Review



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# Beyond the Gut: Unveiling Methane's Role in Broader Physiological Systems

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**Abstract:** Recent interest in breath methane has demonstrated considerable growth, with a 400% increase in publications per year between 2010 and 2020 compared to the previous decade. This surge has been facilitated by advancements in measurement techniques that have improved both the precision and ease of breath methane analysis. Consequently, there has been a growing appreciation for both the routes of production as well as the physiological effects of methane within the human body, shifting from a perspective of methane as an end-product of GI microbiota to viewing it as a potentially biologically active molecule with exciting potential implications for endogenous processes. The breath methane field stands at a pivotal juncture, where new technologies enable real-time, repeated measures of breath methane for the first time, paving the way for novel insights into personalized methane levels and their interplay with health. This review explores the origins, physiological effects, and measurement techniques of breath methane, highlighting potential pathways for future research.

**Keywords:** breath methane; methane physiology; methane diagnostics

## Introduction

Methane has long been regarded as having significance primarily as a greenhouse gas, however, it also plays crucial roles in human physiology. Traditionally, it was believed that methane is primarily produced by methanogenic archaea in the gastrointestinal tract of certain individuals. This methanogen activity not only influences digestive processes but has been reported to impact broader physiological functions, including immune modulation and oxidative stress responses. Recent studies have unveiled additional potential sources of methane production, challenging these traditional views that solely attribute its origins to gut microbiota. Understanding methane's diverse origins and physiological effects is thus pivotal for unraveling its implications for health and disease.

In this review, the origins, physiological effects, and clinical implications of methane are explored in human physiology. By evaluating current research findings, we aim to better understand the mechanisms underlying methane production as well as the potential impact of these processes on gastrointestinal function, immune modulation, and metabolic pathways.

## Sources of methane in human breath

### *Gut Microbiome*

During the typical metabolism of dietary and endogenous components in the large intestine the gut microbiome can produce many volatile compounds. Some of these have seen widespread interest, including the short-chain fatty acids (SCFAs) acetate ($C_2$), propionate ($C_3$) and butyrate ($C_4$). These SCFAs are normally produced at a ratio of around 60:20:20 with around 5-600 mmol produced per day[1–3]. However, these SCFAs form a relatively minor part of the ~0.2-1.5 L of gas produced per day by the gut microbiota of most healthy people[4–6]. Instead the gases that make up the majority of this volume are hydrogen ($H_2$), carbon dioxide ($CO_2$), and methane ($CH_4$), contributing more than 99% of the intestinal gas volume[7]. These SCFAs form part of the final 1%, which also includes sulfur-

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containing trace gases, such as hydrogen sulfide ($H_2S$), methanethiol ($CH_3SH$), and dimethyl sulfide ($(CH_3)_2S$)[8].

The gastrointestinal (GI) microbiota, encompassing a diverse community of microorganisms inhabiting the human gut, constitutes the oldest and most extensively studied source of methane production in humans. Products of the gut microbiome are well known to be affected by a range of factors, including both internal such as composition, as well as external such as diet and age[9].

At a composition level, methanogenic archaea are recognized as the primary producers of methane through anaerobic metabolism. These methanogens utilize the methylotrophic pathway, reducing $CO_2$ with $H_2$ or formate to form $CH_4$. This process occurs primarily in the colon and to a lesser extent in the small intestine. There is comparatively little diversity regarding specific methanogen species, as illustrated in Table 1. The predominant species (across both healthy and diseased states) is *Methanobrevibacter smithii* with *Methanobrevibacte stadtmanae* occurring to a lesser extent[10–12]. The levels of these archaea can be seen to reflect levels of methane production, with the microbiomes of people classed as high methane emitters ($CH_4$ > 5 ppm) characterized by a 1000-fold increase in *M.smithii* [13].

**Table 1.** Illustrating the four key archaeal species associated with methane production within the GI tract.

| Phylum | Genus/Species | Gas | Reference |
|---|---|---|---|
| Euryarchaeota | *Methanobrevibacter Smithii* | $CH_4$ | Weaver et al., 1986 |
| | *Methanosphaera stadmanae* | $CH_4$ | Fricke et al., 2006 |
| | *Methannobrevibacfter oralis* | $CH_4$ | Scanlan et al., 2006 |
| | *Methannomassiliicoccus luminyensis* | $CH_4$ | Nkamga et al., 2017 |

Exogenous dietary consumption can also affect methane production. This can occur directly, such as through modulation of Methanobrevibacter levels, with studies demonstrating that Methanobrevibacter levels are negatively correlated with the intake of total fat, saturated fat, and omega-3 fatty acids[13]. Diet can also affect methane production indirectly by affecting levels of methanogen substrates. This can be observed in the case of vitamin B12 deficiency which can be linked to altered methane production through the modulation of formate availability[13].

