

# Evaluation of Therapeutic Biocorrection by Exhaled Markers of Metabolism

## - A Pilot Study

Gunther Becher, Stephan Heymann, Oleksandr Marksteder, Roman Purkhart, Jörg Schulz

BecherConsult GmbH, Bernau, ICP HealthCare GmbH, Berlin, Germany

### **Introduction**

In daily clinical practice, it seems not suitable to get a blood sample for monitoring of metabolic parameters a couple of times a day or manifold during one patients test. Nevertheless, especially in elderlies and disabled patients effective monitoring of the disease is needed.

So, there is a great need to make a non-invasive assessment of the metabolism, for example by marker from the exhalate. Previous known publications [1] reported evidence in the breath condensate as a marker of oxidative stress or note on metabolism of more unsaturated fatty acids on MDA. EBC needs a long-lasting sampling of about 15 minutes, and highly complicated laboratory methods for analyses.

There is urgently need for evaluation of Individual Systemic Biocorrection is an adjuvant therapy for patients suffering from Metabolic Syndrome, especially from Diabetes mellitus type 2.

IMS seems to give a chance to detect volatile parameters in exhaled breath. IMS has a detection limit of around ppb and ppt. A pre-settled gas chromatographic column will enhance the sensitivity of the IMS as well as the possibility to differentiate ionized markers with different specific weight [2;4;8]

In a pilot study, it was checked whether MDA or MDA-related markers in breath using MCC-IMS as a single breath method is immersion.

### **Methods**

A training procedure of moderate physical load in a controlled breathing atmosphere of 26 % oxygen (hyperoxia) [3; 5; 6] was performed in diabetic patients and healthy, but overweight, volunteers. The training parameters are adjusted individually by means of the Respiratory Quotient (RQ) to be kept in the range of 0,75 – 0,85. Under these conditions, the cellular energy balance is gradually shifted from sugar to fat utilization. After 10 training units, one hour each, the patient's metabolism normalizes noticeably: blood glucose and

triglyceride concentration are improved, indicators of the oxidative-antioxidative equilibrium assume normal values, and the patient's subjective well-being improves significantly.

RQ and heart rate were checked during repetitive training sessions on treadmill every ten minutes.

A MCC-IMS from STEP Sensortechnik was used for the investigations. The system is a combination of a multi capillary column with an ion mobility spectrometer.

Spectra of the reference substance for MDA and breath samples from subjects with moderate physical exertion were measured. The comparable peaks of the reference and the air were determined by means of cluster analysis-based software. The classification has been carried out with a leave-one-out cross validation and support vector machine. The spectra were analyzed by a statistical program based on cluster analysis and non-parametric statistics (U-test).

Using cluster analysis by support vector machine based software the peaks of all measurements were detected and compared each other. Sensitivity and specificity of differentiation was evaluated by Leave-One-Out cross validation method [7; 8]

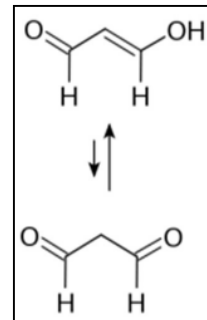
In comparison to previous experimental investigations a Cluster of acetone and three clusters representing MDA-related ions were analysed first.

Fig. 1a

MDA (i.e. Malondialdehyde resp. Propandiale)

a major breakdown product in the oxidation of polyunsaturated fatty acids.

