J. Breath Res. **3** (2009) 027003 (9pp) doi:10.1088/1752-7155/3/2/027003

Breath acetone—aspects of normal physiology related to age and gender as determined in a PTR-MS study

K Schwarz[1,2], A Pizzini[1,2], B Arendacká[3], K Zerlauth[1,2], W Filipiak[1,2], A Schmid[1,2], A Dzien[4], S Neuner[5], M Lechleitner[2,6], S Scholl-Bürgi[7], W Miekisch[2,8], J Schubert[2,8], K Unterkofler[2,9], V Witkovský[3], G Gastl[2,10] and A Amann[1,2,11,12]

[1] Department of Operative Medicine, Innsbruck Medical University, Anichstraße 35, A-6020 Innsbruck, Austria
[2] Breath Research Unit of the Austrian Academy of Sciences, Dammstrasse 22, A-6850 Dornbirn, Austria
[3] Institute of Measurement Science, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic
[4] Bürgerstraße 2, A-6020 Innsbruck, Austria
[5] Peter-Siegmair-Str 6, A-6176 Völs, Austria
[6] Landeskrankenhaus Hochzirl, Anna-Dengel-Haus, A-6170 Hochzirl, Austria
[7] Department of Pediatrics, Innsbruck Medical University, Anichstraße 35, A-6020 Innsbruck, Austria
[8] Department of Anaesthesiology and Intensive Care, University of Rostock, Schillingallee 35, 18057 Rostock, Germany
[9] University of Applied Sciences, Hochschulstrasse 1, A-6850 Dornbirn, Austria
[10] Division of Haematology and Oncology, Department of Internal Medicine, Innsbruck Medical University, Anichstraße 35, A-6020 Innsbruck, Austria

E-mail: anton.amann@i-med.ac.at and anton.amann@oeaw.ac.at

Received 16 December 2008
Accepted for publication 11 March 2009
Published 15 May 2009
Online at stacks.iop.org/JBR/3/027003

Abstract

The present study was performed to determine the variations of breath acetone concentrations with age, gender and body-mass index (BMI). Previous investigations were based on a relatively small cohort of subjects (see Turner *et al* 2006 *Physiol. Meas.* **27** 321–37). Since exhaled breath analysis is affected by considerable variation, larger studies are needed to get reliable information about the correlation of concentrations of volatiles in breath when compared with age, gender and BMI. Mixed expiratory exhaled breath was sampled using Tedlar bags. The concentrations of a mass-to-charge ratio (m/z) of 59, attributed to acetone, were then determined using proton transfer reaction-mass spectrometry. Our cohort, consisting of 243 adult volunteers not suffering from diabetes, was divided into two groups: one that fasted overnight prior to sampling (215 volunteers) and the other without a dietary control (28 volunteers). In addition, we considered a group of 44 healthy children (5–11 years old).The fasted subjects' concentrations of acetone ranged from 177 ppb to 2441 ppb, with an overall geometric mean (GM) of 628 ppb; in the group without a dietary control, the subjects' concentrations ranged from 281 ppb to 1246 ppb with an overall GM of 544 ppb. We found no statistically significant shift between the distributions of acetone levels in the breath of males and females in the fasted group (the Wilcoxon-Mann–Whitney test yielded $p = 0.0923$, the medians being 652 ppb and 587 ppb). Similarly, there did not seem to be a difference between the acetone levels of males and females in the group without a dietary control. Aging was

[11] Author to whom any correspondence should be addressed.
[12] Dr Amann is a representative of Ionimed GesmbH, Innsbruck.

associated with a slight increase of acetone in the fasted females; in males the increase was not statistically significant. Compared with the adults (a merged group), our group of children (5–11 years old) showed lower concentrations of acetone ($p < 0.001$), with a median of 263 ppb. No correlation was found between the acetone levels and BMI in adults. Our results extend those of Turner *et al*'s (2006 *Physiol. Meas.* **27** 321–37), who analyzed the breath of 30 volunteers (without a dietary control) by selected ion flow tube-mass spectrometry. They reported a positive correlation with age (but without statistical significance in their cohort, with $p = 0.82$ for males and $p = 0.45$ for females), and, unlike us, arrived at a p-value of 0.02 for the separation of males and females with respect to acetone concentrations. Our median acetone concentration for children (5–11 years) coincides with the median acetone concentration of young adults (17–19 years) reported by Spanel *et al* (2007 *J. Breath Res.* **1** 026001).

Abbreviations

BMI	body-mass index
GM	geometric mean
GSD	geometric standard deviation
m/z	mass-to-charge ratio
PTR-MS	proton transfer reaction mass spectrometry
ppb	parts per billion
VOC	volatile organic compound

1. Introduction

In the last 10 years, the progress in trace gas analytical techniques has been enormous. The sensitivity has been increased dramatically while the size and weight of instruments have been reduced. This progress has been advantageous for medical applications, such as for biomarker analysis in exhaled breath and exhaled breath condensate [1–4].

The main techniques for exhaled breath analysis are gas chromatography with mass spectrometric detection (GC-MS) [5–10], proton transfer reaction-mass spectrometry (PTR-MS) [11–16], selected ion flow tube-mass spectrometry (SIFT-MS) [17, 18], laser spectrometry [19–21], ion mobility spectrometry [22–24], photoacoustic spectrometry and sensor techniques [25, 26].

GC-MS is the gold standard, resulting in compound identification *and* quantification. Purely mass spectrometric techniques allow on-line quantification of exhaled breath (e.g., during a full night), are easier to handle than GC-MS and do not need elaborate sample preparation. In the present investigation we used PTR-MS, which is a very sensitive technique, measuring accurately even below the ppb concentration level (with a dwell time of 0.5 s).

