# BIOLOGICAL RELEVANCE OF INTESTINAL GASES IN HEALTY HUMANS

Arturo Tozzi
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas 76203, USA
1155 Union Circle, #311427, Denton, TX 76203-5017 USA
tozziarturo@libero.it

Raffaele Minella
Primary Care Pediatrician, ASL NA3 Sud, Torre del Greco, Italy
raffaele-minella@libero.it

## ABSTRACT

Intestinal gases are usually discarded as physiologically inert, useless sub-products of colonic fermentation that must be expelled to prevent discomfort and meteorism. Starting from the observation that many living beings use exogenous and/or endogenous gases to attain evolutionary benefits, we question whether intestinal gases in healthy humans could have underestimated physiological effects, either intestinal or extra-intestinal. We examine gaseous volume, composition, source and local distribution in proximal as well as distal gut, providing extensive data that may serve as reference for future studies. We analyze each one of the most abundant intestinal gases and describe their diffusive patterns, active trans-barrier transport dynamics, chemical properties, intra-/extra-intestinal metabolic effects mediated by intracellular, extracellular, paracrine and distant actions. Discussing the physical properties of the whole intestinal gaseous mixture, we illustrate how changes in volume/pressure can be generated by two different mechanisms, namely, physical muscular gut contraction and biological colonic fermentation, with quite different metabolic outcomes. The experimental gas laws suggest that the gaseous exchanges between lumen and bloodstream are impaired by muscular contraction and improved by muscular relaxation. In turn, the surface-area-to-volume ratio suggests that the gaseous exchanges are impaired by microorganismal overproduction and improved by microorganismal reduction. Further, theoretical stochastic approaches from probability theory indicate that the non-turbulent, random paths of gas molecules inside colonic haustra do not homogenously spread over the whole mucosal surface. This means that the intestinal area available for lumen/blood metabolic exchanges is much less than expected not just in disease states, but also in healthy individuals.

**Keywords**: nitrogen; oxygen; carbon dioxide; methane; hydrogen sulfide; sulfur dioxide; cyanide; gasotransmitter; Boyle's law.

## INTRODUCTION

Many living beings make use of the mechanical and/or chemical properties of gases to increase their likelihood of survival. For example, planktonic heterotrophic prokaryotes (Staley 1980) and certain Haloarchaea (Völkner et al., 2020) modify their overall cellular density by inflating and deflating gas-filled cytoplasmatic micro-structures. These structures, termed gas vesicles, allow microorganisms to float vertically and reach different depths in water columns (Pfeifer 2014), preventing halophilic microorganisms' osmotic shock in high-salted environments and malfunctioning of light-driven proton pumps (Oren 2012; Talapatra et al., 2013). While gas vesicles are highly permeable to gases (i.e., oxygen, nitrogen, hydrogen, carbon dioxide, carbon monoxide, methane and even perfluorocyclobutane) that diffuse from the environment to the cytoplasm in a passive way (Pfeifer 2012), gases can be also produced in an active way by other living organisms. For instance, carbon monoxide is actively produced by the aeriform cells (Larimer et al., 1962) of the deadly Siphonophore Physalia physalis (namely, the Portuguese man of war), which lives at the sea water/air interface and uses an enlarged float filled with gas as a sail to travel by wind (Munro et al, 2019). Exchanges of exogenous gases are crucial for respiration in plants (Ishizaki 2015) and animals supplied with circulatory systems. To provide an example familiar to us, the human body introduces $O_2$ and expells $CO_2$ through the lungs, making chemical use of the inhaled atmospheric gases to attain aerobic cellular respiration.
In this paper, we will focus on one of the major gaseous reservoirs in the human body, i.e., the intestine. Although emphasis has been traditionally given to the gastrointestinal complaints caused by "excess gas" such as belching, flatulence, abdominal bloating and distension, emerging evidence suggests that intestinal gases bring intestinal and extra-intestinal benefits. We will describe the underrated physiological roles of the gases that can be found in the healthy human intestine, trying to answer the following questions:

1) Which types of exogenous and exogenous gases can be found in the human intestine?

2) How are the endoluminal gases generated and/or dispersed?
3) Do intestinal endoluminal gases have physiological roles?

We will proceed as follows. At first, we will analyse the volume, composition, local distribution and source of intestinal gas in healthy human individuals. Then, we will describe the intra-/extra-intestinal physiological roles of every one of the most abundant intestinal gases. Lastly, we will tackle the unexplored issue of the general physical properties of the intestinal gases and their intraluminal/extraluminal effects, stressing that gaseous mixing, transport and trans-barrier exchanges are strictly correlated with the intestinal motor patterns.

## INTESTINAL GASES: GENERALITIES

Quite variable gaseous concentrations can be measured in intestinal lumen, faeces and breath, depending on the quantity and quality of the ingested food, the host/bacterial metabolic processes and the technique used for the assay (Blachier et al., 2021). The total weight of human intestinal gas amounts to just a few grams, while the total gaseous intestinal volume can be quantified, with a certain approximation, in ≈100-500 $cm^3$ (Badley et al., 1993; Schiller et al, 2005; Wang et al., 2021). The source of intestinal gas is twofold (Roccarina et al., 2010):

1) Exogenous swallowed air.
2) Endogenous gases produced by:
   a) Intraluminal fabrication by the host organism.
   b) Intraluminal fabrication by commensal microorganisms.
   c) Trans-membrane diffusion from bloodstream into the intestinal lumen.

The human intestinal gas' composition varies along the different gastrointestinal tracts. See the **Table** for quantitative details. Swallowed air is the major source of nitrogen ($N_2$) and oxygen ($O_2$), while carbon dioxide ($CO_2$) comes both from swallowed air and intestinal production. A large amount of intestinal gases is generated by microorganismal fermentation in the human colon from the dynamic crosstalk between microbiota and host metabolism (Singhal and Shah, 2020). These gases include carbon dioxide ($CO_2$), hydrogen ($H_2$), methane ($CH_4$) (Levitt 1971; Wang et al., 2021). The sum of $N_2$, $O_2$, $CO_2$, $H_2$ and $CH_4$ accounts for more than 99% of the expelled intestinal gas (Roccarina et al., 2010). The remaining 1% is composed of various odoriferous substances including hydrogen sulfide ($H_2S$), sulfur dioxide ($SO_2$), sulphur-containing mercaptans, ammonia ($NH_3$), indole, skatole, volatile amines, short chain fatty acids, plus acetic acid, propanoic acid, butyric acid, isobutyric acid, pentanoic acid, valeric acid, etc (Heresbach et al., 1995; Montalto et al., 2009; Wang et al., 2021). The gaseous production varies according to the age. To provide an example, the intestinal gases and faecal short-chain fatty acids produced by preterms' gut microbiota increase during the first 4 weeks of life (Wang et al., 2021). The dietary uptake deeply affects the amount of some intestinal gases depends on. In particular, some fermenting foods are known to increase intestinal gas production (Scaldaferri et al., 2013):

a) Non-absorbable carbohydrates such as lactulose and mannitol.
b) Incompletely absorbed carbohydrates such as lactose, fructose, sorbitol.
c) Sulfur-rich foods such as beans, pork, onions, cabbage and cauliflower.

A we shall see later, some gaseous substances can be detected after carbohydrate load using breath tests as markers of colonic carbohydrate fermentation (Wang et al., 2021).

| | **Atmosphere** | **Stomach** | **Colon** | **Flatus** | **Breath** |
|---|---|---|---|---|---|
| **Nitrogen ($N_2$)** | 78 | ≈78 | ≈65 | ≈59 | 78 |
| **Oxygen ($O_2$)** | 21 | ≈15 | ≈2 | ≈4 | 16 |
| **Carbon dioxide ($CO_2$)** | 0.04 | ≈7 | ≈10 | ≈9 | 4 |
| **Hydrogen ($H_2$)** | Traces | Traces | ≈3 | ≈21 | Traces |
| **Methane ($CH_4$)** | Traces | 0 | ≈15 | ≈7 | Traces |
| **Hydrogen sulfide ($H_2S$)** | Traces | 0 | Traces | Traces | Traces |
| **Sulfur dioxide ($SO_2$)** | Traces | Traces | Traces | Traces | 0 |

**Table**. Approximate percentage of different gases in various human anatomical districts. Intestinal gases may occur in widely different proportions in each bowel region, depending on manifold factors. Concerning blood gases, they are defined as the mixture of gases dissolved in the fluid fraction of blood or transported by carriers such as., e.g., haemoglobin and urea nitrogen (Walker et al., 1990). Data extracted from: Levitt (1971); Roccarina et al. (2010); Sahakian et al. (2010); Modesto et al. (2022).

**Gaseous exchanges between intestinal lumen and blood**. Most of the intestinal gases cross barrier between the intestinal lumen and the blood by means of passive mechanisms governed by the Fick's law of diffusion (Michenkova et al, 2021). In the human gut, the gradient between the gaseous mixture's partial pressures in the intestinal lumen and in the bloodstream dictates the direction of gases exchanges. The formula provided by Forster (1968) summarizes the physiological basis of passive gas exchange in the intestine:

$$\frac{d(VP_L)}{dt} \frac{1}{P_B - 47} = -k\,(P_L - Pc)$$

Where V is the total intraluminal gaseous volume, $P_L$ and Pc are, respectively, the partial intraluminal and extraluminal gaseous pressures, $P_B$ is the barometric pressure and 47 is the pure pressure of water at 37°. K is a transfer coefficient depending on the type of gas, e.g., $N_2$ diffuses through the epithelial intestinal barrier slowly than $O_2$ and $CO_2$.
The values of mesenteric perfusion and intestinal microcirculation contribute to gaseous exchanges. The total colonic blood flow is ≈18 mL/min per 100 g of colonic tissue, much lower in the muscularis layer (≈11mL /min per 100 g of muscularis tissue) than in the mucosa-submucosa (≈28 mL /min per 100 g of mucosal-submucosal tissue) (Hulten et al., 1976).
Since gases have been traditionally deemed to diffuse freely and passively, previous studies mainly focused on gaseous production/reactivity rather than diffusion/transport (Rodriguez-Grande and Konsman 2018). Still, the discovery of gas pores has raised the intriguing possibility of active cellular modulation of gas diffusion. Indeed, some gases can cross different compartments by means of transmembrane channels. Recent observations suggest that that the highly conserved and widely diffused intramembrane channels termed aquaporins transport not just water molecules, but also gases (Herrera and Garvin, 2011; Zhang et al., 2019). Aquaporin channels represent a major transcellular route for water and gas transport also in the gastrointestinal tract (Zhu et al., 2016). Different aquaporin isoforms have been found in the stomach, small and large intestine, everyone preferentially distributed in distinct gut segments and cell types (Liao et al., 2021). For instance, a water, glycerol and $H_2O_2$ transporting channel expressed in colonic epithelial cells, namely Aquaporin 3, affects epithelial tight junction integrity and permeability (Yde et al., 2021).

