**Online and Offline Breath Analysis of**

**Volatile Organic and Inorganic Compounds**

**with the**

**Mass Spectrometer**

# ***„Airsense“***

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# Contents

# 1) Breath analysis as a possible tool in liver diagnostics: A pilot study

**(submitted for publication)**

**Introduction:** Human exhaled breath contains a wide range of molecules either present as gases or occuring in solubilised form in the humidity of the breath.
In patients with liver diseases, breath analysis might give additional information about the character of the liver disease beyond routine blood tests.Therefore we investigated, whether breath analyses could be helpful in the diagnosis of liver diseases.

Breath was collected from 131 persons having either a liver disease (non-alcoholic fatty liver disease - NAFLD, alcoholic fatty liver disease - AFLD, liver cirrhosis) or being completely healthy. Liver diseases were diagnosed by ultrasound, routine liver tests and the patient's history. Breath samples were collected in glass vials (see figure 1) and analysed by V&F soft ionisation mass spectrometry (Airsense). Several statistical methods were used to calculate statistical significances and positive predictive values for these four groups.

Fig. 1

**Results:** 114 molecules were measured in human breath. Spectra of exhaled breath differ between healthy persons, persons with non-alcoholic fatty liver disease, alcoholic fatty liver disease, and liver cirrhosis. 44 molecules showed a statistically significant difference between the four tested groups. Discriminant analysis based on only five molecules, acetaldehyde, endogenous ethanol, isoprene, nitrogen monoxideand molecular mass 76 which might be carbon disulfide (see figures 2 - 6) provided a positive predictive value of 80% for healthy persons, 67% for NAFLD, 60% for AFLD and 46% for liver cirrhosis.
Results of discriminant analysis based on gammaGT, the most prominent laboratory value for the indication of liver diseases (see figure 7) provided a positive predictive value of 97 % for healthy persons, 82% for NAFLD, 40% for AFLD and 0% for liver cirrhosis.

With this pilot study first steps for a non invasive diagnostic and cheap tool for the assessment of the liver function have been taken. The results suggest that breath analyses might be a useful tool for the diagnosis of liver diseases.