To introduce analytical achievements into clinical settings, it is important to consider age- and gender-related peculiarities as well as biochemical origins of the breath volatile organic compounds (VOCs), which are considered as biomarkers for diagnosis and monitoring of human diseases.

The typical compounds observed in exhaled breath are acetone, ammonia, isoprene, isopropanol, aldehydes such as formaldehyde and acetaldehyde, aromatics such as benzene or toluene, furanes (in smokers), ethane and pentane as markers of lipid peroxidation and small inorganic molecular species such as nitric oxide, carbon monoxide and COS.

Acetone (2-propanone) is the major ketone contained in exhaled human breath being normally detected in exhaled air at levels ranging from a few hundred ppb to several ppm. Acetone is a product of the conversion of acetoacetate by elimination of CO_2 [27, 28]:

$$\begin{aligned} CH_3COCH_2COO^- + H^+ &= CH_3COCH_2COOH \\ &= CH_3COCH_3 + CO_2. \end{aligned}$$

This conversion is either a result of the non-enzymatic decarboxylation of acetoacetate or is catalyzed by acetoacetate decarboxylase. The acetoacetate decarboxylase is induced by starvation and inhibited by acetone itself. High concentrations of blood acetoacetate trigger the acetoacetate decarboxylase, thus draining H^+, while acetone, acting as a competitive inhibitor, helps prevent early acetoacetate decarboxylation of acetoacetate. Acetoacetate is the product of beta-hydroxybutyric acid (=HMG-CoA, an intermediate of the mevalonate pathway) and can be converted either to acetone (see the above reaction) or to D-beta-hydroxybutyrate. Kalapos [28] further reported that a small amount of acetone is converted from isopropanol by class I isoenzymes of the hepatic alcohol dehydrogenase family.

Acetone can be metabolized to lactate, pyruvate and acetyl coenzyme A and subsequently recycled into amino acids, fatty acids and cholesterol [29].

Two C3 pathways have been described, and one of them involves the production of methylglyoxal [29, 30]. Methylglyoxal is further converted to D-lactate by a reversible conjugation with glutathione. Kalapos *et al* [30] investigated the influence of methylglyoxal on metabolism and showed that it inhibits glucose formation at concentrations above 1 mM. Kalapos *et al* also described that the maximum amount of ATP was produced when acetone was metabolized through the L-1,2-propanediol-L-lactate pathway (C3 pathway), yielding 16 ATP for every metabolized acetone molecule [27, 28]. Kalapos [27] also recognized that in a special situation as for example diabetes mellitus, when ketone body production exceeds the degrading capacity, acetone helps to maintain the pH buffering capacity.

We do not expect to see any of the metabolites of acetone in exhaled breath. In principle, methylglyoxal could be a candidate but its Henry constant has a relatively high value

Table 1. Study cohort of adult volunteers.

		Number of subjects	Age		
			Min.	Max.	Mean ± SD
Fasted subjects	Males	83	23	84	52.4 ± 17
	Females	132	19	89	51.4 ± 19
Subjects without a dietary control	Males	12	27	70	51.4 ± 14
	Females	16	23	69	47.3 ± 15

SD = standard deviation.

Table 2. Investigated group of children.

Age	5		6		8		9		10		11	
	F	M	F	M	F	M	F	M	F	M	F	M
Number of children	1	0	5	8	6	7	0	2	8	6	0	1

F = female, M = male.

of ∼3.2 × 10^4 M atm^{-1} [31]. The Henry constant of 1,2-propanediol is even higher with ∼3 × 10^5 M atm^{-1} [32]. Acetone, in comparison, has a comparatively low Henry constant ∼30 M atm^{-1} [33] and will therefore appear in exhaled breath.

Mork *et al* [34] showed that acetone can be exchanged not only between the air in the alveolae and arterial blood, but also between the air in the bronchioles and the mucosa and between the mucosa and arterial blood. In addition, the transfer rate between the bronchial mucosa and bronchial air increases during physical exercise.

The relationships between the concentrations of exhaled acetone, age, gender and body-mass index (BMI) have been investigated by Turner *et al* [35] with SIFT-MS using a cohort of 30 test persons. We increased the study population to a cohort of 243 adult volunteers and 44 children, using PTR-MS instead of SIFT-MS. We did not focus exclusively on acetone, but also analyzed methylglyoxal, which may be formed from acetone and also from acetoacetate (which is the usual source of acetone).

The present study was performed to investigate the variations of breath acetone concentrations with age, gender and BMI in adults.

2. Methods

2.1. Subjects

The investigated cohort consisted of a group of 215 adults, 83 males and 132 females, undergoing a general health check-up after overnight fasting, and of a group of 28 adults, 12 males and 16 females, without a dietary control. The subjects did not suffer from diabetes (which is known to influence the acetone concentrations in breath). The age distribution is shown in table 1. In addition, we investigated the acetone levels in 44 children, 20 girls and 24 boys, aged between 5 and 11; see table 2. Our studies were approved by the local Ethics Committee of Innsbruck Medical University.

