

Chapter 23

Non-invasive Assessment of Exhaled Breath Pattern in Patients with Multiple Chemical Sensibility Disorder

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Abstract Multiple chemical sensitivity (MCS) is a complex disorder initiated by chemical exposure, particularly through the airways. MCS patients report sensitivity or intolerance to low levels of a wide spectrum of chemicals. Symptoms could include asthma-like signs, rhinitis, fatigue, cognitive dysfunction, psycho-physiological alteration, and other specific tissue reactions resembling hypoxic and oxidative stress effects. To recognize physiological signs that would allow the diagnosis of MCS in a non-invasive way we investigated the potential application of a new sensor system. In healthy volunteers, we measured exhaled breath content in the control condition and under exposure to olfactory stressors that mimic hypoxic or pollutant stressors playing a potential role in the generation of the MCS disorder. The recording system used is based on metal oxide semiconductor (MOS) sensor having a sensing range of 450–2,000 ppm CO₂ equivalents, which is able to detect a broad range of compounds playing a potential role in the generation of the MCS disorder, while correlating directly with the CO₂ levels. The results indicate that the recording system employed was suitable for the analysis of exhaled breath content in humans. Interestingly, the system was able to detect and discriminate between the exhaled breath content taken from the control condition and those from conditions under stress that mimicked exposures to pollutant or hypoxia. The results suggest that chronic hypoxia could be involved in the MCS disorder.

Keywords Breath analysis • Exhaled breath content • Multiple chemical sensitivity syndrome • Smell • Volatile organic compounds

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23.1 Introduction

The recognition of exhaled breath in order to achieve a diagnosis has a long history. Ancient Greek physicians already knew that the smell of human breath could provide clues of pathology. Characteristically, the sweet and fruity odor of acetone is a sign of diabetes, the musty, fishy reek is the evidence of advancement of a liver disease, the urine-like smell accompanies failing kidneys, while the putrid stench is that of lung abscess (Phillips 1992). Nowadays, breath markers are known to be released in broad range of diseases, e.g., lung diseases, metabolic disorder, and exposure to volatile organic compounds (VOCs), which could be used for the monitoring of the respiratory CO_2/O_2 ratio and the detection of drug abuse or pollutant exposure (Folke et al. 2003; Cao and Duan 2006). In oxidative stress diseases, like asthma, COPD, bronchiectasis, or during lipid peroxidation the following compound are released: methylated alkane (Moretti et al. 2004), H_2O_2 (Kostikas et al. 2003), pentane and ethane (Aghdassl et al. 2003). In lung cancer, pulmonary allograft dysfunction, lung transplant recipient with acute rejection, and in cystic fibrosis the exhaled compounds are: NO, CO, H_2O_2 , isoprostanes, nitrite/nitrate, eicosanoids (leukotrienes, prostanoids, isoprostanes), and exhaled carbonyl sulfide (Fisher et al. 1998; Phillips et al. 2003a; Studer et al. 2001; Montuschi et al. 2000). In diabetes, acetone is exhaled (Phillips et al. 2004), while exposure to VOCs provokes a blow-out of the vinyl chloride and cis-1,2-dichloroethene, chloroform, bromodichloromethane, and trichloroethene (Miekisch et al. 2004).

Breath micro-assays give the possibility to detect markers of pathologies linked to oxidative stress, because reactive oxygen species (ROS) oxidize polyunsaturated fatty acids in membranes to alkanes, such as ethane and pentane, which are excreted in the breath as VOCs (Phillips et al. 1999, 2000). This breath test demonstrates that the intensity of oxidative stress varies with age (Phillips et al. 2003a) and it is significantly increased by breathing oxygen (Phillips et al. 2003b), normal pregnancy and preeclampsia of pregnancy (Moretti et al. 2004). Oxidative stress, originated by a cascade of ROS leaking from mitochondria, plays a central role in onset of a number of diseases (Knight 1998). A biological concentration of oxygen keeps the cell enzymatic machinery functional. However, when oxidative stress and hypoxia are established, physiological reactions induce multiple changes in ventilation and in the cardiocirculatory and psychophysiological systems (Schumacker 2003). A cumulative result of oxidative damage induced in cells by ROS derives from aerobic metabolism (Finkel and Holbrook 2000). Physiological antioxidant defenses maintain ROS at harmless levels preventing damage; the balance is strictly related to a constant oxygen concentration (Cataldi and Di Giulio 2009). When this balance falters, for instance in aging or chronic exposure to pollutants, a reduction in homeostatic adaptation to metabolic requirements occurs through the activity of such enzymes as endothelial nitric oxide synthase (eNOS) (Drew and Leeuwenburgh 2002). Chronic hypoxia, *per se*, promotes a remodeling of the structure and function of the bodily systems (Cataldi and Di Giulio 2009).