Figure 2: acetaldehyde

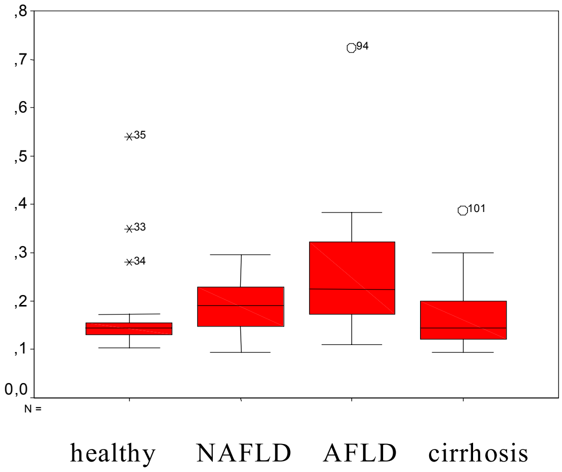



Figure 3: ethanol

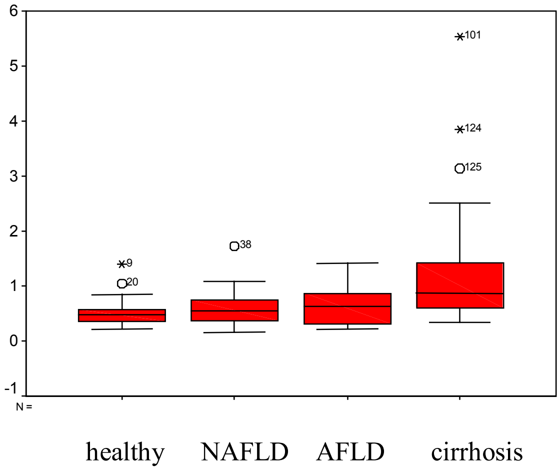



Figure 4: isoprene

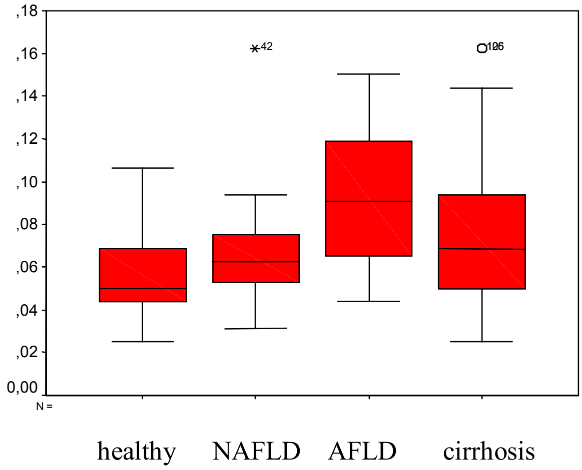



Figure 5: nitrogen monoxide

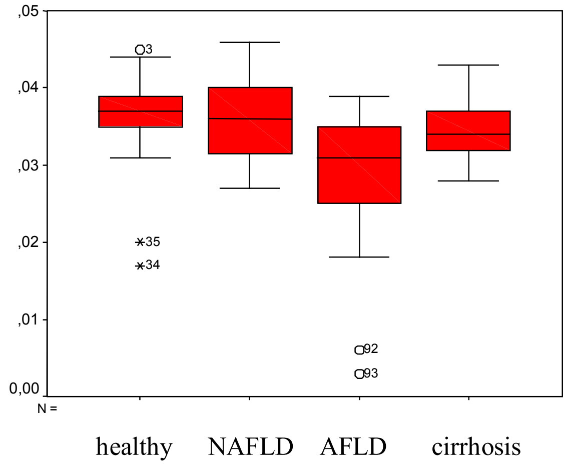



Figure 6: molecular mass 76

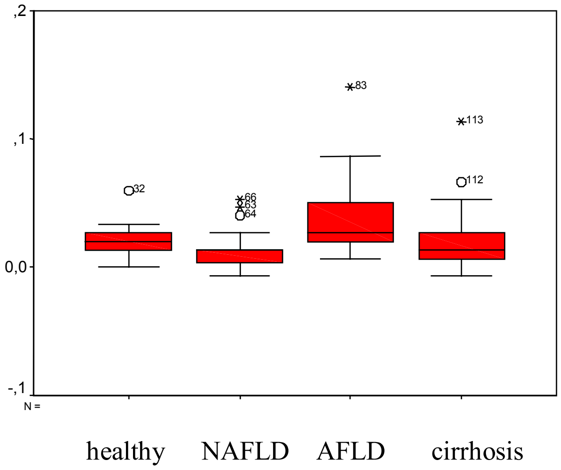
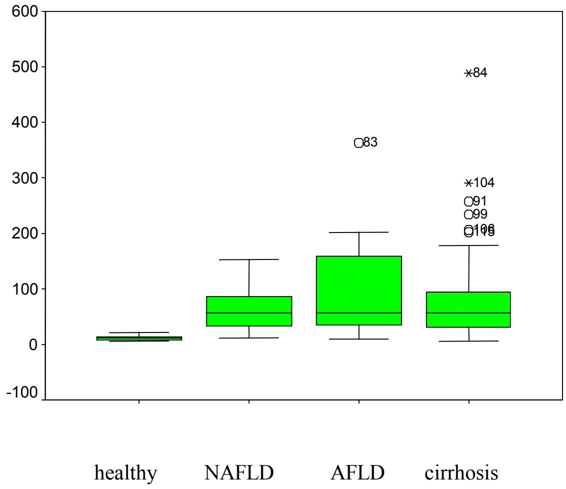



Figure 7: gammaGT



## 2) Monitoring of a detoxification-diet according to Dr. F.X. Mayr

**Introduction:**
One female and one male both aged in their midthirties and in good physical condition were on a diet according to Dr. F.X. Mayr for two weeks in an Austrian health resort.
The man only drank tea and ate rolls, whereas the woman was also allowed to eat light side dishes.
During the first four days of this period both of them exhaled three times a day into glass vials at defined times per day (with an empty stomach in the morning, before and after lunch at midday). After these four days the samples were analyzed by the V&F soft ionisation mass spectrometry.

**Results:**

Probably because of the more intensive fasting of the man there are several molecules which show the deep purification of the body. Interestingly enough the component propanol (figure 8) starts to increase in the exhaled breath on day 2 whereas acetic acid (figure 9) starts to increase on day 3.

