

Is exhaled carbon monoxide level associated with blood glucose level? A comparison of two breath analyzing methods

Thomas Fritsch

Universität Düsseldorf
Institut für Lasermedizin
Universitätsstraße 1
40225 Düsseldorf, Germany

Maarten van Herpen

Golo von Basum

Philips Research
High Tech Campus 34
5656 AE Eindhoven, Netherlands

Peter Hering

Manfred Mürtz

Universität Düsseldorf
Institut für Lasermedizin
Universitätsstraße 1
40225 Düsseldorf, Germany

Abstract. The level of exhaled carbon monoxide (eCO) is considered a marker of oxidative stress in diabetes. Previous findings indicated that eCO levels correlated with blood glucose level. The aim of this work was to apply and compare two independent analyzing methods for eCO after oral glucose administration. Glycemia, eCO, and exhaled hydrogen were measured before and after oral administration of glucose. Six healthy nonsmoking volunteers participated. For eCO analysis, we used two methods: a commercially available electrochemical sensor, and a high-precision laser spectrometer developed in our laboratory. The precision of laser-spectroscopic eCO measurements was two orders of magnitude better than the precision of the electrochemical eCO measurement. eCO levels measured by laser spectrometry after glucose administration showed a decrease of $4.1\% \pm 1.5\%$ compared to the baseline ($p < 0.05$). Changes in the eCO measured by the electrochemical sensor were not significant ($p = 0.08$). Exhaled hydrogen levels increased by 40% within the first 10 min after glucose administration ($p < 0.05$). The previous finding that the glycemia increase after glucose administration was associated with a significant increase in eCO concentrations was not confirmed. We propose that previous eCO measurements with electrochemical sensors may have been affected by cross sensitivity to hydrogen.

© 2008 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2937215]

Keywords: oral glucose tolerance test; cavity leak-out spectroscopy; CRDS; laser spectroscopy; breath analysis; glycemia; carbon monoxide.

Paper 07176RR received May 16, 2007; revised manuscript received Jan. 14, 2008; accepted for publication Jan. 15, 2008; published online Jun. 5, 2008.

1 Introduction

Exhaled carbon monoxide (eCO) is controversially discussed as a volatile marker of oxidative stress and inflammation that could be measured noninvasively. CO is generated endogenously during heme degradation and catalyzed by the heme oxygenase enzymes.¹ Recent studies showing an activation of heme oxygenase (HO)-1 by agents that cause oxidative stress have generated interest in the study of the CO level as a marker of oxidation. Furthermore, accumulating evidence from animal models suggests that elevated eCO levels may occur in the case of respiratory inflammations (like asthma, etc.), and also with nonpulmonary disorders such as diabetes. However, conflicting studies prevent a firm conclusion on the value of this marker as a diagnostic tool.²

It was previously reported by Paredi et al. that eCO was elevated in diabetic patients and that the level of eCO correlated with glucose concentration in the blood.³ The authors found that eCO concentration was significantly increased af-

ter an oral glucose tolerance test (OGTT) by acute elevations of the blood glucose level. The authors speculated that high eCO levels during OGTT may have been a reflection of HO activation in response to the induction of the lipid peroxidation cascade. In that study, as in many other studies on eCO, an electrochemical sensor (Bedfont EM50 Micro Smokerlyzer) was used. According to the manufacturer, this type of CO sensor is not free of cross sensitivities to other compounds present in exhaled breath, e.g., hydrogen. Alternatively, laser absorption spectrometry-based CO sensors can be used. The application of this technique to biogenic CO production has been demonstrated above vascular cells^{4,5} and to breath CO analysis.⁶

The aim of our present study was to investigate whether the reported increase of eCO levels after OGTT could be reproduced with a novel type of CO analyzer that was recently developed. We used a high-precision mid-infrared laser-spectroscopic methodology that was previously evaluated for breath analysis.^{7,8} For comparison, we employed an electrochemical CO sensor (Bedfont Smokerlyzer Micro 4).