Once produced, methane is able to diffuse across the gut mucosa into the portal circulation where it undergoes gas transfer in the alveolar space and is subsequently exhaled[14]. It is estimated that 20-50%[15,16] of the methane produced in the gut is excreted via exhaled breath. This allows the measurement of methane in breath samples to serve as a non-invasive method used clinically to assess GI health and diagnose methane-related disorders. Elevated methane levels are used as diagnostic markers for conditions such as intestinal methanogen overgrowth-small intestinal bacterial overgrowth (IMO-SIBO). IMO, unlike traditional SIBO, represents the overgrowth of methanogenic archaea rather than bacteria. This overgrowth can occur in either the colon or the small intestine, often requiring unique antibiotics to treat. Of note, there are reported physiological differences between methane-positive and methane-negative SIBO, with methane-positive SIBO correlating with delayed small bowel transit, and colonic transit compared to methane-negative SIBO[17] which will be discussed further in this review.

The threshold for what constitutes a positive IMO result varies. While a level of methane gas greater than 10 ppm is generally considered indicative of IMO[18], some classifications consider baseline levels greater than 1-3 ppm as elevated[19]. A significant challenge for these assessments is the reliance on a single timepoint measurement. Recent developments in the field of breath methane monitoring, such as the OMED device[20], allow for longitudinal measurements of breath methane, and the establishment of a personalized baseline to guide further testing.

The prevalence of methanogens in the gut is also susceptible to external factors, such as increases with age, suggesting an age-related shift in the microbial ecosystem, which favors methane

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production. This trend has been observed in several studies[21–23], indicating that age can be a significant factor in the composition and function of the gut microbiota.

*Human Endogenous Processes*

In addition to the relatively well-characterized production of methane by methanogenic archaea in the gut, emerging data has demonstrated across *in vitro* to *in vivo* settings supporting evidence that there are additional host derived endogenous sources that may contribute to measurable methane levels, particularly in settings of stress. These studies suggest that elevated levels of reactive oxygen species (ROS) can produce methane as illustrated in Figure 1. This process relies on the Fenton reaction to generate hydroxyl radicals from hydrogen peroxide ($H_2O_2$), which can subsequently oxidatively demethylate methylated sulfur or nitrogen compounds (e.g., methionine, dimethyl sulfoxide, or trimethylamine).

