Breath Analysis: Past, Present and Future - A Personal Perspective

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Breath analysis in the past

- Classical medicine, since the time of **Hippocrates**, has used “subjective impressions” of the odors of the body, i.e., sweat, urine, feces, or breath to suggest diagnoses
- Additionally, presence of water vapor in breath has been used to signify the presence of life
- **Lavoisier** in collaboration with **Laplace** first detected carbon dioxide in breath in 1784

- Earliest modern day publications on breath analysis by:

  Davidson; **Chen, Mahadevan & Zieve; Pauling, Robinson, Teranishi & Cary**; and **Riely, Cohen & Lieberman** date from late 40s to the early 70s and mirror the development of modern analytical chemistry particularly chromatography

Breath analysis in the recent past

- **Michael Phillips, MD** – has been the pioneering breath researcher for more than 30 years

- **Lars Gustafsson, MD & Phillip Silkoff, MD** – identified endogenous nitric oxide in human breath; developed the successful human nitric oxide breath test respectively

- **Nandor Marczin, MD** & **Peter Barnes, MD** – were organizers of the first conference devoted to breath analysis: NATO Advanced Study Institute on "*Disease Markers in Exhaled breath: Basic Mechanisms and Clinical Applications,"* Crete, Greece June 2001. Edited the first books devoted to breath analysis: "*Disease Markers in Exhaled breath: Basic Mechanisms and Clinical Applications" IOS Press 2002, and* Edited of "*Disease Markers in Exhaled Breath" Marcel Dekker 2003.*

- **Anton Amann, PhD** – organized the International Association of Breath Research (IABR), organized the Journal of Breath Research and the annual international meetings on Breath Analysis starting in 2004.

What have we learned from this previous research into breath analysis?

- Breath analysis has two critical components- sampling and analysis: neglect of either one and you are analyzing garbage
- Tidal breathing is under autonomic control: asking a study subject to breathe causes them to be aware of their breathing and as a result they hyperventilate
- Breath can be collected: into inert gas sampling bags, or evacuated canisters, or adsorbed onto surfaces, collected breath is limited to molecules that are stable
- Breath is a complex mixture of gases, and aerosols
- Breath contains:
  - molecules or their metabolites originating from inhaled air (current or historical exposure) or from dermal absorption
  - molecules or their metabolites derived from foods and beverages
  - molecules produced by anabolic or catabolic reactions that occur in tissues or cells throughout the body

Typical breathing parameters for a healthy subject

Young male, 75 kg, 1.7 m^2 body surface area, and BMI of 25, whose resting breathing is under autonomic control

- Tidal volume 0.6 l, respiratory rate 10-12 /min
- Anatomic dead space 0.13 l
- Alveolar gas ventilation 4.7 l/min
- Inhales 360 l of ambient air/h when breathing tidally