Address all correspondence to Manfred Mürtz, Universität Düsseldorf, Institut für Lasermedizin, Universitätsstraße 1, 40225 Düsseldorf, Germany, Tel.: +49 2118111372; Fax: +49 2118113121; E-mail: muertz@uni-duesseldorf.de

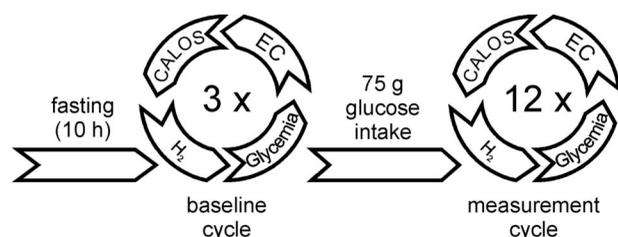


Fig. 1 Measurement procedure. After measuring glycemia, the eCO concentration with the electrochemical (EC) device, the CALOS technique, and the breath hydrogen monitor for three times as a baseline, the subjects took 75 g of glucose solution and repeated the measurement cycle for 12 times. One measurement cycle took approximately 10 min.

2 Materials and Methods

2.1 Subjects

Six healthy nonsmoking volunteers (5 men, 1 woman, ages: 24 to 32) participated who had no diagnosed chronic or acute disease. The subjects had no medication for at least three days before the measurements. This study was conducted in accordance with the guidelines of the local institutional review board. Written informed consent was obtained from all participants.

2.2 General Measurement Procedure

At the beginning of the measurement, all subjects had been fasting for at least 10 hours. During the whole measurement, all subjects were calm and seated. As a baseline, three sets of data were recorded from each subject. The measurement procedure is illustrated in Fig. 1. A set of data consists of a glycemia measurement, one eCO measurement with an electrochemical analyzer, one eCO measurement with our laser spectrometer, and one measurement of breath hydrogen. Glycemia was determined by a commercially available analyzer (Accu-Chek Aviva, Roche Pharma AG). The recording of one data set took 10 min. After recording the baseline, the subjects drank a 75-g glucose solution (Accu-Chek Dextro OGT, Roche Pharma AG) within 2 min. One set of data was recorded afterwards every 10 min for two hours. For all breath

measurements, the subjects inhaled to maximum and exhaled afterwards within 20 s. The last 30 ml of breath was used for analysis.

2.3 Electrochemical eCO Measurements

The electrochemical sensor was a Smokerlyzer Micro 4 (Bedfont Scientific). This device can measure CO fractions from 1 to 500 ppm with a resolution of 1 ppmv. The Smokerlyzer displayed the CO concentration within the last 30 ml of breath. For every data set, the eCO concentration was measured twice within 2 min.

To check the device for cross sensitivity to hydrogen, a certified gas mixture of 3.45 ppm CO in nitrogen was mixed with a certified gas mixture of 1% hydrogen in nitrogen. By varying the mixing ratio, we obtained hydrogen fractions between 0 and 500 ppm.

2.4 Exhaled Hydrogen Measurements

For breath hydrogen analysis, a portable breath hydrogen monitor (GMI Medical Ltd.) was employed with a resolution of 1 ppmv. The sensor's response was read out approximately 1 min after injection of the breath sample when the maximum value was displayed.

2.5 Laser-Spectroscopic eCO Measurements

Cavity leak-out spectroscopy (CALOS) is an extremely sensitive laser absorption spectroscopy technique that uses a high-finesse optical cavity to achieve effective absorption path lengths of several kilometers. Figure 2 shows a schematic of the entire gas system. The gas sample was dehumidified by a Nafion tube (PermaPure, length 2 m). The Nafion tube removed the water but did not affect the CO concentration, which was checked with a certified gas mixture.