2.2. PTR-MS instrument used

A high-sensitivity proton transfer reaction mass spectrometer (three turbopumps, Ionicon, Innsbruck, Austria) with Teflon rings (instead of Viton rings) was used for our measurements. The count rate of primary ions (H_3O^+) was around 10^7 counts per second. The dwell time was 0.5 s for each mass-to-charge ratio (m/z) measured (21–230 m/z). The typical compounds used for the determination of transmission coefficients were acetonitrile, acetaldehyde, acetone, DMS, 2-butanone, benzene, toluene, p-xylene, benzaldehyde, chlorobenzene, 1,2 dichlorobenzene, 1,2,4 trichlorobenzene. These compounds do not show any fragmentation of their respective protonated form. Concentrations of these compounds were chosen in a range leading to ∼10% reduction of primary counts, with subsequent observation of the recovery of primary ion counts (measuring at $m/z = 21$ and the specific mass-to-charge ratio of the respective non-fragmenting compound). The length of the drift tube of our PTR-MS is 9.3 cm, with an applied voltage of 600 V. The usual pressure in the drift tube was ∼2.3 mbar (with slight variations). We computed the concentrations of acetone by considering both H_3O^+ and the first water cluster $H_2O \cdot H_3O^+$ as primary ions.

2.3. Sample collection and mass-spectrometric analysis

Samples of mixed breath gas were collected in Tedlar bags (SKC Inc, Eighty Four, PA) with parallel collection of ambient air (also in Tedlar bags). Breath gas samples were obtained after an ∼5 min sitting of a volunteer by use of a straw (Polypropylen; Art.-Nr. 12718, PAPSTAR 53925 Kall, Germany). All samples were processed within 12 h.

The 'age effect' of volatile compounds in exhaled breath samples during storage of Tedlar bags is negligible (apart from water, which quickly diffuses through the walls of Tedlar bags). Acetonitrile seems to diffuse the quickest through bag walls, with an exponential decay constant $\tau \sim 31$ h [42]. Acetone diffuses slower through the bag walls and its concentration remains almost constant for 12 h.

The acetone concentrations were determined on m/z 59 using PTR-MS. PTR-MS allows on-line monitoring of VOCs

with volume mixing ratios as low as a few parts per trillion (ppt). In our study, an exhaled breath sample was measured at least thrice in the range of m/z ratios from 21 to 230. The concentrations of acetone were determined based on a calibration series (see section 3), leading to a kinetic rate constant of 3.4×10^{-9} cm^3 s^{-1} which is very near the Su–Chesnavich collisional rate constant [36].

Methoxyglyoxal can be observed in PTR-MS measurements at m/z 73, with other compounds such as 2-butanone and tetrahydrofuran appearing at the same mass-to-charge ratio. In GCMS measurements, we sometimes observed 2-butanone (=methylethylketone) but never tetrahydrofuran in exhaled breath. Butanal, on the other hand, whose protonated form has a molecular weight of 73 D, primarily appears at m/z 55 (after loss of water) and a calibration of butanal showed that at m/z 73 only about 7% of m/z 55 appear. Hence, the main compound expected at m/z 73 in PTR-MS is 2-butanone (and possibly the water cluster $H_3O \cdot (H_2O)_3^+$, but at rather low concentration).

2.4. Data pre-processing and statistical analysis

The calibrated concentrations of acetone obtained (ppb), measured repeatedly in each exhaled sample, were log-transformed ($\log_{10}$) before any further analyses. We excluded two samples with outlying standard deviations (after log-transformation), namely the respective geometric standard deviations (GSDs)[13] were 6.39 and 5.89, while the GSD determined from the pooled within-sample variance based on the remaining (adult) samples was 1.012. Without the log-transformation, the standard deviation determined from the repeated measurements in a sample increases with the mean of these measurements. The log-transformation alleviates this dependence, so that the standard deviation determined from the log-transformed values is comparable across the samples and its unusual value may be used as an indicator of a possible instability during the measurement process. In addition, applying the log-transformation leads to a more symmetric distribution of the subjects' concentrations (obtained as described below). However, in the fasted group the Lilliefors test [37] on the subjects' concentrations in a log-scale rejected the hypothesis of a normal distribution (at a 5% significance level). Separated for gender, the hypothesis was rejected for the log-transformed data of women, whereas it was not rejected in the case of men.

In the fasted group, we excluded a subject (male, aged 59) with an extremely high acetone concentration of 25 480 ppb in the only sample he had given. The value is extreme not only in our study, but with respect to other reported data as well.

We also excluded an exhaled breath sample for which the corresponding ambient air sample showed much higher acetone concentration (209 ppb) than all the other ambient air samples (10 ppb–79 ppb) in order to guard against a possible distortion of the exhaled acetone level in the affected sample.

[13] The GSD is determined as 10^{SD} where SD is the usual standard deviation computed from the log-transformed data. Note that GM/GSD, GM*GSD correspond to Mean-SD, Mean+SD computed in the logarithmic scale.

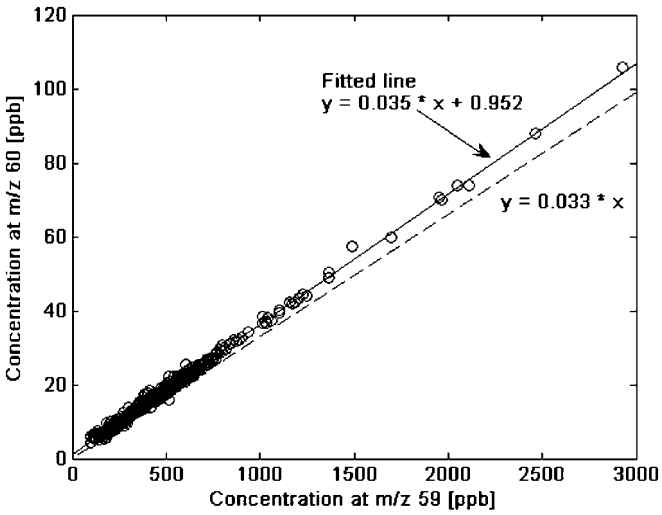

Figure 1. Concentration of ions with $m/z = 60$ against the concentration of ions with $m/z = 59$ in exhaled breath samples. The squared correlation coefficient is computed as $R^2 = 0.9948$.

