Science of the Total Environment 481 (2014) 582–587

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

# Age dependent breath methane in the German population

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## HIGHLIGHTS

- Increase in the percentage of breath methane producers with age within the German population.
- Interruption in the percentage of methane producers in the sixth and seventh decade possibly connected to hormonal factors.
- Predicted increase in percentage of breath methane producers from 30% (2013) to 35% by 2050.

ARTICLE INFO

*Article history:*
Received 23 October 2013
Received in revised form 17 February 2014
Accepted 17 February 2014
Available online 12 March 2014

*Keywords:*
$CH_4$
Methanogens
Gut
Intestinal tract
Gastrointestinal
Demography

ABSTRACT

Methane which can sometimes be found in exhaled breath of humans is known to reflect in situ intestinal methanogenic activity. In recent years, several factors have been studied in order to understand their relevance to methane production in the intestinal tract. However, the relationship between age and methane producing status has hitherto not been sufficiently investigated. In the present study we evaluated the relationship between age and percentage of breath methane producers in the German population in 428 subjects with ages ranging from 4 to 95 years. When subjects were divided into age groups of 15 years, an increase in the percentage of breath methane producers with age was observed. The near linear increase ($R^2 = 0.977$) from 5% for children (1–15 years) to 57% for the elderly (>75 years) may indicate a continuous development in the human gut methanogenic flora throughout lifetime. However, when subjects were compared on 5 year age intervals, an interruption in the percentage of methane producers in the sixth and seventh decade was noted. We further revealed an age dependence on the ratio of female to male producers. This is shown by a dominance in female breath methane producers during the first half of life which afterwards is replaced by a dominance in male breath methane producers with an approximately linear decrease in the ratio between 20 and 65 years ($R^2 = 0.926$). These observations might suggest a relationship between methanogenic activity and hormonal factors. Using our data, we predict that the percentage of breath $CH_4$ producers within the German population will increase from its current value of 30% (2013) to 35% by 2050.



## 1. Introduction

It is well known that part of the human intestinal tract is colonized by methanogenic archaea which are capable of producing methane ($CH_4$). So far, two methanogenic species isolated from human feces have been identified, the hydrogenotrophic *Methanobrevibacter smithii* and the methylotrophic *Methanosphaera stadtmaniae*. 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. Exogenous sources of $H_2$ are associated with the ingestion and incomplete metabolization of carbohydrates (e.g., saccharides) while specific glycoproteins are considered as potential endogenous substrates for methanogens (Flourie et al., 1991). The interaction between methanogens and gut function is an important aspect of the current research (Wilkens et al., 1994; Sahakian et al., 2010). Generally, it is not yet clear if methanogens act as an indicator of in situ gut activity or if they actively contribute to intestinal conditions (Levitt et al., 2006). In humans, $CH_4$ is likely produced in the distal part of the colon (Flourie et al., 1991), but production may also occur in the distal ileum (Bond et al., 1971), especially in the case of bacterial overgrowth in the small intestine (Mello et al., 2012). A fraction of the produced $CH_4$ is excreted via the lungs and can be detected in the breath (Bond et al., 1971). The frequently used term 'breath $CH_4$ producer' refers to those subjects whose exhaled breath $CH_4$ level exceeds the environmental value by 1 ppm. All other subjects are indicated as $CH_4$-non-producers even though they may produce small amounts of $CH_4$. The $CH_4$ producing status of a subject may depend on several interacting factors such as ethnic background (Hudson et al., 1993; Conway de Macario and Macario, 2009), gastrointestinal diseases (Conway de Macario and Macario, 2009; Roccarina et al., 2010; Kunkel et al.,

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http://dx.doi.org/10.1016/j.scitotenv.2014.02.086
0048-9697/

2011), intestinal gas dynamics including transit time (Azpiroz, 2005; Ghoshal et al., 2011), enterobacterial composition with respect to competition for hydrogen (Christl et al., 1992), concentration of bile acids (Florin and Woods, 1995), body mass index (Basseri et al., 2012), intake of specific antibiotics (Peled et al., 1987), sanitary conditions (Mello et al., 2012), genetics (Bond et al., 1971), and sex (Peled et al., 1987; Fernandes et al., 1998). Several of these factors are discussed in terms of general impact, correlation and cause/effect relationship. So far, only a few studies have considered the relationship between $CH_4$ producing status and age (e.g., Bond et al., 1971; Fernandes et al., 1998; Peled et al., 1985). It is widely assumed that the human methanogenic flora begins to develop during infancy and continues until adulthood when the level of methanogenic archaea remains more or less constant (Bond et al., 1971; Peled et al., 1985). However, in contrast, a study by Maczulak et al. (1989) on rats clearly showed a steady increase in colonic methanogens throughout the entire lifetime of the animals. In the case of nitrous oxide ($N_2O$) in human breath, a study undertaken by Mitsui et al. (1997) found that its concentration was highest in children then decreased during adulthood and increased again in the aged. They explained their findings by a change in the immune system which affected the intestinal microbiome. In a study undertaken by Fernandes et al. (1998) it was reported that even though breath $CH_4$ producers were significantly older than non-producers, breath $CH_4$ concentrations were only weakly correlated with age in $CH_4$ producing subjects. They explained the increase in breath $CH_4$ concentration with an age-related increase in colonic transit time and an increase in carbohydrate malabsorption. Unfortunately this study was somewhat limited in that the subject age range did not cover ages below 18 or above 77 years and subjects were only divided into four age groups. In order to reconsider results from previous findings on age dependence of breath $CH_4$ production, we conducted a study with a large group of volunteers from within the German population. In the study we measured breath $CH_4$ concentrations of 428 subjects with ages ranging between 4 and 95 years and compared and contrasted the data when ages were grouped on a 5 or 15 year age basis.

