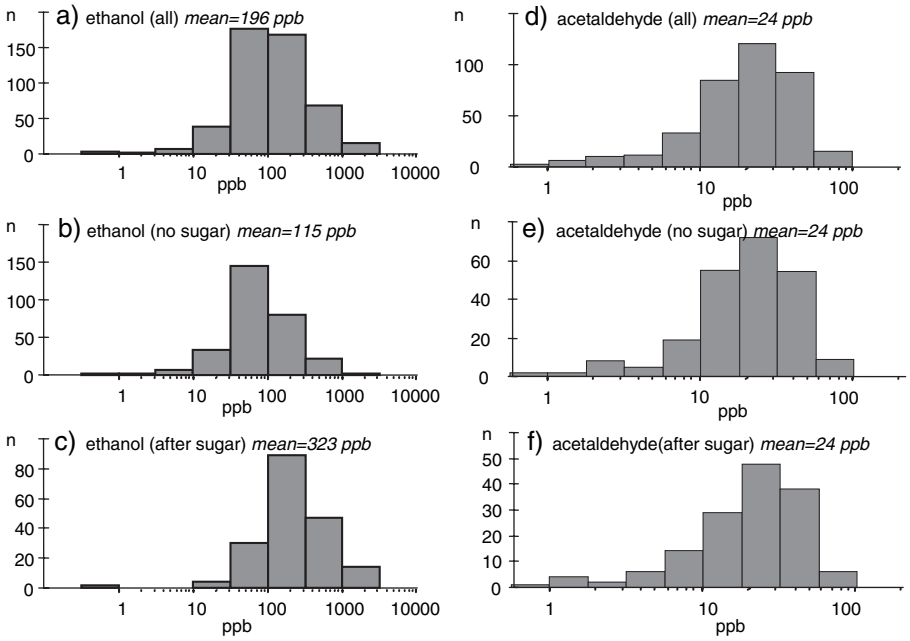


**Figure 3.** Histograms showing the distributions of ethanol and acetaldehyde concentrations in parts-per-billion (ppb) in breath samples from 30 volunteers over a 6-month period. Arranged vertically, the histograms are for all samples (a, d), those samples for which the volunteers had not consumed sweet food or drink less than 2 h prior to the sample being taken (b, e), and the breath samples where sugary food or drink had been consumed within 2 h of sampling (c, f). The median values for data in (a)–(f) are 112, 72, 214, 22, 22 and 23 ppb, respectively.

of 186 breath samples for which sugary food or drink had been consumed within the 2-h period. The mean breath ethanol levels are obviously greater after consumption of a sugary drink or food less than 2 h prior to the breath sample. The implications of this observation are considered below and ask if the enhanced breath ethanol is produced by mouth flora or by gut flora.

Some previous workers have noted an effect of ambient air background levels on the apparent levels of breath volatiles; high background levels may mean that the concentrations of compounds present in breath do not accurately reflect levels of those compounds in blood.[17] The background levels of ethanol measured in the present experiments were readily measurable (although any background acetaldehyde was immeasurably small). This potential problem is addressed below.

Volunteers were not asked to abstain from drinking alcohol during the previous day, and some of the volunteers consumed alcoholic beverages during the evenings before breath samples were obtained. However, no correlation was observed between the level of ethanol in breath and the alcohol consumed the previous evening, although, in all cases, more than 9 h had passed between consumption of alcohol and the breath measurements. This lack of correlation is the case for the entire data set as a whole, and also within data sets for individual volunteers. The decay of ethanol in breath after consumption of different amounts of ethanol in tap water was previously investigated by Smith *et al.*[7] It was found that, after drinking 17 mL of ethanol in 500 mL of tap water on an empty stomach, only 200 min was required for the breath ethanol level to reach the pre-dose level. An extreme example in this study involved a volunteer who consumed the equivalent of six times the above level of ethanol some 20 h (1200 min) prior to the breath test, yet the breath ethanol level had diminished to the usual low level (25 ppb) for this individual (volunteer 1) by the following morning. These limited observations indicate that the breath ethanol levels measured in the morning reflect endogenously produced blood ethanol levels, with a possible additional source due to mouth production as addressed below.