The remaining 289 samples from 243 (215 fasted before sampling, 28 without dietary control) adult subjects were processed as follows. The acetone concentration of each sample (in log-scale) was determined as the median value of all the repeatedly obtained (log-transformed) acetone concentrations within the sample. If a subject gave more than one sample, we considered the respective representative median concentration (i.e. the median of all the sample concentrations of the subject): 202 subjects gave just one sample, 20 gave two samples immediately one after another, 3 gave two samples within an hour and 18 gave several samples (within a time gap of days and months). Note that the medians in both cases (sample concentration, subject concentration) were taken from the log-transformed data, so that the inverse transformation 10^{median} is done when reporting the values in ppb. The same procedure was applied to the 88 samples obtained from the group of 44 children (each child gave two samples within a day).

Throughout the paper, we report acetone concentrations rounded to the nearest integer; however, the number of digits shown does not imply anything about the accuracy of the PTR-MS measurement. To assess the magnitude of the uncertainty connected with a single PTR-MS measurement of acetone concentration in breath, we describe here the values of the coefficients of variation (CoV) computed from the repeated measurements of the acetone concentration for each exhaled breath sample. The mean CoV in all adult exhaled breath samples was 1%; the maximum CoV observed was 5%.

3. Results

3.1. Identification of acetone

Figure 1 plots the concentration of ions with $m/z = 60$ against the concentration of ions with $m/z = 59$ for all 377 exhaled breath samples (289 for adults + 88 for children). The squared correlation coefficient is computed as $R^2 = 0.9948$ and the

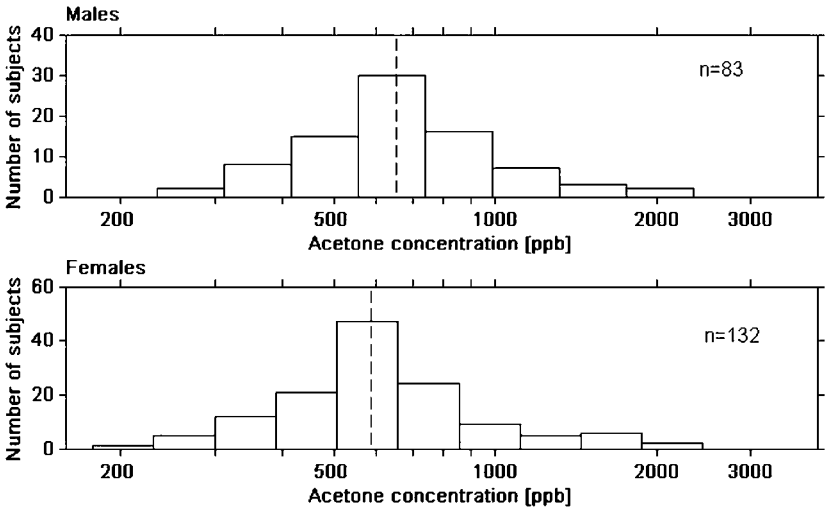

Figure 2. Histograms of the subjects' acetone concentrations for males and females in the fasted group. The dashed lines depict the medians (*n* denotes the total number of male and female subjects in the study).

Table 3. Fragmentation pattern of acetone for PTR-MS measurements. (For details, see section 3.)

m/z	Slope of calibration line	Remarks
59	0.8386	
60	0.0288	Presents the ^{13}C-isotopes from m/z 59
Sum	0.8674	

slope of the fitted line is 0.035 (in this case, the concentrations in ppb were used for fitting). If $m/z = 59$ were only due to acetone (with three carbon atoms) and if $m/z = 60$ only referred to ^{13}C-isotopes of $m/z = 59$, one would expect a straight line with a slope of 0.033 (due to 3.3% of ^{13}C-isotopes).

3.2. Calibration for acetone

The calibration results for the PTR-MS measurements of acetone are presented in table 3. The underlying calibration series used acetone (in nitrogen 6.0, i.e. purity 99.9999%) concentrations of 1.2 ppb, 2.4 ppb, 12.1 ppb, 24.2 ppb, 60.6 ppb, 121.2 ppb and 242.3 ppb. Concentrations of acetone related to m/z 59 have been calculated based on an approximate proton-transfer reaction rate constant of $k = 3.9 \times 10^{-9}$ cm^3 s^{-1} and on a calibration experiment which resulted in a slope of 0.8386 for the calibration line. We therefore corrected all acetone concentrations by multiplying with a factor of $1/0.8386 = 1.1925$. Considering the ^{13}C-isotope of acetone at m/z 60, our calibration corresponds to a reaction rate constant of 3.4×10^{-9} cm^3 s^{-1}, which is approximately equal to the Su–Chesnavich rate constant.

3.3. Acetone concentrations in exhaled breath of adult volunteers

The acetone concentrations of the adult subjects who had undergone an overnight fasting ranged from 177 ppb to 2441 ppb, with the median of 609 ppb, the overall GM being 628 ppb and the overall geometric standard deviation (GSD) being 1.56. The acetone concentrations of the individual samples ranged from 177 ppb to 3490 ppb.

