

# Levels of Hydrogen Sulphide or Nitric Oxide in Induced Belching are Indicators of Adequate Gastric Voiding

Donatini Bruno\*, and Le Blaye Isabelle

Medicine Information Formation (Research), Gastroenterology-hepatology, Cormontreuil, France

\*Corresponding authors: Donatini Bruno, Medicine Information Formation (Research), Gastroenterology-hepatology, Cormontreuil, France, E-mail: donatini@orange.fr

Received: 25 Jan, 2021 | Accepted: 22 Feb, 2021 | Published: 27 Feb, 2021

**Citation:** Donatini B, Le Blaye I (2021) Levels of Hydrogen Sulphide or Nitric Oxide in Induced Belching are Indicators of Adequate Gastric Voiding. J Clin Case Stu 6(1): dx.doi.org/10.16966/2471-4925.216

**Copyright:** © 2021 Donatini B, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Abstract

**Background:** Hydrogen Sulphide (H<sub>2</sub>S) and Nitric Oxide (NO) are gasotransmitters with neuroprotective and antioxidant properties which can be produced by the gastric microbiota.

**Objective:** We investigated whether X-am8000® (a new ambulatory device) is able to detect H<sub>2</sub>S or NO in induced belching and whether these gases are associated with a better gastro duodenal voiding which could signify microbiota diversity, and neuroprotection.

**Methods:** All data were collected during consultations for small gut dysbiosis. A gas test was performed with X-am8000® on breath. Belching was induced by lemon juice plus bicarbonate and osteopathic manoeuvres. A second gas test was performed on belching.

**Results:** 145 patients were included. After at least 10 hours of fasting, gastro duodenal voiding was objectivised by an ultrasound examination in 32 patients (voiding group). No gastro duodenal voiding could be evidenced in 113 patients (non-voiding group). Patients of the voiding group present more frequently with NO>1ppm (75% versus 34.5%; p<0.001) and with H<sub>2</sub>S>0.1ppm (93.8% versus 46.9%; p<0.001) in belching. Patients of the Non-voiding group present more frequently with obesity, excessive alcohol intake, increased glycaemia, pancreatic steatosis, high neutrophil/lymphocyte ratio, and low-molecular-weight hyaluronic acid or uric acid levels.

**Conclusion:** X-am8000® is able to detect H<sub>2</sub>S and NO in induced belching. They are good markers of adequate voiding and perhaps of preserved microbiota or neuronal function of the fore-gut. X-am8000® may help to detect gastric dysbiosis and to select diet able to preserve or facilitate H<sub>2</sub>S or NO-producing bacteria.

**Keywords:** Gastric gas test; Hydrogen Sulphide; Nitric Oxide; Gastric voiding

**Abbreviations:** BMI: Body Mass Index; CMV: Cytomegalovirus; COVID-19: Coronavirus Disease; E-VOCs: Exhaled Volatile Organic Compounds; HPV: Human papilloma virus; H<sub>2</sub>S: Hydrogen Sulphide, LMW-HA: Low Molecular Weight Hyaluronic Acid; NLR: Neutrophil-Lymphocyte Ratio; NO: Nitric Oxide; NPV: Negative Predictive Value; PPM: Particles Par Million; PPV: Positive Predictive Value; RT: Retention Time; Se: Sensitivity; SIBO: Small Intestinal Bowel Overgrowth; Sp: Specificity; T2DM: Type 2 Diabetes Mellitus, US: Ultrasound Examination

## Introduction

Hydrogen Sulphide (H<sub>2</sub>S) and Nitric Oxide (NO) are gasotransmitters with neuroprotective and anti-oxidative properties [1-5].

H<sub>2</sub>S increases autophagy and protects many organs such as liver [6,7], kidney [8], lungs [9], or heart and vessels [10]. It contributes to mucosal and immune defence against infection [11].

