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Long-term monitoring of breath methane

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HIGHLIGHTS

- long-term monitoring of human breath methane levels reveals significant variations within short time periods
- deviations from average breath methane values coincide with a change in health status of individual subjects
- Results also indicate aerobic methane formations possibly related to cellular oxidative-reductive stress reactions

GRAPHICAL ABSTRACT

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ABSTRACT

In recent years, methane as a component of exhaled human breath has been considered as a potential bioindicator providing information on microbial activity in the intestinal tract. Several studies indicated a relationship between breath methane status and specific gastrointestinal disease. So far, almost no attention has been given to the temporal variability of breath methane production by individual persons. Thus here, for the first time, long-term monitoring was carried out measuring breath methane of three volunteers over periods between 196 and 1002 days. Results were evaluated taking into consideration the health status and specific medical intervention events for each individual during the monitoring period, and included a gastroscopy procedure, a vaccination, a dietary change, and chelate therapy. As a major outcome, breath methane mixing ratios show considerable variability within a person-specific range of values. Interestingly, decreased breath methane production often coincided with gastrointestinal complaints whereas influenza infections were mostly accompanied by increased breath methane production. A gastroscopic examination as well as a change to a low-fructose diet led to a dramatic shift of methane mixing ratios from high to low methane production. In contrast, a typhus vaccination as well as single chelate injections resulted in significant short-term methane peaks. Thus, this study clearly shows that humans can change from high to low methane emitters and vice versa within relatively short time periods. In the case of low to medium methane emitters the increase observed in methane mixing ratios, likely resulting from immune reactions and inflammatory processes, might indicate non-microbial methane formation under aerobic conditions. Although detailed reaction pathways are not yet known, aerobic methane formation might be related to cellular oxidative-reductive stress reactions. However, a detailed understanding of the pathways involved in human methane formation is necessary to enable comprehensive interpretation of methane breath levels.

1. Introduction

Methane (CH_4) is the simplest alkane and a well-known potent greenhouse gas. It is mainly produced by microbes (archaea) in oxygen

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free environments (Conrad, 2009), by chemical processes such as geological formation and burning of organic matter (Etiope and Lollar, 2013), and as recently proposed in eukaryotes under aerobic conditions (Keppler et al., 2009; Wang et al., 2013; Liu et al., 2015). In the late sixties, CH_4 was also detected as a component in the exhaled breath of humans (Calloway and Murphy, 1968; Levitt and Ingelfinger, 1968). In this respect CH_4 is assumed to mainly reflect intestinal gas formation by microorganisms in the gut (Bang et al., 2014). So far, two methanogenic species isolated from human feces have been identified, the hydrogenotrophic *Methanobrevibacter smithii* and the methylotrophic *Methanosphaera stadtmanae*. Possible contributions from other methanogenic species are not yet clear (Mihajlovski et al., 2010). Both species identified require H_2 for the reduction steps leading to CH_4 formation. A fraction of the CH_4 produced is excreted via the lungs and can be detected by specific gas analysis (Mürtz and Hering, 2008; Dryahina et al., 2010; Tuboly et al., 2013; Keppler et al., 2016). The proportion of CH_4 in exhaled breath has been investigated in numerous studies (Bond et al., 1971; Pitt et al., 1980; McKay et al., 1985; Peled et al., 1987; Wilkens et al., 1994; Levitt et al., 2006; Sahakian et al., 2010; Fernandes et al., 2013; Polag et al., 2014). However, most of these studies only differentiate between breath CH_4 producers and breath CH_4 non-producers. Subjects are defined as CH_4 producers when they emit breath CH_4 at least 1 ppm parts per million by volume (ppmv) above background level. Based on this definition, between 25% (Polag et al., 2014) and 77% (Hudson et al., 1993) of subjects examined were identified as CH_4 producers. Methane producing status depends on a number of factors such as age (Hopkins et al., 2002; Polag et al., 2014), ethnic background (Pitt et al., 1980; Mello et al., 2012), gender (Triantafyllouand et al., 2014) and exercise status (Szabo et al., 2015). Potentially some diseases might also be related with CH_4 producing status (Haines et al., 1984; Montes et al., 1993; Conway de Macario and Macario, 2009; Di Stefano and Corazza, 2009; Hwang et al., 2010; Roccarina et al., 2010; Kunkel et al., 2011; Furnari et al., 2012; de Lacy Costello et al., 2013). Recently, Keppler et al. (2016) unambiguously demonstrated using high precision concentration and stable carbon isotope measurements that all humans exhale CH_4. In that study, a large fraction of subjects (~ 80%) who would normally be classified as non-emitters were clearly shown to release CH_4 with a median of 118 parts per billion by volume (ppbv). Based on this observation, these authors differentiate between low (<1 ppmv above background), medium (1–4 ppmv above background) and high emitters (>4 ppm above background). They also hypothesized that next to intestinal microbial sources of CH_4 formation there might exist another but as yet unidentified process producing CH_4 in the human body. This finding adds to an increasing body of evidence that next to CH_4 production by obligate anaerobic methanogens in the human gastrointestinal tract a portion of CH_4 might also be produced under aerobic conditions on a cellular level without the contribution of microorganisms (Tuboly et al., 2012; Boros et al., 2015; Tuboly et al., 2017).