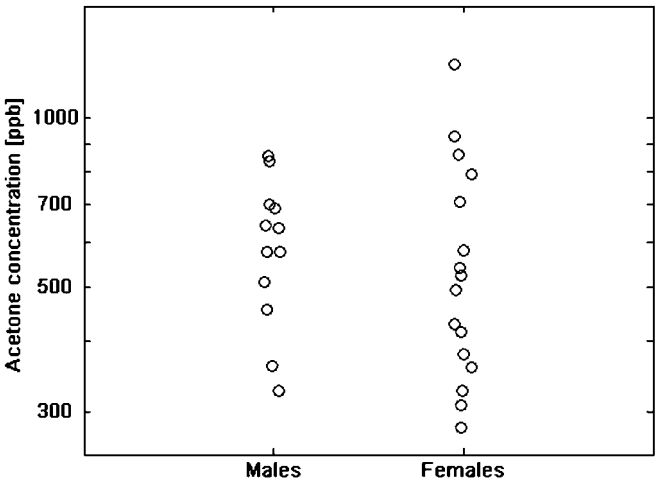

Figure 3. Subjects' acetone concentrations of males and females observed in the group without a dietary control.

In the group without a dietary control, the subjects' concentrations ranged from 281 ppb to 1246 ppb, with the median of 559 ppb, the overall GM of 544 ppb and the overall GSD of 1.46. The individual sample concentrations ranged from 280 ppb to 1269 ppb.

The within-sample GSD calculated from the pooled within-sample variance of all exhaled breath samples collected from adult subjects was 1.012.

The acetone concentrations in ambient air samples (medians of the repeated measurements of each sample) ranged from 10 ppb to 52 ppb in the fasted group, and from 31 ppb to 79 ppb in the group without a dietary control. They were always at least four times lower than the corresponding exhaled breath concentrations.

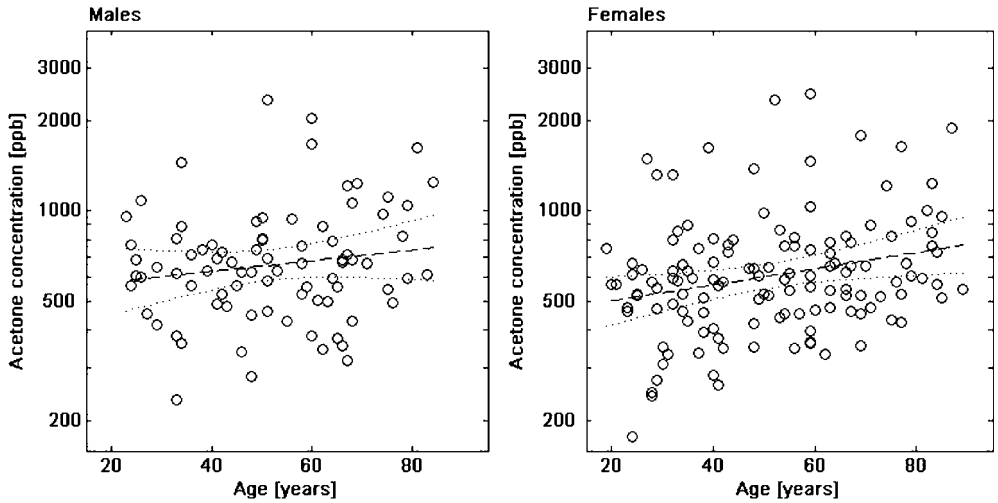

Figure 4. Concentrations of acetone in the breath of fasted male and female volunteers (ppb) as dependent on age. The fitted regression lines are shown together with their 95% confidence bands.

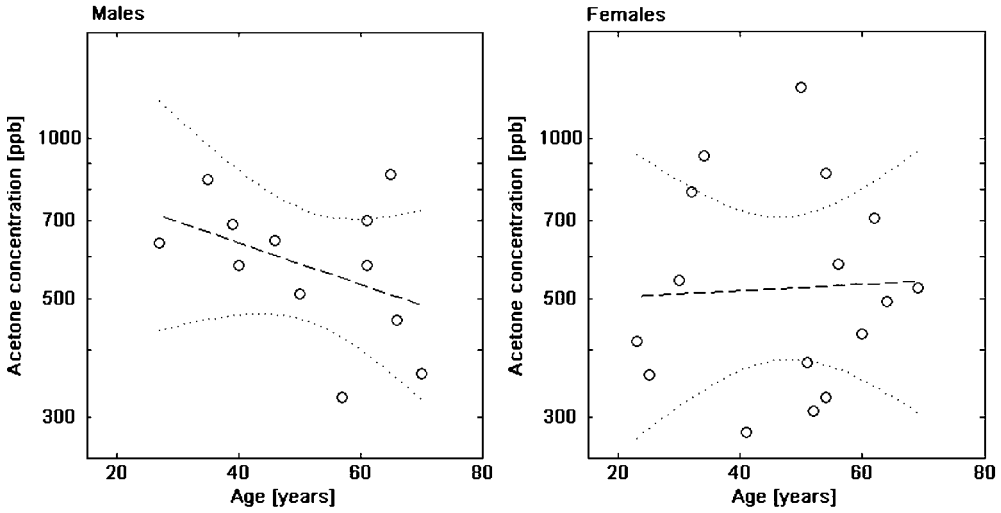

Figure 5. Concentrations of acetone in the breath of male and female volunteers (ppb) under no dietary control as dependent on age. The fitted regression lines are shown together with their 95% confidence bands.

Table 4. Estimated slopes of the regression lines describing the dependence of the log-transformed acetone concentrations on age, for males and females, together with the *p*-values obtained when testing that the slope is zero.