In the mid-infrared spectral region near $4.969 \mu\text{m}$ (2012 cm^{-1}), CO shows a characteristic "fingerprint" absorption spectrum [Fig. 3(a)], which leads to the outstanding specificity of absorption spectroscopy techniques. We recently reported the technical details of this spectroscopic setup.^{8,9} The noise-equivalent CO concentration was 7 ppb with a sub-second time resolution. For calibration, a certified gas mixture of 3.45 ppm CO in nitrogen was used. The corresponding

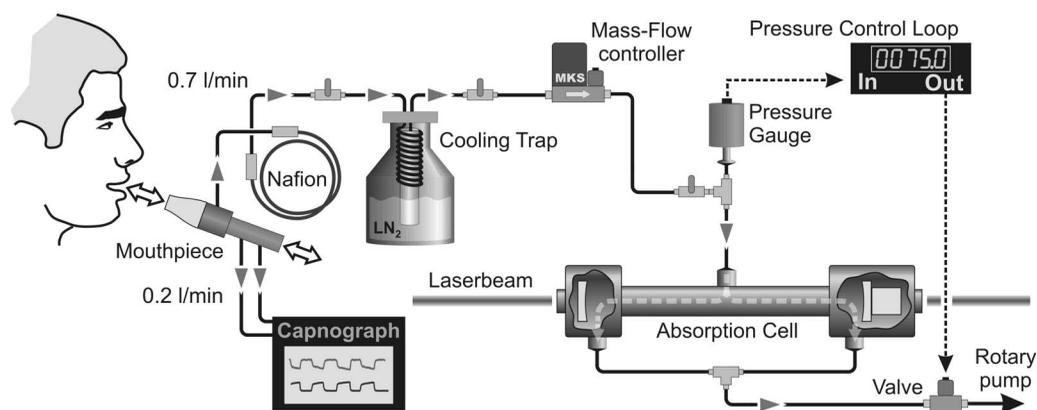


Fig. 2 Schematic of the gas sampling and analyzer setup for laser-spectroscopic eCO analysis. The breath sample was dried and cleaned with a Nafion tube and a cooling trap before entering the absorption cell. The pressure inside the absorption cell was stabilized via a pressure control loop.