Anyone or all of the elements above outlined may be involved in the generation of the symptoms of multiple chemical sensitivity (MCS) disorder. Before Randolph Theron, an allergologist who scientifically described in 1945 the first cases of MCS disorder, its depiction existed only in the non-scientific literature, “The Fall of the House of Usher”, written by Edgar Allan Poe (1839). Today, MCS disorder is also known as chemical sensitivity or intolerance, toxic, or chemical injury syndrome, environmental illness, sick building syndrome, toxicant-induced loss of tolerance, or idiopathic environmental intolerance emphasizing the role of chemicals in initiating the disease. Epidemiological studies report a discrepant MCS prevalence, ranging from less than 5% up to 20% of the population, revealing a pandemic distribution with a potentially strong inference on public health (Kreutzer et al. 1999; Caress and Steinemann 2004; Joffres et al. 2001). The discrepancy could be explained by the fact that MCS is of a controversial diagnosis because of the debate between those who contend that it is a psychogenic disorder (Gots 1996) and those who believe it is a chemical-caused illness (Pall 2009).

From the point of view of the somatic-psychiatric origin, the phenomenon of MCS is defined as a peculiar manifestation of a technophobic and chemophobic lifestyle. MCS is an ailment in which

the patient defines both the cause and the manifestations of his own condition (Gots 1996). It has been rejected as an established organic disease due to the arguing that chemicals are not involved in generating MCS disorders, because of the lack of a mechanism explaining the puzzling characteristics of it, and the lack of diagnostic physiological tests for scientific validation (Davidoff et al. 2000; Das-Munshi et al. 2007). From the standpoint of a chemically induced illness, MCS is defined as a multi-complex syndrome ascribable to chronic low level exposure to chemicals (Pall 2009). MCS patients are affected by sensitivity or intolerance to low levels of a wide range of chemicals. They report qualitative and quantitative variable symptoms ranging in number from 41 to 74 and affecting nervous, neuromuscular, musculoskeletal, gastrointestinal, cardiac, and airway systems (Miller 2001). Three categories, depending on the site of chemical contact, are distinguished: ingestion, dermal contact, and breathing, all able to produce several levels of irritation, pain, fatigue, sensorial magnification, and anxiety.

Chemoreception is involved at different levels in this syndrome. In MCS patients, there has been shown chemoreceptor activation of sensory nerve C-fibres leading to the release of inflammatory mediators and to alterations in the odor processing as an expression of the lower brain responses to odorants (Hillert et al. 2007; Orriols et al. 2009). MCS sufferer exhibit changes in brain function seen in brain-imaging studies with positron emission tomography (PET), single photon emission computed tomography (SPECT), and electroencephalography (EEG) (Heuser and Wu 2001; Hillert et al. 2007; Simon et al. 1994; Heuser et al. 1994; Bell et al. 1999; Lorig et al. 1991). Genetic studies demonstrate a role of genetic polymorphisms influencing MCS susceptibility. These studies suggest that the interaction between the genetic pool and the environment gives the probability that a person will become ill. Therefore, genetic evidence reinforces the position that MCS is a toxicological phenomenon (Schnakenberg et al. 2007).

Six consensus criteria were identified for the MCS diagnosis (Pall 2009): symptoms are reproducible with repeated chemical exposures; the condition has persisted for a significant period of time; low levels of exposure lower than previously or commonly tolerated result in manifestations of the syndrome, i.e., in increased sensitivity; the symptoms improve, or resolve completely, when the triggering chemicals are removed; responses often occur to multiple, chemically unrelated substances; symptoms involve multiple-organ symptoms.