**Flatus' patterns**. As the healthy human individual generates 0.6-1.8 L of gas per day, it follows that discard must be continuous. The mechanisms of removal are manifold: microorganismal consumption, host consumption, absorption into the systemic circulation and ensuing expulsion mainly through the breath, belching, eructation and, above all, flatus (Hasler 2006; Montalto et al., 2009; Sahakian et al., 2010). The data concerning flatus' frequency and volume vary widely, with some subjects passing gas more often than others. The mean total volume ranges in different studies from ≈260 (Manichanh et al., 2013) to ≈705 ml/die, with an upper limit of 1800 (Tomlin et al., 1991). On their usual diet, subjects pass gas from ≈7 (Manichanh et al., 2013) to ≈10 daytime evacuations, with an upper limit of 20 (Furne and Levitt, 1996). The record of abdominal symptoms is rare in healthy subjects (≈0.4 discomfort/pain per day) (Manichanh et al., 2013). Gender, age, and methane production had no significant influence on flatus frequency and volume (Furne and Levitt, 1996). Flatus is produced also during the sleeping period, but the rate is significantly lower than the daytime (median: 16 and 34 ml/h, respectively) (Tomlin et al., 1991). Larger volumes of flatus are produced after meals. Fiber-free diet reduces the total daily volume, suggesting that fermentation gases make the highest contribution to normal flatus volume (Tomlin et al., 1991). The addition to the diet of 10 g/day of the nonabsorbable disaccharide lactulose increases flatus frequency to ≈19 times/die (Furne and Levitt, 1996). On flatulogenic diet, increased gas production leads to increase in number not just of gas evacuations (≈22/day), but also of abdominal symptoms (≈3 mean discomfort/pain per day) (Manichanh et al., 2013).

**Intestinal motility**. Gaseous mixing, transport and trans-barrier exchanges are strictly correlated with bowel movements. Gas is actively propelled by the inner circular and longitudinal musculature, moving along the gut independent of solids and liquids (Azpiroz 2005). Two main motor patterns have been detected, especially in the healthy human colon:

a) Peristalsis followed by propagation. Wave-like muscular contractions along the intestinal length drive strong fluid flows both forward and backward, with the average movement pushing from the proximal to the distal gut (Cheng et al., 2020). The following quantitative data are taken from Chen et al. (2017). **Simultaneous pressure waves** are the most prominent propulsive motor pattern, since their fast-propagating contractions lead to gas expulsion and anal sphincter relaxation. These transient increases in intraluminal pressure covering the entire colon length are characterized by ~30 episodes/h frequency, ~15 sec pressure duration, ~32 cm propagation distance, ~15 mmHg average amplitude, fast rate of rise up to ~20 mmHg in ~8 s, slower rate of return to baseline in ~12 s. Passing of intraluminal material takes a few seconds from one end of the colon to another, with speeds

up to 10 cm/s. Rhythmic low frequency of ~0.5–1 cycle/min occurs, suggesting the presence of a stimulus-dependent pacemaker, likely the interstitial cells of Cajal endowed in the deep muscular plexus (Sanders et al., 2006; Huizinga et al., 2021).

b) Segmentation followed by mixing and absorption. Non-propagating muscular contractions drive weak flows that lead to increased mixing (Codutti et al., 2022). The following quantitative data are taken from Chen et al. (2017). **Isolated haustral boundary pressure transients** are common, short-lasting pressure increases ranging in amplitude 5–230 mmHg, lasting 30–180 min and occurring simultaneously in many haustral segments spaced 4-5 cm apart, but not in adjacent segments. These pressure transients display a typical 3 cycles/min rhythmicity in the human colon, likely associated with electrical slow wave activity generated by the interstitial cells of Cajal endowed in the submucosal plexus (Huizinga et al., 2014). **Intra-haustral segmentation** activity is characterized by isolated pressure transients covering the size of an haustra, i.e., 3–5 cm. A distinctive, synchronized intra-haustral cyclic motor pattern at 3–6 cycles/min is exhibited. These waves occur for 2 to 10 min, 0–6 times /hour and abruptly alternate with erratic patterns resembling the small intestine' segmentation motor pattern. Their propagation at $2 \pm 1$ cm/s in antegrade or retrograde direction suggests once again the presence of a dominant pacemaker. The **antegrade** and **retrograde pressure waves** roughly display the same features: ~2/h frequency, ~20 seconds pressure duration, ~29 cm propagation distance, ~16 mmHg average amplitude and ~5 cm/s propagation velocity.

Intestinal transit and the correlated intraluminal pressure can be also modified by a series of viscero-somatic reflexes triggered by intraluminal lipidic nutrients and by mechanical stimulations like rectal distension, intra-abdominal volume load, etc (Azpiroz 2005). Besides gut musculature contractions, also boosts in abdominal pressure caused by coughing, body movements, talking and drinking lead to increases in luminal pressure (Chen et al., 2017). Symptoms perception depends on intraluminal gas distribution and gut motor response to gas loads (Harder et al., 2003). Similar magnitude of gas retention produces more abdominal symptoms during jejunal than rectal infusion, whereas abdominal distension is similar. The colon is more compliant during quasi-static distensions than during rapid barostatic balloon distensions at 10 ml/s (Barucha et al., 2001).
In sum, the healthy intestine is characterized by two major motor patterns, i.e., high-amplitude, lumen-obliterating neurogenic waves and rhythmic trains of shallow, low-amplitude myogenic waves (Amedzrovi Agbesi and Chevalier, 2022). We will see in the sequel that these different patterns lead to different outcomes regarding not just gaseous fluid dynamics, but also gaseous metabolic outcomes.

**Gaseous volumetry in gut segments**. The intestine has an oro-anal length of ≈5 m, two-third of which refers to the small intestine. The gut surface area is ~32 $m^2$, of which about two refers to the colon (Helander and Fändriks, 2014). In the undisturbed gut of healthy subjects, extreme volumetric variability can be appreciated in every compartment depending on extra-intestinal pressure, intestinal walls contraction and quantity/quality of ingested foods. In presence of food and fluid, the small bowel, which is empty and closed most of the time, moves more frequently than the colon (Fang et al., 2009). With a large degree of approximation (Pritchard et al., 2014; Nilsson et al, 2015; van Meegdenburg et al., 2015), the ascending colon has a mean volume of ≈200 mL, the transverse colon of ≈185 mL, the descending colon of ≈175 mL and the rectosigmoid colon of ≈200 mL (including the feces). In the ascending colon, 10% increases occur 90-240 minutes after feeding as the meal residue enters the cecum (Schiller et al., 2005). The human mean fluid volume is lower in fasting colon that in fasting small bowel (≈13 versus ≈54 mL, respectively) (Schiller et al., 2005). Fluid is not homogeneously distributed, rather it is provisionally confined in separated fluid pockets that increase in number after meals (mean number ≈5) (Schiller et al., 2005). The total colonic fluid volume in almost entirely stored (~95%) in the few fluid pockets (~10%) larger than 1 mL (Goelen et al., 2021).
In conclusion, since fluids and faeces occupy just a relatively small volume (a few dozen mL and ≈200 mL, respectively), it can safely be said that the partially relaxed colon's intraluminal volume is filled by gases for the most part.

## PHYSIOLOGY OF INDIVIDUAL INTESTINAL GASES

Here we will describe the physical/biological features and metabolic effects of each one of the main intestinal gases. We will show how intestinal gases, conventionally believed as biologically inert, provide instead countless physiological effects. Our main goal will be to point out that gas production, consumption, excretion and disposal in different gut compartments take part in the hemostasis of manifold physiological processes involving not only the gut, but also extra-intestinal organs (Montalto et al., 2009). For example, we will show how four small gaseous molecules previously considered as toxic gases, namely hydrogen sulfide ($H_2S$), nitric oxide (NO), carbon monoxide (CO) and sulfur dioxide ($SO_2$), have been recently assigned to the mammalian family og gasotransmitters (Huang et al., 2021). Gasotransmittes are signalling molecules that freely diffuse through the intestinal cellular membranes, playing crucial physiological and pathophysiological roles in many processes such as stomach acid release, smooth muscles relaxation, local blood flow adjusting, inflammation activation, angiogenesis, heart contractility control, etc (Verbeure et al., 2021).

