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Effect of temperature control on the metabolite content in exhaled breath condensate

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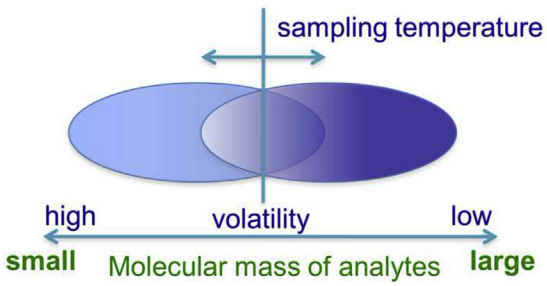
HIGHLIGHTS

- Effect of temperature control and sample physical phase on the metabolomic content of exhaled breath condensate.
- Concentration of volatile compounds detected with GC-MS and non-volatiles detected with HILIC LC-MS are temperature influenced.
- Detection of specific types of compounds may be more efficient at a specific temperature.
- The importance of temperature control relative to saliva filtering and surface coatings needs to be further investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

The non-invasive, quick, and safe collection of exhaled breath condensate makes it a candidate as a diagnostic matrix in personalized health monitoring devices. The lack of standardization in collection methods and sample analysis is a persistent limitation preventing its practical use. The collection method and hardware design are recognized to significantly affect the metabolomic content of EBC samples, but this has not been systematically studied.

Here, we completed a series of experiments to determine the sole effect of collection temperature on the metabolomic content of EBC. Temperature is a likely parameter that can be controlled to standardize among different devices. The study considered six temperature levels covering two physical phases of the sample; liquid and solid. The use of a single device in our study allowed keeping saliva filtering and collector surface effects as constant parameters and the temperature as a controlled variable; the physiological differences were minimized by averaging samples from a group of volunteers and a period of time. After EBC collection, we used an organic solvent rinse to collect the non-water-soluble compounds from the condenser surface. This additional matrix enhanced metabolites recovery, was less dependent on temperature changes, and may possibly serve as an additional pointer to standardize EBC

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sampling methodologies. The collected EBC samples were analyzed with a set of mass spectrometry methods to provide an overview of the compounds and their concentrations present at each temperature level.

The total number of volatile and polar non-volatile compounds slightly increased in each physical phase as the collection temperature was lowered to minimum, 0 °C for liquid and −30, −56 °C for solid. The low-polarity non-volatile compounds showed a weak dependence on the collection temperature. The metabolomic content of EBC samples may not be solely dependent on temperature but may be influenced by other phenomena such as greater sample dilution due to condensation from the ambient air at colder temperatures, or due to adhesion properties of the collector surface and occurring chemical reactions. The relative importance of other design parameters such as condenser coating versus temperature requires further investigation.

1. Introduction

The non-invasive, quick, and safe collection of exhaled breath condensate (EBC) makes it a candidate as a diagnostic matrix for use in personalized health monitoring devices. Single-use, sterile EBC collection device components can be mass produced cheaply and used safely in non-medical settings. The recent advances in microfluidic lab-on-a-chip analysis and cloud-based data analysis algorithms may make prescreening of a number of diseases possible in a short time period at a small fraction of the current cost.