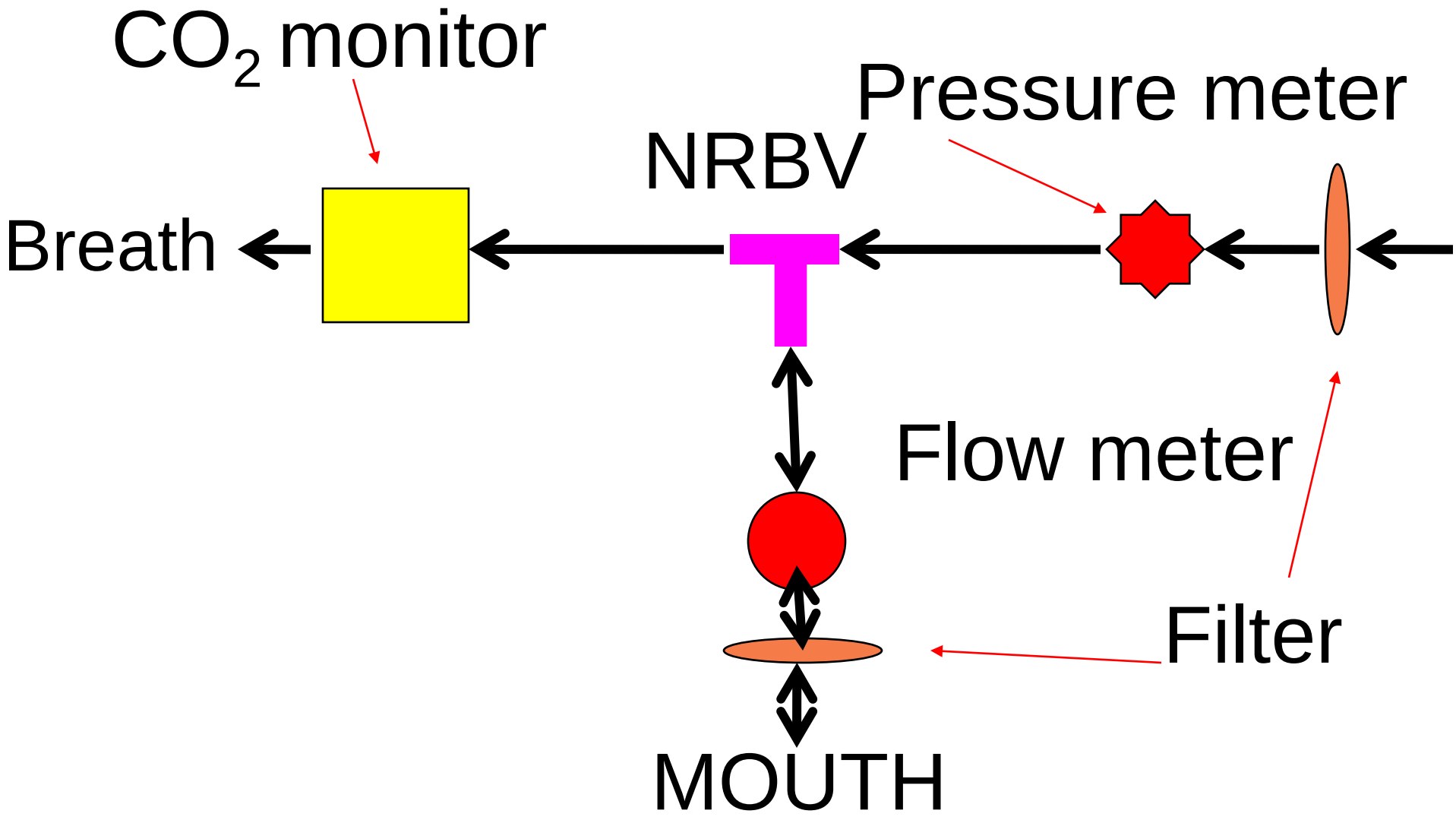
- Breath components/composition will change during a breathing cycle (inspiratory air, airway gas, mixed expired gas.
- Pure end tidal gas can only be sampled with a bronchoscope

- Endogenous breath molecules originate from biochemical processes occurring in cells within oral/nasal cavities, pulmonary system and organs and tissues throughout the body