		Males	Females
Estimated slope (*p*-value)	Fasted subjects	0.0018 ($p = 0.1554$)	0.0027 ($p = 0.0032$)
	Subjects under no dietary control	−0.0039 ($p = 0.1790$)	0.0006 ($p = 0.8701$)

3.4. Acetone concentrations in exhaled breath of children

In the group of 44 children aged between 5 and 11, the acetone concentrations of the subjects ranged from 120 ppb to 728 ppb with the median of 263 ppb and the overall GM of 275 ppb. The ambient air acetone concentration (ranging from 14 ppb to 77 ppb) was always at least 1.9 times lower than the corresponding concentration of acetone in exhaled air.

3.5. Relationship of the breath acetone concentration with age and gender in adult volunteers

The age profile of the subjects (in both adult groups) is shown in table 1, the acetone concentrations observed for males and females in the fasted group are shown in figure 2 and those for the subjects under no dietary control are shown in figure 3.

In the fasted group, the GM and the median for males were 661 ppb and 652, respectively; for females, the GM

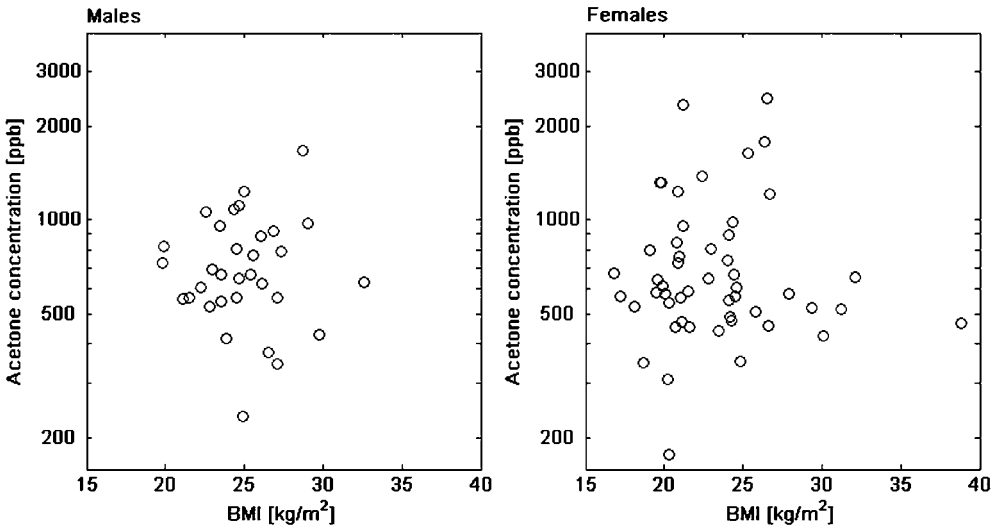

Figure 6. Acetone concentrations plotted against the BMI for subjects from the fasted group. No significant correlation was found between the two quantities.

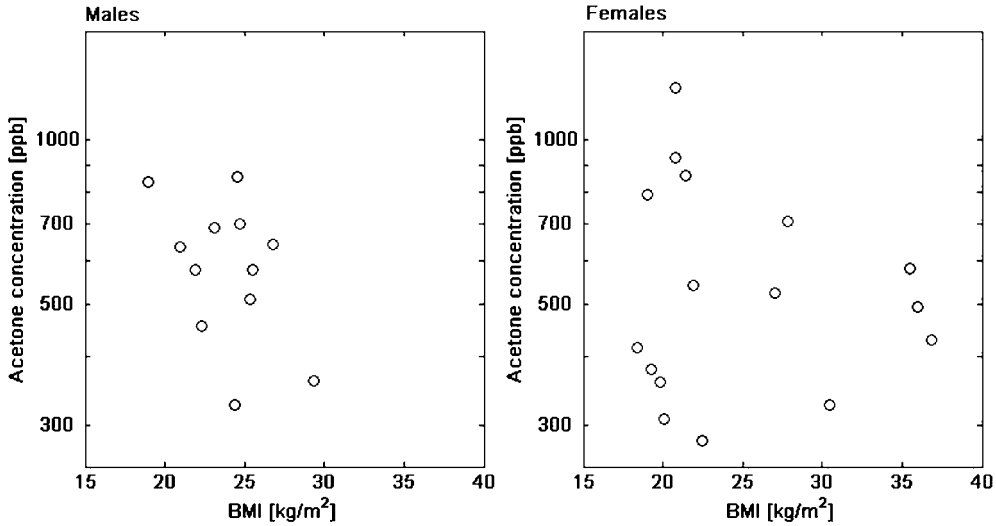

Figure 7. Acetone concentrations plotted against the BMI for subjects under no dietary control. No significant correlation was found between the two quantities.

was 608 ppb and the median was 587 ppb. The Wilcoxon-Mann–Whitney test did not find a significant (at a 5% level) shift between the distributions of the acetone concentrations of males and females (p-value = 0.0923).

Similarly, we found no significant difference between the mean (log-transformed) acetone levels of males and females in the group of subjects without a dietary control, the GM being 575 ppb and 523 ppb, respectively.

The dependence of the acetone concentrations on age was examined by means of linear regression (for the log-transformed concentrations); see figures 4 and 5. In the fasted group, the estimated slope was not significantly different from zero in the case of males; in the case of females, the increase of acetone concentration with aging was found significant at a 5% level (p-value = 0.0032), see table 4. However, the estimated slope was only 0.0027 in the logarithmic scale, which corresponds to a change by a factor $10^{0.0027} = 1.006$ per year or $10^{0.0027\times 10} = 1.064$ per 10 years. Considering the apparently large variation of acetone concentrations (see figure 4), such a relationship between acetone and age would be too weak to be relevant for practical purposes.

