Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study

DAVID SMITH,1 PATRIK SPANEL,2 AND SIMON DAVIES3

1Centre for Science and Technology in Medicine, School of Postgraduate Medicine, Keele University, Keele ST5 5BG, United Kingdom; 2) . Heyrovsky Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, 18223 Prague 8, Czech Republic; and 3Department of Medicine, School of Postgraduate Medicine, Keele University, Keele ST5 5BG, United Kingdom

Smith, David, Patrik Spanel, and Simon Davies. Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. J. Appl. Physiol. 87(5): 1584–1588, 1999.—The selected ion flow tube technique was used to quantify in breath the trace gases acetone, ammonia, ethanol, isoprene, and methanol during single exhalations while fasting and in response to feeding. Six normal volunteers were fasted for 12 h, and, after baseline breath samples were obtained, were fed a liquid protein-calorie meal to provide 0.47 g/kg of protein (Fortisip). Further breath samples were obtained at 20, 40, and 60 min, and then hourly for a further 5 h. Breath acetone concentrations fell from a maximum during fasting, reaching their nadir between 4 and 5 h. Breath ammonia concentrations fell immediately to one-half their fasting levels before a steady increase to two or three times baseline values at 5 h. There was a brief increase in breath ethanol concentrations after feeding, reflecting detectable ethanol contamination of the food. Subsequently, breath ethanol levels remained low throughout the experimental protocol. Isoprene concentrations did not change significantly, whereas changes in methanol concentrations reflected those in the ambient air. This preliminary study indicates that the selected ion flow tube technique may be used to detect changes in the trace gases present in breath and define their concentrations in the fasting and replete state. Of particular interest is the biphasic response of the breath ammonia concentration after feeding.

m = mass spectrometry; ammonia; ethanol; acetone; isoprene

The selected ion flow tube (SIFT) method represents a remarkable breakthrough in the science of trace gas analysis (13, 16). It enables identification, both simultaneously and in real time, of gases present in complex mixtures and quantifies them precisely at concentrations presently as low as 10 parts per billion (ppb) (14, 17). As such, it lends itself to exploitation in several biological contexts, including the quantitative analysis of trace gases in human breath. Until now, the value of breath analysis as an analytic tool has been limited to a few specific applications [e.g., 13C breath tests for Helicobacter pylori infection (3)] due to the relative insensitivity of the available methods in which preconcentration of the breath sample is usually required (12), and because of the difficulty in analyzing such complex mixtures (8). Consequently, there has been considerable doubt as to the nature and concentrations of the most abundant breath trace gases. In principle, any volatile with a molecular mass under 200 Da, present on breath in concentrations above 10 ppb, can be quantified by using the SIFT technique at present; examples, in addition to those measured in this study, would include propanol, acetaldehyde, and dimethyl sulfide.

Initial studies using the SIFT technique have established that it can be used to identify a large number of different molecules in breath (13, 17), but they exogenous or products of normal or abnormal metabolism. For example, breath ammonia is elevated in patients with renal failure, such that it is quantitatively related to the uremic state, its concentration decreasing exponentially during a hemodialysis treatment (1, 2). The concentrations of these trace gases in normal breath by using the SIFT method (1, 2) having been established, the purpose of the present study was to examine them in the fasting state and then to see how they were influenced by a moderate-sized, liquid protein-calorie meal. Five gases were selected, partly on the basis of their ease of detection and partly because their concentrations were likely to change after feeding. Acetone, ammonia, and ethanol are all naturally occurring products of human metabolism (8), which could potentially be influenced by a meal containing calories and protein. Isoprene is also an endogenous compound present in breath, being a product of terpene or lipid biosynthesis (10). In contrast, methanol is considered not to be an important product of normal human metabolism; however, it can appear in breath after ingestion or inhalation. In the present study, these five compounds were monitored in the breath of six healthy volunteers in the fasting state and for several hours after the meal.