The MCS begins in response to exposure to one or more chemicals of seven classes. These include three groups of pesticides (organophosphorus, pyrethroid and organochlorine), a large assortment of volatile organic compounds (VOCs) and related compounds. In addition, mercury and its compounds, e.g., dental amalgams, hydrogen sulphide, and carbon monoxide are implicated as initiators. All these classes of chemicals produce a common response, such as excessive activity of the NMDA receptor. Organophosphorus and carbamate are acetyl cholinesterase inhibitors producing an increase in acetylcholine, which stimulates the muscarinic receptors, which in turn increases glutamate release leading to an increase in NMDA receptor stimulation. Organochlorine, chlordane, lindane, dieldrin, and aldrin lower GABAA receptor activity, which produces elevated NMDA activity. Pyrethroid acts on sodium channel opening probability, which increases NMDA stimulation and similarly acts on GABAA as do other pesticides. Organic solvents and VOCs act on the transfer receptor potential (TRP) family, leading to an increase in NMDA activity. Mercury and its compounds, hydrogen sulphide and carbon monoxide produce increased NMDA activity (Pall 2009). Furthermore, animal studies have demonstrated that toxic responses to chemicals belonging to each of these seven classes are greatly decreased by using drugs that inhibit the NMDA response. Enhanced NMDA activity also produces an increase in NO and its oxidant ONOO⁻ realizing a vicious cycle. These compounds act to generate local sensitivity in regions of the brain and in peripheral tissues, including the lungs, the skin and the upper respiratory and the gastrointestinal tracts (Pall 2009). Apart from the above outlined factors, we hypothesized the existence of a linkage between the MCS syndrome and the oxidative stress induced by inhaled VOCs stressors which could be detected by a new metal oxide semiconductor (MOS)-sensor based system.

23.2 Methods

Healthy volunteers ($n = 10$), who provided written informed consent, were enrolled into the study. The procedures were performed in agreement with the Ethical Standards of the Helsinki Declaration and were accepted by a local Ethics Committee. We measured the exhaled breath content in the control condition and under exposure to the olfactory stressor n-propanol which mimics the hypoxic or pollutant stressors. The recording system used in this experiments was an iAQ-2000 (AppliedSensor, Warren, NJ) equipped with a metal oxide semiconductor (MOS) having a sensing range of 450–2,000 ppm CO₂ equivalents which is able to detect a broad range of volatile compounds (both organic and inorganic, e.g., alcohols, aldehydes, aliphatic hydrocarbons, amines, aromatic hydrocarbons, ketones, organic acids and CO), while correlating directly with the CO₂ levels. The MOS sensor is based on chemical reaction which occurs between the surface of the sensor and the volatile compounds. This reaction cause closure of an electrical circuit, which sends a raw electrical signal in Ohm to the acquisition system. The signal is recorded, saved, and elaborated by the system algorithm, which expresses the VOCs concentration. A Wohler A600 gas analyzer (Wohler USA Inc. Danvers, MA) was used to verify the O₂ and CO₂ measurements done by the iAQ-2000. The exhaled breath was collected into a tight-sealed mask that completely covered the nose, which leaved free inspiration through the mouth, and was directly measured by the iAQ-2000 system. Data treatment and statistical analysis was done by Excel and Origin software.

23.3 Results

In order to recognize physiological characteristics which would allow to diagnose the MCS or exposure to VOCs in a non-invasive procedure we recorded breath exhale using an MOS-sensor system. The normalized breathing raw recordings in a representative subject, presented as a comparison of the control *vs.* n-propanol stressor conditions are superimposed in the consecutive panels of Fig. 23.1. In this and the following figures there are normalized data on the vertical axis according to the formula: $N = (x - \min) / (\max - \min)$.

The recordings from the Wohler A600 gas analyzer, serving as a reference for verification are shown in Fig. 23.1a and c, while those obtained from the MOS-sensor system are shown in Fig. 23.1b and d. Statistical comparisons of the corresponding grand averages of the raw data obtained in the control *vs.* n-propanol stressor conditions are presented in Fig. 23.2; panels a and c concern the data from the Wohler A600 gas analyzer and panels b & d concern those obtained from the MOS-sensor system. In response to the olfactory stressor, the mean fraction of CO₂ increased and that of O₂ decreased in the exhaled breath, which was accompanied by an increase in VOCs ($p < 0.05$, *t*-test for all comparisons).