The endogenous production of H<sub>2</sub>S is primarily mediated by cystathione β-synthase, cystathione γ-lyase, and 3-mercaptopyruvate sulfurtransferase. These enzymes are widely expressed in the liver tissues and regulate hepatic functions [7].

H<sub>2</sub>S is also produced by mucolytiques bacteria such as *Akkermansia multocida*, *Helicobacter pylori*, *Desulfovibrio species*, *Bacteroidetes fragilis*, *Bacteroidetes thetaiotaomicron*, *Prevotella species* or *Fusobacterium nucleatum* [12]. H<sub>2</sub>S favours NO synthesis [3].

NO supplementation has demonstrated cardiovascular benefits by normalizing blood pressure, enhancing blood flow, and reducing inflammation, immune dysfunction or oxidative stress [13,14]. It may decrease the risk of cancer and may slow down senescence [15]. NO demonstrates potent cytoprotective effects on neurons [16], gastrointestinal mucosa [17,18] or skin [19,20].

NO favours gastroduodenal voiding by decreasing sphincter tonus, and by increasing gastric tone and phasic contractions [21-24].

NO is a strong anti-herpes-simplex agent [25-28] and also attenuate CMV infection [29]. NO is produced by NO synthetases which are ubiquitous. They exist in endothelia, smooth muscles, platelets, macrophages, lymphocytes, myocardial cells and neurones. They are classified in nNOs (neuronal origin), eNOs (endothelial origin) or iNOs (inducible NO of mainly immune origin) [30,31].

However, a substantial amount of NO is produced by bacteria through the sequential reduction of inorganic nitrate to nitrite. NO produced from inorganic nitrate supplementation has been found to have the same cardio protective benefits as NO produced by NO synthetases [32]. The enzymatic reduction of nitrate to nitrite depends on a unique set of bacterial nitrate reductase enzymes possessed by specific bacterial populations localised in the mammalian mouth and gut [33-36]. Nitrate shapes oral microbiome communities and may be a nutrient for the lower gut microbiome. Furthermore, nitrate may act as a respiratory substrate for the existing communities, ensuring the production of bacterial metabolites such as short chain fatty acids [36].

Gastroparesis frequently complicates Type 2 Diabetes Mellitus (T2DM) [37-39] or obesity [40]. Gastrointestinal voiding disturbances are possibly due to the alteration of vagal tone or of myenteric plexus activity [40-43].

NO levels are decreased in obesity or T2DM [43,44]. Bariatric surgery enables the recovery of NO synthesis and bioavailability within 3 months [45,46]. Metformin the most currently prescribed drug in T2DM increases NO levels [47,48]. Women are more susceptible to gastroparesis after menopause because of the decrease in gastric NO levels after the drop of estradiol synthesis [49-51].

Imbalanced intestinal microbiota may favor over weight or obesity, Type 2 Diabetes Mellitus (T2DM) [52,53], chronic inflammation/destruction of mucosa, vagal impairment, as well as decreased immunity [54,55].

Intestinal microbiota can be studied by the analysis of exhaled gases such as hydrogen or methane [56-59] after the intake of sugars. However, Volatile Organic Compounds (VOCs) appear to be more interesting markers and many authors reported links between specific faecal, urine or exhaled-VOCs (E-VOCs) with T2DM [60-62] or overweight/obesity [40,63,64]. Gastric NO or H<sub>2</sub>S levels have never been studied in T2DM, overweight or obesity. We investigated whether a new ambulatory device (X-am8000®) was able to detect NO and H<sub>2</sub>S firstly in exhaled breath and secondly in induced belching.

Abdominal Ultrasound Examination (US) may evaluate gastroduodenal and jejunal movements [39,40] and is routinely performed in patients presenting with Small Intestinal Bacterial Overgrowth (SIBO). We investigate whether movements are different according to NO or H<sub>2</sub>S levels.