MATERIALS AND METHODS

Subjects and study design. Six normal volunteers (5 men), all nonsmokers, were investigated [mean age 40 yr (range 24–61 yr); see Table 1 for details]. None had evidence of liver or kidney disease, diabetes mellitus, or nutritional abnormalities (mean body mass index 25.9, range 22.9–28.4), and none took regular medication. Each volunteer had fasted for 12 h overnight before commencement of the protocol. After a baseline breath sample was obtained, all the volunteers simultaneously drank a liquid protein-calorie meal in the form of the dietary supplement (time 0 on Figs. 1 and 2) Fortisip (Nutricia, Zoetermeer, The Netherlands) over a period of 5 min. Fortisip, a commercial dietary supplement containing casein protein, sugars, fat (90% unsaturated), vitamins, and trace elements, is designed to provide all requirements. Six to eight hundred milliliters were consumed...
Table 1. Individual concentrations of breath isoprene

<table>
<thead>
<tr>
<th>Subject No. (Gender:Age, yr)</th>
<th>Time, h</th>
<th>1 (M:58)</th>
<th>2 (M:24)</th>
<th>3 (F:27)</th>
<th>4 (M:40)</th>
<th>5 (M:60)</th>
<th>6 (M:30)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>156</td>
<td>68</td>
<td>114</td>
<td>115</td>
<td>137</td>
<td>34</td>
<td>104</td>
</tr>
<tr>
<td>0.33</td>
<td></td>
<td>35</td>
<td>41</td>
<td>66</td>
<td>82</td>
<td>111</td>
<td>45</td>
<td>63</td>
</tr>
<tr>
<td>0.67</td>
<td></td>
<td>55</td>
<td>70</td>
<td>56</td>
<td>89</td>
<td>93</td>
<td>50</td>
<td>69</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>160</td>
<td>81</td>
<td>66</td>
<td>157</td>
<td>79</td>
<td>79</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>81</td>
<td>57</td>
<td>8</td>
<td>140</td>
<td>71</td>
<td>54</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>68</td>
<td>69</td>
<td>33</td>
<td>74</td>
<td>92</td>
<td>33</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>95</td>
<td>50</td>
<td>31</td>
<td>113</td>
<td>55</td>
<td>39</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>71</td>
<td>37</td>
<td>48</td>
<td>110</td>
<td>113</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>132</td>
<td>50</td>
<td>60</td>
<td>141</td>
<td>79</td>
<td>46</td>
<td>85</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>95</td>
<td>58</td>
<td>54</td>
<td>113</td>
<td>92</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

Values are in parts/billion. M, male; F, female.

According to body weight, providing 30–40 g of protein (0.47 ± 0.02 g/kg, equivalent to a substantial protein meal) and 900–1,200 kcal, of which 48% was carbohydrate, 40% fat, and the rest protein. Breath samples were then taken at 20, 40, and 60 min and subsequently hourly until 6 h postprandial. Subjects gave informed consent, and the project had been approved by the local ethics committee.

The SIFT analytic technique. The new development, which allows the detection and accurate quantification of the trace gases on human breath in real time without the need for preconcentration and water removal from the breath sample, is the SIFT method of gas analysis, described in detail previously (2–4). This technique was conceived by two of the authors (D. Smith and P. Spanel) (1, 4) and, in a transportable form, will soon be available commercially as a self-contained analytic instrument. It combines gas kinetics, chemical ionization, flow techniques, and mass spectrometry. The essence of the new technique is simple. It exploits the chemical ionization of the trace gases in the breath sample (e.g., ammonia, acetone, ethanol) to the exclusion of the major breath gases (nitrogen, oxygen, carbon dioxide, argon) by judiciously chosen precursor ions. These precursor ions are selected by mass, using the upstream quadrupole mass filter, and injected into fast-flowing helium carrier gas along a flow tube (as depicted in Fig. 1). The breath is sampled by complete exhalation of the lungs through a wide-bore (diameter 1 cm) tube, in which a calibratable capillary leak into the helium carrier gas is heated to 100°C to prevent condensation of volatiles from the breath. The product ions that result from the chemical ionization reactions of the trace gases in the breath sample are detected and counted by a sampling mass spectrometer system located downstream. The precursor ions of choice are H₃O⁺, NO⁺, and O₂⁺, variously chosen to detect particular trace gases, M. H₃O⁺ precursor ions usually result in a single product ion, M⁺, for each reactive molecular species present in the sample, but also some association of the H₂O⁺ precursor ions with the water molecules in the breath sample occurs, producing the so-called hydrated hydronium ions, H₃O⁺·(H₂O)₁,₂,₃ (18). The relative magnitudes of the H₂O⁺ precursor ion and the H₂O⁺·(H₂O)₁,₂,₃ ion signals provide a measure of the humidity of the breath sample. O₂⁺ and NO⁺ precursor ions usually result in M⁺ and sometimes (M⁺)₂ product ions (19). Because only one or two product ions result from the reaction of each trace gas in the breath sample (unlike conventional electron impact ionization for which several ions are formed from each trace gas), the detected ion mass spectra and hence the identification and quantification of the trace gases present in the sample are greatly simplified.