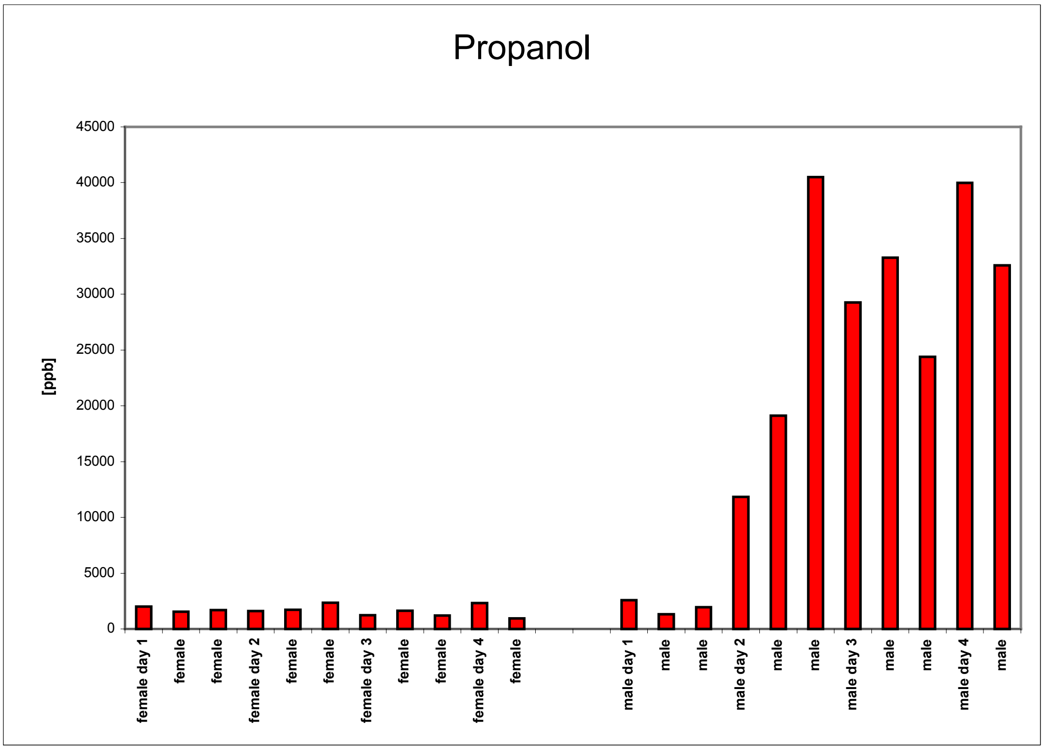



Fig. 8

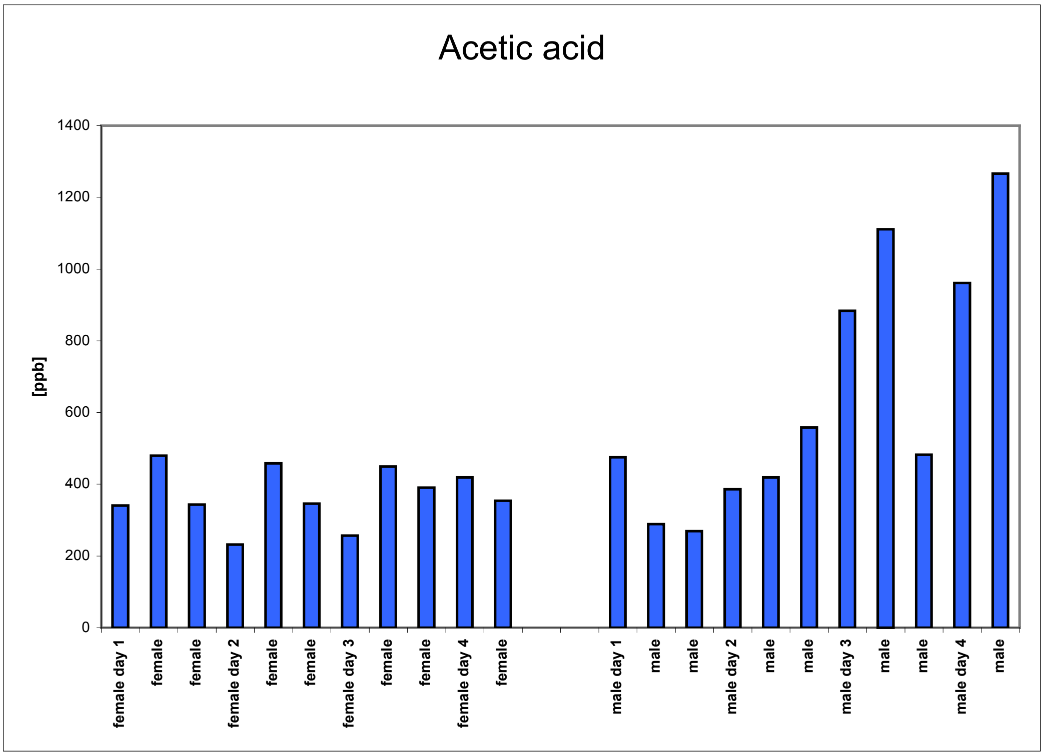



Fig. 9

Beside these two molecules also other compound classes like alcohols, aldehydes and ketones show similar tendencies. The biggest difference showed the compound acetone (figure 10).