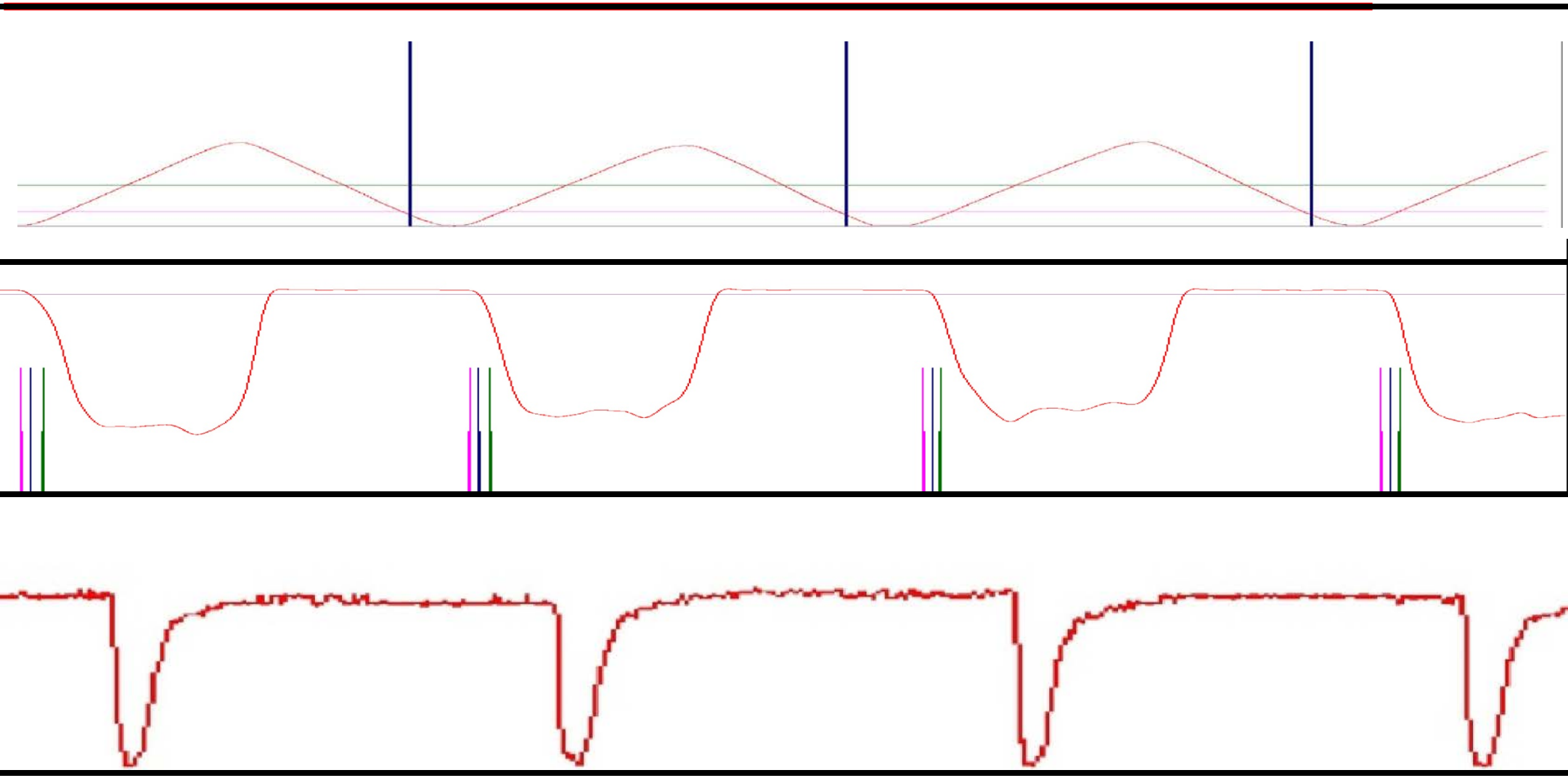
Sampling multiple breaths from spontaneously breathing subjects

- Monitor tidal volume of each breath and breathing frequency
- Monitor the concentration of carbon dioxide continuously, *i.e.*, determine end-tidal and steady state concentrations of each breath
- Monitor mouth pressure continuously
- Monitor pulse