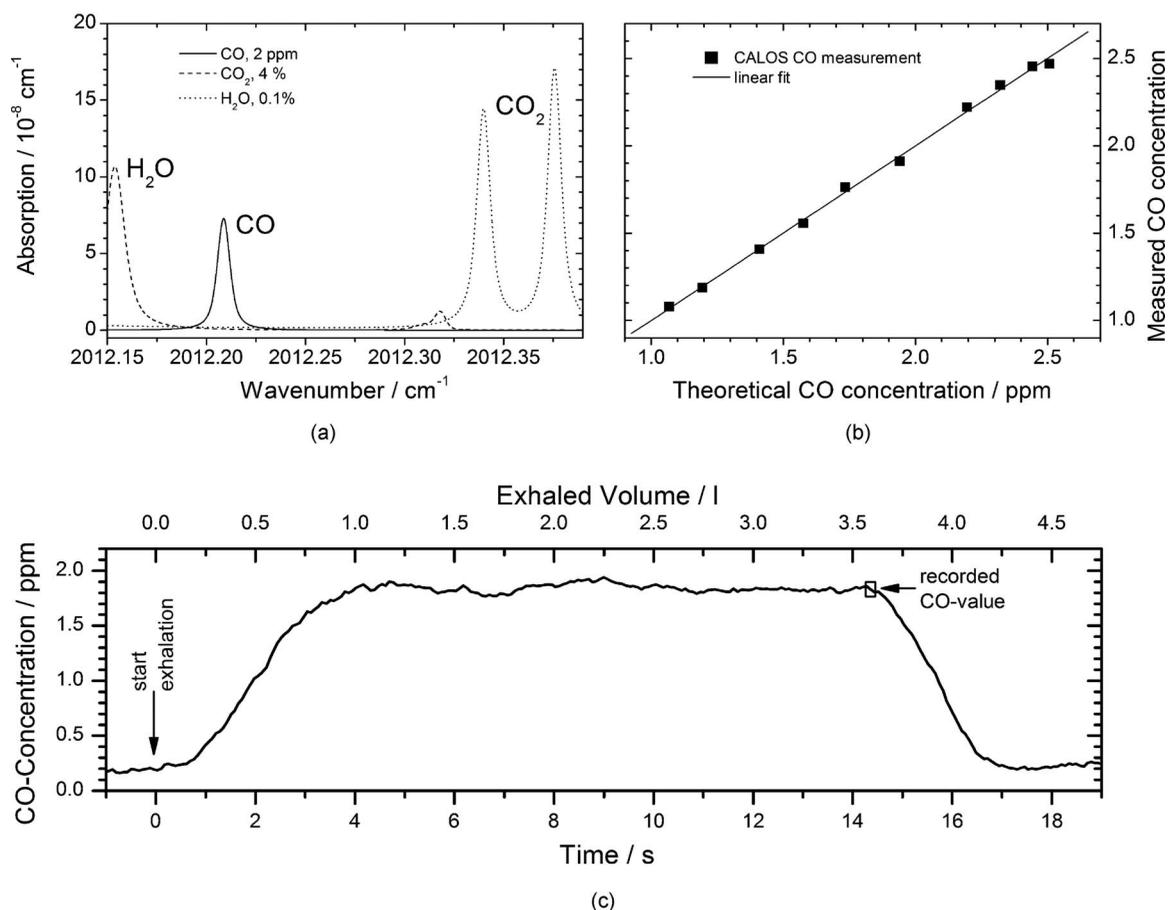


Fig. 3 (a) Calculated absorption spectra of water vapor, carbon dioxide, and carbon monoxide near the laser frequency (HITRAN2000 data). The concentrations displayed are average values of a typical exhalation after drying with a Nafion tube. (b) Calibration plot obtained by a standard addition method using a certified gas mixture of CO in nitrogen. (c) Raw data of a single exhalation. The exhalation starts at $t=0$ and $V=0$, respectively. To copy the sampling procedure used with the Smokerlyzer, only the last 30 ml of the expirogram was used for the laser-spectroscopic eCO analysis.

calibration plot is shown in Fig. 3(b). The accuracy of the spectrometer derived from this calibration series was approximately 1%.

The CO level of the expired air was recorded for two exhalations. Simultaneously, the breath flow rate, CO₂, and O₂ concentrations were measured by a capnograph (Capnomac Ultima, Datex Ohmeda). Since the gas sample traveled about 6 m from the mouthpiece to the absorption cell through the NAFION tube, the cooling trap, and the flow controller, the CO measurement was delayed by a few seconds, which was corrected via data acquisition software (homemade, Lab-View 7.0 programming language). From the raw data, plots of the eCO concentration over the exhaled volume (expirograms) were extracted [see Fig. 3(c)]. The expirograms exhibited three phases. The exhalation started with phase I, where the eCO concentration equaled the ambient CO concentration. During phase II the CO concentration rose rapidly up to phase III. The described breathing procedure resulted in a nearly constant CO level during phase III. To copy the breath sampling procedure used with the Smokerlyzer, we used only the last few data points corresponding to the last 30 ml of breath for CO analysis.

2.6 Statistical Analysis

For analysis differences between basal values ($-30 \text{ min} < t < 0 \text{ min}$) and values in the phase of maximum glycemia level ($20 \text{ min} < t < 60 \text{ min}$), we used a paired student's t-test with the significance set at $p < 0.05$.

3 Results

The laser spectrometer we used is capable of measuring eCO level changes down to 7 ppb at a time resolution of 1 s. This sensitivity is two orders of magnitude better than the electrochemical device, which has a resolution of 1 ppm.

After intake of glucose, the glycemia level increased within 30 min by 75% and decreased to about 30% above the initial value during the following 40 min. The mean initial glycemia was 83 mg/dl, and the standard deviation (SD) was 9.1 mg/dl. Initial measurements spread from 74 to 96 mg/dl.