Figure 23.3a and b demonstrate comparison of breathing frequency traces between the control *vs.* n-propanol stressor conditions. As is convincingly seen, breathing frequency increased in response to the olfactory stressor. In Fig. 23.4, in turn, the grand averages of breath frequency are presented as a power spectrum analysis. For comparison, the frequency spectra obtained in the control condition are superimposed on those from the n-propanol stressor condition; Fig. 23.4a and c shows the spectra pertaining to the Wohler A600 gas analysis, while the remaining panels (Fig. 23.4b, d) pertain to the recordings based on the MOS-sensor system ($p < 0.05$, *t*-test for all comparisons).

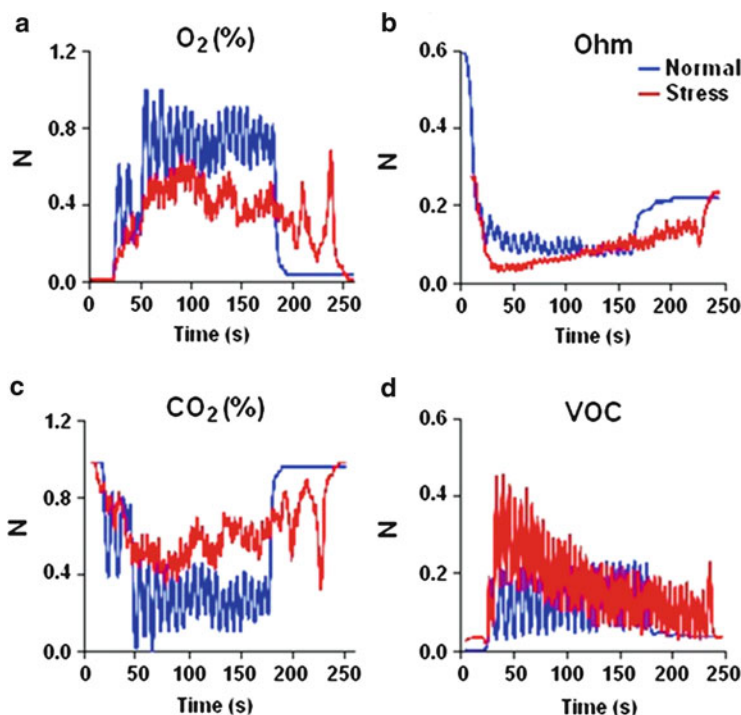


Fig. 23.1 Representative breathing raw data comparison in control vs. stressor (n-propanol): (a) O_2 from Wohler A600 analyzer; (b) Ohm is the electrical signal recorded by the MOS-sensor as a direct measure of the volatile compounds reacting with the sensing surface of it; (c) CO_2 from Wohler A600 analyzer; (d) VOC is the signal elaborated by the MOS-sensor algorithm which express the VOCs concentration. 'N' stands for normalized data: $N=(x-\min)/(\max-\min)$. Note different scales in Y axes

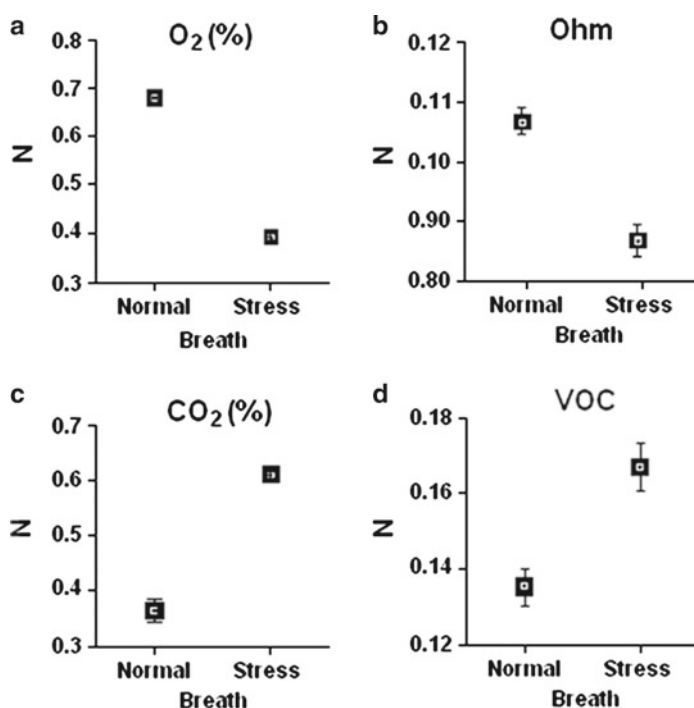


Fig. 23.2 Comparisons of the grand averages of raw breathing data in control vs. stressor (n-propanol): (a) O_2 from Wohler A600 analyzer; (b) Ohm from MOS-sensor; (c) CO_2 from Wohler A600 analyzer; (d) VOC from MOS-sensor. 'N' stands for normalized data, note different scale in Y axis ($p < 0.05$ for all comparisons)