Sampling paced tidal breathing



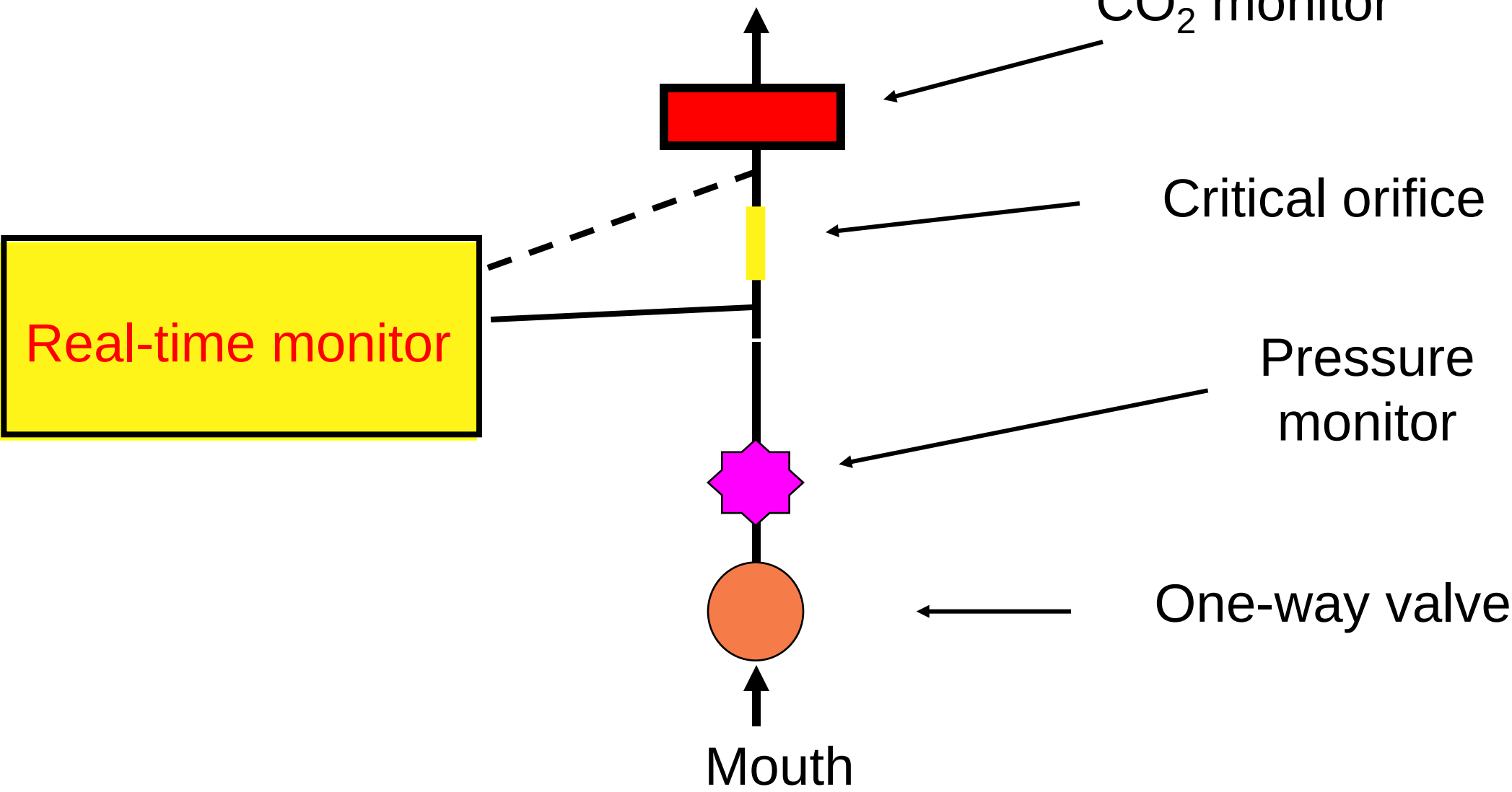
Paced breathing as a function of time



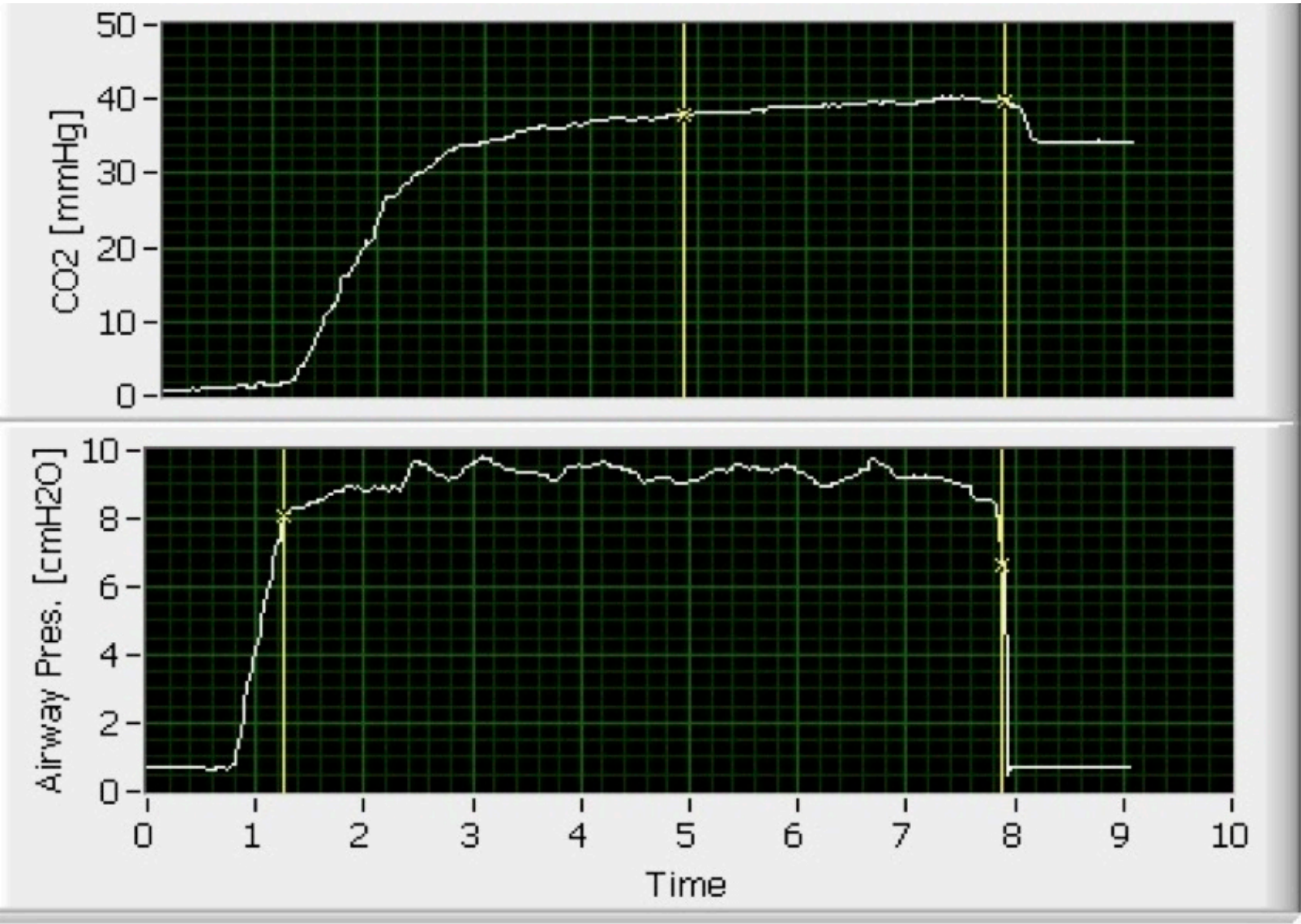
Sampling a single breath from a spontaneously breathing subject

- Control pressure (flow using critical orifice)
- Monitor pressure continuously
- Monitor the concentration of carbon dioxide continuously

Monitoring a single breath



Breath parameters as a function of time



Plateau Report

CO2 [mmHg]

39,2 ± 0,7

Pressure [cmH2O]

9,2 ± 0,4

Duration [sec]

6,6

Exogenous molecules in breath

- Samples of exhaled breath are contaminated with inspiratory gas (immediate or previous)
  - There is no accepted method to background correct for room air - alveolar gradient method assumes contaminants in current room air
- Samples of exhaled breath are contaminated with molecules absorbed through the skin
  - There is no accepted method to correct for these molecules
- Samples of exhaled breath are contaminated with molecules derived from foods and beverages
  - There is no accepted method to correct for these molecules