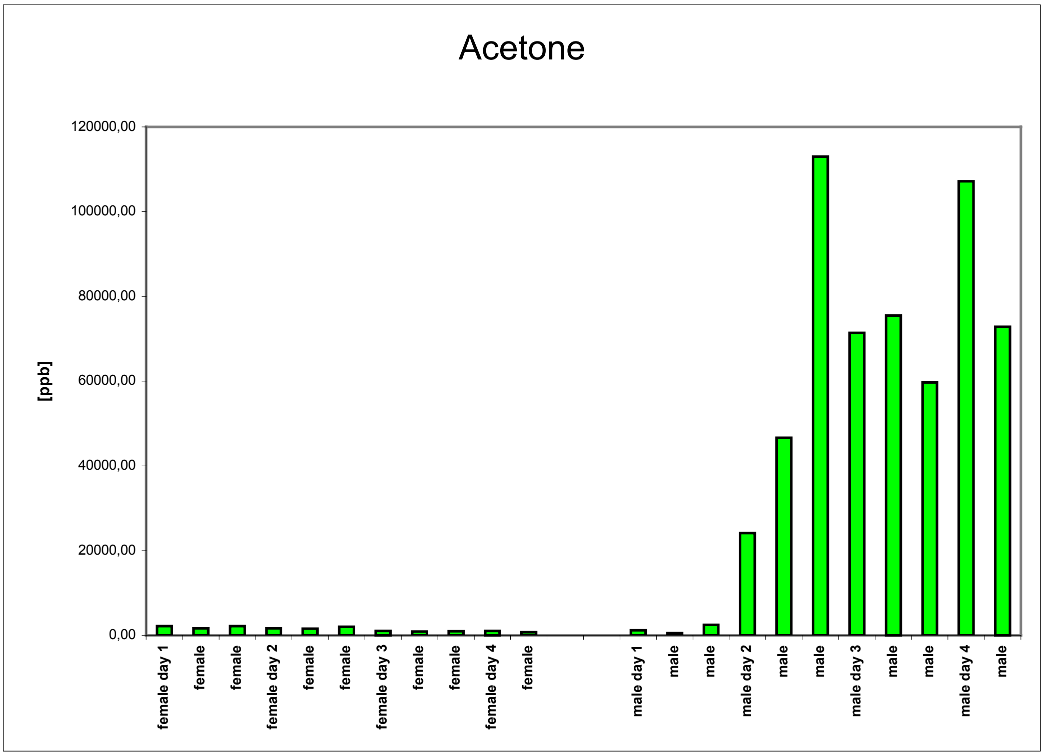



Fig. 10

## 3) Breath to breath monitoring of pharmacokinetic profiles

After the ingestion of tablets containing 300 mg thymol, the quantitative inline gasphase analysis of thymol, isoprene, mass 61 and ethanol was performed in the exhaled breath of a 65 kg male.

Over 90 minutes the concentrations were measured with four-second cycle time by the V&F-mass spectrometer "Airsense".

Mass spectrometer adjustments:

| compound | lower detection level [ppb] | resolution [ppb] | detector sampling time per cycle [msec] |
|---|---|---|---|
| thymol | 0,2 | 0,1 | 2500 |
| isoprene | 50 | 10 | 300 |
| mass 61 | 50 | 10 | 300 |
| ethanol | 100 | 20 | 150 |

Figure 11 shows the input function of the released drug, reaching a maximum of 3 ppb 36 minutes after the ingestion of the tablets followed by a slow decline of the concentration, describing the disposition of thymol. 30 minutes after the ingestion of the tablets the ethanol level began to rise in the exhaled breath starting from 0,5 ppm reaching 7 ppm after 90 minutes.