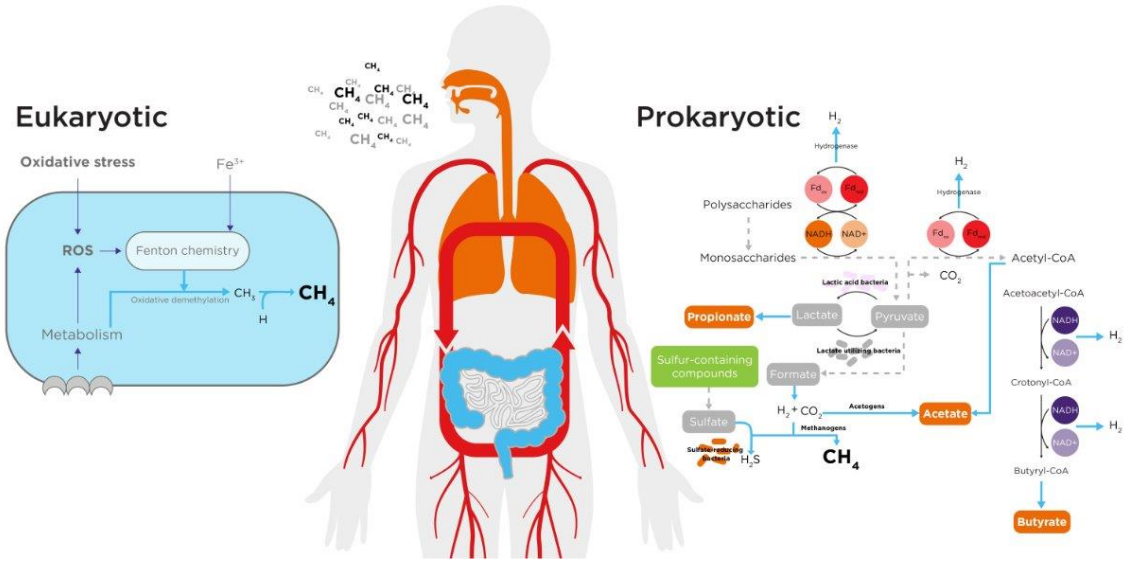


**Figure 1.** Demonstrating the proposed routes for both exogenous and endogenous methane production. Exogenous methane production relies on the generation of methane by prokaryotic species in anaerobic conditions within the GI tract. Endogenous methane production by contrast relies on oxidative demethylation of methane containing moieties by reactive oxygen species.

Methane production as a result of oxidative stress *in vitro* has been demonstrated, with methane being produced after the application of 2M $H_2O_2$ to a variety of endogenous compounds. Of these compounds choline chloride was the most potent, generating 4-25 µM methane, but methionine and ethanolamine were also capable of producing measurable amounts of methane[24,25]. Of note, in these settings compounds that generated appreciable concentrations of methane also demonstrated some anti-oxidant activity, with reductions in the generation of ROS[25].

These findings can be extended from these *in vitro* settings to an *ex vivo* setting as demonstrated through the application of oxidative stress (induced via $H_2O_2$ and ascorbic acid) to isolated mitochondria. In this setting, oxidative stress induced methane production, the rate of which was proportional to both the level of oxidative stress as well as to the quantity of mitochondrial protein added. Levels of production at 100 mM $H_2O_2$ and pH 7.4 were 0.3 nmole methane in 60 minutes per mg mitochondrial protein[25] and the application of catalase prevented these effects, lending further weight to oxidative stress being required for this methane production[24]. Using the liver as an example organ, and extrapolating rates based on this observation one can obtain approximate estimated rates of methane production between 58-118 µmole from a liver in 60 minutes (taking the average liver weight of between 968-1860g[26], of which around 20% is mitochondria[27]). Making the approximation of negligible loss during this time, and a blood volume of 5L, one could estimate blood concentrations

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between 11.6-23.6 μM placing values within an order of magnitude of predicted levels in blood of around 2 μM in blood under normal conditions[28].

Taking this one step further, moving from isolated mitochondria to a cultured endothelial cell setting, Adamczuk *et al.* demonstrated that cultured cells produce methane even at baseline (2 nmol/mg), and that this can be increased by exposure to agents associated with elevations in ROS[29] such as sodium azide ($NaN_3$) (15 nmol/mg) or 2,4-dinitrophenol (DNP) (23 nmol/mg)[25]. This phenomenon has been observed both in mammalian cells, as well as plant cells, with grapevine demonstrating a similar increase in methane production following exposure to $NaN_3$ [30].

The final piece of the puzzle, translating these *in vitro/ex vivo* effects to an *in vivo* setting was first provided by Tuboly *et al.* who demonstrated an elevation in methane production following $NaN_3$ administration[31]. This was further supported by evidence from Keppler *et al.* first identifying the production of methane from leaves (0.3 ng/g dw)[32] and then from humans, demonstrating methane release from radiolabeled methionine both in blood and from the skin (headspace)[33]. Two additional aspects to these experiments stand out as providing potential additional insights into this phenomenon, firstly Tuboly *et al.* demonstrated that this elevation in methane could be prevented by co-administration of α-glyceryl phosphorylcholine, a protectant against lipid peroxidation, further supporting that methane production is linked to oxidative stress. Secondly, both Tuboly *et al.* and Keppler *et al.* took steps to remove microbial-linked methane production, either via the administration of rifaximin or UV irradiation (respectively). There is therefore good evidence that observed effects were due to extra-microbial production of methane.

The relevance of these findings to human physiology had its first indications in 2013, when Tuboly *et al.* demonstrated that administration of LPS in mice (an acute sepsis setting) corresponded to a 2-3 fold increase in methane production[34]. These data suggested that infection, and associated elevations in inflammation/oxidative stress, may provide a real-world setting for elevations in non-microbial methane production. Whilst further work validating this finding in sufficiently powered studies is required, there are some preliminary indications that this may hold for human infection as well, with Keppler *et al.* demonstrating elevations above baseline (on a similar order of magnitude to Tuboly) in response to COVID-19[35].