Typical molecules found in human breath

Compound	Conc (v/v)	Physiological Basis
acetaldehyde	ppb	ethanol metabolism, lipid peroxidation
acetone	ppb	fatty acid metabolism
ammonia	ppb	protein metabolism
carbon dioxide	%	respiration
carbon monoxide	ppm	heme catabolism catalyzed by *heme oxygenase*, cytoprotective role
carbonyl sulfide	ppb	gut bacterial oxidation of reduced sulfur species
ethane	ppb	lipid peroxidation
ethanol	ppb	gut bacterial metabolism of sugars
ethylene	ppb	lipid peroxidation, molecular signaling
hydrogen	ppm	gut bacterial metabolism of carbohydrates

Typical molecules found in human breath

Compound	**Conc (v/v)**	**Physiological Basis**
hydrogen cyanide	ppb	synthesized by *P. aeruginosa*
hydrogen sulfide	ppb	bacterial metabolism of thiol proteins; mediator of brain, gastrointestinal and liver function
isoprene	ppb	cholesterol biosynthesis; may be involved in regulation of *HMG CoA reductase*
methane	ppm	gut metabolism of carbohydrates
methanethiol	ppb	methionine metabolism
methylamine	ppb	protein metabolism
nitric oxide	ppb	catalyzed by *nitric oxide synthases*; involved in vasodilation or neurotransmission
1-pentane	ppb	lipid peroxidation
water	%	respiration

Analytical methods for analysis in human breath

Analysis of collected breath -- molecular profiles

- Gas solid chromatography with various detectors (TSD, FID, etc)
- Capillary gas chromatography with various detectors (FID, ECD, mass spec, etc)
- 2 Dimensional gas chromatography with various detectors

Real time analysis of molecules in breath

- Chemiluminescence reactions
- Electrochemical sensors
- Absorption of infra-red radiation
- Solid state sensors (quartz crystal microbalance, surface acoustic wave)
- Thermoelectric sensors

Analysis of collected or real-time -- breath profiles (electronic nose)

- Nanomaterial sensor arrays (metal-oxide materials, conducting polymers, carbon nanotubes, organic dielectrics, organic conductor)

Do unique breath biomarkers for diseases exist?

- Unique biomarkers in breath ***can only*** originate from the ingestion, inhalation or dermal absorption of foreign substances
- Or unique biomarkers in breath ***can only*** originate from bacterial, viral, or fungal metabolism

- The onset of disease results in changes in the concentrations of breath molecules and ***not the production of unique breath biomarkers - disease does not produce novel biochemistry it inhibits or induces enzyme systems***

Oxidative Stress Status: balance between oxidative stress and antioxidant defenses

- Oxidative stress is caused by reactive oxygen species such as as superoxide anion, hydrogen peroxide, and hydroxyl radical which are generated in all cells. A typical adult (75 kg) generates approximately **0.3 mole of ROS*/*day** based upon the utilization of approximately **14.7 mole of O_2*/*day**
- Antioxidant defenses: enzymes (*superoxide dismutase*, *glutathione peroxidase*, *catalase*), vitamins (vitamins E & C, beta-carotene, polyphenols (flavonoids, flavones), and proteins (albumin, ferritin, transferrin, metallothionein)
- Oxidative stress status can be quantified in breath indirectly by quantifying stable products of damage by ROS i.e., lipid peroxidation: hydrocarbons (ethane**,** ethylene, pentane, branched chain hydrocarbons), aldehydes, or arachidonic acid metabolites.

What does increased oxidative stress mean

- Bad personal habits – poor diet, smoker
- Exposure to solvents, ionizing radiation
- Diseases such as: cancer, Alzheimer's disease, amyotrophic lateral sclerosis, scleroderma, pulmonary disease, diabetes, liver disease, Parkinson disease, cardiovascular disease, airway reactivity (asthma) etc.,
- Having an active infection - viral, fungal, or bacterial (host response to infection)
- Being premature
- Growing old
- Surgery – ischemia/reperfusion injury - also observed during sickle cell anemia crises.