The results of the laser-spectroscopic eCO measurements are shown in Fig. 4(a). Initial eCO fractions varied from 1.3 ppm to 3.8 ppm (mean=2.4 ppm, SD=0.72 ppm). The eCO level significantly decreased by $4.2 \pm 1.4\%$ during the

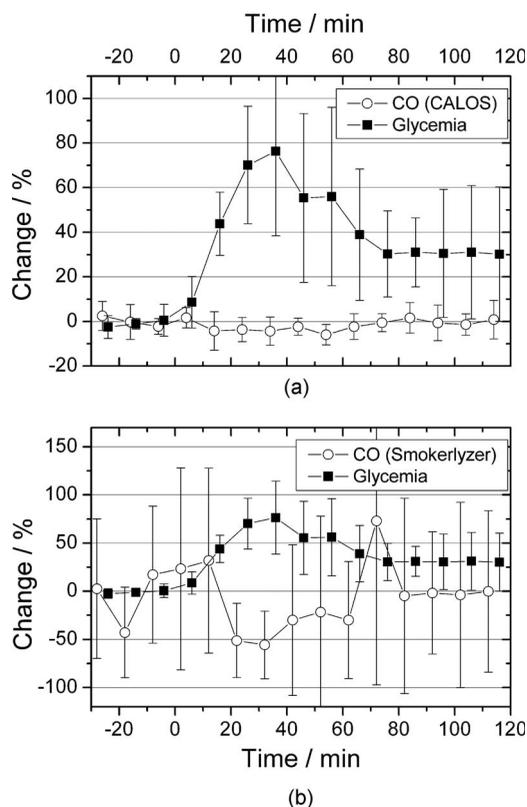


Fig. 4 Glycemia level and simultaneous measurements of eCO with (a) the CALOS analyzer, and (b) the Smokerlyzer Micro 4. The graphs show the average of six healthy volunteers. The CALOS analyzer shows a significant decrease ($p < 0.05$) of the eCO level during the phase of maximum glycemia (20 min $< t < 50$ min), while the change of eCO measured by the smokerlyzer is not significant ($p = 0.08$).

maximum increase of glycemia in the time between 20 and 60 min after glucose administration ($p < 0.05$).

The results of the eCO measurements with the Smokerlyzer Micro device are shown in Fig. 4(b). In contrast to the results obtained with the CALOS analyzer, the change in eCO levels measured by the electrochemical sensor after glucose administration was not significant ($p = 0.08$). The initial eCO concentrations ranged from 0 to 4 ppm; the peak concentrations did not exceed 4 ppm; the mean initial concentration was 1.6 ppm (SD=1.3 ppm).

We found that the Smokerlyzer Micro 4 exhibited a slight cross sensitivity to hydrogen. Figure 5(a) shows the H_2 dependence of the response of both the Smokerlyzer and the laser spectrometer for different $H_2/CO/N_2$ mixtures, normalized to a pure CO/N_2 mixture. According to the results displayed in Fig. 5(a), the response of the Smokerlyzer to hydrogen (in the range up to 500 ppm) was nearly linear with a slope of 0.014, whereas the CALOS analyzer was inherently insensitive to hydrogen fractions in the gas sample.

The measurements of exhaled hydrogen during the OGTT are shown in Fig. 5(b). Initial H_2 concentrations ranged from 3 ppm to 97 ppm (mean=23 ppm). The breath hydrogen level increased by 40% within the first 10 min after glucose administration ($p < 0.05$).