## Detection and Measurement of Methane in Breath

### *Breath Sampling and Analytical Techniques*

Breath sampling techniques largely fall into 3 separate categories: direct exhalation into collection bags, breath sampling via tubes, and real-time breath analysis with direct analyzers. These sampling techniques each have advantages and disadvantages[36], as summarized in Table 2. In a similar fashion to sampling, there are a number of possible analytical techniques for methane detection, including GC-FID (Gas chromatography-flame ionization detection), IR (infrared spectroscopy) and MOS (metal oxide sensors) as summarized in Table 3. The relative strengths of each collection, and analytical method, are key considerations when designing a study, especially one investigating personalized methane levels as highlighted in Figure 2.

**Table 2.** An overview of collection methods for breath methane analysis.

| | **Collection bags** | **Tubes** | **Handheld real-time analyzer** |
|---|---|---|---|
| **Overview** | One of the most common methods used, where patients exhale directly into a bag. | Uses tubes through which the patient exhales. Tubes are then sealed awaiting analysis. | Involves the use of portable devices that analyze breath in real-time with no need for separate sample collection/storage |
| **Different forms** | Mylar bags (made from a type of polyester film impermeable | Vacuum tubes (draw in the breath sample | The OMED device. |

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| | | | |
|---|---|---|---|
| | to gases) or Tedlar bags (made from PVF). | automatically) or glass/plastic tubes. | |
| **Procedure** | Patients take a deep breath and exhale completely into the bag. The bag is subsequently sealed for later analysis. | The patient exhales through a mouthpiece connected to the tube, which is then sealed after collection. | Patient breathes directly into the analyzer through a mouthpiece. Gas concentrations are fed back in real-time. |
| **Advantages** | Simple and cost-effective. | Easy to use and transport. | Provides instant results and allows for simple repeat measures. |
| **Considerations** | Bags can be challenging to handle post-collection and can lead to sample contamination/loss. | Tubes may require specific storage to prevent sample degradation. | Device calibration is crucial for accurate readings. |

**Table 3.** An overview of analytical methods for breath methane.

| | **GC-FID** | **IR** | **MOS** |
|---|---|---|---|
| **Overview** | GC-FID combines GC for separation of components of a breath sample and flame ionization detection for methane quantification. | IR measures the absorption of IR light by methane to determine its concentration. | MOS detect gases based on changes in electrical resistances of a metal oxide sensor when it interacts with methane. |
| **Advantages** | High sensitivity, high specificity and quantitative analysis. | Real-time analysis and easier than GC-FID. | Cost-effective, durable and provides real-time analyses. |
| **Limitations** | Complexity and cost, requiring sophisticated equipment and trained personnel. Time-consuming, requiring lab prep. | Unlikely to reach the level of GC-FID for low concentrations. Subject to interference from other gases/water vapor if not properly calibrated. | Unlikely to reach the level of GC-FID for low concentrations. Requires appropriate calibration. |