## Acetaldehyde

A total of 391 mean breath values, obtained from 1173 single breath exhalations, are under consideration here. The mean breath acetaldehyde concentrations (in ppb) for each volunteer over the 6-month observational period, together with their SDs and the spread in these values for the breath samples analysed, are given in Table 2. Again, the data are listed in increasing age of the volunteers and separated according to gender, and the BMI of each volunteer is recorded. The concentrations are much smaller than those for ethanol, ranging from 0 to 104 ppb with a mean of 24 ppb and a SD of 17 ppb; the median value is 22 ppb. A histogram showing the distribution of 391 acetaldehyde concentrations is given in Fig. 3(d). As for the ethanol case, the data were divided into breath samples given when the volunteer had not consumed sweet drinks or food within 2 h of the sample, and those that had. Figure 3(e) is a histogram of 238 breath acetaldehyde samples for which no sugary food or drink had been consumed within the previous 2 h (mean 24 ppb,

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**Table 2.** Summary of acetaldehyde concentrations in all the breath samples from 30 volunteers over a 6-month period

| Volunteer no. | Age | BMI | Gender | Mean ppb | SD | Low ppb | High ppb | No. of samples |
|---|---|---|---|---|---|---|---|---|
| 21 | 24 | 22.2 | F | 21 | 24 | 0 | 90 | 13 |
| 22 | 29 | 29.1 | F | 12 | 9 | 1 | 17 | 3 |
| 1 | 39 | 22.9 | F | 31 | 14 | 2 | 58 | 18 |
| 4 | 39 | 24.3 | F | 26 | 13 | 5 | 59 | 14 |
| 23 | 42 | 23 | F | 25 | 13 | 0 | 45 | 15 |
| 27 | 43 | 18.4 | F | 23 | 20 | 0 | 76 | 14 |
| 3 | 44 | 30.6 | F | 23 | 12 | 9 | 46 | 12 |
| 13 | 44 | 28.7 | F | 14 | 12 | 0 | 38 | 16 |
| 16 | 46 | 23.6 | F | 36 | 17 | 0 | 61 | 13 |
| 19 | 47 | 30.1 | F | 24 | 21 | 1 | 63 | 15 |
| 15 | 56 | 28 | F | 32 | 20 | 7 | 61 | 12 |
| 29 | 27 | 20 | M | 17 | 7 | 5 | 27 | 9 |
| 8 | 33 | 22.6 | M | 21 | 13 | 3 | 46 | 12 |
| 17 | 39 | 20.7 | M | 31 | 10 | 16 | 52 | 16 |
| 26 | 41 | 23.6 | M | 19 | 15 | 0 | 47 | 15 |
| 7 | 41 | 27.5 | M | 26 | 10 | 10 | 41 | 13 |
| 30 | 45 | 24.2 | M | 16 | 18 | 0 | 54 | 10 |
| 25 | 48 | 25.4 | M | 29 | 17 | 12 | 63 | 9 |
| 28 | 49 | 25.7 | M | 22 | 14 | 0 | 47 | 15 |
| 12 | 49 | 26.5 | M | 28 | 25 | 9 | 97 | 11 |
| 5 | 50 | 22.8 | M | 20 | 13 | 6 | 46 | 15 |
| 2 | 52 | 24.6 | M | 24 | 18 | 0 | 60 | 12 |
| 14 | 52 | 25.3 | M | 17 | 13 | 2 | 44 | 14 |
| 20 | 52 | 24.2 | M | 37 | 29 | 8 | 104 | 15 |
| 6 | 55 | 27.6 | M | 22 | 14 | 2 | 43 | 13 |
| 10 | 55 | 23 | M | 24 | 18 | 0 | 68 | 13 |
| 24 | 56 | 27.6 | M | 23 | 14 | 3 | 44 | 12 |
| 11 | 58 | 22.6 | M | 24 | 13 | 12 | 54 | 13 |
| 9 | 59 | 25.3 | M | 19 | 12 | 8 | 44 | 12 |
| 18 | 59 | 25.7 | M | 15 | 13 | 0 | 36 | 15 |