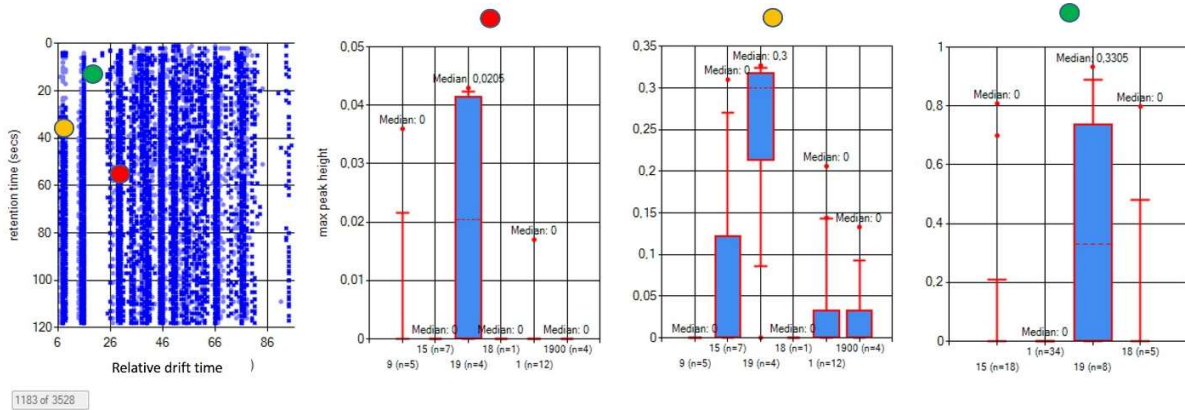
MDA serves as biomarker of oxidative stress mostly elevated in plasma of diabetics.



Similar chemical structures with barbituric acids are the reason for comparative peaks of these agents, but can be differentiated by calculating the complementary peaks. The following figures show identical and different peaks of the substances, enabling the calculation of the specific peaks of one substance.

Fig. 1b

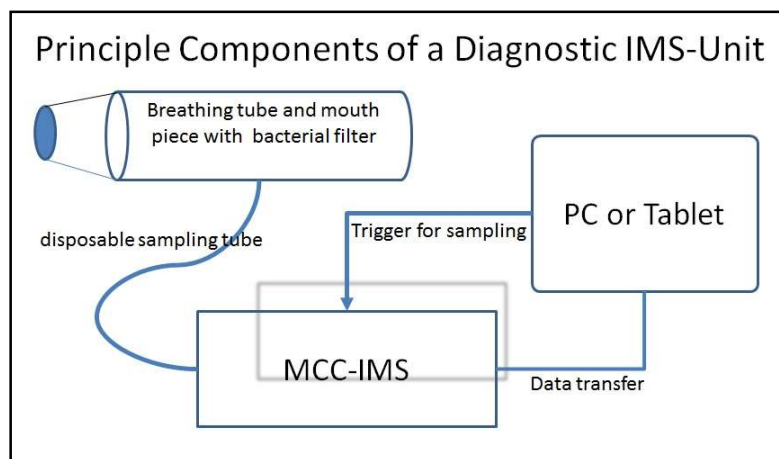
Experimental identification of clusters which represent MDA from related chemicals or controls spectrograms (MDA 19; mint oil 9; Barbituric acid 15, *thio-barbituric acid* 18, *cooled MDA* 1900, *clean air* 1)



Clusters representing experimental investigated reference-substances were used for further investigations on exhaled breath for detection of VOC's of fatty acid metabolism.

Breath samples were taken as a single breath test. The volunteer has had to perform a slowly exhalation for at least 20 secs following a deep nearly complete inspiration. During the second half of expiration a 2.5 cc sample of breath was taken by IMS.

Fig. 2 Schematic diagram of setup for breath sampling



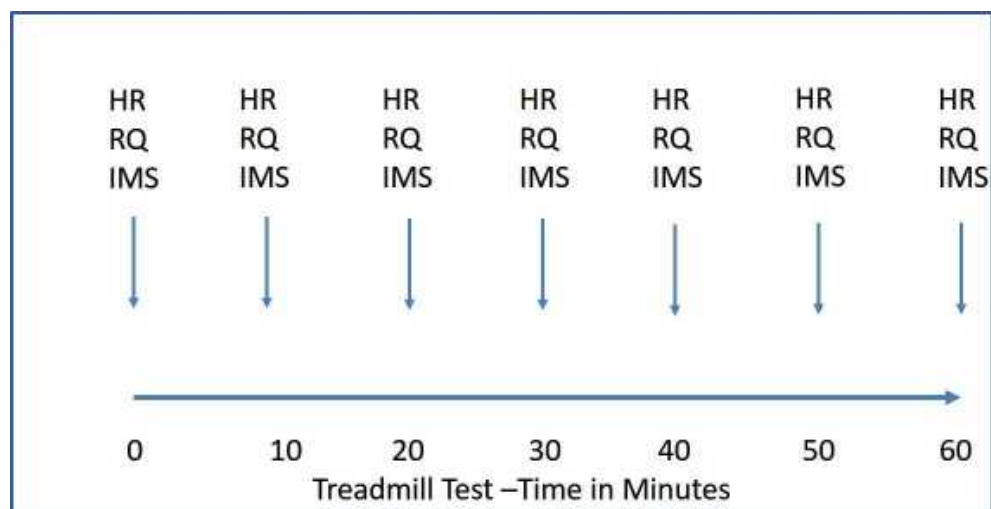
### Volunteers:

The study included 6 volunteers, (3 female, 3 male) which performed overall 26 treadmill tests in hyperoxide conditions with a moderate effort. Each test last 60 minutes.

Mean age was  $69 \pm 3$  (Diabetes) and  $69,5 \pm 2$  (control), respectively. The body weight was  $82 \pm 29$  kg in Diabetics and  $99 \pm 8$  kg in healthies.

During each test at point zero and every ten minutes – up to 7 times the following parameters were measured: Heart rate, Respiratory quotient (RQ), and a single breath test for IMS was taken.

Fig 3: Treadmill Test – time course and points of parameters sampling



The initial values were taken at the onset of the test, for standardized conditions in all volunteers.

### Results

All volunteers were able to perform the one hour treadmill tests without break or premature stops during a time-course of up to 4 weeks.

Fig 4 and 5 shows the tracings of Heart rate (HR) and RQ as mean of all tests  $\pm$  SD. There was a visible difference between diabetic patients and healthy controls. HR of diabetics was even at the beginning of a test higher than in healthy volunteers.

The differences represent the specific training effect of the volunteers.

Diabetic patients with overall higher heart rate showed a lower increase of HR during exercise tests at mean than the healthy controls.

Fig. 4: Heart Rate [mean  $\pm$  SD] during the test

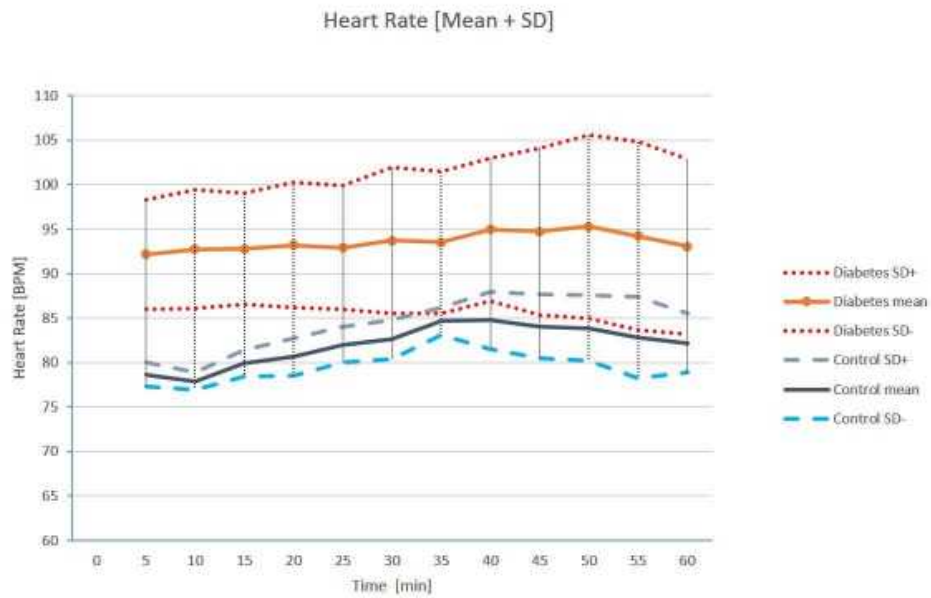
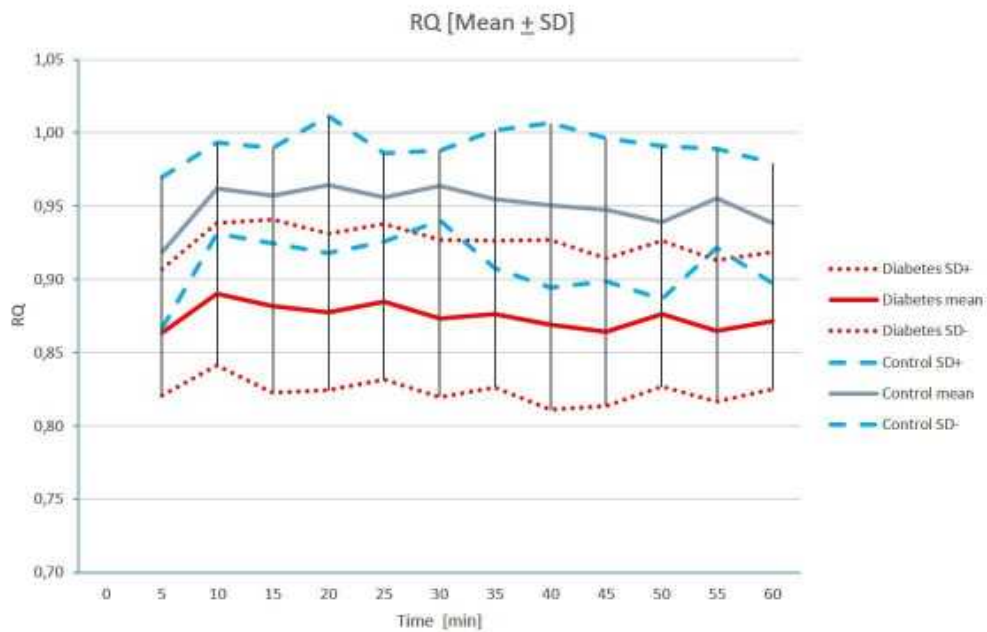


Fig. 5: RQ [mean  $\pm$  SD] during the test



RQ showed a higher physical limit of healthy controls than in diabetics within the threshold under the hypoxic limit.

After initial increase by beginning of each treadmill test a slowly decrease during the test occurred, but remaining above the level at rest.

The physiological parameters demonstrated that the physical exercise in all tests was in a range below the individual hypoxic threshold which shows the correct test level due to the described target.

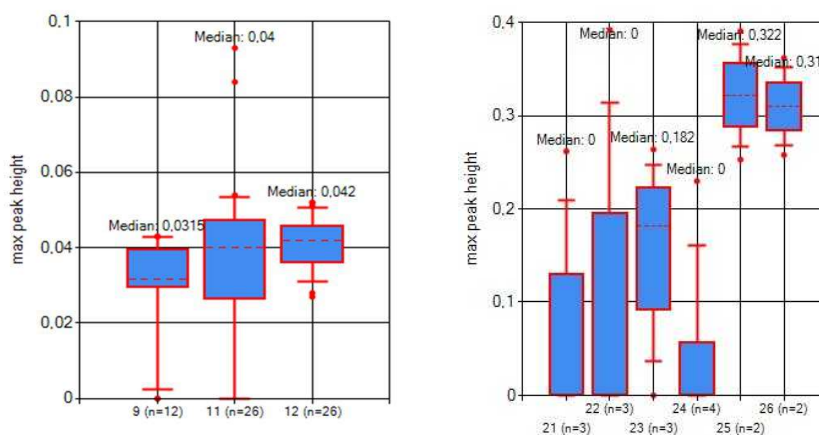
In IMS analyses could be shown that identical peaks such as for reference measurements even in exhaled air of subjects are identifiable. Different height of peaks represents the concentration in air.

Fig. 6

Specific clusters in experimental investigations and exhaled breath for detection of MDA related peaks, due to the yellow-indicated peak in Fig. 1b.