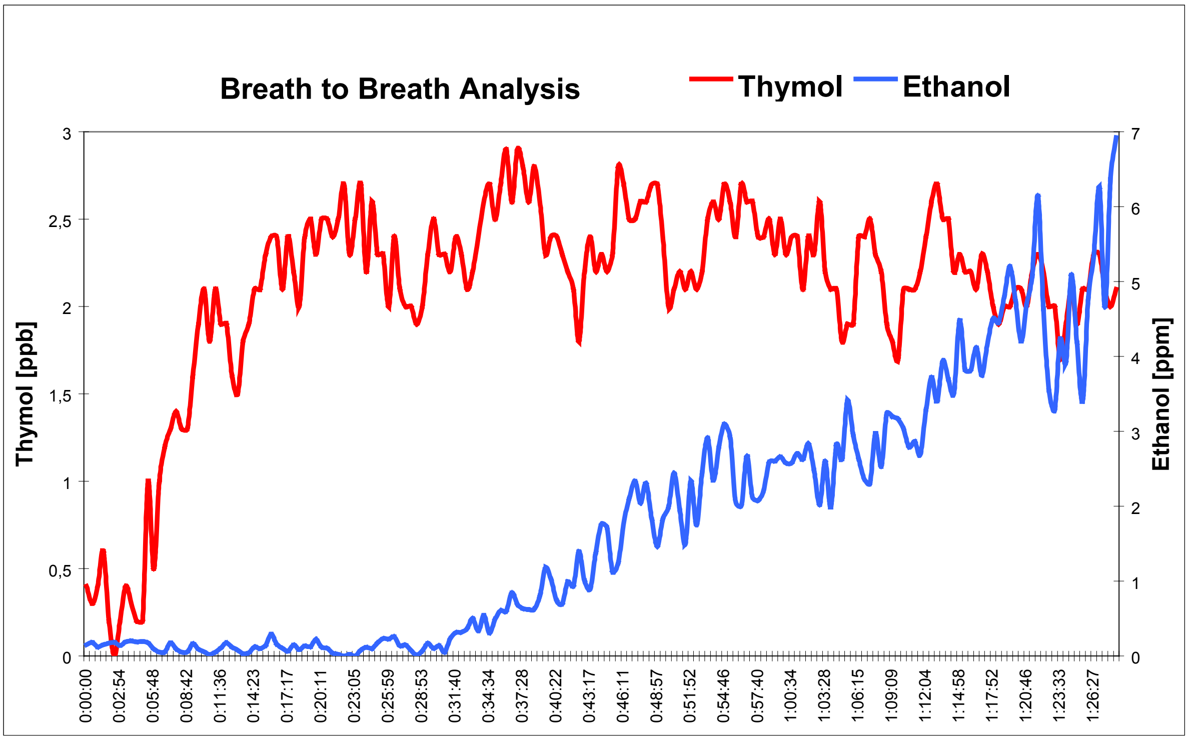


Fig. 11

## 4) Blood - lactate in correlation to off-line exhaled breath monitoring under bicycle ergometry: A promising non invasive diagnostic tool in performance function diagnostics

In a pilot study three volunteers agreed to participate in a classical blood lactate determination with bicycle ergometry.
Starting at 50 watt the power was increased with an additional 50 watt every three minutes until the volunteers reached their individual maximum power. Whenever the power was increased blood was taken from the ear to determine lactate and simultaneously the test persons exhaled into vials, which then were analyzed by V&F's mass spectrometer technology.

From 114 analyzed molecules, the exhaled value of carbon dioxide divided through the value of the compound isoprene showed the best correlation to blood lactate (figure 12). For a better overview both blood lactate and carbon dioxide/isoprene have been calculated to a maximum relative value of 1 (i.e. 100%).

Beside carbon dioxide/isoprene also the compound methane tends to result in a positive correlation to the blood lactate gradient.