**Nitrogen ($N_2$)**. Nitrogen supplies the main fraction of intestinal gases. Its concentration varies greatly with the diet, especially in the distal gut (Modesto et al., 2022). It has long been believed that the intestinal $N_2$ entirely came from swallowed air. However, the gut commensals Klebsiella and Clostridiales strains might produce ~0.01% of the standard nitrogen requirement for humans (Igai et al., 2016). Also, Helicobacter pylori produces $NH_3$ using uric acid as a substrate to achieve local gastric acid neutralization (Naito et al., 2018). Compared with other gases like $O_2$ and $CO_2$, $N_2$ gradient diffusion between lumen and blood is much slower (Levitt 1971). This means that most of the intestinal N is not absorbed, rather is propelled towards the distal intestinal tracts. While a $N_2$ partial pressure gradient from the intestinal lumen to the blood does exist in the duodenum and upper small bowel, downhill gradients occur from blood to intestinal lumen in the colon, in particular after beans meals. The gradient established by $CO_2$, $CH_4$ and $H_2$ produced by commensal bacteria drives $N_2$ diffusion from the bloodstream into the colon (Levitt 1971). Therefore, the gastric $N_2$ comes entirely from swallowed air, while a certain amount of $N_2$ in flatus comes from blood diffusion.
Contrary to the inert nitrogen from the inhaled/exhalated atmospheric air, the intestinal $N_2$ plays a role in nitrogenous compound metabolism. In the lumen of the small intestine, amino acids from alimentary sources and endogenous proteins from enterocytes are deaminated, hydrolysed, incorporated or degraded by the microbiota, in particular by Bacteroidetes (Davila et al., 2013; Bergen and Wu, 2009; Reese et al., 2018). $N_2$ supply and recycling from the small intestine is crucial also for colonic digestion, building and absorption of endoluminal proteins/aminoacids (Reese et al., 2018). Nitrogen-derived molecules like nitrated short-chain fatty acids are energy substrates for both colonocytes and peripheral tissues (Davila et al., 2013). When the dietary supply of $N_2$ for the synthesis of dispensable amino acids is deficient, urea nitrogen absorption from the large intestine is used for increasing body protein synthesis and deposition (Mansilla et al., 2015). Residual undigested luminal proteins and recovered amino acids, rather than being absorbed, can play the role of precursors for the synthesis of numerous metabolic end products (Davila et al., 2013). These bioactive products affect physiological processes, locally in the gut as well as systemically after absorption (Lundberg and Weitzberg 2013; Carlström et al., 2020). For example, reactive nitrogen oxides such as nitric oxide (NO), nitrite, nitrate, nitrated fatty acids, N-nitrosamines peroxynitrite, S-nitrosothiols are continuously manufactured in the colon (Lundberg and Weitzberg 2013; Carlström et al., 2020). The $N_2$-derived ammonia ($NH_3$), produced by colonic intestinal bacterial urease from uric acid, is used not just by the surrounding bacteria as a nitrogen source for amino acid synthesis (Naito et al., 2018), but also by enterocytes via glutamate, glutamine, citrulline, and urea synthesis (Bergen and Wu, 2009). Able to diffuse through the pores of the human Aquaporin 1 (Michenkova et al, 2021), $NH_3$ can be systemically absorbed and cause hepatic encephalopathy in patients with liver cirrhosis.
In sum, intestinal $N_2$ is crucially involved in the nitrogenous compound metabolism that is mandatory for the survival of both intestinal host cells and microorganismal commensals.

**Oxygen ($O_2$)**. Oxygen concentration progressively declines throughout the gut. The atmosphere contains about 21% $O_2$, while the stomach approximately 15-16%. This means that some of the swallowed $O_2$ is adsorbed through the intestinal vessels. Most of the $O_2$ has been removed in the colon, falling to ≈2% of the total gaseous amount (Modesto et al., 2022). Growing evidence points toward a facilitating role for the human Aquaporin 1, abundantly distributed in the endothelial cells of the gastrointestinal tract, in $O_2$ transport (Zhu et al., 2016; Zwiazek et al., 2017). In the colon, $O_2$ tends to diffuse from the bloodstream into the lumen, due to its low pressure. However, at very high $O_2$ intraluminal pressures, mammals can absorb $O_2$ through their intestines. Experiments in rodent and porcine models, inspired by loaches that use intestinal air breathing to survive under extensive hypoxia, demonstrated that intra-rectal $O_2$ delivery attains significant systemic oxygenation (Okabe et al., 2021; Mountford et al., 2021).
The role of the atmospheric oxygen is mainly correlated with the aerobic respiration, which requires $O_2$ transport from the lungs to the peripheral tissues via haemoglobins. In turn, the role of $O_2$ in the intestinal lumen is manifold. First, the scarce amount of intraluminal colonic $O_2$ favours the proliferation of essential anaerobic commensals (Levitt 1971). In turn, the microbiome contributes to maintain the hypoxic environment of the intestine that is critical for mucosal cells' nutrient absorption, intestinal barrier function and innate/adaptive immune responses (Singhal and Shah, 2020). Genes

dependent on hypoxia-inducible factors contribute to epithelium maintenance, nutrient absorption and immune regulation (Singhal and Shah, 2020). Further, the hypoxic condition of the large intestine makes various fermenting bacteria able to produce acetic acid, $CH_4$ and HS2 as energy sources (Naito et al., 2018).

Oxygen is also a crucial component to build active molecules with profound intestinal and extra-intestinal effects. For example, the $O_2$-derived hydrogen peroxide ($H_2O_2$) is a major redox signaling molecule with effects on growth and differentiation. Produced by cell-surface NADPH Oxidase enzymes, $H_2O_2$ shapes both the colonic epithelial surface environment and the colonic bacterial growth, in particular Citrobacter's growth (Miller et al., 2020). The process is favoured by the water channel Aquaporin-3 that accelerates $H_2O_2$ uptake and intracellular accumulation, leading to downstream intracellular signalling (Miller et al., 2010). Moreover, the epithelial release of reactive oxygen species such as $H_2O_2$ toward the intestinal lumen provides an innate mucosal defensive mechanism after chronic inflammation, as well as after exposure to dysbiotic microbiota (Singh et al., 2018; Burgueño et al., 2019).

Oxygen enters the composition of the a gaseous signalling molecule's endothelial biosynthesis, namely the nitric oxide (NO), the first of the four known gasotransmitters to be discovered. NO is generated from $O_2$, L-arginine and NADPH by the enzyme nitric oxide synthase (NOS) that reduces organic nitrates (Carlström et al., 2020). Three different NOS isoforms exist: the neuronal, the endothelial and the inducible NOS, the latter mainly produced by macrophages to cope with inflammatory stress factors (Verbeure 2021). Displaying a half-life time of a few seconds, the extremely active NO freely diffuses across membranes. Supposed to be mostly active at the site of production and the surrounding cells, NO engenders transient paracrine and autocrine effects (Verbeure et al., 2021). NO is a key regulator in processes like blood vessel dilatation, anti-inflammatory activity, feeding behaviour, glucose metabolism. Acting as a neurotransmitter for non-adrenergic, non-cholinergic inhibitory neurons, NO promotes relaxation of the fundic area after a meal. Via intracellular signalling in enteroendocrine cells, NO (together with other gasotransmitters) plays a role in the release of gut peptides such as gastrin, cholecystokinin, secretin, motilin, ghrelin, glucagon-like peptide 1 and 2 (Verbeure et al., 2021). Indeed, gut peptides regulate a number of physiological processes in different gut segments through NO-mediated pathways. For instance, NO promotes CCK-mediated prevention of oesophageal acid reflux during digestion, gastrin release, motilin-mediated contractility of gastric smooth muscles, CCK-mediated neurogenic vasodilatation in mesenteric and cerebral arteries, GLP-1-mediated endothelium-dependent vasodilatation, CCK2Rs-mediated inhibition on motor activity in distal colon, etc. For a survey, see Verbeure et al. (2021). Further, bacteria-dependent NO formation from dietary nitrate has been associated with blood vessels' endothelium vasodilation and increasing blood flow in the cardiovascular system (Carlström et al., 2020).

In sum, intestinal $O_2$ contributes to gut homeostasis in a roundabout way. By one side, its shortage in the distal gut promotes the homeostasis of anaerobic commensals essential to host's survival. By another side, $O_2$ is one of the main constituents of powerful active molecules that affect physiological phenomena well beyond the gut.

**Carbon dioxide ($CO_2$)**. Carbon dioxide is generated in various intestinal segments. The $CO_2$ content in the stomach is much higher than the swallowed air (5-9 vs 0.04%), since carbon dioxide is locally produced during the periods of high gastric acid secretion via HCl neutralization by dietary bicarbonates (McIver et al., 1926; Levitt 1971). Carbon dioxide partially diffuses from the proximal intestinal lumen into the blood, but its absorption rate is not enough to prevent accumulation in the duodenum and proximal jejuneum. More jejunal $CO_2$ is generated by the degradation of dietary triglycerides to fatty acids (Ritz et al., 1993). $CO_2$ movements across cellular membranes are not just passive, but also depend to a small extent on active transport performed by aquaporin channels (Michenkova et al, 2021). The amount of $CO_2$ has increased to ≈10.0% in the colon, with large variations depending on the diet (Modesto et al., 2022). Although $CO_2$ diffuses much more rapidly than $H_2$, $CH_4$, $N_2$, and $O_2$ (Scaldaferri et al., 2013), the volume of the intestinal-produced $CO_2$ is greater than the volume passed in flatus, since a part of the intraluminal gas diffuses into the bloodstream (Levitt 1971). Colonic $CO_2$ is a fermentative subproduct of the bicarbonate/acid reaction performed by commensals such as Bifidobacteria and butyrate-producing Clostridial clusters (Heresbach et al., 1995; Rivière et al., 2016). Short-chain fatty acids (acetate, propionate and butyrate) are the dominant fermentation acids that accumulate to high concentrations in the colon (Louis et al., 2022). The extreme acid load associated with high colonic p $CO_2$ is partially counteracted by the proximal colon epithelium's apical membrane, that provides a significant resistance towards $CO_2$ diffusion and confers cellular protection (Endeward and Gros, 2005).

The intestinal $CO_2$ enters red blood cells and is converted to carbonic acid, which dissociates to hydrogen ion and bicarbonate. Two-thirds of the bicarbonate diffuses out into the plasma, is converted back to $CO_2$, in the lungs and expelled by exhalation. Apart from the well-known roles in cellular respiration and peripheral chemoreceptors' activation during hypoxia (Oikawa et al., 2003; Akaishi et al., 2019), $CO_2$ is involved in manifold metabolic reactions. For instance, $CO_2$ enters the composition of Carbon monoxide (CO), one of the four known gasostransmitters. Contrary to the very short-lived and labile NO, $H_2S$ and $SO_2$, the hemoglobin-binded CO is a relatively stable molecule with half-life time up to 4 h (Verbeure et al., 2021). Its biological functions are mainly related to the activation of soluble guanylyl cyclase and, to a less extent, cytochrome P450 inhibition (Verbeure et al., 2021). CO is synthesized by two enzymes. The inducible heme oxygenase 1, located in gastric epithelial cells/lamina propria's inflammatory cells, is activated during inflammation/oxidative stress. For example, HO-1 macrophages' production is stimulated by the bacterial lipopolysaccharide. The CO produced by HO-1 acts on intestinal bacteria to cause release of adenosine triphosphate, which in turn activates inflammasome response and interlukin-1β production (Naito et al., 2018). In turn, the heme

oxygenase 2 is constitutively active and more broadly expressed: it can be found within the jejuneal myenteric and submucosal plexuses, vascular endothelial cells, liver, brain (Verbeure et al., 2021). CO is an important regulator of acute and chronic inflammation in the gastrointestinal tract.
A crucial intermediate of carbon dioxide and carbon metabolism is the lactate, a readily combusted fuel which can be shuttled throughout the body as energy source (Mahan 2021). A relatively small number of lactate-utilizing colonic species can metabolize lactate to short-chain fatty acids, butyrate, acetate and propionate (Wang et al. 2020; Louis et al., 2022). Some bacteria like Bacteroides and Firmicutes are susceptible to growth inhibition by relevant concentrations of lactate and acetate, whereas other bacteria like the lactate-producer Bifidobacterium adolescentis are resistant (Wang et al., 2020). By increasing expansion of intestinal stem-cells, Paneth and goblet cells, the lactic acid produced in anaerobic conditions by Bifidobacterium and Lactobacillus spp. contributes to intestinal epithelial development (Lee at al., 2018). Further lactate may work as a whole-body metabolite acting as a potent signaling molecule in the central nervous system, impacting neuron/astrocyte activity in brain areas well beyond the neuronal diffusion zone (Mahan 2021)
In sum, intestinal $CO_2$ is produced in in various intestinal segments in different ways. Carbon dioxide's metabolites play direct roles in pH homeostasis, energy production and intestinal anti-inflammatory responses.