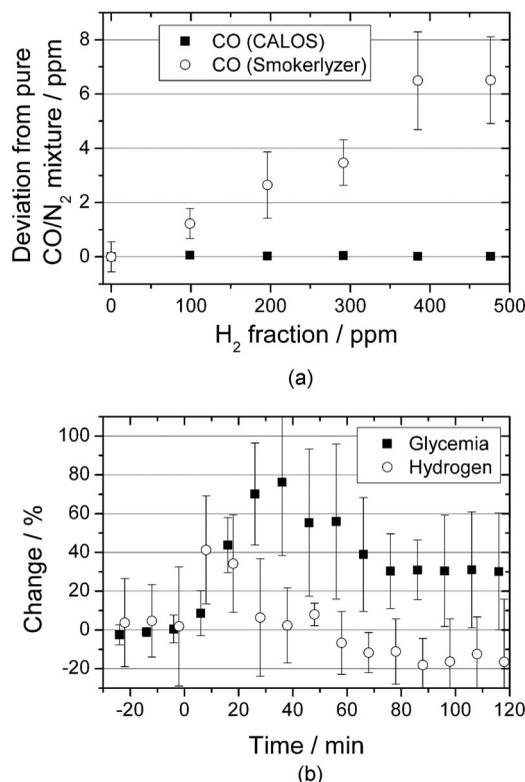


Fig. 5 (a) Analysis of CO in various $H_2/CO/N_2$ mixtures with increasing H_2 fraction from 0 to approximately 500 ppm. The deviation from the theoretical CO value, which is given by the analysis result of the zero-hydrogen mixture, is plotted over the hydrogen concentration. (b) Change in exhaled hydrogen and glycemia during the OGTT. The graphs show the average of six healthy volunteers. The maximum increase occurs 10 min after glucose ingestion.

4 Discussion and Conclusion

In comparison with the electrochemical eCO analysis, the laser-spectroscopic eCO measurement is an extremely sensitive and precise method for analyzing eCO in human breath. The Smokerlyzer has a resolution of 1 ppm, so the systematic measurement error is 0.5 ppm. For typical eCO levels of about 2 ppm, this error leads to a relative uncertainty of 25%. The uncertainty of the laser-based analyzer is around 1%. Also, the laser spectrometer is highly specific to CO due to the use of its “fingerprint” absorption spectrum in the mid-infrared spectral region around 5 μm . Homonuclear compounds like nitrogen, oxygen, and hydrogen cannot affect this method due to the absence of infrared absorption of such molecules.

Using an electrochemical Smokerlyzer Micro 4 for eCO analysis, we did not find any significant change in eCO after glucose ingestion. This is in opposition to the observed strong elevation of eCO (i.e., 50% change) after glucose ingestion that was previously reported by Paredi et al.³ They used a Smokerlyzer EM50 for eCO analysis, which is an earlier version of the device that was used in our study.

Using our laser-spectroscopic technique, we confirmed that eCO levels are not elevated after glucose ingestion. In contrast, we found that eCO levels decreased a few percent after glucose intake. Due to the lower sensitivity and precision of the

electrochemical sensor, this slight decrease could not be observed with the Smokerlyzer.

What are the possible reasons for the found discrepancy? We propose that the eCO measurements with the Smokerlyzer EM50 device reported by Paredi et al. may have been considerably affected by this electrochemical device's well-known cross sensitivity to hydrogen. Hydrogen is generated by bacteria in the colon from carbohydrates that escaped digestion in the small intestine,¹⁰ but also in the small intestine itself. For example, hydrogen breath tests are used to diagnose small intestine bacterial overgrowth.¹¹ Our speculation is strengthened by our measurements of breath hydrogen after glucose ingestion. The maximum increase of exhaled hydrogen was observed during the first 10 min after glucose ingestion. This characteristic course is almost identical with the course of the eCO measurement reported by Paredi et al.

Generally, electrochemical sensors are sensitive to hydrogen, but the Smokerlyzer Micro 4 device used in our study has been considerably improved in this regard (private communication with manufacturer). This explains why we did not reproduce the findings of Paredi et al. A measurement series with a CO/H₂/N₂ mixture still showed a slight cross sensitivity to hydrogen. However, this resulted in a measurement error of only 1.4% of the hydrogen concentration. For breath hydrogen concentrations of up to 100 ppm, this results in only 1 to 2 ppm offset to the CO measurement. According to the manufacturer, aging of the sensor might increase this cross sensitivity to hydrogen. If we assume that the crosstalk reaches 1 ppm CO per 10 ppm hydrogen (i.e., 10%) in an older or aged version, a hydrogen increase from 25 to 35 ppm observed after glucose administration would appear as a considerable eCO increase by 1 ppm, corresponding to a 50% increase of eCO for a baseline eCO value of 1 ppm.