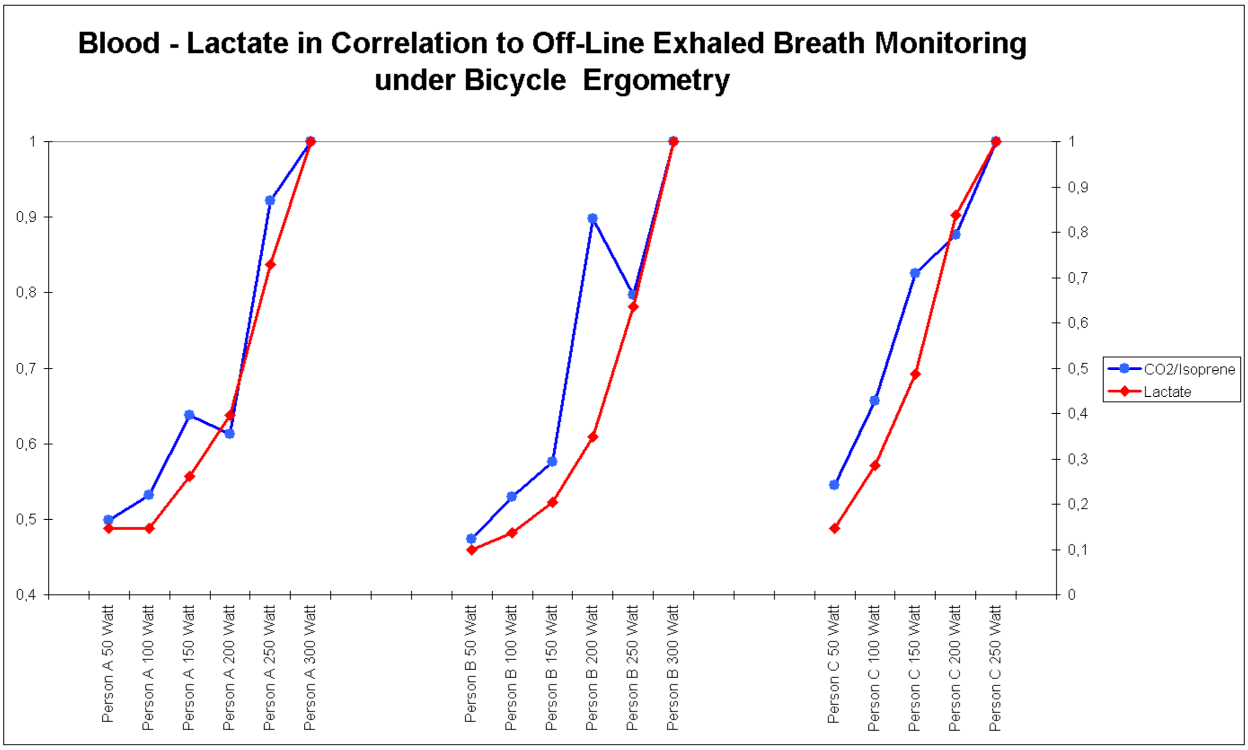



Figure 12

## 5) Acetone and diethyl ether vapor exposure monitoring

Offline measurement of the exhaled breath of people working in a binocular producing company were performed. The main solvents for cleaning the lenses and prisms during the fabrication of the binoculars are acetone and diethyl ether. Despite ventilation systems in the factory the workers are exposed to average gas concentrations of 30 ppm acetone and 40 ppm diethyl ether. Inhaled volatile compounds readily obtain high concentrations in well perfused organs like brain, lung and heart whereas the disposition in muscle and tissue is slow. For this reason these VOCs are exhaled unchanged.

The exhaled breath was collected in glass vials and while sampling a second vial was placed in the surronding to collect the ambient air. The analysis was performed by the V&F-Airsense in combination with the V&F-Autosampler under consideration of the ambient air.

Figure 13 shows the natural logarithm of the acetone and diethyl ether concentrations versus the time of exposure of six people after two days of regeneration.
The concentration of diethyl ether in exhaled breath rises at 5 ppm/h and discharges fast to zero at 10 to 15 ppm/h (lunch break). Acetone starts with offset levels due to the inner acetone contents in every person and thus rises and discharges more slowly than diethyl ether.