**Methane ($CH_4$)**. Methane is produced by the human enteric microflora through anaerobic fermentation of both endogenous and exogenous carbohydrates (Sahakian et al., 2010). The human colonic concentration of methane is ≈14% (Modesto et al., 2022), with differences observed according to the amount of ingested fermentable dietary residues, time of the day and individual variations (Naito et al., 2018). To make a comparison, cattle produce ≈250-500 Lt of methane every day (Johnson and Johnson, 1995), with an estimated emission rate of 95-150 g/animal/day (Sejian et al., 2011). Just one/two third of healthy human adults produce methane (Roccarina et al., 2010; Ishaq and Peter. 2016). Children start producing $CH_4$ at about two years (Levitt 1971). Contrary to $H_2$, the colonic $CH_4$ production is relatively constant throughout the day (Levitt 1971). Human $CH_4$ is produced in extremely anaerobic conditions not by bacteria, but by methanogenic microorganisms of the Archea domain (Gandhi et al., 2021). The Archea Methanobrevibacter smithii is able to reduce $CO_2$, methanol, or acetate to $CH_4$, using $H_2$ as an electron donor (Miller et al., 1982 Hylemon et al., 2018). Tiny amounts of $CH_4$ can be produced under hypoxic conditions to counteract intracellular oxygen radical production not just by Archea microorganisms, but also by host components such as, e.g., rat mitochondrial subfractions and bovine endothelial cell cultures (Boros et al., 2015). In this case, $CH_4$ is generated from phosphatidylcholine metabolites containing both electron donor and acceptor groups (Ghyczy et al., 2008). Like $H_2$, the intraluminal colonic $CH_4$ diffuses to the blood for gradient concentration, enters the splanchnic circulation and is excreted through the breath (Bond et al., 1971; Zaorska et al., 2021). Analysis of respiratory methane provides approximative measure of $CH_4$ production (Sahakian et al., 2010; Schneider et al., 2020; McKay et al., 1985), revealing that methane concentration varies from 1 to 20 ppm as measured by exhaled $CH_4$ (Naito et al., 2018)
Evidence suggests that $CH_4$ may not be inert as previously thought (Sahakian et al., 2010). Methane significantly influences the enteric nervous system's cholinergic pathway, increasing contraction amplitudes (Park et al., 2014). In guinea pig ileum, $CH_4$ delayed ileal peristaltic conduction velocity by increasing contractility (Jahng et al., 2012). In radiolabelling experiments of small intestinal infusion, $CH_4$ slowed dogs' and guinea pigs' small intestinal transit, by increasing bowel contractions oral and aboral to the stimulus (Pimentel et al., 2006; Lee at al. 2013). The increases in contractile activity correlated with $CH_4$ production have been associated with constipation-predominant irritable bowel syndrome (Pimentel et al., 2006; Sahakian et al., 2010). Further, abundance in methanogenic bacteria has been positively correlated with slowed intestinal transit time (Attaluri et al., 2010; Triantafyllou et al., 2014) and chronic intestinal pseudo-obstruction (Khan et al., 2022). In turn, Rifaximin has been shown to improve chronic constipation by altering methane production (Ghoshal et al., 2018). It is noteworthy that patients with irritable bowel syndrome are characterized not just by reduction of methane producing microorganisms, but also by reduction of butyrate producing bacteria, known to improve intestinal barrier function (Pozuelo et al., 2015).
In mammals, during hypoxic events such as haemorrhagic shock, $CH_4$ production and subsequent mitochondrial redox regulation/oxidative phosphorylation improves basal respiration (Boros et al., 2015; Mészáros et al., 2017; Bársony et al., 2020). Mitochondria themselves can be sources of endogenous $CH_4$ under oxido-reductive stress conditions. During hypoxic condition, various colonic bacteria produce chemical compounds that can be used as energy source, such as acetic acid, $CH_4$ and $HS_2$ (Naito et al., 2018). Also, lines of evidence suggest that exogenous $CH_4$ exerts anti-inflammatory, anti-apoptotic and antioxidative effects (Mészáros et al., 2017; Zaorska et al., 2021). Methane modulates leukocyte activation (Boros et al., 2012) and plays shielding roles in hepatitis (He et al., 2016), acute lung injury (Sun et al., 2017), diabetic retinopathy, spinal cord ischemia/reperfusion injury and sepsis (Li et al., 2019).
In sum, intestinal $CH_4$ is produced by intestinal commensal anaerobia in approximately one-two third of healthy humans. Methane delays intestinal transit and compensates oxido-reductive stress conditions. Its extra-intestinal effects are so extended, that a few researchers are starting to suggest that $CH_4$ could stand for a fifth gasotransmitter apart from NO, CO, $H_2S$ and $SO_2$.

**Hydrogen ($H_2$)**. In healthy individuals, $H_2$, which accounts for ≈3% of the colonic gases, is almost entirely produced by the dietary fiber's intraluminal fermentation performed by anaerobic commensals in the large intestine (Modesto et al.,

2022). Hydrogen is a by-product of ingested fermentable substrates including non-absorbable oligosaccharides such as beans/lactulose (90%) and poorly absorbed proteins, short chain fatty acids and alcohols (10%). The amount of lactose in a glass of milk generates 500–1,000 ml of $H_2$ after bacterial fermentation. Hydrogen-producer species are abundant in the gut microbiota and include not just the two major colonic phyla, i.e., Firmicutes and Bacteroidetes, but also members of the genera Roseburia, Ruminococcus, Eubacterium (Hylemon et al., 2018; Naito et al., 2018). It is remarkable that many hydrogen cross-feeding microbes have evolved. The three main hydrogenotrophic colonic groups are the sulfate-reducing bacteria (such as Desulfovibrio), the methanogenic archaea and the reductive acetogens (Carbonero et al., 2012; Scaldaferri et al., 2013; Smith et al., 2021). Luminal colonic $H_2$ freely diffuses between the lumen and the blood, the net movement depending on the pressure gradient. Colonic $H_2$ absorption is highly effective at low colonic hydrogen accumulation rates, but not at higher accumulation rates (Hammer 1993). Therefore, about 15% of $H_2$ diffuses back into the bloodstream, with the rest passing as flatus. The colonic $H_2$ absorbed by the blood is cleared by the lungs. The time taken for $H_2$ to appear in the breath after ingestion of a standard load of glucose or lactose is used to determine whether the upper gastrointestinal tract has been colonized by $H_2$ producing bacteria (Hammer 1993; Carbonero et al., 2012; Wilder-Smith et al., 2018).

Colonic hydrogen leads to the production of short-chain fatty acids like butyrate. When $H_2$ is not fully metabolized, fermentation may be incomplete and intermediates such as lactate, succinate, and ethanol may accumulate (Scaldaferri et al., 2013). Physiological $H_2$ concentrations have been shown to have antioxidant properties, protecting the healthy colonic mucosa from oxidative insults and preventing inflammation/carcinogenesis (Carbonero et al., 2012). Also, roles on local intestinal motility have been suggested for $H_2$. It shortened guinea pigs' colonic transit by 47% in the proximal colon, but just by 10% in the distal colon (Jahng et al., 2012).

In sum, intestinal $H_2$ is major byproduct of colonic fermentative metabolism. Hydrogen preserves the healthy colonic mucosa from oxidative insults and might have a role in shortening colonic transit.

**Hydrogen sulfide ($H_2S$)**. In healthy humans, the most of $H_2S$ is a by-product of colonic bacterial metabolism (Linden 2014). Cysteine catabolic bacteria and, to a lower extent, sulfate-reducing bacteria produce $H_2$ using as substrates both dietary and endogenous compounds of organic and inorganic nature (Guo et al., 2016; Blachier et al., 2021). Two enzymatic trans-sulfuration pathways are involved in $H_2S$ production, i.e., the cystathionine gamma-lyase in the vascular system's smooth muscles, and the cystathionine beta-synthase in both central and intestinal nervous systems (Guo et al., 2016; Verbeure et al., 2021). To generate $H_2S$ in an anaerobic watery environment like the human colon, some requirements must be satisfied, e.g., high-concentration of sulfate ions and organic substances as carbon source and sulfate-reducing bacteria like Desulfovibrio bacteria (Naito et al., 2018). Sulfate reduction and methanogenesis seems to be mutually exclusive in the colon and this is possibly linked to sulfate availability, which favours $H_2S$ instead of $CH_4$ production (Scaldaferri et al., 2013). Hydrogen sulfide is produced also by endogenous cellular enzymes expressed in intestine, liver, kidney and brain (Sahakian et al., 2010). In particular, $H_2S$ is synthetized by specific enzymatic pathways in different intestinal cell types, including neurons and smooth muscle (Jimenez et al., 2017). Vegetables like garlic and onions contain the natural $H_2S$ donor allicin and therefore generate a large amount of colonic $H_2S$ (Benavides et al., 2007). Intraluminal and faecal colonic $H_2S$ concentrations are rather variable and may reach 3.4 mmol/L in human stools (Guo 2016; Blachier et al., 2021).