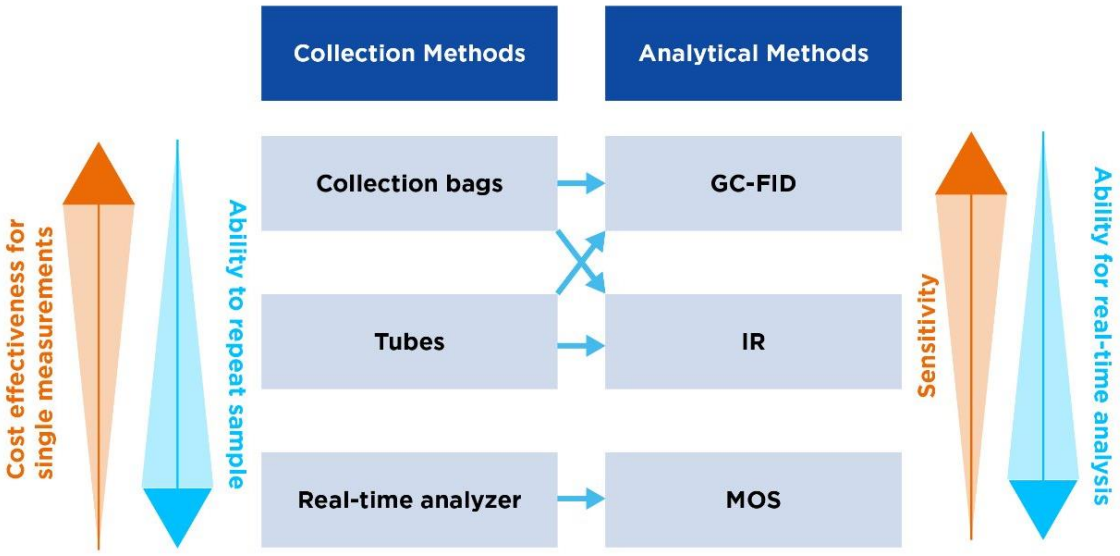


**Figure 2.** Summarising the advantages and limitations of the collection and analytical methods used.

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**Potential Effects of Methane**

With both the potential routes for methane production considered, as well as the value and mechanism for testing methane concentrations on breath, the next section will focus on the local and systemic potential effects of methane, with an emphasis on delineating correlation from causation. The conclusions are summarized in Figure 3.

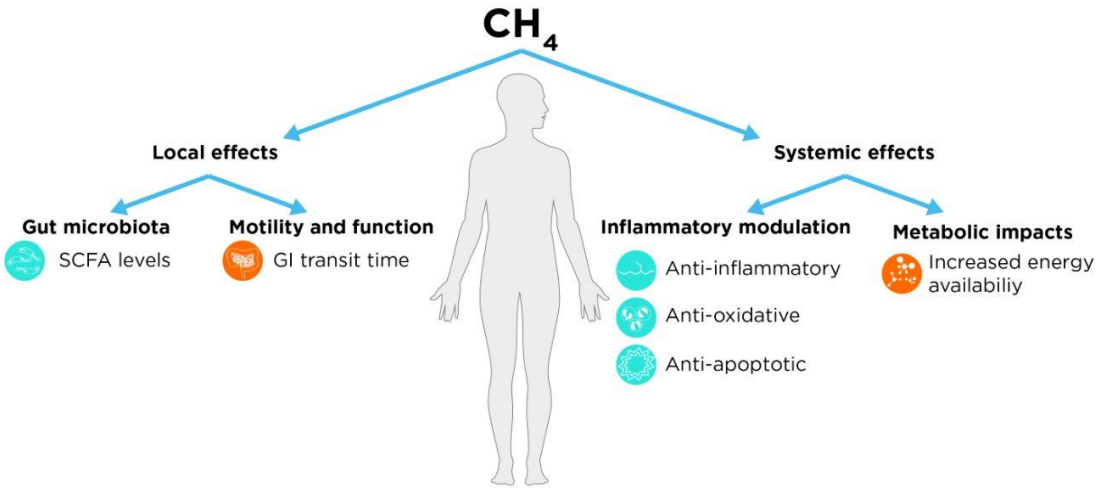


**Figure 3.** Summarising both the local and systemic effects proposed for methane within the field. Green icons indicate an effect with positive associations, orange icons indicate an effect with negative associations.

*Local (GI) Effects*

Motility and Function

Data from animal studies suggests that methane can have a profound impact on GI transit time. In experimental settings, exogenous methane gas applied *ex vivo* has been shown to directly inhibit intestinal transit by 59% in dogs[37] and decrease peristaltic velocity in guinea pigs[38]. This can be translated to observations in human populations, with methane levels correlating strongly with slower intestinal transit times[39–43]. Work from Soares *et al.* supplemented these findings with additional detail, demonstrating that total colonic transit time averages 80.5 hours in methane producers compared to 61.0 hours in non-methane producers, and providing a breakdown of these transit times. This revealed substantial delays in specific sections of the colon: 17.5 hours versus 10.5 hours in the right colon, 29.5 hours versus 10.5 hours in the left colon, and 31.5 hours versus 27.0 hours in the rectosigmoid region[44].

These data universally support a link between elevated methane production and increased GI transit times, however data so far has been limited to correlations. Work from Pimentel *et al.* extended these findings towards an *in vivo* model through direct administration of methane (via intestinal fistulae). This model removed other potential confounders (such as dietary effects) from human studies, demonstrating a 59% increase in transit times in the presence of methane (at a concentration equivalent to a breath methane level of 50 ppm)[37]. The work of Park *et al*. provided the first potential mechanism to explain these observations. Namely, they demonstrated in studies involving the infusion of methane under electrical field stimulation, that methane increased the amplitude of ileal contractions across all tested frequencies (1-16 Hz)[45].

