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Reproducibility and verification of real time breath VOCs concentration data by modeling

104 – Biomedizin Innovativ – patientInnenfokussierte, anwendungs-orientierte sowie interdisziplinäre Forschung am Puls der Zeit

Abstract

Human breath contains over 200 volatile organic compounds (VOCs), appearing as result of normal metabolic activity or pathological disorders. These molecular species can be detected and quantified in concentrations down to ppb range and have proven as potentially useful parameters for medical diagnosis, therapeutic monitoring, and drug testing. To relate breath gas concentrations of VOCs with their corresponding blood levels a deeper understanding of this correspondence is necessary which can be achieved by mathematical models. King et al. [1] developed a three compartment model consisting of an alveolar, a richly perfused tissue, and a peripheral muscle compartment to describe the exhalation patterns of a prototypical endogenous trace gas found in human breath. This model was applied to isoprene the major hydrocarbon in the breath of humans. Isoprene exhibits pronounced rest-to-work transitions in response to physical activity. At the beginning of an exercise phase there is a pronounced peak of the breath isoprene concentration that quickly diminishes. This cannot be explained using the Farhi equation which would predict a decrease in the breath isoprene concentration due to dilution. Typically, at a moderate workload of 75 Watts the cardiac output doubles and the breath flow increases 4-fold. King et al. [1] suggest that this peak originates from a peripheral compartment producing and storing isoprene. Due to an increased peripheral blood flow during exercise, it is washed out and delivers a short peak at the start of exercise. To test the predictions of this model we set up a modified ergometer experiment. We used the same experimental set up as in [1], but filled the room air with different levels of isoprene-D5. Considering that both isoprene compounds can be assumed to have the same blood to gas partition coefficient, the observed concentration profiles show that the exercise peak for normal isoprene cannot be explained by changes in ventilation and perfusion. If such changes would cause that peak then also inhaled isoprene-D5 should demonstrate the same behavior. However, these experiments perfectly match the prediction of.

Keywords:

Breath gas analysis, volatile organic compounds (VOCs), isoprene, compartment modeling

Introduction

Due to its broad scope and applicability, breath gas analysis holds great promise as a versatile framework for general bio-monitoring applications. As a biochemical probe, volatile organic compounds (VOCs) in exhaled breath are unique in the sense that they can provide both non-invasive and continuous information on the metabolic and physiological state of an individual. Apart from diagnostics and therapy control, this information might potentially be used for dynamic assessments of normal physiological function (e.g., by a stress test on a cycle ergometer, in an intra-operative setting, or in a sleep lab), pharmacodynamics (drug testing), or for quantifying body burden in response to environmental exposure (e.g., in occupational health). Furthermore, as has been demonstrated on the small inorganic molecule nitric oxide, trace gases can actively participate in the regulation of physiological events. This renders breath VOCs as an intriguing tool for examining more fundamental endogenous processes.

With the advent of powerful new mass spectrometric techniques over the last years, these molecular species can be detected and quantified in concentrations down to parts per billion (ppb) range.

While sufficiently accurate and fast instrumental techniques are vital for fully exploiting this huge potential, also several methodological issues need to be addressed before breath gas analysis can become the ``new blood test''. Specifically, a crucial yet underestimated aspect of breath gas analysis concerns the blood-gas kinetics of the VOCs under scrutiny as well as their systemic distribution and physiological flow within the human body. Much can be learnt in this context from the cumbersome development of the exhaled nitric oxide breath test for asthma, which nowadays ranks among the most successful breath tests based on an endogenous compound. This paradigmatic example shows that a purely explorative search for VOC biomarkers is not sufficient: in order to make breath tests operational, a thorough quantitative understanding of the underlying gas exchange mechanisms and their sensitivity with respect to physiological factors is required.

Due to the current lack of definite sampling recommendations for other trace gases, end-tidal breath collection is usually adopted as best measurement practice. Accordingly, the first part of the exhalation - corresponding to the gas volume filling the anatomical dead space and hence not participating in gas exchange according to classical pulmonary inert gas elimination theory - is discarded and only the last exhalation segment (supposed to contain ``alveolar air'') is collected for analysis. In more sophisticated sampling systems, end-tidal extraction is triggered by virtue of simultaneously measured CO_2- and/or flow-data. While such setups are an important contribution to current standardization efforts in breath sampling, they generally give little insight into a series of major experimental questions:

- What is the quantitative relationship between breath VOC levels and their underlying endogenous (e.g., blood) VOC levels?
- Does the end-tidal VOC concentration level reflect the alveolar level?
- How does the variability of breath VOC concentrations relate to varying physiological conditions?