$H_2S$ is an important energy substrate in colonocytes because its mitochondrial oxidization results in ATP synthesis (Blachier et al., 2021). However, when the intracellular $H_2S$ concentration is pushed locally above the healthy limit and exceeds the colonocyte capacity for its oxidation, the mitochondrial respiratory chain is inhibited and the energy metabolism is impaired (Blachier et al., 2021). Too high luminal $H_2S$ concentration affects the integrity of the mucosal layer, leading to increase in inflammatation.

Together with CO, NO and $SO_2$, $H_2S$ is regarded as an endogenous gasotransmitter (Wu et al. 2017). Its biology is a still developing area of research. Although less studied that the other three, $H_2S$ has been found to act as a signalling molecule immediately after release. It has been demonstrated that $H_2S$ affects intestinal motility, promoting colonic transit (Nalli et al., 2017). It has been recently suggested that exogenous $H_2S$ might exert an excitatory effect on colonic motility, through Substance P release from afferent nerves together with activation/deactivation of different Ca2+ channels in smooth muscle cells (Quan et al., 2022). Inhibition of $H_2S$ biosynthesis increases motility, while $H_2S$ donors cause smooth muscle relaxation and inhibition of propulsive motor patterns (Jimenez et al., 2017). Recent reports provide evidence for crosstalk between NO and $H_2S$ in colonic smooth muscle. NO generates $H_2S$ via cGMP/PKG pathway, while $H_2S$ increases NO-induced cGMP levels. Further, $H_2S$ and its oxidation product polysulfide can activate nociceptors expressed in sensory nerves, displaying effects on visceral nerve hypersensitivity (Chassard et al., 2012). After high protein meals, the $H_2S$ donor amino acid l-cysteine suppresses ghrelin release from the rat stomach, reducing appetite for a long time (Verbeure et al., 2021). Colonic $H_2S$ stimulates GLP-1 release, improving glycemia in male mice. Considering its short half-life time, $H_2S$ possibly achieves this effect by stimulating nearby colonic cells instead of ileal cells after plasmatic transport (Pichette et al., 2017). High concentrations of hydrogen sulfide produced by bysulfate-reducing bacteria are involved in gut inflammation through detrimental mechanisms of toxicity, pH lowering and inhibition of the beneficial lactic acid bacteria (Dordević et al., 2021).

Recent, still controversial findings suggest that endogenous $H_2S$ might play roles in angiogenesis and smooth muscle vascular relaxation (Linden 2014; Wu et al. 2017). Abnormal $H_2S$ metabolism is associated with diseases including heart

failure, hypertension, atherosclerosis, asthma, diabetes and neurodegenerative diseases (Wu et al. 2017). Increased expression of various $H_2S$-producing enzymes could be correlated with ulcerative colitis and human colonic cancer development (Cai et al., 2014; Guo et al., 2016). $H_2S$ displays a bell-shaped pharmacology, whereby lower (endogenous) $H_2S$ production promotes, while higher (generated from exogenous $H_2S$ donors) inhibits colorectal cell tumoral proliferation.
In sum, $H_2S$ is an endogenous gasotransmitter produced almost exclusively in the colon as a by-product of colonic bacterial metabolism. Hydrogen sulfide produces ambivalent physiological effects, depending on its intracellular concentration.

**Sulfur dioxide ($SO_2$)**. Previously regarded as a highly toxic gas smelling of burnt matches detectable in atmospheric pollutants, sulfur dioxide is not harmful if ingested in low concentration with food. One of the main sources of $SO_2$ in the human body comes from the addition of sulfites to food products due to their bacteriostatic, bactericidal and antioxidant properties. Sulfites are regarded as safe for consumption at concentrations up to 5000 parts per million (Irwin et al., 2017). Sulfur dioxide is used as a preservative termed E220 for dried fruits, food starches, wine/beer fermentation and medications to prevent oxidation and changes in pigment (Zamboni et al., 2011). Also, $SO_2$ or its conjugate base bisulfite is endogenously produced during intestinal fermentation. Generated through cysteine metabolism and ingested sulphur's conversion, $SO_2$ is an intermediate product of sulfur-oxidizing bacteria and sulfate-reducing organisms, in particular Desulfovibrio genus. Variation in the distribution of sulfate-reducing microbial communities have been detected in healthy mice (Kushkevych et al., 2019). Usually, the healthy individuals' colonocytes are able to absorb and detoxify the gas. Sulfur dioxide is generated also in mammalian cardiovascular tissues from sulfur-containing amino acids (Huang et al., 2016). Interactions occur between $SO_2$ and the other gasotransmitter $H_2S$, the latter regulating some $SO_2$ pathways (Huang et al., 2021).
Although its biological role in mammalian biology is not well understood, $SO_2$ is regarded as the fourth gasotransmitter. Very small amounts of $SO_2$ display cytoprotective, antioxidant and anti-inflammatory properties that ameliorate colitis in rats, reversing inflammatory features like oxidative stress, NF-κ B and inflammasome activation, endoplasmic reticulum autophagy, p53 activation and apoptosis (Banerjee et al., 2019). Endogenous sulfur dioxide in low concentrations regulates cardiac and blood vessel function, triggering endothelium-dependent vasodilation and myocardial antioxidant defense reserve (Want et al., 2011). Relatively recent studies showed that $SO_2$ ameliorates systemic and pulmonary hypertension, prevents atherosclerosis development and protects against myocardial ischemia-reperfusion injury (Huang et al., 2016).
At high concentrations, $SO_2$ displays manifold adverse effects, especially on the colonocytes. High $SO_2$ levels cause colonocyte's cell death, goblet cell loss, crypt architectural distortion and superficial mucosal ulceration, leading to permeability and barrier function shortfall. Still controversial data suggest that high $SO_2$ levels may be linked with ulcerative colitis (Teigen et al.,2019). One of the key deleterious effects of $SO_2$ consists of impairment of short chain fatty acids metabolism. Competition for the available intestinal hydrogen occurs between sulphur-reducing bacteria and short chain fatty acids-producing bacteria, causing reversible inhibition of butyrate oxidation. This leads to decreases in butyrate acid, that is vital in providing up to 70% of the energy metabolism required by the colonocytes. High $SO_2$ concentrations cause endothelium-independent vasodilation mediated via calcium channels, leading to harmful inotropic effects on cardiac output function. An association has been found between sulfur dioxide and increase in ischemic heart disease, heart failure and arrhythmia, mainly due to mitochondrial dysfunction in cardiac muscles (Qin et al., 2016).
In sum, intestinal $SO_2$ is both ingested with the food and produced by intestinal bacteria via sulfur conversion. Like a two-faces Janus, sulfur dioxide is a beneficial antioxidant and anti-inflammatory molecule at low doses and an extremely dangerous poison at high doses.

In conclusion, the gaseous mixture is composed by a large number of gases, each one characterized by its own sources, dynamics, metabolism, biological effects.

## PHYSICAL PROPERTIES OF THE INTESTINAL GASEOUS MIXTURE

Here we will analyze the effects of physical laws on intestinal gases physiology, describing how the physical interplay between gaseous volume and luminal surface impacts absorption and mixing. We will consider just the whole mixture of different gases, leaving apart the differences among each gas. According to the Dalton's law of partial pressures in ideal gases, the pressure of a mixture of non-reactive gases equals the sum of the pressures of all the constituent gases. Further, the Amagat's law states that the volume of a gas mixture equals the sum of the volumes of the component gases, when the temperature and the pressure are held constant. Taken together, the two theorems suggest that gas particle identity plays no role in determining both final pressure and volume of the intestinal gaseous mixture.

Modifications in intestinal gaseous volumes may have a twofold cause:

1) Physical factors, e.g., changes in luminal pressure and temperature.
2) Biological factors, e.g., changes in diet and intestinal microorganisms' metabolism.

We will argue that the two distinct causes of gaseous volumetric modification lead to fully different absorptive outcomes.

**Gaseous volumetric changes due to intestinal physical factors: Boyle's law**. Trans-barrier gaseous exchanges do not depend on the intraluminal pressure, rather on the difference between the partial gaseous pressures in lumen and in blood. Though, the intraluminal pressure retains an indirect, deep effect on gas exchanges. It is well-known that variations in the three main physical gaseous parameters, i.e., pressure, volume and temperature, cause modifications in gut diameter, flow volume, fluids velocity (Uno 2018). For instance, analytical Poiseuille-flow simulations suggest that oppositely directed contractile waves are useful for mixing and favouring nutrient absorption. Indeed, two colliding contractile waves moving toward one another cause blobs of silicone injected into the embryonic chicken's intestine to be squeezed together (Amedzrovi Agbesi and Chevalier, 2022).
Pressure, volume and temperature are linked to each other through the experimental gas laws (Webster 1965; Randsoe and Hyldegaard, 1985). Even though these laws hold for a given mass of an ideal gas within a closed system, they can be also used to describe the behavior of real gases such as the intestinal gaseous mixture in the confined environment of colonic haustra.
Charles's law states that the temperature and the volume are in direct proportion when the gas pressure is held constant. One of its formulations reads:
$V/T = k$
Where V is the gaseous volume, T is the gaseous temperature and k is a constant such that every 1°C increase in temperature leads to 1/273 increase in volume.
Gay-Lussac's law states that the temperature and the gas pressure are in direct proportion when the gas volume is held constant. One of its formulations reads:
$P/T = k$
Where P is the gaseous pressure, T is the gaseous temperature, and k is a constant such that every 1°C increase in temperature leads to 1/273 increase in pressure.
About the intestinal gases, the two laws can be formulated as follows:

a) Charles' law: keeping the gaseous pressure constant, slightly decreases (1/273) in the gaseous volume occur when the gut temperature decreases of 1°C.
b) Gay-Lussac' law: keeping the gaseous volume constant, slightly decreases (1/273) in the gaseous pressure occur when the gut temperature decreases of 1°C.

Being the range of gut temperatures very narrow (about 37-40°C) (Dokladny et al., 2005; Ruddock et al., 2014), increases or decreases in temperature will produce very small changes in volume and pressure. This means that the effects of the Charles' and Gay-Lussac' laws are negligible in the colon.