In the group without a dietary control, acetone was not found to depend on age.

3.6. Breath acetone and BMI in adult volunteers

In the fasted group, the BMI was measured only for 83 subjects, 32 males and 51 females. For males the BMI ranged from 19.8 to 32.6, with the mean of 24.9 and the standard deviation of 2.81. For females the range was 16.8–38.9, with the mean of 23.2 and the standard deviation of 4.14. Neither for males nor for females, were the acetone concentrations found to be correlated with the BMI; see figure 6.

No significant (at a 5% level) correlation between acetone and the BMI was found in the group without a dietary control either, see figure 7. There, the BMI for males ranged from 18.9 to 29.3, with the mean of 24 and the standard deviation of 2.77; for females, the range was 18.4–36.8, and the mean and the standard deviation were 24.9 and 6.53, respectively.

4. Discussion

We used a comparatively large cohort of 215 fasted adult volunteers to determine the variability of acetone concentrations in exhaled breath. Turner *et al* [35] observed a significant difference between the acetone concentrations of males and females, with males having higher mean concentration of acetone. They hypothesized that the difference may be related to the greater average energy consumption of males and called for a larger cohort under a dietary control to further investigate the issue. In our data, we did not observe a significant difference between the acetone concentrations of fasted males and females.

We found a very slight rise of acetone concentrations with age for fasted female volunteers ($p = 0.0032$), but the estimated slope was very small compared to the large variation in the data and thus practically irrelevant (see figure 4). However, it may give rise to differences when comparing the acetone levels in distant age groups. The BMI did not influence the acetone concentration at all.

Overall, for the variables sex, age and BMI, the influence on the breath concentration of acetone in adults is almost negligible. However, note that our adult group did not include many subjects aged 18–25 and it is probably in this age group where the shift from children to adult levels occurs. Children show lower acetone concentration in breath than adults, their median acetone concentrations being about half of the adults' concentrations. Spanel *et al* [38] investigated 26 young adults (17 and 19 years) and found a median concentration of 263 ppb. This median concentration coincides with our median concentration for children (5–11 years).

The acetone metabolism is rather complex, with different mechanisms of production and metabolization, as well as regulatory feedback mechanisms such that, e.g., acetoacetate decarboxylase is induced by starvation and inhibited by acetone itself [27–30, 39–41]. It would be ideal if some of the metabolites of acetone—such as methylglyoxal or 1,2-propanediol—could be observed, but these compounds are rather hydrophilic and therefore not expected to be observed in exhaled breath. The median concentrations of m/z 73 (methylglyoxal and butanone appear at this mass-to-charge ratio) were almost identical in inspiratory and expiratory air, namely 3.5 and 3.6 ppb, respectively, in the group of adults. Also, the correlation between (log-transformed) the adult subjects' concentrations at m/z 59 and m/z 73 in exhaled air was low, $R^2 = 0.0262$. We therefore conclude that we could not observe methylglyoxal in exhaled breath of our cohort.

Acknowledgments

This project has been supported by the European Commission (project BAMOD, project no LSHC-CT-2005-019031), the Austrian Ministry for Science and Research (BMWF, Vienna, Austria, grants GZ 651.019/1-VI/2/2006, GZ 651.019/3-VI/2/2006) and the Slovak Research and Development Agency (grant APVV-RPEU-0008-06). We greatly appreciate the generous support of the Member of the Tyrolean regional government Dr Erwin Koler and the Director of the University Clinic of Innsbruck (TILAK) Mag. Andreas Steiner.

References

[1] Amann A and Smith D 2005 *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* (Singapore: World Scientific)

[2] Risby T 2005 Current status of clinical breath analysis *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 251–65

[3] Amann A, Spanel P and Smith D 2007 Breath analysis: the approach towards clinical applications *Mini. Rev. Med. Chem.* **7** 115–29

[4] O'Reilly P and Bailey W 2007 Clinical use of exhaled biomarkers in COPD *Int. J. Chron. Obstruct. Pulmon. Dis.* **2** 403–8

[5] Miekisch W, Schubert J K and Noeldge-Schomburg G F 2004 Diagnostic potential of breath analysis—focus on volatile organic compounds *Clin. Chim. Acta* **347** 25–39

[6] Schubert J, Miekisch W and Geiger K 2003 Exhaled breath markers in ARDS *Lung Biology in Health and Disease. Disease Markers in Exhaled Breath* ed N Marczin and S Kharitonov (New York: Dekker) pp 363–80

[7] Schubert J, Miekisch W and Nöldge-Schomburg G 2005 VOC breath markers in critically ill patients: potentials and limitations *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 267–92

[8] Schubert J K, Miekisch W, Geiger K and Noldge-Schomburg G F 2004 Breath analysis in critically ill patients: potential and limitations *Expert Rev. Mol. Diagn.* **4** 619–29

[9] Ligor T, Ligor M, Amann A, Ager C, Bachler M, Dzien A and Buszewski B 2008 The analysis of healthy volunteers' exhaled breath by the use of solid-phase microextraction and GC-MS *J. Breath Res.* **2** 046006

[10] Buszewski B, Kesy M, Ligor T and Amann A 2007 Human exhaled air analytics: biomarkers of diseases *Biomed. Chromatogr.* **21** 553–66

[11] Lindinger W, Hansel A and Jordan A 1998 On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical applications, food control and environmental research *Int. J. Mass Spectrom. Ion Processes* **173** 191–241