Mathematical modeling

In this context, mathematical modeling and simulation can be employed to establish mechanistic descriptions of the gas exchange processes governing the VOC under scrutiny. Once such a model has been developed it becomes possible to understand the key relationships underlying the physiological behavior of the compound and to identify its defining parameters. Such a quantitative treatment provides

a sound conceptual framework for experiment design as well as decision making and can aid substantially in preventing misinterpretations of empirical results. Furthermore, these analyses can guide the standardization and establishment of new sampling protocols, avoiding potential confounding factors and maximizing the information content of experimental results.

King et al. developed a three compartment model consisting of an alveolar, a richly perfused tissue, and a peripheral tissue (containing skeletal muscles) compartment to describe the exhalation patterns of a prototypical abundant, endogenous trace gas found in human breath. This model is based on the physical law of mass balance. For that matter the body is divided into different compartments of different physiological activities. Each compartment must obey a mass balance equation. This yields a system of ordinary differential equations for the concentrations in different compartments. Thereby breath flow and blood flow are given (measured) functions.

This model was applied to isoprene which is the major hydrocarbon in the breath of humans. Isoprene can be regarded as the prototype of an exhaled breath VOC exhibiting pronounced rest-to-work transitions in response to physical activity. Isoprene, which has a low solubility in blood (as reflected by a small blood to air partition coefficient (h = 0.95), is lipophilic and presumed to be correlated with cholesterol biosynthesis.

Isoprene reacts extremely sensitive with respect to changes in ventilation or pulmonary perfusion due to its small blood to air partition coefficient. It is exchanged exclusively in the alveoli. During exercise on an ergometer the breath concentration profile exhibits a peak-shaped behavior reaching a new steady state after about 15 minutes. After a break a typical wash-out effect is recognizable, where the peak height recovers fully after a break of one hour. This cannot be explained using the simple Farhi equation which would predict a decrease in the breath isoprene concentration due to dilution. Typically, at a moderate workload of 75 Watts the cardiac output doubles and the breath flow increases 4-fold. This would lead to a breath concentration decrease by a factor of approximately two for endogenous produced VOCs in breath during pedaling with 75 Watts.

The model of King et al. suggests that this peak originates from a peripheral (muscle) compartment producing and storing isoprene. Due to an increased peripheral blood flow during exercise it is washed out and delivers a short peak at the start of exercise. The observed breath concentration patterns can be fitted well by the three compartment model. The interpretation of this model is in contrast to the predominant hypothesis that isoprene might be a byproduct of cholesterol synthesis which would take place in the richly perfused tissue compartment (containing the liver).

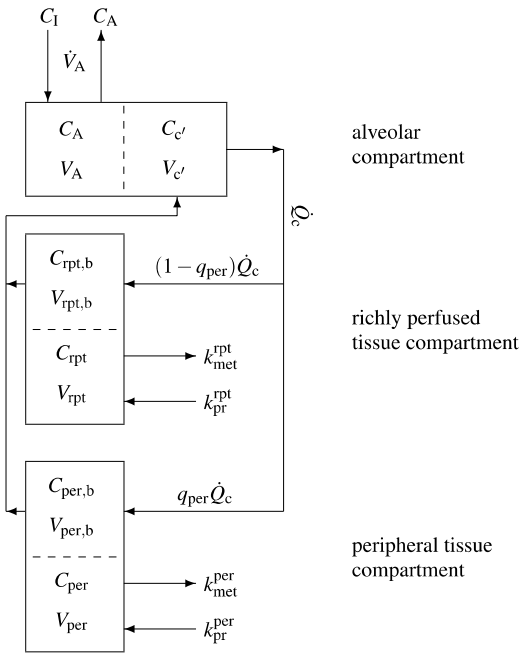

Figure 1: Sketch of the model structure. The body is divided into three distinct functional units: alveolar/end-capillary compartment (gas exchange), richly perfused tissue (metabolism and production) and peripheral tissue (storage, metabolism and production). Dashed lines indicate equilibrium according to Henry's law; k_{met}...metabolic rate, k_{pr}...production rate, C_X...concentration.

New experiments

To test the predictions of this model we set up a *modified* ergometer experiment. We took the same experimental setup as described in King [2], but used a specific back ground level of isoprene.

Three volunteers were asked to perform several ergometer exercises at different room air levels (back ground) of deuterated isoprene-D5 according to the following protocol (see Figure [2]):

minutes 0--9: the volunteer rests on the ergometer with head-mask on,

minutes 9--12: deuterated isoprene-D5 is released and the room air is mixed by a fan,

minutes 12--22: volunteer rests on the ergometer,

minutes 22--40: volunteer pedals at 75 Watts,

minutes 40--46: volunteer rests on the ergometer,

minutes 46--58: volunteer pedals at 75 Watts,

minutes 58--63: volunteer rests on the ergometer,

minutes 63--68: mask is taken off and the room air concentration is measured.