median 22 ppb), and Fig. 3(f) is a histogram of 153 breath acetaldehyde samples for which sugary food or drink had been consumed (mean 24 ppb, median 23 ppb). These histograms show that acetaldehyde levels are essentially unaffected by the consumption of sugar prior to providing the breath sample, and indicates that our acetaldehyde data are probably unaffected by the action of mouth and throat bacteria as was suggested in another study.[6]

It is important to note that, from the more limited study of Diskin *et al.*,[1] mean values for breath acetaldehyde for just five volunteers were apparently within the range 2 to 5 ppb. However, it must be appreciated that this was close to the limiting concentration that can be quantified by the particular SIFT-MS instrument used for that study, and thus the derived values are subject to much larger uncertainties than those obtained in the present experiments. Nevertheless, the earlier results[1] indicate that the breath acetaldehyde levels for the five volunteers involved in that study fall into the lower part of the concentration distribution for healthy individuals derived from the more statistically significant data from the present study (Fig. 3).

## DISCUSSION

Notwithstanding the known link between breath ethanol and its metabolite acetaldehyde following the ingestion of ethanol,[7] examination of the present rather large set of ethanol and acetaldehyde data reveals that there is no statistically significant correlation between these two compounds in the exhaled breath of these volunteers. So, while the relationship between consumed ethanol and acetaldehyde production has now been well documented,[3] endogenously produced ethanol (no ingestion) and acetaldehyde are not obviously correlated in exhaled breath. This may be due to the binding of acetaldehyde to endogenous molecules in blood or tissue prior to partitioning into air, or it is also possible that they may, at least in part, have different biochemical origins. If so, then what are the origins of these compounds *in vivo*? Are they a result of human physiological or biochemical processes, or of microbial processes in the body? This question is clearly in need of further investigation.

There are identifiable sources other than endogenous sources for the ethanol contained in the exhaled air, including significant ambient levels of ethanol and mouth production of this compound.

### Ethanol and acetaldehyde in ambient air

As stated previously, during the breath inhalation cycles the levels of the targeted compounds in the ambient air are determined to investigate any possible distortion of the levels of targeted compounds that are tacitly assumed to be generated endogenously. For acetaldehyde, the vast majority of samples show 0 ppb during the inhalations, i.e. if acetaldehyde is present in the ambient air it is at a level below the threshold of measurement and so no distortion of the breath acetaldehyde levels can result. However, ethanol

was present in the ambient air at levels that varied from day-to-day, but fell within the range 0 to 145 ppb, which is sometimes a significant fraction of values measured during the exhalations. The level does not change significantly during the inhalation profiles between exhalations for each volunteer, indicating that it is probably not caused by between-breath contamination of the ambient air. As examples, for volunteer 25 with a mean ethanol concentration in breath of 1091 ppb, the mean background level during breath inhalations was 61 ppb (n = 28, range 17–145 ppb, SD 38 ppb), which is an insignificant fraction of the endogenous ethanol. For volunteer 1, with a mean breath ethanol concentration of 43 ppb, the mean background ethanol during the same session was measured as 43 ppb (n = 51, range 0–115 ppb, SD 32 ppb). While this ambient level might be interfering with the quantification of endogenous ethanol in this case, this seems unlikely because any ethanol introduced into the lungs during the inhalations can quickly be absorbed at the breath/blood interface, and it is probable that at such very low inhaled concentrations the emission of endogenous ethanol will dominate the exhaled ethanol concentration.