Left: Cluster 49 of 391 compared the MDA reference (9) to air of patients before (11) or to moderate exercise stress (12) in diabetics.

Right: Diabetes mell. - treadmill-training 3 times a week, (21, 22 and 23 first measurement at beginning of a test series, 24, 25 and 26 measurement during the last test of an individual series.



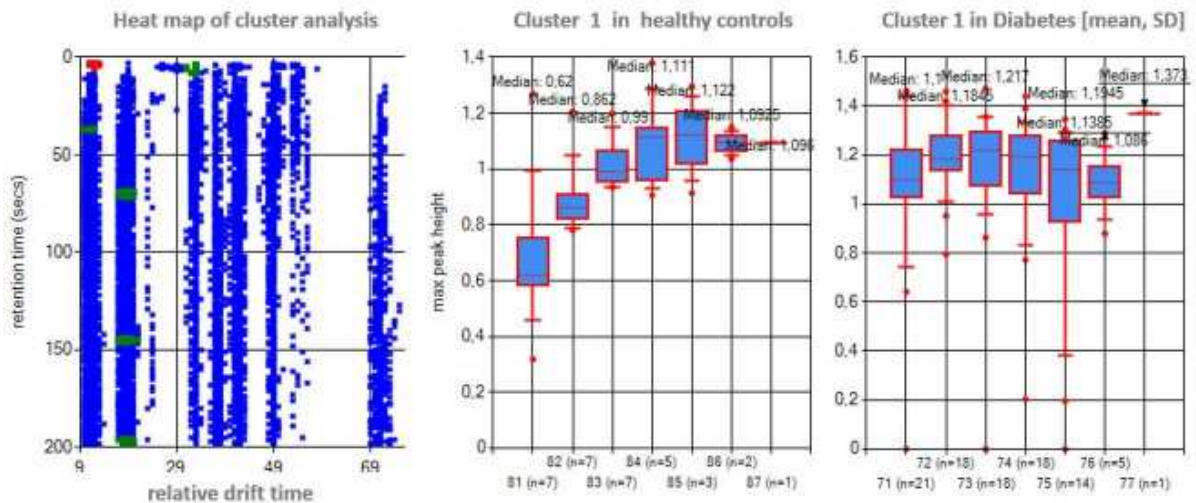
The right part of figure 6 shows the difference of MDA related VOC-exhalation between first and fifth exercise test. The results may serve as indicator for effective metabolic switching due to moderate exercise tests in diabetics within a few weeks of training.

The following figures indicate the occurrence and height of different clusters in IMS-tracings between diabetics and healthy controls. Each left diagram give the sum of all identified clusters in all measurements, the specific cluster is indicated red.

The middle and right diagrams give this cluster for healthies (middle) and diabetics (right).

Fig. 7

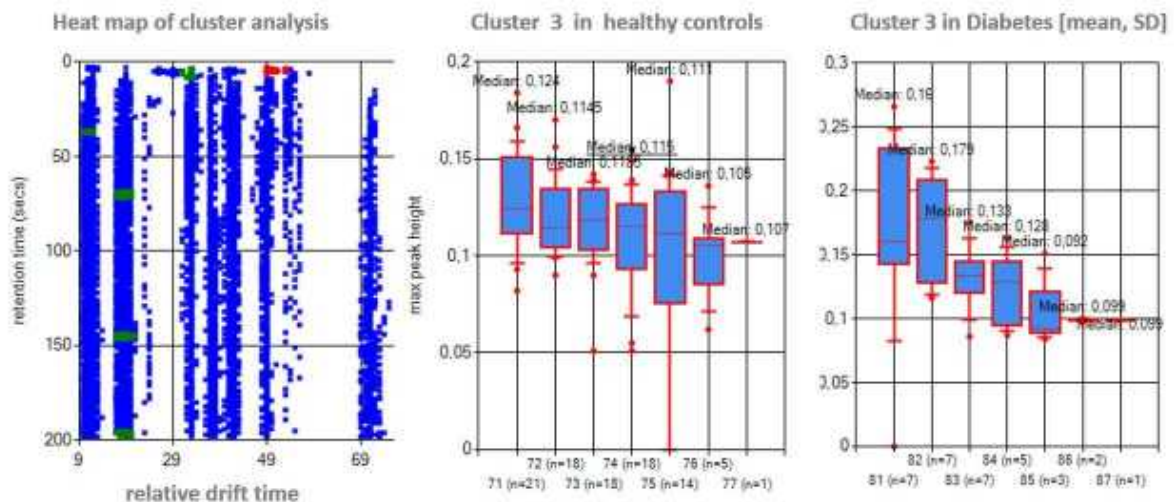
Time Course of Cluster 1 (acetone-related) during treadmill-tests in diabetics and healthy controls [mean + SD, median]