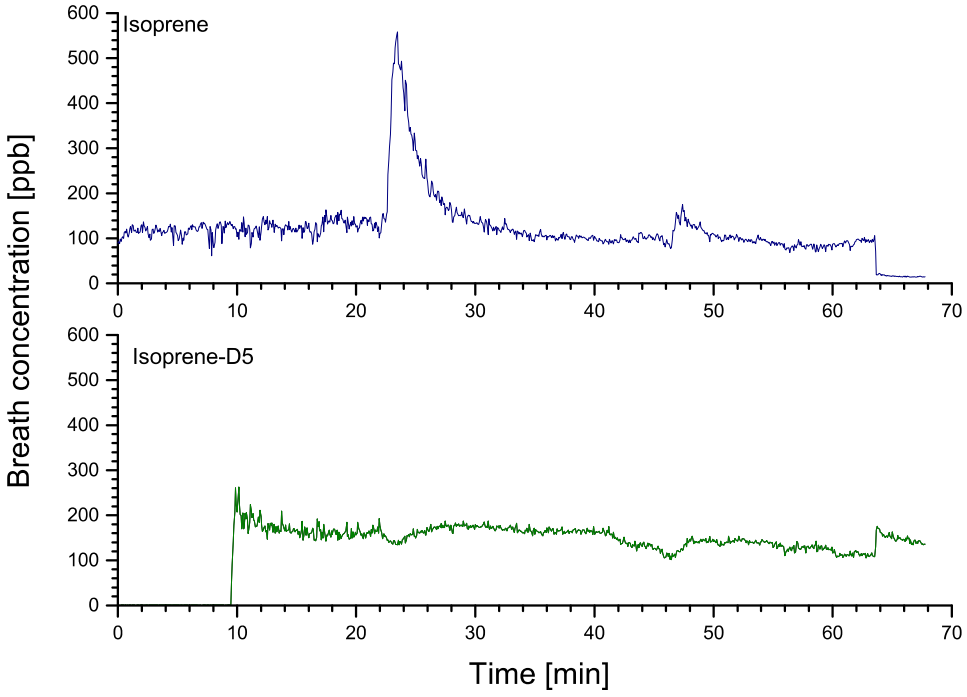

Figure 2: Typical results of a single ergometer session with inhalation of deuterated isoprene-D5: rest for 9 min - release of deuterated isoprene-D5 into the sealed laboratory and waiting for 13 min -75 Watts for 18 min - rest for 6 min – 75 Watts for 12 min - rest for 5 min; deuterated isoprene-D5 breath concentration: green; normal isoprene breath concentration: blue.

Discussion of the experimental results

These experiments (see Figure 2 for a typical test result of such an experiment) show as predicted by the model that inhaled deuterated isoprene-D5 enters the arterial blood stream quickly. It takes only about two minutes until it appears in exhaled breath and an equilibrium is achieved in the room air and the blood of the volunteer. At the onset of exercise normal (endogenous) isoprene shows the typical peak. This peak presumably originates from a high concentration in muscle blood caused by the production in the peripheral (muscle) compartment. Deuterated isoprene-D5 is brought into the body only through inhalation and distributed by the arterial blood into every body compartment. Hence in every part of the body its concentration is similar (zero at the beginning of the experiment). At the onset of exercise, the ventilation-perfusion ratio goes up and the deuterated isoprene-D5 in exhaled breath declines in accordance with the Farhi equation since the venous blood still has an unaltered isoprene level for 1 to 2 minutes. However then, due to the increased inhalation of deuterated isoprene-D5, the venous blood gains a higher concentration level too, and the exhaled concentration of deuterated isoprene-D5 reaches its former level.

Conclusions

Considering that both isoprene compounds can be assumed to have the same Henry constant (blood to gas partition coefficient), the observed concentration profiles show that the exercise peak for normal isoprene cannot be explained by changes in ventilation and perfusion. If changes in ventilation and perfusion would cause that peak then inhaled isoprene-D5 should demonstrate the same behavior. However, these experiments perfectly fit within the prediction of the model developed in [1] and hence

support the hypothesis that isoprene is produced in the muscle compartment by an unknown metabolic pathway.

References

J. King, et al., Physiological modeling of isoprene dynamics in exhaled breath, J. Theor. Biol. **267** (2010) 626-37.

J. King, et al., Isoprene and acetone concentration profiles during exercise on an ergometer. J. Breath Res. **3** (2009) 027006.