[12] Lindinger W, Hansel A and Jordan A 1998 Proton-transfer-reaction mass spectrometry (PTR-MS): on-line monitoring of volatile organic compounds at pptv levels *Chem. Soc. Rev.* **27** 347–54

[13] Kushch I *et al* 2008 Breath isoprene—aspects of normal physiology related to age, gender and cholesterol profile as determined in a proton transfer reaction mass spectrometry study *Clin. Chem. Lab Med.* **46** 1011–8

[14] Kushch I *et al* 2008 Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS *J. Breath Res.* **2** 026002

[15] Amann A, Poupart G, Telser S, Ledochowski M, Schmid A and Mechtcheriakov S 2004 Applications of breath gas analysis in medicine *Int. J. Mass Spectrom.* **239** 227–33

[16] Amann A, Telser S, Hofer L, Schmid A and Hinterhuber H 2005 Exhaled breath as a biochemical probe during sleep *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 305–16

[17] Smith D and Spanel P 2005 Selected ion flow tube mass spectrometry, SIFT-MS, for on-line trace gas analysis of breath *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 3–34

[18] Smith D and Spanel P 2005 Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis *Mass. Spectrom. Rev.* **24** 661–700

[19] Thelen S, Miekisch W, Halmer D, Schubert J, Hering P and Murtz M 2008 Intercomparison of infrared cavity leak-out spectroscopy and gas chromatography-flame ionization for trace analysis of ethane *Anal. Chem.* **80** 2768–73

[20] Wysocki G, McCurdy M, So S, Roller C and Tittel F 2005 Exhaled human breath analysis with quantum cascade laser-based gas sensors *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 75–84

[21] von Basum G, Halmer D, Hering P and Mürtz M 2005 Laser spectroscopic on-line monitoring of exhaled trace gases *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 67–74

[22] Baumbach J, Sielemann S, Xie Z and Schmidt H 2003 Detection of the gasoline components methyl tert-butyl ether, benzene, toluene, and m-xylene using ion mobility spectrometers with a radioactive and UV ionization source *Anal. Chem.* **75** 1483–90

[23] Baumbach J, Vautz W, Ruzsanyi V and Freitag L 2005 Metabolites in human breath: ion mobility spectrometers as diagnostic tools for lung diseases *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 53–66

[24] Ruzsanyi V, Baumbach J I, Sielemann S, Litterst P, Westhoff M and Freitag L 2005 Detection of human metabolites using multi-capillary columns coupled to ion mobility spectrometers *J. Chromatogr.* A **1084** 145–51

[25] Machado R F *et al* 2005 Detection of lung cancer by sensor array analyses of exhaled breath *Am. J. Respir. Crit. Care. Med.* **171** 1286–91

[26] Di Natale C, Macagnano A, Martinelli E, Paolesse R, D'Arcangelo G, Roscioni C, Finazzi-Agro A and D'Amico A 2003 Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors *Biosens. Bioelectron.* **18** 1209–18

[27] Kalapos M P 1999 Possible physiological roles of acetone metabolism in humans *Med. Hypotheses* **53** 236–42

[28] Kalapos M P 2003 On the mammalian acetone metabolism: from chemistry to clinical implications *Biochim. Biophys. Acta* **1621** 122–39

[29] Mandl J, Banhegyi G, Kalapos M P and Garzo T 1995 Increased oxidation and decreased conjugation of drugs in the liver caused by starvation. Altered metabolism of certain aromatic compounds and acetone *Chem. Biol. Interact.* **96** 87–101

[30] Kalapos M P, Garzo T, Antoni F and Mandl J 1991 Effect of methylglyoxal on glucose formation, drug oxidation and glutathione content in isolated murine hepatocytes *Biochim. Biophys. Acta* **1092** 284–90

[31] Zhou X and Mopper K 1990 Apparent partition coefficients of 15 carbonyl compounds between air and seawater and between air and freshwater; implications for air–sea exchange *Environ. Sci. Technol.* **24** 1864–9

[32] Saxena P and Hildemann L M 1996 Water-soluble organics in atmospheric particles: a critical review of the literature and application of thermodynamics to identify candidate compounds *J. Atmos. Chem.* **24** 57–109

[33] Staudinger J and Roberts P V 1996 A critical review of Henry's law constants for environmental applications *Crit. Rev. Environ.* **26** 205–97

[34] Mork A K and Johanson G 2006 A human physiological model describing acetone kinetics in blood and breath during various levels of physical exercise *Toxicol. Lett.* **164** 6–15

[35] Turner C, Spanel P and Smith D 2006 A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS *Physiol. Meas.* **27** 321–37

[36] Su T and Chesnavich W J 1982 Parametrization of the ion–polar molecule collision rate-constant by trajectory calculations *J. Chem. Phys.* **76** 5183–5

[37] Conover W J 1999 *Practical Nonparametric Statistics* (New York: Wiley)

[38] Spanel P, Dryahina K and Smith D 2007 The concentration distributions of some metabolites in the exhaled breath of young adults *J. Breath Res.* **1** 026001

[39] Kalapos M P 2008 Methylglyoxal and glucose metabolism: a historical perspective and future avenues for research *Drug Metabol. Drug Interact.* **23** 69–91

[40] Kalapos M P 2008 The tandem of free radicals and methylglyoxal *Chem. Biol. Interact.* **171** 251–71

[41] Kalapos M P, Mandl J, Banhegyi G, Antoni F and Garzo T 1994 Net glucose production from acetone in isolated murine hepatocytes. The effect of different pretreatments of mice *Int. J. Biochem.* **26** 1069–79

[42] Herbig J 2007 private communication