In the present study, only H₃O⁺ and O₂⁺ precursor ions were used. After their introduction into the helium gas, the absolute concentrations of the trace gases, M, present in the air sample are determined from the count rate of each product ion, M⁺ and M₂, relative to the precursor ion count rate, H₂O⁺ or O₂⁺, by using the known rate coefficients for the reaction of the particular precursor ions with M (17). To achieve this, the downstream mass spectrometer is switched rapidly between selected product ions to allow the quantification of several trace gases simultaneously. The time response of this technique (presently 20 ms) is fast enough to allow the concentrations of several trace gases, to be followed during the breathing cycle, typically in 5 s. Each gas reaches a plateau concentration between 3 and 5 s of the exhalation, and this was taken as the equilibration with alveolar air.

Details of this mass spectrometric technique (17), its calibration by using a standard syringe technique (14), and the reproducibility of repeated measurement of the equilibrated exhalation concentration have already been published (15). The SIFT technique readily determines partial pressures of trace gases in breath from 10 ppb to 100 parts/million with the same efficiency for all trace gases, independent of their molecular weight. These values may be converted to molar concentrations (22.4 ppb are equivalent to 1 nmol/l at normal temperature and pressure).

RESULTS

In the present study the concentrations of five trace gases (acetone, ammonia, ethanol, isoprene, and methanol) were determined immediately before and several

Fig. 1. Schematic diagram of selected ion flow tube (SIFT) apparatus. See text for further explanation.
hours after subjects ingested the protein meal. \( \text{H}_3\text{O}^+ \) and \( \text{O}_2^+ \) were used separately as precursor ions, which therefore required two breath exhalations from each subject at each sampling time. \( \text{H}_3\text{O}^+ \) ions are ideal for the detection of acetone, ammonia, ethanol, and methanol, and \( \text{O}_2^+ \) ions also detect acetone, ammonia, and, additionally, isoprene. So the acetone and ammonia were detected and quantified by both \( \text{H}_3\text{O}^+ \) and \( \text{O}_2^+ \) with essentially identical results (coefficient of variation <3%), and this acted as a valuable consistency check on the concentrations of these vapors (15).

The concentrations in the laboratory air were also measured immediately before the breath samples were taken from the six subjects. However, it is very important to stress that the essential point of our direct breath-sampling method is that the breath totally displaces the ambient air at the entrance to the sampling leak, and therefore the breath sample is not directly contaminated with the ambient air. The purpose, therefore, of sampling room air was not to make a direct comparison between air and breath but rather to demonstrate any changes that were occurring with time in the ambient environment that could account for the observations. This was certainly the case for methanol, which is constantly being released into the air from adjacent laboratories and often exceeds the breath concentrations (see Fig. 3E). However, with this exception, none of the changes we observed with time in the remaining trace gases could be explained by their inhalation from laboratory air. The relatively large fluctuation in the ammonia background was the result of the constant exchange and mixing of air in the ventilated laboratory, coupled with the changes in humidity that occurred during the observation period.

A remarkable feature of the concentrations of these breath trace gases is that they show very similar trends in all six subjects, although the initial, premeal concentrations are different for each person. These differences are well illustrated by the results for acetone and ammonia, as shown in Fig. 2, A and B. The premeal acetone levels range from \( \sim 200 \) to 600 ppb (9–27 nmol/l), but despite the scatter within the data points they all decrease with time after the meal, reaching similar values, \( \sim 200 \) ppb, after 4–5 h; then, they show some tendency to increase. This increase is most obvious in the breath of the subject with the highest initial acetone level. The mean values of the acetone concentrations for all six subjects at each sampling time are shown in Fig. 2A, where these trends can clearly be seen. Note that the ambient air acetone levels are obviously lower than the breath levels.

Figure 2B shows the individual ammonia concentrations for each subject as a function of time. The premeal levels range from 300 to 600 ppb (14–28 nmol/l), but then they all unexpectedly decrease after the meal, reaching minimum values of \( \sim 200 \) ppb after \( \sim 30 \) min, before increasing toward maximum values, which range from 600 to 1,800 ppb (80 nmol/l), at 5 h. Again, the mean values at each time are shown in Fig. 3B, which clearly indicate the "dip" in the ammonia level and the subsequent rise with a tendency to fall again after 5 h (note the corresponding increase in the acetone levels).