In conclusion, if an electrochemical sensor is used for eCO analysis, it is essential to make sure that no other constituents of exhaled breath, especially hydrogen, interfere with the measurement. Laser-based absorption spectroscopy techniques like CALOS are excellent methods to detect eCO with extremely high specificity, sensitivity, and speed. Ongoing projects in our laboratory seek to develop a more rugged and compact CALOS analyzer that eventually could be used in the doctor's office or at the bedside.

Acknowledgments

We thank Kathrin Heinrich and Rufus Driessen for their helpful collaboration; Leigh Greenham, Uwe Günther, and Wilfried Salmen for providing information and the smokerlyzer device; and Martha Newger and Andreas Erhardt for providing the breath hydrogen monitor. This work is part of the PhD thesis of Thomas Fritsch at the faculty of mathematics and science at Heinrich-Heine Universität, Düsseldorf.

References

1. S. W. Ryter and A. M. K. Choi, "Therapeutic applications of carbon monoxide in lung disease," *Curr. Opin. Pharmacol.* **6**(3), 257–262 (2006).
2. S. W. Ryter and L. E. Otterbein, "Carbon monoxide in biology and medicine," *BioEssays* **26**(3), 270–280 (2004).
3. P. Paredi, W. Biernacki, G. Invernizzi, S. A. Kharitonov, and P. J. Barnes, "Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease?," *Chest* **116**(4), 1007–1011 (1999).
4. A. A. Kosterev, F. K. Tittel, W. Durante, M. Allen, R. Kohler, C. Gmachl, F. Capasso, D. L. Sivco, and A. Y. Cho, "Detection of biogenic CO production above vascular cell cultures using a near-room-temperature QC-DFB laser," *Appl. Phys. B: Lasers Opt.* **74**(1), 95–99 (2002).
5. Y. Morimoto, W. Durante, D. G. Lancaster, J. Klattenhoff, and F. K. Tittel, "Real-time measurements of endogenous CO production from vascular cells using an ultrasensitive laser sensor," *Am. J. Physiol. Heart Circ. Physiol.* **280**(1), H483–H488 (2001).
6. B. W. M. Moeskops, H. Naus, S. M. Cristescu, and F. J. M. Harren, "Quantum cascade laser-based carbon monoxide detection on a second time scale from human breath," *Appl. Phys. B: Lasers Opt.* **82**(4), 649–654 (2006).
7. G. von Basum, H. Dahnke, D. Halmer, P. Hering, and M. Murtz, "Online recording of ethane traces in human breath via infrared laser spectroscopy," *J. Appl. Physiol.* **95**(6), 2583–2590 (2003).
8. D. Halmer, G. von Basum, P. Hering, and M. Murtz, "Mid-infrared cavity leak-out spectroscopy for ultrasensitive detection of carbonyl sulfide," *Opt. Lett.* **30**(17), 2314–2316 (2005).
9. D. Halmer, G. von Basum, M. Horstjann, P. Hering, and M. Murtz, "Time resolved simultaneous detection of (NO)-N-14 and (NO)-N-15 via mid-infrared cavity leak-out spectroscopy," *Isotopes Environ. Health Stud.* **41**(4), 303–311 (2005).
10. A. M. Stephen, A. C. Haddad, and S. F. Phillips, "Passage of carbohydrate into the colon - direct measurements in humans," *Gastroenterology* **85**(3), 589–595 (1983).
11. A. Gasbarrini, E. C. Lauritano, M. Gabrielli, E. Scarpellini, A. Lupascu, V. Ojetti, and G. Gasbarrini, "Small intestinal bacterial overgrowth: diagnosis and treatment," *Dig. Dis.* **25**(3), 237–240 (2007).