Interaction with Gut Microbiota

The gut microbiome is inherently synergistic, and so it is reasonable to hypothesize that in methane producers, with high methanogenic archaeal levels, there may be other changes to gut

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microbiota composition, or products. Indeed, data from Kumpitsch *et al.* identified that high-methane producers (>5 ppm) demonstrate a significantly higher alpha diversity and substantially different microbiome composition compared to low-methane producers[13]. Methane-emitting microbiomes were significantly associated with Euryarchaeota (Methanobrevibacter) as well as signatures of Christensenellaceae R7 group, Ruminiococcus/Ruminococcacaeae, Holdemanella and the Eubacterium ruminatium groups[13], groups which are associated with dietary fiber degradation. These data supports findings that when Christensenella and Methanobrevibacter are co-grown *in vitro* they form dense flocs whereby the $H_2$ generated by the Christensenella supports $CH_4$ production by Methanobrevibacter. In this setting SCFA production is shifted more towards acetate and away from butyrate[46]. Supporting this, high methane producers also show increased levels of formate and acetate in the gut, with these metabolites strongly correlated with dietary habits such as vitamin, fat, and fiber intake.

This association has been investigated *in vivo* by a number of groups, with seemingly conflicting results. Early work in 1984 found no significant difference between the levels of SCFAs in the feces of methane producers compared to non-methane producers[47], which was corroborated by serum findings in 1998[48]. Subsequent work however found significant elevations in both fecal and serum SCFA levels (particularly in propionate, formate, and acetate)[13,49,50]. Finally, Fernandes *et al.* identified a negative correlation between breath methane levels and fecal SCFA levels in patients[51].

Whilst these results at first glance appear conflicting, when the impact of confounders is considered, namely those that may independently correlate SCFA and breath methane levels (e.g. sex, age, or diet) a trend emerges. When these results are considered within the context of age, which is known to correlate with both increased methane production[52] and decreased SCFA levels[53], we can observe that studies with an age mismatch[48,51] demonstrate no change/a decrease in SCFA levels with elevated methane, whilst those that correct for age[13,50] clearly demonstrate elevations in SCFA levels with elevated methane levels. It is noted that, whilst considerations of microbiome level implications of methanogen presence must be considered, these findings are supported from a purely biochemical standpoint whereby removal of $H_2$ by methanogens would be expected to modify, and potentially increase SCFA production through end-product removal[54,55].

*Systemic Effects*

Much of the work around methane's potential bioactivity, and the focus of this review so far has been around the potential local effects of methane in the GI system. However, data have emerged largely via the exogenous application of methane, supporting a potential systemic effect. This includes potential activity as an anti-inflammatory, anti-apoptotic, antioxidant, or metabolic regulatory molecule. In this section, we will focus on some of these effects and their context.

Inflammatory Modulation

The most common systemic effect attributed to methane is its potential as a cytoprotective compound. Studies have associated methane with three potential cytoprotective effects.

1. Anti-inflammatory effects, that manifest as reductions in TNF$\alpha$, IL-6, and IL-1B levels following intraperitoneal (IP) dosing of methane-rich saline (MRS). These effects appear to be mediated via IL-10, and upstream through the PI3K-AKT-GSK-3B pathway[56–66].
2. Anti-oxidative effects, presenting as reductions in MDA or 8-OHdG levels, as well as the prevention of loss of antioxidant activity (SOD/CAT levels)[58–65,67–70].
3. Anti-apoptotic effects, manifesting as reductions in TUNEL staining, as well as reduced caspase 3/9 activation[59–63,67,68,71].

These effects have been observed across a wide range of diseases, including ischemia/reperfusion injury[67–69], inflammatory disease[56–58,72], neuronal disease[65,73] and others.

These studies generally leverage methane-rich saline (MRS) (at 0.99 mM), first used by Zhouheng *et al.*[59], with doses between 0.5-20 ml/kg demonstrating efficacy (with rough end-dosage of around 9 μmol/kg). Assuming total blood volume of a rat at ~64 mL/kg, full displacement of methane into the blood, and minimal methane loss, this would be expected to give ~ 140 μM, or

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around 70x the levels expected from microbiome production and 14x levels expected from endogenous production during sepsis. Therefore, the dose-dependent observation of effects in these studies brings into question comparisons between observations within these MRS dosing experiments and their impact in a real-world setting.