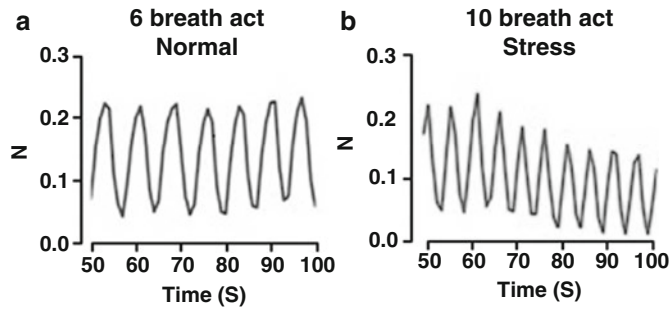


Fig. 23.3 Representative comparison of the breath traces in control (a) vs. stressor (n-propanol) (b) from MOS-sensor system in a subject freely breathing through a nose mask. ‘N’ stands for normalized data

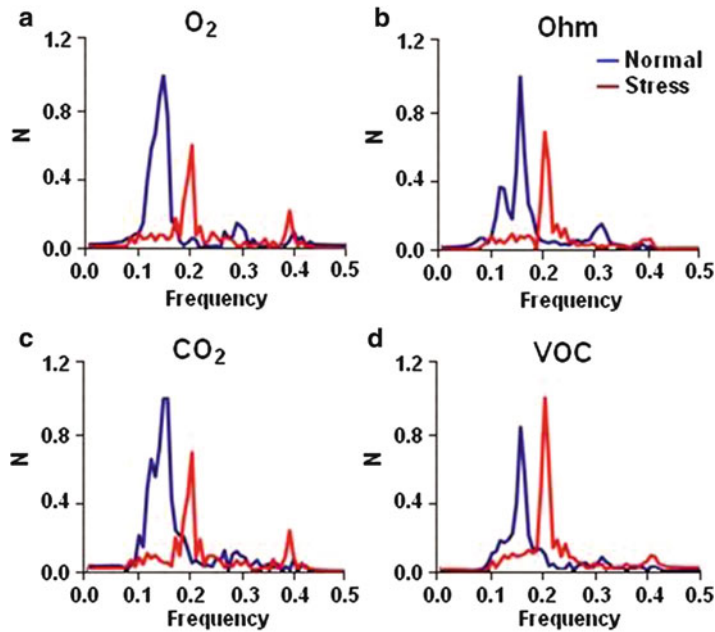


Fig. 23.4 Power spectrum analysis of grand averages of breath frequency in control vs. stressor (n-propanol): (a) O₂ from Wohler A600 analyzer; (b) Ohm from MOS-sensor; (c) CO₂ from Wohler A600 analyzer; (d) VOC from MOS-sensor

On the other hand, the volume component of ventilation, represented by breath amplitude, decreased in response to the olfactory stressor. The decrease can be seen in the recordings based on the MOS-sensor system comparing the breath amplitude in the control vs. n-propanol stressor conditions (Fig. 23.5) ($p < 0.05$ for all, except the VOCs). The opposing alterations in breathing frequency and amplitude were confirmed by the Ohm signals recorded with the MOS sensor (Figs. 23.4 and 23.5).