For a third experimental gas law, i.e., the Boyle's law, the story is different. The law states that the absolute pressure exerted by an ideal gas is inversely proportional to the volume it occupies, when the temperature and the amount of gas remain unchanged. One of its formulations reads:
$P \cdot V = k$
Where P s the gas pressure, V is the gaseous volume and k is a constant such that the volume doubles when the volume halves.
About the intestinal gases, the Boyle's law can be formulated as follows: Keeping the colon temperature constant, the gut gaseous volume halves when the gut pressure due to gut contraction doubles (**Figure 1A**). Among the three examined experimental laws of gases, the Boyle's law has the deepest physiological implications. It suggests that every change in gut pressure leads to significant changes in intraluminal gaseous volume, and vice versa. When the muscular relaxation

causes increases in gut diameter and decreases in intraluminal pressure, the gaseous mass tends to fill a larger intraluminal volume. This means that the amount of fixed gaseous mass per unit volume, namely the gaseous density, has been modified. See **Figure 1B** for further details.
Intestinal movements have a deep effect on nutrients' intraluminal persistence and absorption. Small intestine's fluid dynamics simulations suggest that the average flow velocity is the key feature to control absorption efficiency and bacterial growth (Codutti et al., 2022). Alternating the two contraction patterns of peristalsis and segmentation, the gut switches between different flow regimes, optimizing nutrient absorption and minimizing bacterial overgrowth (Codutti et al., 2022). The slower the flow speed, the longer the gaseous mixture persists in the gut, the more the intraluminal gases are exchanged with the blood. When the gaseous volume is augmented due to muscular relaxation, a larger amount of mucosal surface is available, leading to increases in trans-barrier metabolic exchanges between the lumen and the bloodstream.
Summarizing, the Boyle's experimental gas law suggests a twofold effect on the gut function: gaseous exchanges between intestinal lumen and blood are impaired by muscular contraction and improved by muscular dilation.

**Gaseous volumetric changes due to intestinal biological factors: surface-area-to-volume ratio**. A puzzling phenomenon has been reported, i.e., the colonic absorption of $H_2$ is highly effective at low colonic accumulation rates, but ineffective at the higher accumulation rates occurring after ingestion of non-absorbable carbohydrates (Hammer 1993). An unnoticed physical/biological factor might shed new light on this experimental observation. When the volume of colonic gas increases due to microorganismal fermentative reactions, the surface area per unit volume cannot be overlooked. This factor, termed surface-area-to-volume ratio (henceforward SA:V), suggests that body size and shape are mathematically interconnected. Changes in volume are not linearly correlated with changes in surface, since volumes tend to increase much more than areas. For instance, when the sphere's radius increases, the volume expansion is proportional to the radius' cube ($r^3$) while the surface expansion is proportional just to the radius' square ($r^2$). Therefore, the smaller sphere displays relatively more surface (and a higher SA:V) compared with the bigger sphere. To provide a rough example, an eukaryotic cell with radius 2 displays a surface of 4 and a volume of 9, while an eukaryotic cell with radius 3 displays a surface of 9 and a volume of 29. The phenomenon is ubiquitous: a wide range of living systems exhibits robust SA:V homeostasis at different coarse-grained scales, from neurons to elephants (Harris and Theriot 2018; Beaulieu-Laroche al., 2021). SA:V has been widely utilized to evaluate cellular packing, animal water loss, heat transfer during thermoregulation, etc (Glazier 2010; Hales at al., 2017; Nguyen et al., 2019).
The large surfaces (relative to their volumes) displayed by tiny organisms permit plentiful exchanges of nutritive substances between the internal and the external, compared with the small surfaces (relative to their volumes) displayed by larger organisms. About the colonic gas produced by fermentation, every intraluminal gaseous bubble displays a surface available for lumen/blood trans-barrier gaseous exchanges. SA:V dictates that enhanced microorganismal production of intraluminal gases leads to large increases in gaseous bubble's volumes and small increases in their surface (**Figure 1C**). The bigger gaseous bubbles have less capability of performing lumen/blood metabolic exchanges, compared with the smaller ones. This means that a high volume of colonic gas (corresponding to a small SA:V) stands for a detrimental outcome that impairs gaseous exchanges, adsorption, catalysis and reactions across the intestinal barrier. Despite the SA:V mechanism and the Boyle's law mechanism may both lead to increases in gaseous volumes, they exert opposite metabolic effects. While the increases in gaseous volume due to Boyle's law mechanism cause decreases in gaseous density (**Figure 1B**), the increases in gaseous volume due to SA:V cause increases in gaseous density (**Figure 1C**).
Summarizing, SA:V ratio suggests that the gaseous exchanges between the intestinal lumen and the bloodstream are impaired by microorganismal overproduction and improved by microorganismal decreased production.

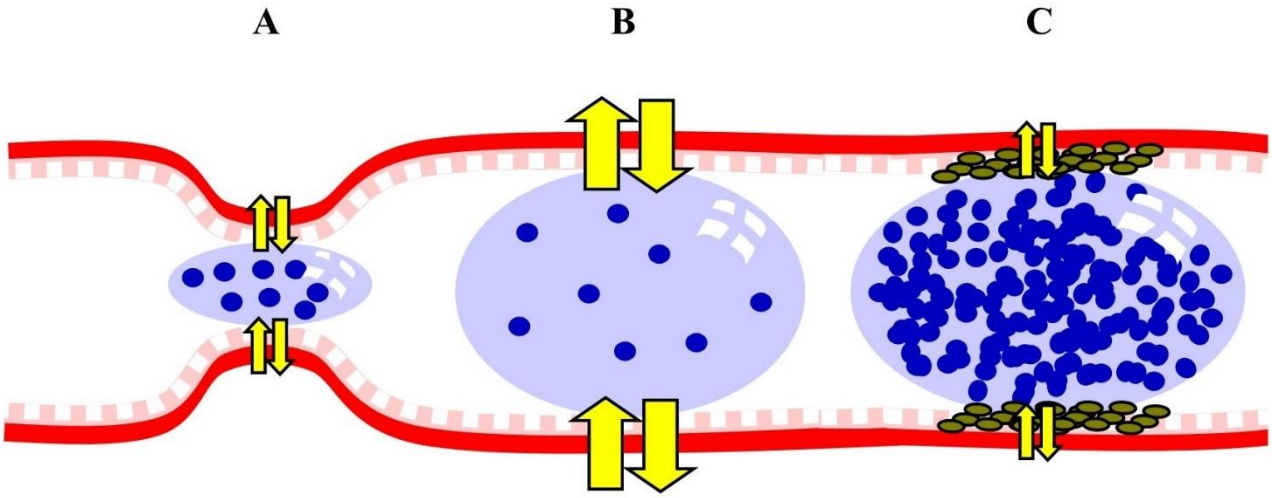


**Figure 1**. Effects of intraluminal volumetric modifications on intestinal gaseous absorption. Intraluminal gas bubbles with varying surfaces and volumes are illustrated inside a contractile intestinal segment. **Figure 1A**. Due to the Boyle's law, muscular contraction leads to decrease in gaseous volumes. The subsequent decrease in gaseous surface leads to impaired lumen/blood metabolic exchanges (small yellow arrows). **Figure 1B**. Due to the Boyle's law, muscular dilation leads to increased gaseous volumes. The subsequent increase in gaseous surface leads to improved lumen/blood metabolic exchanges (big yellow arrows). Note that the gaseous density, illustrated by the blue dots standing for single gas molecules, is lower, compared with Figure A. **Figure 1C**. Large amounts of fermenting microorganisms (green ovals) in a relaxed gut lead to increase in gaseous volumes. Despite the increases in gaseous surface and gaseous density, SA:V dictates that the lumen/blood metabolic exchanges are less effective (small yellow arrows) than in Figure B.

**Gaseous random walks in colonic haustra**. An overlooked factor in gut dynamics research is the hydrodynamic flow. Are intestinal gaseous movements laminar or turbulent? The best available quantitative approach to tackle the complexity of turbulent flows consists of the dimensionless Reynolds number (henceforward Re), which describes the ratio of inertial/viscous forces:

$$\mathrm{Re} = \frac{\rho \cdot L \cdot u}{\mu}$$

where ρ is the fluid density (kg/m$^3$), L is the characteristic linear dimension (the cylinder's diameter in m), u is the fluid velocity (m/s) and μ is the dynamic viscosity (kg/m · s). Re differentiates between laminar (Re < 2,100) and turbulent flows (Re > 3,000) (Wygnanski and Champagne, 2006). Simulations can be performed through freely available calculators, such as, e.g., https://www.omnicalculator.com/physics/reynolds-number. Reynolds number simulation of intestinal gas produces the following result: gaseous density ρ = ≈1.18 kg/m$^3$, dynamic viscosity μ = ≈0.000018 Kg/m · s, colonic fluid velocity u = ≈ 0.01 m/s, while the characteristic linear dimension L equals the intestinal diameter, which ranges from ≈ 0.08 m in the caecum to ≈ 0.055 m in the rectum (Uno, 2018). Since the Reynolds number turns out to be ≈ 30.0 in all the intestinal segments, gaseous flows in the gut are always laminar. Therefore, gaseous flows do not display the vorticity that is the hallmark of turbulent flows (Sturm et al., 2012; Kalmár-Nagy and Bak, 2019).
Unconstrained, random movements of gaseous molecules occur in the undisturbed environment of the segmented haustra. In probability theory, a "random process" (the term "random" is roughly interchangeable with "stochastic") is a sequence of variables whose outcomes do not follow a deterministic pattern, rather their evolution is described by probability distributions (Paul and Baschnagel, 2013). Since no preferential flow direction does exist, the state of a stochastic process cannot be accurately predicted by the knowledge of its previous and current states. A "random walk" is a path consisting of a succession of random steps in a mathematical space (Codling et al., 2008). A "Brownian motion" depicts the random fluctuations of particles inside a fluid at thermal equilibrium (Blomberg et al., 2020). "Ergodicity" is a random process in which all the accessible microstates in a phase space are equiprobable over a long period of time (Walters, 1982). The concepts of random processes, random walks, Brownian motion and ergodicity are used to approach classic, relativistic and quantum systems (Gong et al., 2021) such as, e.g., light, liquids/gases motion, genome organization's heterogeneity, protein concentrations' fluctuations, signal processing, gambling, evolution, ecological processes, etc (Elowitz et al., 2002; Finn and Misteli, 2019; Pontes-Filho et al., 2020; Klosin et al. 2020; Casacio et al., 2021; Wooller et al., 2021).