Metabolic Impacts

There has been a focus on gut dysbiosis within obesity for over 20 years now, and early work from Turnbaugh *et al.* demonstrated that the gut microbiomes of obese (*ob/ob)* mice have increased representation of archaea compared to their control weight (*ob/+)* littermates[74]. This was attributed to an increased ability to degrade polysaccharides, a phenomenon which was demonstrated to be transmissible, resulting in greater weight gain in lean germ-free mice following fecal microbiome transplant[74]. Supporting increased energy harvesting driving this phenomenon, data demonstrated that co-colonization of mice with the symbiotic pairing of *M.smithii* and *B.thetaiotaomicron* resulted in significantly greater adiposity compared with colonization of either organism alone[55].

Given the known, and well-demonstrated association of dysbiosis with metabolic syndromes[75], data surrounding correlations between methane and BMI must be approached cautiously. Despite this, there are two effects of methane that could be expected to contribute towards additional weight gain, and therefore provide a rationale for a positive correlation between BMI and methane production. Namely, slowed GI transit time, providing greater time for nutrient absorption across the GI tract, and increased production of SCFAs increasing calorie availability from food (responsible for ~10% of calorie availability in humans[76]).

Translating this to a real-world setting, the majority of data support a correlation between elevated methanogen presence, and therefore breath methane levels, and a higher BMI. This has been demonstrated at baseline in obese patients, where those with breath $CH_4$ > 3 ppm display a BMI ~7 higher than those without[77] as well as in obese compared to lean children[78], and, although not reaching statistical significance (potentially due to study power) also by Fernandes *et al*[48]. Of note, in addition to baseline levels, correlations have also been observed between elevated methane levels following a lactulose challenge and BMI, firstly by An *et al.*[79] and also by Matur *et al.* who demonstrated a correlation only if both breath methane and hydrogen were elevated[80].

Despite this evidence for a positive correlation between methane and BMI there is some disagreement amongst the field. Ozato *et al.* found no significant difference between methane and BMI, but demonstrated a lower visceral fat area in methane producers vs non-producers[81]. Wilder-Smith *et al*. even found that people who had detectable methane in their breath following a lactose/fructose challenge had a lower BMI compared to non-methane producers[82]. Of note, a key difference here was that Wilder-Smith *et al.* were the only group to study specifically patients with a functional gut disorder (irritable bowel syndrome, as diagnosed by Rome III criteria). On balance, the data above suggests that in the general population, higher breath methane levels are associated with a higher BMI, however, in a subset of people with functional gut disorders this may not hold to be true, potentially due to the presence of additional factors that drive methane levels.

Following from this, methane producers also had worse glucose tolerance compared to non-methane producers[83], and pharmacologically reducing breath methane (through antibiotic use) in obese patients improved glucose tolerance[84]. Patients who were positive for both methane and hydrogen also displayed reduced (prorated) percentage changes in BMI following bariatric surgery[85]. Whilst these data suggest that methane correlates with increased BMI and altered glucose handling, there is also data suggesting an overall potentially cardioprotective effect of methane. Wu *et al*. found that the transition from pre-diabetes to type 2 diabetes was associated with a downregulation of bacterial methanogenesis[86]. Ozato *et al*. also found that higher methane levels were associated with decreased visceral fat area, a key contributor for cardiometabolic risk[81]. Finally Laverdure *et al.* found that, in an *in vitro* setting, GLP-1 secretion could be stimulated by methane[87].

*Potential Role of the Vagus Nerve and Cholinergic Pathway*

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There is limited data focused on elucidating the proposed mechanisms by which methane may elicit both these local and systemic effects. An emerging potential mechanism however involves its interaction with the vagus nerve. The vagus nerve, the longest and most extensively distributed autonomic nerve, originates in the brainstem and extends through the neck into the thoracic and abdominal cavities. This nerve carries both motor and sensory fibers, providing innervation to numerous systems and influencing critical aspects of human physiology, including heart rate, blood pressure, sweating, digestion, and even vocalization[88].