EBC is a complex matrix which has been shown to have a chemical composition resembling that of the extracellular lung fluid [1–5]. This biological sample is rich with a wide variety of compounds including: non-volatile biomolecules aerosolized from the airway lining fluid and water-soluble volatile compounds, proteins, lipids, antibodies, and carbohydrates. In total, in humans, EBC contains up to 2000 different compounds [6–8]. EBC analysis may not precisely measure solute concentrations in native airway fluid; however, if concentrations of certain compounds differ enough between a healthy and diseased state, EBC analysis can be a potential diagnostic tool [7]. Some individual compounds or a set of compounds can be reflective of diseased state and are called biomarkers. Currently, nitric oxide (NO) [9–12], hydrogen peroxide (H_2O_2) [13–16], and acetone, measured from breath condensate, are the three most studied and used compounds for diagnostic biomarkers of inflammatory responses in the respiratory system. Concentrations of lipids can also be measured from EBC, including fatty acids, steroids, eicosanoids, and their subclasses, such as prostaglandins or isoprostanes [17,18]. For instance, 8-isoprostane detected in EBC, is considered a biomarker of oxidative stress and antioxidant deficiency, showed differences between healthy smokers and patients with COPD [19]. Plasma lysozyme was found to be significantly higher in patients with adult respiratory distress syndrome (ARDS) as compared to healthy patients [20]. Other low-volatility compounds such as enzymes have been considered as effective biomarkers of illness diagnosis. The pH of EBC can also serve as a simple but robust biomarker of various lung diseases [21–23]. The compounds, including volatile organic compounds (VOCs) present in EBC are not limited to the respiratory system but may originate from blood borne biogenic compounds, and can be representative metabolites of a wide range of systemic processes [24,25]. Patients with and without lung cancer, regardless of the cancer stage, were discriminated using 22 VOCs including alkanes, alkane derivatives, and benzene derivatives [26]. A combination of eight VOCs was sufficient to discriminate between asthmatic and healthy children [27].

EBC analysis has some current limitations; the persistent problem is the lack of standardization in the collection methods, the collection devices, and the sample analysis [28–30]. The collection procedure and hardware design are known to significantly affect the metabolomic content of the EBC sample [31,32]. A number of parameters were examined: effect of sampling duration, breathing pattern, collected fraction of the exhaled breath (alveolar end tidal versus total expired volume), collection device material, condensation temperature, contamination from saliva, sample transfer, and storage [33–35]. The design and performance of commercially available EBC samplers such as the Rtube™ (Respiratory Research, Inc., Austin, TX, USA), EcoScreen® (Erich Jaeger GmbH, Hoechberg, Germany), and TurboDECCS (MEDIVAC, Parma, Italy) were compared to answer some of the questions about sample collection procedure and device choice [36–38].

In our previous work [38], we compared the performance of an engineered EBC collection device with that of RTube™ and TurboDECCS®. Though the three devices equilibrated in the volume of collected EBC sample, the EBC samples differed in the metabolomic content. The engineered device collected EBC samples that contained less saliva but higher number of compounds. There were some design differences responsible for that; different collection temperatures, different surface materials, and different saliva filtering mechanism.

The engineered device had a PTFE housing (duct) and a glass condenser surface cooled by dry ice pellets; it warmed up from −56 °C to - 30 °C in a 10 min sampling period. RTube™ and TurboDECCS® had polypropylene condenser surfaces and significantly differed in collection temperature and its stability. RTube™ warmed from −56 °C to 0 °C during a 10 min sampling period and TurboDECCS® warmed from −7 °C to 6 °C during the first minute of breath sampling [38]. The surface properties of the collector surface are also known to have an effect on the recovery of the metabolomic content of EBC.

Rosias et al. [35,37] studied the effect of the condenser surface coatings on measurement of biomarkers in EBC. Five condenser coatings (silicone, glass, aluminum, polypropylene, and Teflon) were compared using the EcoScreen® device. Adhesive properties of different condenser coatings influenced the eicosanoids and proteins measurements in EBC. Silicone and glass coatings were shown to be more efficient for measurement of 8-isoprostane or albumin in EBC. The relative importance of hardware parameters, e.g. temperature level versus surface material, and their effects on the content of EBC samples needs to be quantified with a rigorous set-up where both tested parameters are highly controlled.

The contamination with saliva needs to be minimized because oral microbiome contributes a wide variety of metabolites that may obscure biomarkers originating in the lungs [39]. The level of saliva contamination in collected EBC samples was different; the engineered device had the least level of saliva contamination.

The previous studies that used different devices are informative and give some common points for estimation but lack to define the

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