It might be tempting to believe that the gaseous random walks tend to uniformly spread inside the segmented colonic haustra, disrupting the initially clumpy distribution of gas bubbles. Though, we will show here that gaseous random walks in colonic haustra cannot spread homogeneously. An unappreciated factor must be regarded when coping with random walks, i.e., the number of dimensions of the phase space where the movements take place. Stochastic approaches are not profitable when random walks occur in phase spaces of dimensions higher than two: the higher the phase space's dimensions, the less the possibility that random walks return to the starting point. Indeed, random walks in two-dimensional lattices (**Figure 2A**) have unity probability of reaching any point (say, the starting point) as the number of steps approaches infinity (**Figure 2B**). Consider a random walk on a lattice of dimension $D^d$. The probability $p^{(d)}$ that a random walk returns to the origin is: $p(1) = p(2) = 1$ (McCrea and Whipple, 1940). This means that a particle almost surely will get back to the starting point in case of two-dimensional random walks. To provide an example, it has been stated that "a drunk man will find his way home, but a drunk bird may get lost forever". A drunk man randomly walking around the streets arranged in a square grid will always go back home. Yet, in case of random walks in phase spaces equipped with dimensions higher than two (**Figure 2D**), the probability to reach the starting point decreases with increases in number of dimensions (Domb, 1954; Finch 2003) (**Figure 2E**). Indeed, $p(d)<1$ for $d>2$, so that, for example, $p(3)=0.3405$, $p(5)=0.1351$, $p(8)=0.079$, and so on (Montroll, 1956). A drunk bird, that, unlike the drunk man, can fly in three dimensions instead of just two, will not get back to its nest. Since three-dimensional random walks exhibit an extremely low probability to return by chance to the starting point, we are allowed to state that the (apparently stochastic) trajectories occurring in three-dimensional phase spaces are hampered and constrained. In case of two-dimensional random walks, ergodicity is guaranteed by the fact that all the accessible microstates are equiprobable over a long period of time (**Figure 2C**). In turn, random walks taking place in high dimensions cannot be fully ergodic, because every point (say, the starting point) cannot be easily crossed more than once (**Figure 2F**). This means that the trajectories in three-dimensional systems are unable to homogenously fill the whole phase space, leading to a loss of the same ergodicity that is fully guaranteed in two dimensions. In touch with this observation, significant deviations from Brownian motion have been uncovered in a variety of animate and inanimate systems such as, e.g., the movements of avian predators or bird flocks (Metzler 2014; Vilk et al., 2022). Ergodicity breaking has been observed uniquely at the micro-scale of observation, while it is hidden at large-scale interpatch observation (Vilk et al., 2022).

About the gut, this means that the undisturbed random diffusion of gas (i.e., the gaseous random walks) inside a colonic pocket (i.e., inside a three-dimensional phase space) impacts gaseous permeability rates and trans-membrane exchanges. The gaseous mixture's three-dimensional random paths do not diffuse ergodically, therefore they cannot reach all the regions of the haustral mucosa (i.e., all the partitions of the phase space). Since the particle probability to reach the starting point is 0.3405 for three-dimensional random walks, this means that the amount of colonic area available for lumen/blood metabolic exchanges has fallen by ≈two thirds. Therefore, changes in gut permeability could be caused not just by well-studied noxae like, e.g., intestinal epithelium's increased apoptosis, altered permeability, impaired tight junctions' porosity, zonulin-mediated diseases, leaky gut syndrome and so on (Camilleri, 2019; Obermüller et al., 2020; Tajik et al., 2020), but also by the physiological stochastic dynamics of the gases confined in the colonic haustra of healthy individuals.

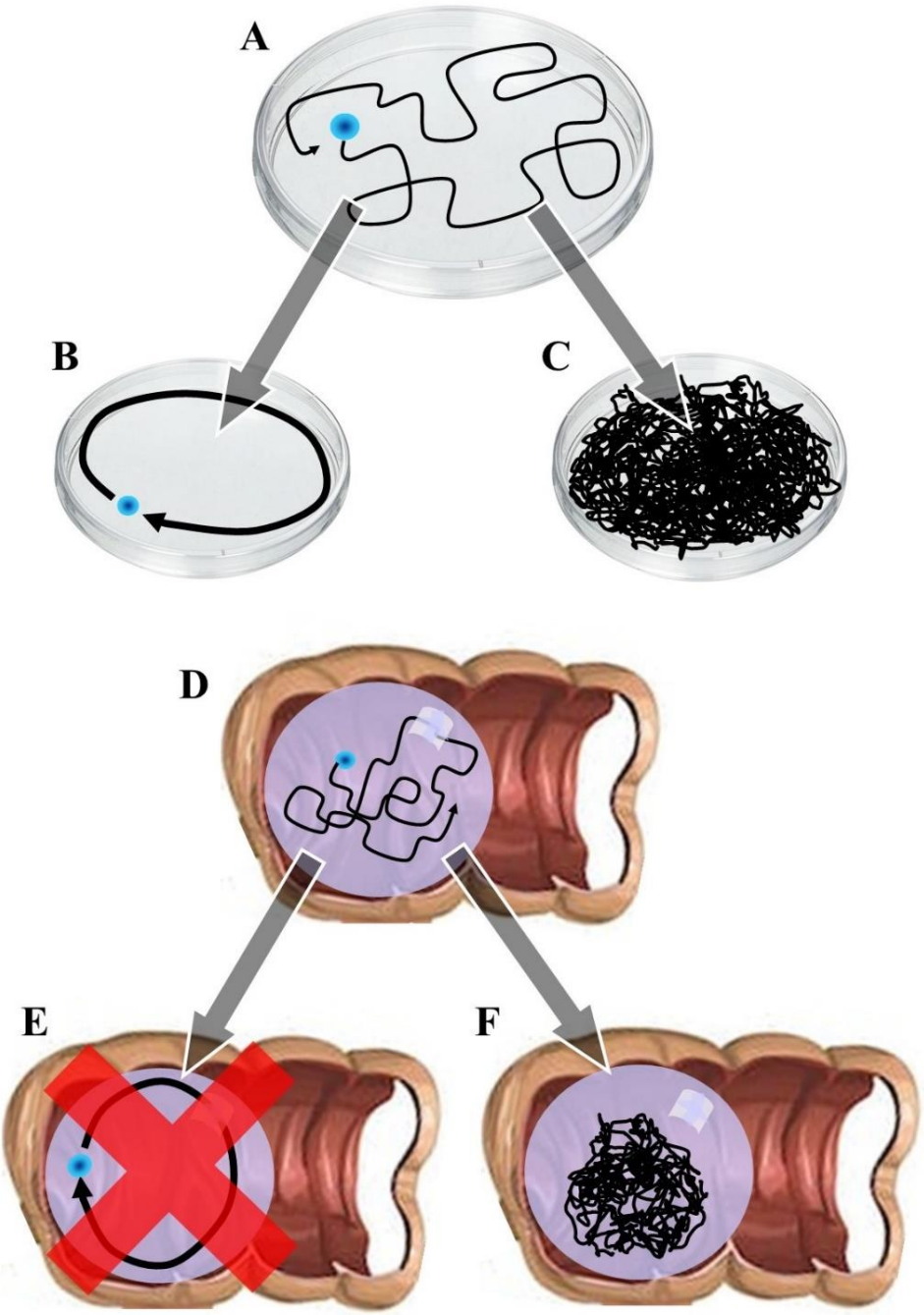


**Figure 2**. Random walks in phase spaces with different dimensions. **Figure 2A** illustrates a two-dimensional random walk with its starting point (blue dot). Two dimensional random paths tend to form recurrent closed paths (**Figure 2B**) and to fill the whole available surface (**Figure 2C**). **Figure 2D** illustrates a three-dimensional random walk of a gaseous particle confined in a gaseous bubble inside a colonic haustrum. Three-dimensional random paths cannot form recurrent, closed paths (**Figure 2E**) and cannot fill the whole available volume (**Figure 2F**).

## CONCLUSIONS

We discussed the production and storage mechanisms of intestinal gases, emphasizing their biological roles and growing importance in human physiology and pathology. Rather than waste material discarded by host's and microbiome's biological reactions, the intestinal gaseous mixture affects energy metabolism, gut transit regulation, immunity, paracrine and eccrine regulation, bacterial proliferation, blood musculature control, gut metabolic exchanges with blood and breath, etc. Being intestinal gas' volume and composition important factors for the experimental assessment of gut microbiome, functional disorders, bowel perforation diagnostics, etc (Modesto et al., 2022), the quantitative data provided in this review may be helpful in basic research and translational medicine. An important operational feature of intestinal gases is that some of them are detectable in exhaled breath. Measurement of breath hydrogen and methane excretion after ingestion of test-carbohydrates is a valuable, non-invasive and inexpensive diagnostic test that provides crucial information concerning gastrointestinal disorders (Hammer, 2022). Breath test is useful in the diagnosis of carbohydrate maldigestion syndromes, small intestinal bacterial overgrowth, methane-associated constipation, evaluation of bloating/gas, etc (Rezaie et al., 2017). $^{13}$C-breath tests is used to detect Helicobacter pylori infection, quantify gastric emptying time and screen pancreatic exocrine and liver function (Keller et al, 2021). Orally administered urea containing isotopically labelled $CO_2$ is hydrolysed by the urease produced in large quantities by Helicobacter pylori. Urea is then hydrolysed to ammonia and carbon dioxide, which diffuses into the blood and is excreted by the lungs (Savarino et al., 1999). Yet, more standardization is required regarding indications for breath tests' methodology, performance and results interpretation (Keller et al., 2021).