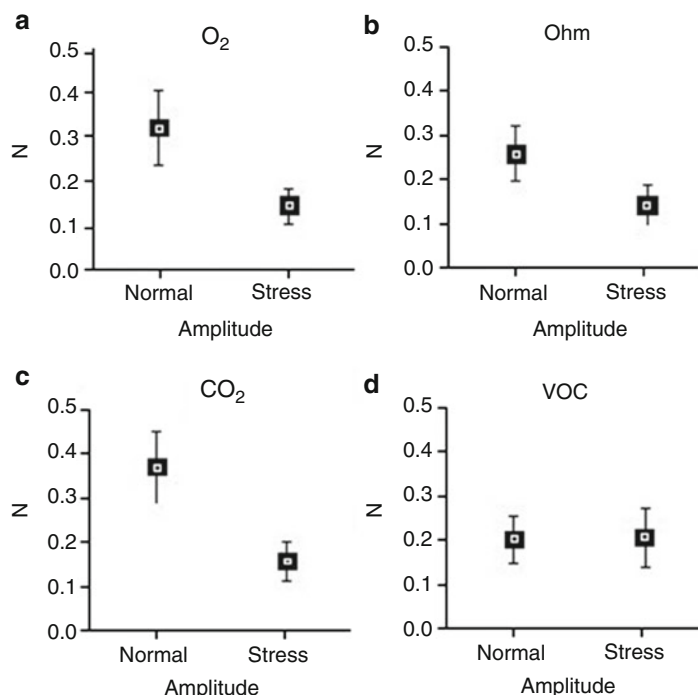


Fig. 23.5 Comparison of breath amplitude in control vs. stressor (n-propanol): (a) O₂ from Wohler A600 analyzer; (b) Ohm from MOS-sensor; (c) CO₂ Wohler A600 analyzer (p < 0.05); (d) VOC from MOS-sensor (p > 0.05)

23.4 Discussion

Our findings indicate that the recording system employed in this study was suitable for the analysis of the breath exhale content in humans. Interestingly, the system was able to detect and discriminate between the exhaled breath content taken from the control condition and that from the stressful condition which mimicked exposures to pollutants or hypoxia known to lay a groundwork for the development of multiple chemical sensitivity disorder (MCS). Figure 23.6 demonstrates the hypothetical links between the oxygen supply cascade and NO/NOO⁻ vicious cycle. The key steps are the formation of NO and ROS, both of which increase directly or indirectly NMDA receptor activity as results of oxidative stress, which could create the basis for the subsequent effects of pollutant exposures or hypoxia resulting in MCS symptoms.

The bulk matrix of exhale breath is a combination of nitrogen, oxygen, CO₂, H₂O, and inert gases. The remaining fraction consists of numerous VOCs with concentrations in a range of parts per million (ppm) to parts per trillion (ppt) by volume (Miekisch et al. 2004; Phillips et al. 1999; Mukhopadhyay 2004). The VOCs are generated in the body (endogenous) or may be absorbed as contaminants from the environment (exogenous). The composition of VOCs in breath varies widely from person to person and alters in pathological conditions, both qualitatively and quantitatively. Although the number of VOCs found to-date in human breath is more than 1,000, only a few VOCs are common to all humans or characteristic of a given disease. These markers, which include isoprene, pentane, acetone, ethane and methanol, etc., are products of core metabolic processes and are especially informative for clinical diagnostics (Mukhopadhyay 2004). Notable exception is NO, which is released into the airway in case of airway inflammation. Testing for variation in the quantity of the endogenous mix

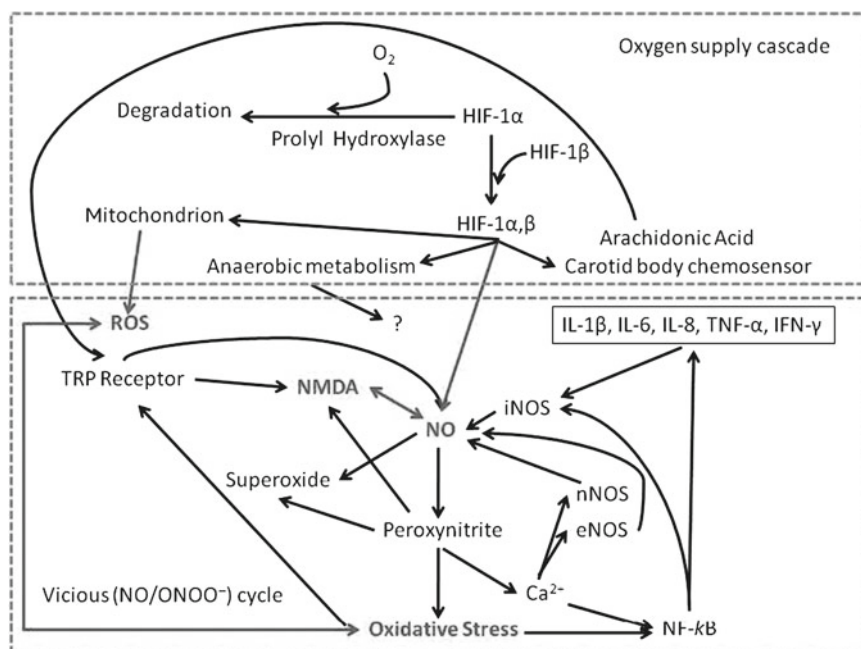


Fig. 23.6 Physiological link between oxygen supply cascade and NO/NOO⁻ vicious cycle. Key steps are the formation of NO and ROS, both increase NMDA receptor activity as a result of oxidative stress which could be a trigger for the multiple chemical sensitivity symptoms due to pollutant exposure or hypoxia