We eventually investigated whether overweight/obesity or signs in favour of immunosuppression, mucosal destruction or altered vagal tone were associated with NO or H<sub>2</sub>S levels.

We therefore collected data which may be related to:

- 1) TH1-immunosuppression (cancer or precancerous lesion, opportunistic infections such as herpetic flares, IgG against *Cytomegalovirus* (CMV) or mild COVID-19.
- 2) Mucosal destruction (serum LMW-HA levels, Neutrophil/Lymphocyte Ratio (NLR), and *Helicobacter pylori* infection).
- 3) Vagal impairment (gastroparesis, arrhythmia, osteopenia).

## Materials and Methods

This work is a descriptive retrospective epidemiological study.

Data were collected during the normal course of routine gastroenterological consultations for Small Intestinal Bacterial Overgrowth (SIBO), from 2019 July 1<sup>st</sup> to 2020 June 30<sup>th</sup>.

There was no hypothesis testing before data collection, no data collection beyond that which is part of routine clinical practice, no scheduled data analysis before the work has already been done. This retrospective analysis of case series cannot therefore be qualified as "research" and does not require approval from ethics boards designed to protect humans involved in clinical research, according to the International Committee of Medical Journal Editors (ICMJE).

### Inclusion criteria

Patients consulting for SIBO and who underwent a breath test.

Patients should provide with a full medical history, especially regarding cancer and precancerous lesions, *Herpes simplex*, *Human Papilloma Virus* (HPV) infections, thyroid pathologies, allergic reactions, vagal syncope, arrhythmia, osteoporosis, body weight and height, as well as T2DM.

CMV serology, NLR, serum uric acid and LMW-HA levels, and transabdominal plus thyroid ultrasound examinations are routinely performed inpatients consulting for SIBO.

Patients signed a written consent for the possible retrospective use of the collected data.

### Exclusion criteria

Ongoing tobacco abuse (which may interfere with E-VOCs); lack of CMV serology or of serum hyaluronic acid dosage; lack of transabdominal ultrasound; lack of signed consent for possible retrospective epidemiological use of data; uncontrolled diabetes mellitus; lack of breath test or recent intake of antibiotic therapy or of essential oils leading to massive destruction of the digestive flora or less than 2ppm of E-VOCs at the first measure, after 10 hours of fasting; uncontrolled endocrine disease (including thyroid insufficiency); incomplete data on drug or food complement intake; less than 18 years of age; ulcerative colitis or Crohn's disease; refusal of the protocol inducing belching.

### Medical history of cancer or precancerous lesions

All types of cancer or dysplasia were included. Lesions should have been histologically documented. As a consequence, non-dysplastic polyps were not included in the cancer group. Gallbladder polyps diagnosed by ultrasound examination were therefore not graded as dysplastic polyps.

### Ultrasound examination (US)

Gastroparesis was diagnosed when the surface of the stomach reached 10cm<sup>2</sup> after 10 hours of fasting. Ileal distension was diagnosed as soon as ileal diameter reached 2.2cm at the ileocecal junction. Lack of gastro-duodenal voiding was diagnosed when no evacuation of bubbles between the superior mesenteric artery and the aorta was observed after 2 minutes of osteopathic abdominal manoeuvres.

Jejunal hypotonia was diagnosed when jejunal diameter reaches 19.4mm. In that case, the jejunum contains few bubbles, the mucosa is thin ( $\geq$  1mm) and no peristalsis is visualized.

Decreased jejunal diameter (jejunal spasm attributed to vagal hypertonia) was considered when the measure drops below the threshold of 11.4mm [65,66].

### Gas measurement

The patient comes after at least 10 hours of fasting. He/she exhales the air of the lungs in a neutral plastic bag (Contralco®; Gignac; France; [www.contralco.com](http://www.contralco.com)).