In 2006 it was shown that plants are able to produce CH_4, per se under aerobic conditions (Keppler et al., 2006). Several subsequent investigations identified other eukaryotes such as fungi (Lenhart et al., 2012), mosses (Lenhart et al., 2015), and algae (Lenhart et al., 2016) as CH_4 producers. The work by Ghyczy et al. (2008) using in vivo experiments with eukaryotic cells also provided an alternative pathway to methanogenesis. In that study CH_4 production was observed in animal mitochondria and cell cultures under hypoxic conditions. Thus, it seems most likely that human CH_4 production is not restricted to microbial formation within the gastrointestinal tract but CH_4 might additionally be formed by non-microbial processes occurring within cells. Although the processes leading to aerobic CH_4 formation in eukaryotes remain speculative (Benzing et al., 2017) some precursors with sulfur and nitrogen bonded methyl groups might be involved (Ghyczy et al., 2003; Keppler et al., 2009; Althoff et al., 2014; Wang et al., 2013). Several studies including experiments with mitochondria of animals, plant tobacco cells, and a chemical model system led to the hypothesis that biomolecules such as choline and methionine are involved in CH_4 formation by eukaryotes (Wishkerman et al., 2011; Althoff et al., 2014). However, it would appear more likely that formation of CH_4 is associated with the production of reactive oxygen species (ROS) (Ghyczy et al., 2003; Tuboly et al., 2012) and iron oxo species (Benzing et al., 2017). Tuboly et al. (2012) observed CH_4 formation during sodium azide-induced hypoxia in rats. These researchers found that when biological CH_4 formation was inhibited with antibiotics an increase in CH_4 was still observed suggesting non-microbial CH_4 formation associated with a mitochondrial dysfunction.

In addition to the emerging importance of breath analysis as a non-invasive tool to detect various diseases (Manolis, 1983; Nakhleh et al., 2017), CH_4 might also play an important role as a gasotransmitter in the human body (Wang, 2014; Boros et al., 2015). For a detailed overview of our current knowledge on CH_4 formation in humans we refer the reader to a recent article by Boros and Keppler (in press). It is becoming more apparent that basic knowledge of the general variability of human breath CH_4 levels is of crucial importance when interpreting specific values of production. So far, fundamental studies with respect to long-term monitoring of CH_4 for individual persons are scarcely available. Kinoyama et al. (2006) tested diurnal variation in the concentration of CH_4 and H_2 in the breath of methane producers ($CH_4 > 5$ ppmv). They observed four diurnal variation patterns when CH_4 concentrations were correlated with H_2 concentrations. To the best of our knowledge, this is the first long-term monitoring of breath CH_4 for individual humans. Variations in breath CH_4 mixing ratios were studied for three individuals over time periods varying in length from 196 to 1002 days. Moreover, ratios were compared with changes and temporary events that occurred in the health status of each of the subjects during the monitoring period.

2. Material and methods

2.1. Subjects

Long term monitoring of breath CH_4 was studied for three individuals, here indicated as subjects A, B, and C. Age, sex, monitoring period, and physical states of the subjects are summarized in Table 1. Monitoring was carried out at high frequency (every 1 to 3 days) for periods varying in length between 196 and 1002 days. The three subjects (2 female and 1 male) were volunteers from the Institute of Earth Sciences in Heidelberg (Germany) aged between 35 and 54 years at the beginning of monitoring period. Body mass indices (BMI's) of the subjects were in a normal range (18.5 to 25) and no prescribed medication or drug intake was reported prior to commencement of the experiment. During monitoring, subject B was positively tested for fructose malabsorption and undertook a low-fructose diet for a time period. Subject C suffered from multiple sclerosis (MS) showing specific symptoms during monitoring, i.e. muscular pain, and fatigue. The health condition of each of the subjects was also recorded at every sampling interval. To quantify the actual health status, subject C was evaluated subjectively on a numerical basis where zero represented average health conditions with higher well-being indicated by positive numbers and worse than average by negative numbers. Thus, the higher the absolute numbers the greater the deviation from subjective average health conditions.

Table 1
Subjects for breath CH_4 analysis.

Subject	Monitoring period	Age	Sex	Annotation
A	Nov2012–Jul2013	35	f	
B	Nov2012–May2013	45	m	Fructose malabsorption
C	Nov2014–Jul2017	54	f	Multiple sclerosis