Cluster one is due to acetone and correlates with the glucose-concentration and degradation. Acetone is one volatile product of the “Krebs-Cycle”.

Fig. 8

Time Course of Cluster 3 during treadmill-tests in diabetics and healthy controls [mean + SD, median]



Cluster 3 represents a short chain Carbohydrate like two or three carbon-atoms. This marker is reduced in breath in diabetics more than in healthy volunteer during exercise.

Fig. 9

Time Course of Cluster 198 during treadmill-tests in diabetics and healthy controls  
[mean + SD, median]

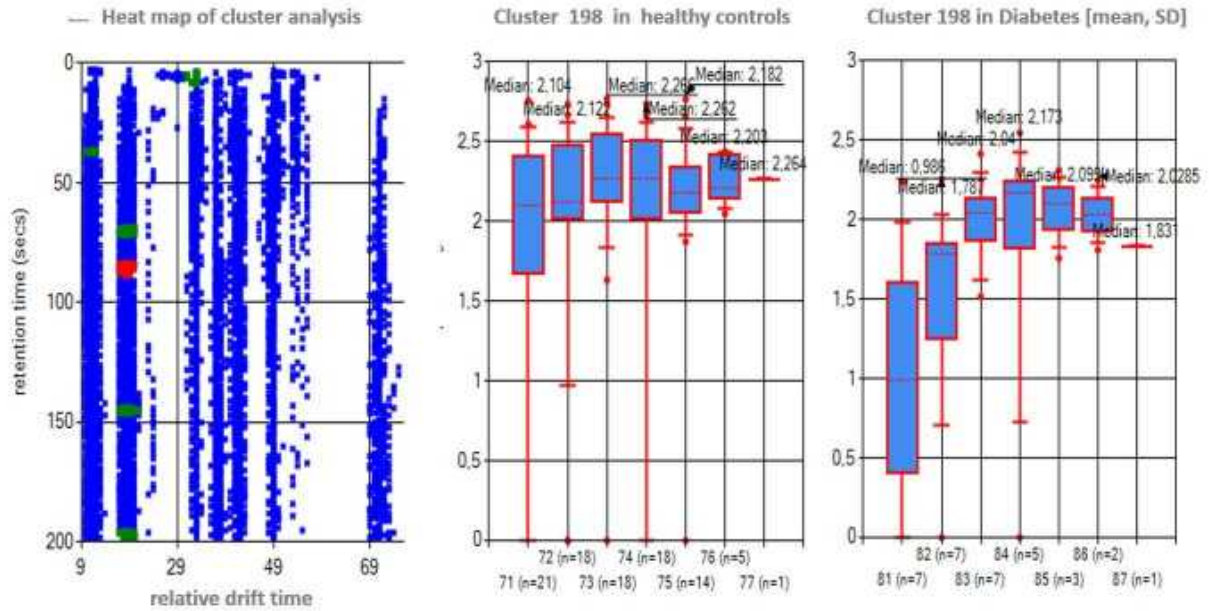
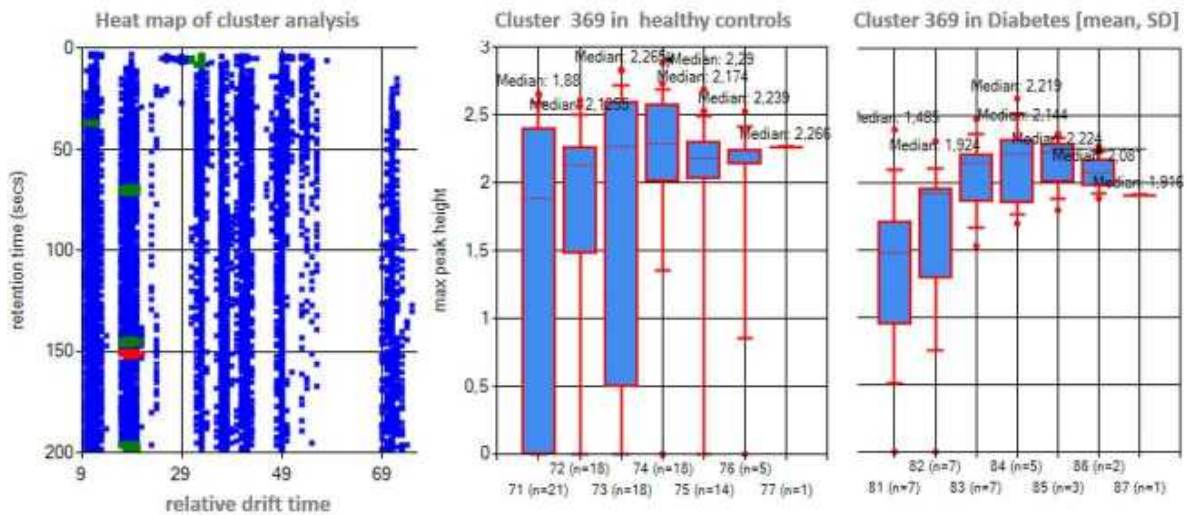