Nitric oxide and hydrogen sulphide were measured by the X-am8000®, an ambulatory device associated with photoionization detection technology [Dräger; Lubeck; Germany; [www.draeger.com](http://www.draeger.com) › Products › Multi-Gas-Detectors]. X-am8000® detects NO or H<sub>2</sub>S concentrations as low as 0.1particle per million (ppm).

The device is portable and equipped with a powerful pump. Patients could be placed in separate rooms when necessary. The setup is basic and only requires a short neutral tube to connect the bag and the device.

### Gastric gases collection

Belching is induced by lemon juice+water (½ glass) followed by sodium bicarbonate (1g in ½ glass of water). Bubbles quickly blow up the stomach. Belching occurs within 5 to 20 minutes either spontaneously or after few osteopathic manoeuvres such as trigger points on the great curvature of the stomach, on the second duodenum, on the first jejunum or on the duodenojejunal flexure.

The patient expels the gas coming from the stomach directly into a new bag, without exhaling air from the lung. The patient is required to refrain from breathing out when he/she feels the burp and to contract or press on the abdomen. The physician may also help with mild abdominal pressures. The patient remains sited during the procedure.

### Statistics

Comparisons of percentages or means used two-sample t-tests. The voiding group was compared with the non-voiding group for each collected variable. Because of the large number of tests necessary for this specific analysis the threshold of statistical significance was set to p<0.001.

Sensitivity, false positive ratio, negative predictive value and positive predictive value were calculated for the most relevant gas.

### Control/voiding group

All consulting patients were pre-included in the study and no case was discarded except when at least one exclusion criteria was identified. As a consequence no recruitment or selection bias is expected. The voiding group is stated to be the healthy control group since the pace-maker of the stomach is expected to be active within fasting periods. The non-voiding group is equal to the total number of included patients minus the voiding group. Classical demographic data will be compared and are expected to be similar.

### Results

This descriptive epidemiological study includes 145 patients. Gastroduodenal voiding was visualized by US in 32 patients (voiding group). 113 patients belong to the non-voiding group.

NLR, LMW-HA and uric acid, which are supposed to be markers of chronic inflammation, chronic oxidation or tissue destruction, were higher in the non-voiding group.

Excessive alcohol intake, increased glycaemia (5.3 ± 0.6 versus 4.8 ± 0.5mmol/l; p<0.001) and pancreatic steatosis mainly characterize the

non-voiding group (Table 1) and can be explained by the same above-mentioned physiopathological mechanisms. Age, gender and BMI are similar in both groups (Table 2).

However, all obese patients belong to the Non-voiding group. Jejunal hypertonia (which is a marker of overweight/obesity) follows the same trend as obesity (Table 1).

NO is not detectable by X-am8000® in exhaled breath. However, in belching, NO levels are higher in the voiding group (5.1 ± 4.0 versus 1.6 ± 2.8 ppm; p<0.001) and 75% of patients from the voiding group have NO levels above 1ppm versus 34.5% in the non-voiding group (p<0.001). NO was detectable in belching in 105 patients.

H<sub>2</sub>S levels in breath or in belching are low and similar. In addition, concentrations of H<sub>2</sub>S are not statistically different between the two groups. However, regarding belching, the percentage of patients with H<sub>2</sub>S levels above 0.1ppm is statistically different between the voiding and the non-voiding group (93.8% versus 46.9%; p<0.001). In 69 patients, H<sub>2</sub>S levels were higher in belching than in breath.

NO and H<sub>2</sub>S levels in induced belching are therefore highly discriminant parameters between the two groups (Table 1). In 112 patients, NO or H<sub>2</sub>S levels were higher in belching than in breath. The 33 remaining patients have NO or H<sub>2</sub>S levels close to 0ppm in breath or in belching and all present with gastroparesis.

Except for CMV IgG (Table 1), infection rate (herpetic flares, HPV infection, COVID-19, *Helicobacter pylori*) were similar in both groups (Table 2).