## 2. Materials and methods

A total number of 428 breath samples were collected from volunteers visiting the Max-Planck-Institute of Chemistry in Mainz at an open day and from members of the Institute. Samples were collected from 190 female and 238 male subjects with ages ranging from 4 to 95 years. In order to exclude possible ethnic factors, breath samples were collected only from Caucasian subjects. No data on body mass index or the health status of the subjects, including bowel complaints or known gastrointestinal diseases was recorded. Breath methane sampling was conducted in the non-fasting state and a disinfection of the oral cavity was not performed. For collection of breath samples, 1 l-Tedlar-gas sampling bags were used. During sample collection the subjects were required to breathe normally, stop breathing for 5 s and then exhale into the Tedlar-bag. Subsequently, a sample of 5 ml was removed from the Tedlar-bag and injected into a Shimadzu gas chromatograph (GC-14B) coupled with a flame ionization detector (FID). Methane mixing ratios were calculated by integrating the $CH_4$ peak area of the breath sample and comparing this value to the peak area of a known $CH_4$ standard (8.9 ppm). Analytical precision was 0.01 ppm and $CH_4$ mixing ratios usually varied within 10% between single consecutive measurements ($c_v = 0.1$ for $i = 15$). Although not strictly correct, for the rest of this manuscript we use the term $CH_4$ concentrations instead of $CH_4$ mixing ratios. Subjects with a breath $CH_4$ concentration above 1 ppm of the atmospheric value were denoted as breath $CH_4$ producers (Bond et al., 1971). As the laboratory air had a $CH_4$ mixing ratio of around 2 ppm the threshold was set to 3 ppm, a value which is in agreement with the majority of previous studies (e.g., Basseri et al., 2012). Changing the threshold by $\pm 0.5$ ppm resulted in a decrease/increase of $CH_4$ producers of less than 2% indicating that the selected threshold of 3 ppm is suitable to distinguish between the status of 'non-producer' and 'producer'.

## 3. Results

Of the 428 subjects (190 female and 238 male) 107 (25%) were found to be breath $CH_4$ producers, including 51 female (26.8%) and 56 male (23.5%) with an average $CH_4$ concentration of 15 ppm. Table 1 provides a summary of results reported in age groups of 5 years and includes the number of subjects together with the number of male and female $CH_4$ producers. The bar graph in Fig. 1 shows the percentage of breath $CH_4$ producers for age intervals of 15 years. Here, an approximate linear increase in percentage of breath $CH_4$ producers with age can be observed ($R^2 = 0.977$) with values ranging from 5% for the age range 1–15 to 57% for the age range >75. Fig. 2 shows a box–whisker-plot highlighting the distribution of breath $CH_4$ values for the different age groups. $CH_4$ values lie in the range between laboratory background (2 ppm) and a maximum observed $CH_4$ value of 42 ppm. For increasing age groups a greater variation of $CH_4$ values can be observed. However, when the percentage of breath $CH_4$ producers are divided into groups with 5 year age intervals (Fig. 3), a somewhat different pattern emerges. From the first to the fifth decade the number of breath $CH_4$ producers steadily increases from 3% in the 6–10 age group to 45% in the 46–50 age group. The increase is more pronounced in the age groups between 31 and 50 years with an increase of approximately 8% between each adjacent group in comparison to the first 25 years where the increase

**Table 1**
Breath methane producers for subjects aged between 4 and 95 years in 5 year age groups.

| Age group | Number of subjects | Number of $CH_4$ producers | Number of male subjects | Number of male $CH_4$ producers | Number of female subjects | Number of female $CH_4$ producers |
|---|---|---|---|---|---|---|
| 1–5 | 3 | 0 | 2 | 0 | 1 | 0 |
| 6–10 | 29 | 1 | 17 | 1 | 12 | 0 |
| 11–15 | 22 | 2 | 9 | 0 | 13 | 2 |
| 16–20 | 33 | 3 | 21 | 2 | 12 | 1 |
| 21–25 | 39 | 6 | 21 | 2 | 18 | 4 |
| 26–30 | 42 | 6 | 22 | 2 | 20 | 4 |
| 31–35 | 37 | 7 | 16 | 2 | 21 | 5 |
| 36–40 | 16 | 4 | 11 | 2 | 5 | 2 |
| 41–45 | 27 | 9 | 14 | 4 | 13 | 5 |
| 46–50 | 33 | 15 | 19 | 7 | 14 | 8 |
| 51–55 | 30 | 4 | 22 | 3 | 8 | 1 |
| 56–60 | 34 | 9 | 18 | 5 | 16 | 4 |
| 61–65 | 24 | 5 | 12 | 3 | 12 | 2 |
| 65–70 | 14 | 9 | 13 | 8 | 1 | 1 |
| 71–75 | 24 | 15 | 14 | 11 | 10 | 4 |
| >75 | 21 | 12 | 7 | 4 | 14 | 8 |
| Total | 428 | 107 | 238 | 52 | 190 | 51 |