Fig. 10

Time Course of Cluster 369 during treadmill-tests in diabetics and healthy controls  
[mean + SD, median]





Cluster 198 and 369 represents longer chained VOC's which may be an indicator of degradation of fatty acids. Whereas in healthy controls there is no significant change of these markers in diabetics there was found in all patients an increase during the tests. This increase is a hint for a shift in metabolic processes due to the exercise.

## Discussion

The results give some evidence that moderate exercise training in diabetic patients may induce a shift between carbohydrate and fatty acid metabolism. This is the effect which was expected by so-called Biocorrection Methods [9; 10].

It was demonstrated that MDA is measurable also bypassing the Breath condensate directly out of the exhaled air, using MCC-IMS. For detection was suitable a air-sample of 2.5 cc.

The data of increasing levels of acetone (Cluster 1), as well as cluster 198 and 369 in diabetics show significant changes in metabolic processes during the tests. The differences between healthy controls and diabetics are evident.

It became evident that there is a sufficient sensitivity for measuring in air at least for MDA with the MCC IMS. Further investigations must show if the found parameters of oxidative metabolism and the metabolism of unsaturated fatty acids can be used to track metabolic alterations during exercise non-invasive.

Unless not fully understood peaks in exhaled breath it was shown that between healthy volunteers and diabetic patients seems to be distinguished differences in metabolism of carbohydrates and fatty acids for cellular energy balance.

Healthy volunteers shift their energy metabolism during exercise tests by increased exhalation of acetone, indication high glucose metabolism, while diabetics already at rest are in different metabolic status due to glucose metabolism.

In the opposite diabetics seems to induce more fatty-acid degradation during exercises (Cluster 198), approaching the levels of the controls.

Further investigations must show if the found parameters of oxidative metabolism and the metabolism of unsaturated fatty acids can be used to track metabolic alterations during exercise non-invasive.

#### Summary:

There is a great need to make a non-invasive assessment of the metabolism, for example by marker from the exhalate. Previous known publications reported evidence in the breath condensate as a marker of oxidative stress or note on metabolism of more unsaturated fatty acids on MDA. In a pilot study, it should be checked whether MDA using GC-IMS as a single breath method is immersion.