Evidence supporting the role of methane in modulating vagal nerve/cholinergic pathway activity was first demonstrated by Park *et al.* who identified that the application of tetrodotoxin or atropine can abolish methane-induced increases in contraction amplitude in guinea pig ileal muscle strips[45]. It can be noted that whilst not directly investigated further, there is data supporting that this interaction may occur indirectly, via associated changes in serotonin production[89]. Supporting the implication of the vagus nerve, the effects of methane appear to correlate closely with the outcomes associated with vagal nerve and cholinergic pathway activation. This relationship is evident in the shared anti-inflammatory effects[56–66], alterations in heart rate[90,91], modifications in gastrointestinal transit time[37–45], and the secretion of pancreatic polypeptide following sham feeding[92], as summarized in Figure 4 below.

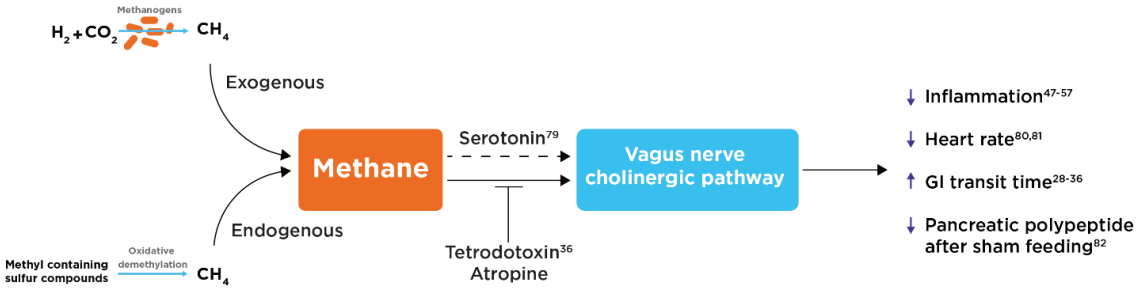


**Figure 4.** Highlighting the potential role of the vagus nerve/cholinergic pathway in mediating the effects of methane, with focus on potential regulators of this pathway, as well as shared effects.

## Clinical Implications and Future Research

Breath methane measurements have received attention in the past as part of their use in the clinical diagnosis of various gastrointestinal conditions, such as SIBO. These tests largely involve fasting (to minimize baseline sample variance) followed by administration of a challenge substrate (e.g. lactulose, glucose, or fructose) and subsequent breath measurements at timed intervals.

These challenge tests help to pinpoint changes in methane levels that specifically originate in the GI tract, allowing breath methane to be used as a test for SIBO without interference from other potential sources of methane. In contrast, using endogenously generated methane as a biomarker is more complex. Here, longitudinal measurements and comparisons to an individual's baseline could support breath methane as a reliable biomarker by minimizing or controlling for other potential confounders.

This shift has been observed in recent years across medical fields, with tests moving away from static measures towards more dynamic, continuous and comprehensive monitoring. This has been reflected in assays such as blood glucose monitoring, where technological advancements have brought with them a shift from static fingerstick blood glucose readings to the widespread use and adoption of continuous glucose monitoring. The same can be envisaged for breath methane readings, whereby technological advancements, such as the development of the OMED device, can allow for affordable, at-home, real-time monitoring of breath methane levels.

The field is positioned at an exciting time whereby for the first time there is the potential for population-based longitudinal studies on breath methane levels and so the opportunity to better understand the role of breath methane in health beyond the GI system.

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## Conclusion

Over the past 20 years, research on methane measurement in human physiology has grown rapidly, with 359 publications from 2005-2014 and 948 from 2015 to mid-2024. As the field expands, so does the understanding of methane's physiological associations.

Methane's roles in human physiology are diverse and complex, spanning from GI health to systemic inflammatory and oxidative responses. As research progresses, the potential of methane as both a diagnostic marker and a therapeutic target becomes increasingly evident. Addressing the current research gaps and standardizing methodologies will be pivotal in harnessing the full clinical potential of breath methane measurements.

The advent of handheld, real-time breath methane monitors has enabled large-scale, longitudinal data collection. This opens the door to population studies on the role of breath methane in infection markers, correlations with lifestyle factors (e.g., alcohol intake, exercise, BMI, and heart rate), and how methane levels change with shifts in the gut microbiome, such as after antibiotics or other GI treatments. Exploring methane's origins, mechanisms, and health effects promises new insights for diagnostics and treatments.

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