The ethanol levels for each subject are shown in Fig. 2C. After the meal there is a clear increase in the ethanol levels in all but one of the subjects toward peaks after \( \sim 1 \) h. The initial values are within the 50- to 100-ppb range, but the peak values are much more varied, ranging from \( \sim 100 \) to 400 ppb. From these peaks they all decrease to low values \( \sim 50 \) ppb (2–3 nmol/l). The peaks in the ethanol levels are clearly reflected in the mean values represented in Fig. 3C, where it can also be seen that the peak values are

![Fig. 2. Individual absolute breath concentrations (in parts/billion (ppb)] of acetone (A), ammonia (B), and ethanol (C) in 6 volunteers. ▲ Data for female subject. Fasting concentrations are those at time 0, with subsequent measurements having been taken 20 and 40 min, then hourly until 6 h, after feeding.
generally much above the ambient air levels of ethanol and converge to the air levels after ~2 h. (Note that ethanol is also being released into the atmosphere by the adjacent chemistry laboratories but at a much lower partial pressure than methanol; see Fig. 3E.)

The isoprene concentrations are, on average, the lowest among the five trace gases. Within the scatter of the data points there is no apparent time variation of the concentrations in the breath of any of the subjects, the time-averaged mean values among the subjects being within the 50- to 110-ppb range (2–4 nmol/l). The scatter in the individual time measurements can be seen in Table 1. Again, the average values for all six subjects at each sampling time are shown in Fig. 3D (isoprene is not detected in the ambient air).

The mean values of the breath methanol levels, together with the ambient air values, are shown in Fig. 3E. Note that the breath values “track” the air values except when the air value reaches its maximum, ~600 ppb.

DISCUSSION

This paper is the first to report the true, absolute concentrations of a number of trace gases in the breath, including acetone, ammonia, ethanol, and isoprene in the fasting and replete state in normal human subjects, as determined simultaneously and by direct sampling from single breath exhalations. These concentrations represent the plateau found toward the latter part of the exhalation cycle and as such will be close to equilibration with those present in alveolar breath. The robust nature of the sampling technique was also confirmed, with identical values being obtained from duplicate samples by using different precursor ions (15).

The concentrations of acetone in normal breath reported in this study are similar to those reported previously by using the SIFT method and high-performance liquid chromatography (6, 17). As would be anticipated after feeding, there is a steady decrease in the breath acetone concentration to levels about one-half those in the fasting state, reaching the nadir at 4–5 h. Acetone is present in the breath because of the spontaneous decarboxylation of acetoacetate, a slow reaction at physiological pH (9). Acetoacetate accumulates in the circulation both during the starving state and in diabetic ketoacidosis. However, the concentrations of acetone observed in breath in the present study were considerably lower than those seen in patients with insulin-dependent diabetes, even when the individual is apparently adequately controlled on treatment, where values are typically 2,000–5,000 ppb (2, unpublished observations).

Perhaps the most unexpected finding of this study was the clear and significant decrease in the breath ammonia concentration immediately after the ingestion of the meal. This seems to imply that there is an increased removal of ammonia at this stage, although the alternative possibility, that there is reduced production, cannot be completely excluded. The principal biochemical pathways involved in removal of ammonia from the circulation are the urea cycle in the liver and transamination pathways in skeletal muscle, and, to a lesser extent, brain (7). The latter are unlikely to be the explanation of the observed drop in breath ammonia as muscle mass and physical activity remained constant throughout the experiment. If anything, blood flow to muscle will have decreased after feeding. The most likely explanation of this apparent removal of ammonia would be the enhanced clearance by the liver after the
increase in portal blood flow consequent to feeding. If this were the case then it might be anticipated that patients with significant liver disease, in particular those with portal hypertension, may fail to exhibit this phenomenon. The subsequent rise in breath ammonia concentrations over the following 4-h period is most likely derived from the nitrogen in the meal protein, being transported in the form of amino acids to the liver, where they are then degraded. It should be pointed out that the relationship between breath and blood ammonia concentrations does not appear to be straightforward. For example, the breath ammonia is considerably elevated in patients with advanced renal impairment, to levels 10–20 times those seen in normal subjects, and yet hyperammonemia is not a feature of various physiological and pathological conditions, and it offers an important opportunity for clinical diagnosis and therapeutic monitoring.

We thank Tracy Holland, David Simpson, and John Bicknell for participating in this study. We gratefully acknowledge financial support from the North Staffordshire Medical Institute.

Address for reprint requests and other correspondence: S. J. Davies, Dept. of Nephrology, North Staffordshire Hospital Trust, Princes’ Rd., Hartshill, Stoke-on-Trent ST4 7LN, United Kingdom (E-mail: SimonDavies1@compuserve.com).

Received 30 October 1998; accepted in final form 24 June 1999.

REFERENCES


