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FACTORS AFFECTING THE VARIABILITY OF BREATH COMPOSITION

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Breath analysis is considered a promising tool for the non-invasive monitoring of health conditions relying on the almost instantaneous equilibrium of volatile chemicals in blood and lung air across the alveolar-capillary membranes. Breath composition contains information concerning the present and past exposure to xenobiotics, food and beverage consumption, normal and abnormal physiology as well as the presence of bacteria. Such richness of information becomes a limitation when the purpose is to diagnose a pathology, as intra- and inter-individual variabilities hinder the classification of subjects into healthy or pathologic and the identification of specific biomarkers or “breathprints”. In addition, the lack of standardized procedures for breath sampling and analysis makes the comparison of results obtained from different research groups complicated.

For these reasons, in this study we tried to assess how factors such as sampling conditions, circadian rhythms and diet affect the inter- and intra-individual variability. The assessment of such variability and the weight of the different factors is important to evaluate the number of subjects to enrol and the statistical power of tests using breath biomarkers to distinguish patients and nominally healthy individuals. Breath composition was analysed in 20 healthy subjects following an omnivorous (n= 10) or a vegan (n= 10) diet under different conditions. After acquaintance of the subject with the sampling device, during which the CO₂ profile in breath was acquired, 25 mL of mixed breath were collected at 15 mL/min into needle trap devices loaded with an internal standard. Volatile organic compounds (VOCs) were analysed by needle trap micro-extraction coupled to gas chromatography tandem mass spectrometry. The chromatographic separation was carried out by a DB-624 ultra-inert capillary column and the mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Qualifier and quantifier transitions were monitored for each compound.

The experiments showed that the effect of respiratory rate and ventilation on the composition of breath differs among VOCs, where the largest variations were observed with isoprene and acetone. Vegans showed lower values of VOCs related to oxidative stress compared to omnivorous subjects, but differences were not very large and a higher number of volunteers is needed for significance. Individual behaviours concerning smoke or consumption of specific foods were also mirrored in breath. Additional experiments, performed in the framework of a multicentre study organized from the breath community to compare different measurement approaches (Peppermint experiments), showed the individual variability in the washout of menthol after the ingestion of a peppermint.