### Mouth and gut production of ethanol

The development of more sophisticated techniques for the measurement of trace volatile compounds in exhaled breath is propagating breath analysis towards clinical diagnosis of disease and therapeutic monitoring.[13] Therefore, it becomes imperative that any extraneous sources of compounds (that are also metabolites) do not confuse the analyses. In this regard, mouth production is an obvious focus of attention. Over many years of breath analysis, we have occasionally recognised unexpectedly large concentrations of 'breath ethanol', which we have gradually realised is probably due to the consumption of sweet drinks prior to analysing the breath. This raises the obvious question as to how the extra ethanol is produced, by mouth flora or due to increased activity of gut flora and/or salivary enzymes on the ingested sugar? Therefore, we have begun a systematic study of this interesting phenomenon involving the production of several compounds in the mouth. It is sufficient to state here that initial exploratory experiments, involving simply washing sugar solution around the mouth, certainly result in the elevation of ethanol in the exhaled air but no apparent production of acetaldehyde. We are not yet in a position to quantify these effects, but a careful examination of the data obtained in the present study clearly shows that breath ethanol is enhanced when sugary food or drinks have been consumed within 2 h of the breath test (cf. Fig. 3(b) with Fig. 3(c)). However, for longer times after sugar ingestion, a steady-state endogenous production of ethanol is restored.

In the more limited earlier experiments[1] the volunteers had taken nothing but water by mouth during the morning of the measurements, which effectively rules out distortions due to production by mouth flora acting on sugars. It is also perhaps significant that the mean breath ethanol level for the five volunteers in this study (87 ppb) was very similar to the mean level for the present cohort who had not consumed sugar (115 ppb, see Fig. 3). Our planned research programme will be designed to identify the separate contributions of mouth and gut flora in producing ethanol in the exhaled air following sugar ingestion, on the one hand by mouth washing using sugary drinks and on the other using tablets that by-pass the mouth.

## CONCLUSIONS

The present study represents the first concerted effort to measure the distributions of endogenously produced ethanol and acetaldehyde simultaneously in the exhaled breath of a group of healthy volunteers over a period of several months. The volunteers had not consumed alcohol during the 9-h period prior to providing the breath samples. The results reveal that the apparent mean breath ethanol levels for the complete cohort are typically some 200 ppb, and that the mean acetaldehyde breath levels are typically an order-of-magnitude lower at about 20 ppb. The distributions of ethanol and acetaldehyde levels for the complete cohort are close to log-normal, as shown in Fig. 3. However, the ethanol levels in the exhaled air are clearly increased after consumption of sugars and the action on it by either mouth or gut flora/enzymes. There may also be a small increase in the apparent endogenous ethanol due to a contribution from the inhaled ambient air, but this is unlikely. Acetaldehyde is not detected in the ambient air, and, from our preliminary (unpublished) work, this compound is not produced in the mouth at measurable concentrations. The close similarity of the three histograms for acetaldehyde given in Fig. 3 gives credence to these observations, in that there are no significant additional sources to distort the acetaldehyde levels in the exhaled breath.

As previously indicated, these studies are closely linked to the development of breath analysis as a clinical tool for disease diagnosis, and as such they demonstrate the importance of carefully evaluating breath data to ensure that what is being measured is fully understood. It is known that bacterial overgrowth in the gut results in elevated breath ethanol to much greater levels than the mean levels seen in these healthy volunteers.[13] Therefore, any interferences by ambient ethanol and by mouth production, at the levels suggested by the present experiments (Fig. 3), need not detract from use of breath ethanol levels as a detector of excessive gut bacteria as long as simple rules are observed; these include avoiding sweet drinks prior to breath analysis and rinsing the mouth out with water prior to sampling.

Following the observations that acetaldehyde is emitted by cancer cells *in vitro*,[9] there is the possibility that its detection in breath might offer a detection method for tumours *in vivo*, uncomplicated by effects from sugar ingestion since there is no correlation between ethanol and acetaldehyde levels in exhaled breath when alcohol has not been ingested. The present measurements are an essential prelude to such studies, in that they establish the normal levels of acetaldehyde in the breath of healthy individuals and it seems that there are no interferences that can distort these measurements, at least at the 20 ppb level.

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