Cancer or precancerous lesions- a second marker of immunosuppression-were not over-represented in the Non-voiding group.

Surprisingly, vagal impairment (vagal syncope, arrhythmia, osteopenia) was not more frequent in the non-voiding group. Similarly, atopy, eosinophil count or nodular thyroiditis seems to have no influence on gastric voiding in clinical practice (Table 2).

**Table 1:** Relevant clinical and biological data related to gastroduodenal voiding.

	Voiding group(32 patients)	Non-voiding group(113 patients)	P value
BMI>30	0%	7.1%	<0.001
NLR	1.02 ± 0.19	1.86 ± 1.02	<0.001
CMV IgG+	6.3%	19.5%	<0.001
LMW-HA (µg/l)	24 ± 7	57 ± 52	<0.001
Uric acid (µmol/l)	232 ± 49	280 ±71	<0.001
Excessive alcohol intake	6.3%	15.0%	<0.001
Pancreatic steatosis	6.3%	23.0%	<0.001
Glycaemia (mmol/l)	4.8 ± 0.5	5.3 ± 0.6	<0.001
Decreased jejunal diameter	6.3%	30.0%	<0.001
NO level in belching (ppm)	5.1 ± 4.0	1.6 ± 2.8	<0.001
% of patients with NO>1ppm in belching*	75%	34.5%	<0.001
% of patients with H <sub>2</sub> S>0.1ppm in belching**	93.8%	46.9%	<0.001

\*NO level in belching was detectable in 105 patients.

\*\*H<sub>2</sub>S levels were higher in belching than in breath in 69 patients. In 112 patients, NO or H<sub>2</sub>S levels were higher in belching than in breath. The 33 remaining patients present with gastroparesis.

**Table 2:** Non-relevant clinical and biological data related to gastroduodenal voiding.

	Voiding group (32 patients)	Non-voiding group (113 patients)	P value
Eosinophil count/mm <sup>3</sup>	156 ± 92	207 ± 156	<0.02
Liver steatosis	21.9%	35.4%	<0.05
Jejunal hypotonia	28.1%	45.1%	<0.05
Female	75.0%	69%	>0.05
Age	47.9 ± 11.6	51.3 ± 12.8	>0.05
BMI	22.0 ± 3.0	23.0 ± 4.4	>0.05
Vagal syncope; Arrhythmia	18.8%	20.4%	>0.05
Herpetic flares	46.9%	34.5%	>0.05
HPV infection	9.4%	8.0%	>0.05
Osteoporosis	12.5%	13.3%	>0.05
Nodular thyroiditis	37.5%	32.7%	>0.05
Helicobacter pylori+	28.1%	21.2%	>0.05
COVID-19 infection	6%	5%	>0.05
Cancer or precancerous lesion	12.5%	11.5%	>0.05
Atopy	34.4%	28.3%	>0.05
NO level in breath (ppm)	0.0	0.0	>0.05
H <sub>2</sub> S level in breath (ppm)	0.2 ± 0.25	0.27 ± 1.4	>0.05
H <sub>2</sub> S level in belching (ppm)	0.4 ± 0.2	0.3 ± 0.2	>0.05

We calculated the sensibilities, the specificities, the positive predictive values and the negative predictive values of H<sub>2</sub>S levels>0.1ppm (Table 3), NO levels>1ppm (Table 4) or H<sub>2</sub>S levels>0.1ppm plus NO levels>1ppm (Table 5) in induced belching, regarding gastroduodenal voiding.

The specificity (Sp) of the H<sub>2</sub>S levels>0.1ppm plus NO levels>1ppm is equal to 78.8% and the predictive positive value (PPV) is equal to 89.9%, in favour of gastroduodenal voiding.

Only three factors (in extension: obesity, increased glycaemia and gastroduodenal voiding) are associated with H<sub>2</sub>S>0.1ppm plus NO>1ppm in induced belching. All other recorded parameters do not appear to modify or to be modified by gastric H<sub>2</sub>S and NO production in ambulatory clinical practice (Table 6).