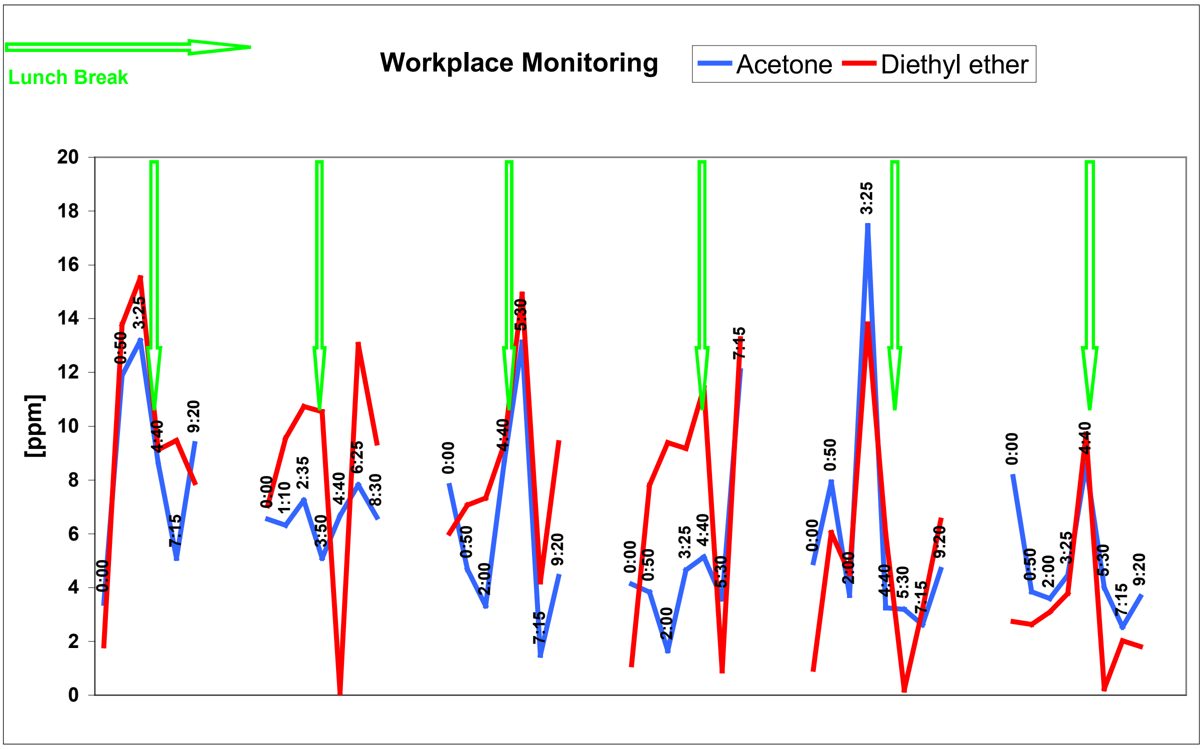



Fig. 13

# 6) Lactose/Fructose intolerance and malabsorption monitoring

## Introduction

Lactose and fructose intolerance or malabsorption are frequent disorders in civilized countries. In the USA more than 40 Mio people suffer from one or both of these disorders. In Europe the incidence is suspected to be around 15 to 20 percent. Lactose intolerance is due to a disorder in the lactase gene leading to a decreased production of lactase in heterozygous patients whereas lactase is missing in homozygous patients. Fructose intolerance is associated with alterations in the aldolase B gene. Clinical presentation is characterized by bloating, diarrhea, abdominal pain. In fructose intolerance additional symptoms not associated to the gastrointestinal tract like headache and chronic fatigue are frequently reported. Usually patients tend to avoid either lactose- or fructose-containing meals. In fructose intolerance vitamin-deficiency is seen whereas lactose intolerant patients have an elevated risk to develop osteoporosis due to reduced calcium intake and absorption.

## Patients and methods

48 Patients – 33 female, mean age 41, 9 years – complaining of gastrointestinal symptoms were enrolled into the study. 41 patients completed lactose and fructose breath tests. When both tests were performed, there had to be a wash out phase of at least 48 hours betweeen the tests. Patients were given 50 g of fructose or lactose dissolved in water and breathing samples were collected in glass vials before and every 30 minutes up to 2 hours after administration of fructose or lactose and subsequently analyzed by the V&F-Airsense. In addition patients had to answer a questionnaire on symptoms including bloating, abdominal pain, rumors, headache, nausea, flatulence on each breath sample collection.

## Results

### Lactose breath test

Some of more than a hundred of analyzed substances show different reaction patterns after lactose administration in normal patients not complaining symptoms (group 1, n=19) and patients complaining on bloating (group 2, n=2), abdominal pain (group 3, n=2) or nausea (group 4, n=5). Methanol, propane, nitrogen monoxide, mass 102 and mass 90 (ev. lactic acid) remain unchanged in normal individuals whereas they decrease in patients of group 3 and 4.

Free water and mass 100 is increasing in non-normal patients while they remain stable in normal controls (group 1)

Whether the decrease of methanol, propane and lactic acid and the increase of mass 100 is due to different bacterial colonization of the small intestine in normal and lactose intolerant patients has to be clarified in further studies. The increase in free water seems to reflect the osmotic effect of higher sugar content in the small intestine and is furthermore associated with diarrhea following lactose administration in these patients.
Decrease in NO in lactose intolernat patients (only group 3 and 4) is associated with more severe symptoms. Whether it reflects induced bacterial metabolism or a crude reaction type of patient energy metabolism has to be elucidated.

## Fructose breath test

In fructose breath test, the reaction pattern of analyzed substances is more uniform than in lactose breath test. Formaldehyde, methanol, nitrogen dioxide, mass 85 and 86 show an increase within 60 minutes after fructose administration in normal controls (group 1, n=6) whereas those substances decrease in all other patient groups.

Mass 90 (ev. lactic acid) shows a similar increase in normal patients but is decreasing only in patients complaining of nausea (group 4, n=11), whereas it remains stabel in group 2 (bloating, n=6) and group 3 (abdominal pain, n=7).

Formaldehyde is a substrate of pentose and methane cycle in human cellular pathway and the decrease in non-normal patients seems to reflect the missing substrate. Whether lactic acid reflects changes in intestinal bacterial metabolism due to fructose overfilling remains under suspicion.

**Summary**

Intolerant patients show different reaction patterns of some analyzed substances after fructose or lactose administration than normal controls. Some of these reaction patterns are associated with specific symptoms like nausea or abdominal pain. Whether some of these changes are due to altered bacterial colonization of the small intestine is to be clarified. In fructose intolerance formaldehyde could be associated with missing substrate in the pentose and methane cycle of the patient.