and the presence of new compounds can provide valuable information concerning a possible disease state. Furthermore, exogenous molecules, such as halogenated organic compounds, can indicate recent exposure to drugs or environmental pollutants (Miekisch et al. 2004).

The advantages of breath analysis over hitherto existing serum or urine analysis include noninvasiveness, ease of repetition, no discomfort associated with blood and urine tests; let alone the samples closely reflect the arterial concentrations of biological substances. Breath analysis could be particularly advantageous when a number of arterial blood samples are required, e.g., in monitoring a patient. Breath exhalation also is a much less complicated mixture than serum or urine and is amenable to complete analysis of all compounds present, and no work-up of a breath sample is required, in contrast to many analyses performed on serum or urine samples. Breath analysis can dynamically in real-time monitor the decay of volatile toxic substances in the body (Mukhopadhyay 2004; Phillips 1992). However, breath testing has its limitations. A considerable problem hampering the use of breath tests in clinical practice is the lack of standardization of analytical methods and the wide variation in results obtained in different studies and sample collection and pre-concentration are usually necessary because most substance concentrations in exhaled breath fall in the nmol/l to pmol/l (ppb to ppt by volume) range. Pre-concentration can be achieved by adsorption on sorbent traps, coated fibers (solid-phase microextraction), or by direct cryofocusing. Standardization of breath sample collection and pre-concentration is more difficult than that for serum. Different analytical methods may give very different results. Another critical issue is a high water content of breath samples, which may affect pre-concentration, separation, and detection of single compounds. This is especially true for mechanically ventilated patients if active humidifiers are used in the respiratory circuit. Compared with the simple chemical tests widely used in serum and urine analysis, instruments for breath analysis are expensive. At present, the most commonly used method, GC-MS, requires bulky instrumentation, is time-consuming, and needs skilled operators. The lack of established links between breath substances and disease is also a problem. Therefore, in some case, the breath test is not a conclusive

diagnostic tool, but can be used as a part of a range of diagnostics. However, new MOS-sensor based systems could solve part of these problem and are helpful in diagnosis of diseases and the assessment of exposure to VOCs. These sensors are activated at ppm concentration by several classes of VOCs (both organic and inorganic, e.g.: alcohols, aldehydes, aliphatic hydrocarbons, amines, aromatic hydrocarbons, ketones, organic acids, and CO), while correlating directly with the CO₂ levels (Mazzatenta et al. 2011). As our data show, an iAQ-2000 sensor identifies breath characteristics as Ohm resistance signal and transduces them into VOCs equivalents. These values are directly comparable to the measurements obtained with the laboratory control system such as a gas analyzer. Furthermore, the signal includes physiological parameters such as breath frequency and amplitude. In addition, the test may be self performed by the patient. Thus such test seems advantageous compared with other exhale breath tests.

The present study demonstrates that the MOS-sensor can discriminate qualitative and quantitative variables in the breath concentrations of VOCs and physiological variables. We showed that by comparing the variables recorded in healthy subjects in the control non-stimulated condition with those obtained from the same subjects exposed to the olfactory n-propanol stressor. The MOS-sensor was able to discriminate between the two conditions and showed an increase in VOCs in body response to a bad quality of the inhaled air and impending hypoxic condition. The results also help advance a biological hypothesis based on the cross interaction between the NO/ONOO⁻ vicious cycle (Pall 2009) and the oxygen supply cascade linked by two possible pathways, a direct one *via* NO and an indirect one through the oxidative stress-mediated release of ROS resulting in neural sensitization *via* NMDA over activation and MCS induction.

In conclusion, we submit that the MOS sensor recordings may be useful in as diagnostic, non-invasive tests able to detect physiological variables which can validate the existence of an MCS disorder as well as for the measurement of VOCs concentrations in threatened environmental conditions.

Conflicts of interest: The authors declare no conflicts of interest in relation to this article.

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