A MCC-IMS was used for the investigations. The system is a combination of a multi capillary column with an ion mobility spectrometer. Spectra of the reference substance for MDA and breath samples from subjects with moderate physical exertion were measured. The comparable peaks of the reference and the air were determined by means of cluster analysis-based software. The classification has been carried out with a leave-one-out cross validation and support vector machine.

It could be shown that identical peaks such as for reference measurements even in exhaled air of subjects are identifiable. Different height of peaks represents the concentration in air.

Thus, it appears that MDA is measurable directly out of the exhaled air. Furthermore, differences in metabolism between glucose and fatty-acids seems to be detectable by VOC analyses in breath.

It became evident that there is a sufficient sensitivity for measuring in air at least for MDA with the GC IMS.

Further investigations must clarify if the found parameters of oxidative metabolism and the metabolism of unsaturated fatty acids can be used to track metabolic alterations during exercise.

#### Abbreviations:

EBC	Exhaled Breath Condensate
IMS	Ion Mobility Spectrometry
MDA	Malone Dialdehyde
ppb	Parts per billion
ppt	Parts per trillion

RQ	respiratory quotient $RQ = V_{CO_2} / V_{O_2}$ (relationship between expired CO <sub>2</sub> and inspired O <sub>2</sub> )
SD	Standard deviation
VOC	volatile organic compounds

## References

1. Becher, G.: Atemkondensat-Diagnostik (Exhalat-Diagnostik); In: „Spezielle Lungenfunktionsprüfung“, Hrsg.: Prof. K.-H. Rühle, München: Dustri Verlag Dr. Karl Feistle, 2013, S. 105-124.
2. Buszewski, B.; Keszy, M.; Ligor, T. and Amann, A.: Human exhaled air analytics: biomarkers of diseases. *Biomed. Chromatogr.* 21: 553–566 (2007)
3. S. Heymann, J. Schulz, O.G. Nikolaev, S.V. Fomchenkov, Fortschritte in der adjuvanten therapie des Diabetes mellitus Typ 2 mit Mineralien, *archiv euromedica* 5 (2015) , 58
4. A. Hillmann: Differenzielle Ionenmobilitätsspektrometrie als Methode zur Messung spezifischer Cluster von volatilen Substanzen in der Ausatemluft des Menschen. Bachelor; Hochschule Mittweida 2010
5. R. Klopp, W. Niemer, J. Schulz, Änderungen des Funktionszustandes der Mikrozirkulation durch eine adjuvante BioKorrektur-Behandlung bei Patienten mit Diabetes mellitus Typ II, *archiv euromedica* 4 (2014), 36-44
6. Klopp R., J. Schulz, W. Niemer, K.J. Ruhnau, Wirkungen einer physikalischen Stimulierung der spontanen arteriolen Vasomotion auf die Mikrozirkulation und das Immunsystem bei Patienten mit Diabetes Typ II und Wundheilungsstörungen, *Zeitschrift für Gerontologie und Geriatrie* 47 (2014), 415-424.
7. R. Purkhart: Klassifikation von Ausatemluft anhand ihrer differenziellen Ionenbeweglichkeitsspektrogramme. Diplomarbeit, HUB 2010
8. R. Purkhart , A. Hillmann, R. Graupner, G. Becher: Detection of characteristic clusters in IMS-Spectrograms of exhaled air polluted with environmental contaminants. *IJIMS*, Feb. 2012 (DOI 10.1007/s12127-012-0090-4)
9. Schulz J., S. Heymann, U. Fuchs, M. Sturm, R. Klopp, Individuelle systemische BioKorrektur – adjuvantes Behandlungsverfahren bei Diabetes mellitus Typ II, *archiv euromedica* 3 (2013), 40–44
10. M. Sturm, Der Einfluss eines Ausdauertrainings unter hyperoxischen Trainingsbedingungen mittels der Steuerung der Herzfrequenz und des

respiratorischen Quotienten auf die individuelle systemische Biokorrektur des metabolischen Syndroms, Masterarbeit im Studiengang M.Sc- Sportwissenschaft – Prävention und Rehabilitation, Universität Leipzig, 2012