Elevated oxidative stress status provides no definitive information it is similar to a temperature measurement

Breath ammonia in humans

Normal catabolism of amino acids in proteins produces ammonia and urea

Urease producing gut flora will convert urea to ammonia

Elevated levels of breath ammonia

- Patients with severe impairment of metabolic liver function
- Patients with genetic disorders of the urea cycle
- Patients with end-stage renal disease i.e., decreased excretion of urine
- Subjects who have just exercised
- Subjects with periodontal disease

Elevated breath ammonia could be due to liver disease, kidney disease, genetic diseases, periodontal disease, exercise

Breath acetone in humans

Acetone together with other ketone bodies is produced by hepatocytes from excess acetyl CoA.

Ketone bodies diffuse from the hepatocytes and are oxidized *via* the Krebs cycle in peripheral tissue

Elevated levels of breath acetone

- Patients presenting with diabetes
- Study subjects dieting (Adkins Diet)
- Study subjects fasting
- Study subjects under stress
- Study subjects after exercise

Elevated breath acetone could be due to a diseases or activities

Breath isoprene in humans

Isoprene is biosynthesized from DL-mevalonate in the liver.
Isoprene is produced during the biosynthesis of cholesterol

Elevated levels of breath isoprene

- Patients presenting with familial hypercholesterolemia
- Patients with familial combined hyperipidemia
- Patients presenting with Duchenne muscle dystrophy
- Subjects who have been exercising
- Elderly study subjects
- Smokers

Elevated breath isoprene could be due to a number of diseases or activities

Breath sulfur compounds in humans

Reduced sulfur compounds are produced by the incomplete catabolism of methionine in the liver

Reduced sulfur compounds are produced by bacteria in the gut and mouth.

Elevated levels of breath sulfur compounds

- **Patients presenting with liver diseases**
- **Patients presenting with bacterial overgrowth in the gut**
- **Acute rejection of organ transplants**
- **Subjects who have periodontal disease**

Elevated breath sulfur compounds could be due to a number of diseases or conditions

What are the future directions for breath analysis

- Based upon all available information breath analysis can currently be used to follow therapy/pharmacolic intervention but not diagnosis

- However, breath analysis will have a role in clinical diagnosis if it:
  - Provides novel information or diagnoses
  - Provides information quicker than traditional tests (real time)

- Diagnosis based upon breath analysis will use real time analysis

Requirements for real-time breath analysis

- Real-time -- result available within 5-10 min
- Based upon sampling single breath

Requirements

- Must be able to be performed by people with limited training
- Instruments must be reliable

Current clinical breath tests

Clinical test	**Molecule used in test**
capnography	carbon dioxide
gastrointestinal diagnoses (disaccharide deficiency, GI transit time, bacterial over growth, intestinal statis)	hydrogen, methane
heart transplant rejection	branched chain H/C
carbon monoxide toxicity, smoking cessation	carbon monoxide
airway reactivity (asthma)	nitric oxide
metabolism of labeled drugs or enzyme substrates (*H pylori*, liver function tests, renal function, etc.)	C^{13} carbon dioxide

Potential clinical breath tests

Clinical test	**Molecule to be used in test**
neonatal jaundice	carbon monoxide
oxidative stress (acute or chronic disease)	hydrocarbons, aldehydes
cholesterol biosynthesis	isoprene
renal function	ammonia, alkylamines
hepatic function	ammonia, carbonyl sulfide, methyl sulfide, methanethiol
host response to infection	
oxidative stress	hydrocarbons, aldehydes
induction of antioxidant defenses	carbon monoxide, nitric oxide
Urea cycle disorder, hepatic encephalitis, exercise physiology	ammonia
diabetes, fasting/dieting, weight loss	acetone, (ethanol)

Conclusions

Clinical breath analysis: are we there yet?

- From a clinical standpoint: *progress has been made*
  - There is a demonstrated need for breath analysis particularly real-time portable devices (point-of-care testing, personalized medicine)
- From an instrument standpoint: *progress has been made*
  - Devices suitable for routine clinical use are at various stages of development

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