A few intestinal gases, namely hydrogen sulfide ($H_2S$), nitric oxide (NO), carbon monoxide (CO) and sulfur dioxide ($SO_2$), are classified among the gasotrasmitters. To fall within the definition of gasotransmitter, the gasous molecule must be generated endogenously and must exert regulatory (rather than toxic) effects (Pacher 2021). Interestingly, all these molecules are either produced by mitochondrial enzymes, and/or display significant effects on mitochondria, suggesting ancient regulatory roles in bacterial species (Wareham et al., 2018). It has been recently suggested that another molecule could be counted among the mammalian gasotransmitters, namely, cyanide. We agreed not to talk about cyanide in the previous chapters because its effects on the gastrointestinal tract are still unknown. Exposure to cyanide occurs via cigarette smoking, smoke inhalation during plastics combustion, drinking cyanide-contaminated water or ingestion of large amounts of edible plants like apricot kernel, flaxseed, cassava, almonds, Chinese cabbage, radish (Lee and Kwon, 2009). Further, endogenous cyanide production has been demonstrated by various mammalian enzymes from endogenous and exogenous substrates (Cipollone and Visca, 2007). For instance, apricot cyanogenic glycosides can be converted into thiocyanate by human hepatic and colonic cells (Lee and Kwon, 2009). Some bacteria like Pseudomonas aeruginosa are cyanide-producing too. Hydrogen cyanide is readily soluble in water and in biological fluids, where it usually exists in the volatile undissociated form. While cyanide at high concentrations is a cytotoxic agent exerting harmful effects via inhibition of the mitochondrial Complex IV, cyanide at low concentrations stimulates mitochondrial electron transport and promotes ATP production, resulting in cell proliferation stimulation (Randi et al., 2021). A few, tenuous clues point towards a possible involvement of cyanide in the physiology of the intestinal tract. Studies evaluating dietetic regular intake of $^{14}$C-labelled cyanide in rats suggest that the stomach accounts for 18% of the total injected radioactivity. Most of the cyanide is excreted in the urine and only small amounts are found in the faeces, indicating intestinal absorption into the body fluid (Okoh et al., 1982).

Intestinal motility is emerging as a deal-breaker in a variety of unexpected topics. Traditionally, the wide inter-individual heterogeneity in microbiota composition has been associated with physiological variables and population-wide differences in human lifestyle (Vujkovic-Cvijin et al., 2020). For example, industrialized infants have a paucity of microbiotic gene cassettes involved in human milk utilization, compared with Tanzanian hunter-gatherers' infants (Olm et al., 2022). Still, bowel movement quality is recently emerging as a strong source of heterogeneity in human gut microbiota profiles (Vujkovic-Cvijin et al., 2020). It is becoming increasingly clear that different intestinal contraction patterns may cause changes in fluid dynamics. Propulsion, reflux and mixing lead to modifications in the intestinal microbiota, since obliterating waves induce considerable bolus mixing due to an upstream vortex, while colliding waves create a high-pressure region characterized by rapid fluid flow, high shear stress and radial mixing upon annihilation (Amedzrovi Agbesi and Chevalier, 2022). We provided an effort to correlate intestinal gaseous changes in volume or density with modifications in gaseous mixing and trans-barrier diffusion. We showed that increases in gas volume due to two different mechanisms (namely, physical muscular gut contraction and biological colonic fermentation) lead to different metabolic outcomes. The Boyle's experimental gas law suggests that the gaseous exchanges between lumen and bloodstream are decreased by muscular contraction and augmented by muscular relaxation. The surface/volume ratio suggests that these exchanges are reduced by microorganismal overproliferation and increases improved by microorganismal decline.

It is noteworthy that the two mechanisms may also exert synergic effects. For instance, volumetric gaseous increases during intestinal obstruction are caused by both muscular relaxation and increased colonic fermentation, allowing the two simultaneous mechanisms to provide counterbalanced effects on the inter-barrier gaseous exchanges. Therefore, at least to some extent, the physiological gaseous turnover tends to be neither increased nor decreased in case of intestinal dilation. The distinction between physical and biological causes of intestinal volumetric changes might shed new light also on the

puzzling phenomenon of pneumatosis intestinalis, i.e., the radiographing finding of gas in the mucosa, submucosa, muscular layers or mesentery (Ho et al., 2007; Albright 2019). A variety of contributing factors have been suggested to explain the pathogenesis of intramural gas accumulation (St Peter et al., 2003; Blair et al., 2015; Sugihara and Okada, 2017):

a) Mechanical pressure from pulmonary diseases like chronic obstructive pulmonary disease.
b) Progressive diffusion of high-pressure intraluminal gas inside the bowel wall.
c) Infiltration of gas-producing microorganisms inside the impaired bowel wall. The mucosal derangement and the subsequent increase in mucosal permeability would allow gas-forming bacteria to enter the bowel wall and locally produce gas.
d) Intraluminal bacterial overgrowth.
e) Excessive $H_2$ excretion due to lack of $H_2$-consuming intestinal bacteria (Levitt and Olsson, 1995). It has been suggested that rapid $H_2$ diffusion from the lumen into intramural gas bubbles would cause $N_2$, $O_2$, and $CO_2$ to diffuse from the bloodstream into the bubbles. As a result, the bubble would expand and persist indefinitely as long as intraluminal $H_2$ continues to diffuse (Levitt and Olsson, 1995). The phenomenon, termed gas cysts' counterperfusion supersaturation, occurs mainly near the blood vessels of the colonic mesenteric border (Florin and Hills, 1995).

Our account suggests that intraluminal increases in gaseous volume due to microbial fermentation could be the main factor that increases the gaseous pressure exerted in the haustral pockets. In the confined haustral volumes, local increases in gaseous pressure may reach high intraluminal values, overtaking the counter-pressure exerted by the blood flow and impacting the allometric relationships governing the biophysics of the mucosal cells. Therefore, we speculate that the best mechanism to explain intestinal pneumatosis is to consider that high-pressure transitory waves might allow intraluminal gases to passively diffuse inside the gut wall.

Along the "gut-brain-axis", the intestinal microbiota influences functions, development and disease of both the enteric and the central nervous systems. Intestinal bacteria deliver a wide range of mammalian neurotransmitters, including dopamine, norepinephrine, serotonin, gamma-aminobutyric acid (Strandwitz 2018). Some gases that can be found in the gut exert well-known effects on both the peripheral intestinal neurons and the central neurons. For example, NO, apart from the above-described effects on the intestinal neurons, is synthesized "on demand" in the brain from postsynaptic terminals and is involved in neuronal signaling, volume transmission, retrograde modulation of presynaptic electrical activity, transmitter release (Rodriguez-Grande and Konsman 2018; Del-Bel and De-Miguel, 2018). Hydrogen sulfide, apart from promoting colonic transit and affecting intestinal motility/visceral nerve hypersensitivity, is also a neuromodulator that enhances NMDA-induced currents in hippocampal neurons (Abe and Kimura, 1996; Nagai et al., 2004) and mediates the reciprocal brain interactions between glial calcium waves and neuronal activity (Guo et al., 2016). Other gases that can be found in the gut are known to exert effects on the central neurons, but their roles on the intestinal neurons is still controversial. For example, ammonia, due to the $NH_3$ -permeable channels, can be systemically absorbed causing hepatic encephalopathy in patients with liver cirrhosis (Rodriguez-Grande and Konsman 2018). Further, $N_2$ hyperbaric exposure induces narcosis targeting the striatum and the substantia nigra compacta (Rostain and Lavoute, 2016). A role of $H_2O_2$ as intercellular signaling molecule/neuromodulator in the brain is becoming increasingly apparent (Ledo et al., 2022).
Here we suggest a testable hypothesis, i.e., that the intestinal submucosal and myenteric neurons might be sensitive to $NH_3$ and $H_2O_2$ as the central neurons are. Having these gases well-studied metabolic effects on the central neurons, it is theoretically possible that they could have metabolic effects also on the intestinal neurons, considering that cell-produced volatile substances often regulate neighbouring cells in a paracrine fashion (Herrera and Garvin, 2011). This notion is supported by the ability of $H_2O_2$ to diffuse in the extracellular space of the living rodent brain over 100 μm within its 2.2 seconds average half-life (Ledo et al., 2022). The in vivo $H_2O_2$ brain diffusion coefficient of about $2.5 \times 10^{-5}$ $cm^2/s$ makes it theoretically possible for intraluminal-generated $H_2O_2$ to reach the intestinal nervous system. Since $H_2O_2$ can be transported through Aquaporin 3 in colonic epithelial cells (Yde et al., 2021), it might be able to reach at least the neurons of the submucosal plexus.
In sum, we suggest that still unknown effects of intestinal gases on the myenteric and submucosal neurons are (possibly) waiting to be discovered.

The last, but not the least, our physical account indicates that gaseous mixing and absorption are far from optimum even in the healthy gut. Two mechanisms contribute to weaken the gaseous exchanges between the lumen and the bloodstream:

a) The absence of turbulence in the intestinal content's flows. Turbulence is a dissipative phenomenon characterized by mathematically untreatable fluctuating instabilities. The breakdown of the continuum flow assumption that is characteristic of the Navier-Stokes equations (Barber and Emerson, 2002) leads to unsteady flow conditions and to the onset of the hallmark of turbulence, i.e., the occurrence of vortices, eddies and swings at different length scales (Sturm et al., 2012). Overflowed surfaces are subject to counter-rotating foci, separation and saddle points, extinction and even inversion of the velocity (Ma et al., 2020). While the intrinsic

unpredictability of turbulence contributes to enhance fluid mixing and improve diffusive exchanges, the laminar flows displayed by intestinal fluids must generate just a small amount of intraluminal mixing and metabolic exchanges.

b) The random walks' failure to cover the entire phase space. In the (relatively) quiet colonic pockets' environment, the gaseous particles can stochastically reach just one third of the entire mucosal surface, leaving apart large "patches" of colonocytes where metabolic trans-barrier exchanges cannot be fully realized.

Therefore, next-to-come experimental studies concerning intestinal absorption might take into account that a large, quantifiable fraction of the intestinal surface is not available for fluid exchanges between the lumen and the bloodstream.

In conclusion, intestinal gases have always had a bad reputation from the standpoints of aesthetics, etiquette, and ethics. Things have come to such pass that a proposal in Malawi has been brought up in 2011 to criminalize public flatulence (Pfeifer, 2020). Nevertheless, in stark contrast to this negative reputation, intestinal gases have a wide range of physiological as well as pathological effects not just on various intestinal segments, but also on extra-intestinal organs. Viewed as toxic gases and/or environmental toxins until a few years ago, many gaseous molecules have been recently "promoted" to the role of biologic mediators (Pacher 2021). The capability to metabolically interact with the intestinal wall and to cross the barrier between the lumen and the bloodstream makes intestinal gases a versatile tool to achieve intracellular, extracellular, paracellular, paracrine as well as distant actions, both in a state of well-being and in response to manifold noxae.

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