# 7) The V&F-Airsense: Technology – Instrumentation

The technology and the main features can be described as follows.

The detection principle of the instrument is the analysis of the molecular weight of the substances. Sample gas is introduced to a high vacuum chamber and transformed into ions that are subsequently mass selected by electromagnetic fields and counted in a particle detector (figure 14).

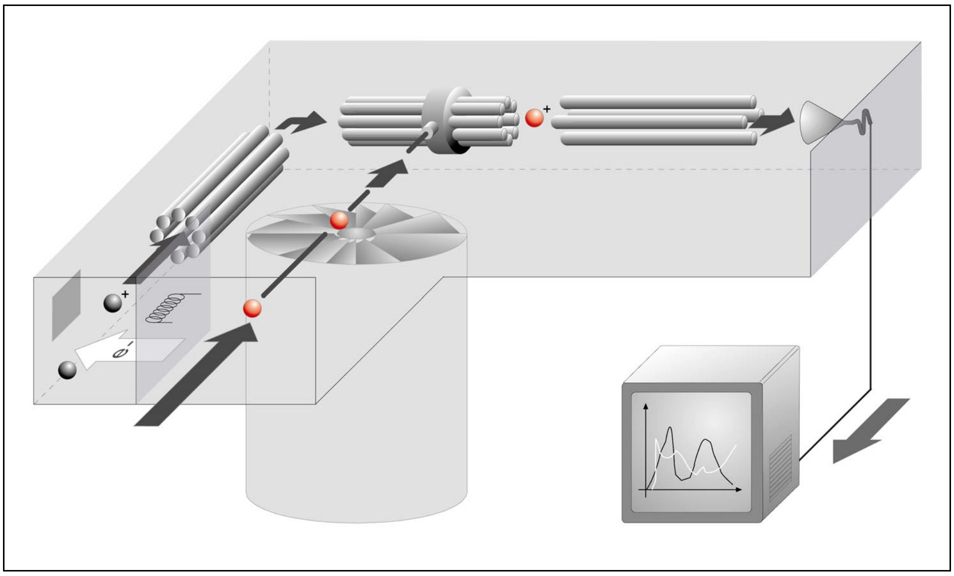


Fig. 14 Schematic setup of the "V&F-Airsense"

As different molecules may carry the identical molecular weight, such as $N_2$ and CO or formaldehyde and NO or $CO_2$ and $N_2O$, the instrument uses different ionization levels (i.e. different primary ion beams) to distinguish between mass identical molecules. Every molecule has an individual energy that is necessary to remove an electron and thus convert the molecule into an ion. An additional separation effect is gained through the formation of defined fragment ions. The interaction of the ion beam with the gas sample results in well-defined product ions. A krypton ion beam with 13,9 eV energy, for instance, separates the mass identical molecules $N_2$ (14.2 eV) and CO (13,7 eV). The mass identity of methanol $CH_3OH$ and oxygen $O_2$ on mass 32 is solved by ionization with xenon (12,2 eV) that generates an $O_2$- ion on mass 32 and a $CH_3O^+$-ion on mass 31. Larger hydrocarbons, for example, need ionization energies in the range of 10 eV created by a mercury ion beam. Table 1 shows the general technical data of the "V&F-Airsense".

**Tab. 1 Rating Data for AIRSENSE**

| | |
|---|---|
| Mass Range | 0 - 500 amu, 0 - 128 amu (depending on application) |
| Mass Separation | min. 1 amu over the mass range |
| Cycle Time | min. 10 msec - depending upon desired precision |
| Detection Limit | 0,1 ppb for benzene – controlled via a molecule library |
| Response Time | < 100 msec tested with 1 ppm benzene |
| Idle Time | < 100 msec from inlet to high vacuum, depending on bypass flow |
| Dynamic | $10^4$ |
| Linearity | +/- 3% |
| Drift (Concentration) | < 2% over one hour |
| Computersystem | Controller Interface, HP-Pentium Computer for control and datahandling |
| Software | ACP APPLICATION CONTROL PROGRAM for Windows<br><br>SCP - SIMS CONTROL PROGRAM for Windows<br><br>programs for controller interface, datahandling, trend analysis (time-/concentration-analysis for unlimited molecules), molecule libraries, bargraph, analog inputs and outputs |
| Analog Outputs | 4-channels on-line outputs |
| Analog Inputs | 16-channels on-line inputs |
| Supplyvoltage | 220 V / 50 Hz, max. 1200 Watt |
| Temperature | 10 °C - 40 °C |
| Humidity | 90% max., not condensing |
| Size | 1 x 19’’ unicab |
| Service-Interval | Routine service once a year |