## Discussion

To our knowledge, results of NO and H<sub>2</sub>S measurement in induced belching has never been published, and their association with gastroparesis has never been reported. In our study, gastroparesis is associated with a decrease in NO or H<sub>2</sub>S levels in induced belching.

### NO

NO is a peripheral mediator synthesized by small parasympathetic nerve fibres, localized in all hollow organs including gastric or pyloric walls [67,68]. NO is also synthesized by local bacteria with nitric reductase activities [33-36].

NO is not transported to the stomach by vagal afferent nerve. It is exclusively produced locally [24]. In addition, endogenous nitrates are recycled by oral bacteria into NO which regulates gastric mucosal thickness, blood flow and defence [69,70].

**Table 3:** Sensitivity (Se), Specificity (Sp), Positive Predictive Values (PPV), Negative Predictive Values (NPV) of H<sub>2</sub>S levels>0.1ppm in induced belching regarding gastroduodenal voiding.

	H <sub>2</sub> S levels>0.1ppm (83 patients)	H <sub>2</sub> S levels ≤ 0.1ppm (62 patients)	Se§ Sp PPV NPV
Voiding	30 (a)	2 (c)	93.8% 53.1% 36.1% 96.8%
Non-voiding	53 (b)	60 (d)	

§ Se=a/(a+c); Sp=d/(b+d); PPV=(Se\*prevalence)/(Se\*prevalence+(1-prevalence)\*(1-Sp)); NPV=Sp\*(1-prevalence)/(Sp\*(1-prevalence)+prevalence\*(1-Se)); Prevalence=(a+c)/(a+b+c+d)

**Table 4:** Sensitivity (Se), Specificity (Sp), Positive Predictive Values (PPV), Negative Predictive Values (NPV) of NO levels>1ppm in induced belching regarding gastroduodenal voiding.

	NO levels>1ppm (63 patients)	NO levels ≤ 1ppm (82 patients)	Se§ Sp PPV NPV
Voiding	24 (a)	8 (c)	93.8% 53.1% 36.1% 96.8%
Non-voiding	39 (b)	74 (d)	

§ Se=a/(a+c); Sp=d/(b+d); PPV=(Se\*prevalence)/(Se\*prevalence+(1-prevalence)\*(1-Sp)); NPV=Sp\*(1-prevalence)/(Sp\*(1-prevalence)+prevalence\*(1-Se)); Prevalence=(a+c)/(a+b+c+d)

NO decreases sphincters' tonus and enables the stomach to void [23]. H<sub>2</sub>S is probably necessary to nNO synthesis [3]. The specificity of these two combined gasotransmitters regarding the detection of impaired gastroduodenal voiding is very high (78.8%) when NO exceeds 1ppm and H<sub>2</sub>S exceeds 0.1ppm simultaneously. X-am8000<sup>®</sup> is able to detect these gases in belching within a few minutes.

The combination of H<sub>2</sub>S and NO detection in induced-belching by X-am8000<sup>®</sup> provides a painless and inexpensive opportunity to detect the gases physiologically involved in gastroduodenal voiding and perhaps to confirm the local bacterial diversity which is require for its occurrence. Breath does not contain detectable levels of NO when X-am8000<sup>®</sup> is used.

In asthma, NO levels are increased. However, they do not exceed 0.1ppm [71-73] which is the detection limit of X-am8000<sup>®</sup>. Due to its limited lifetime and diffusion distance, NO has been mainly believed to act in autocrine/paracrine fashion. However, the recognized pharmacological effect of endogenous NO at distant sites has changed the conventional wisdom [74].

NO and H<sub>2</sub>S are small and hydrophobic molecules. They can cross cellular unhindered membranes and their diffusion is unimpeded by cellular membranes [75]. NO appears to operate in two main modes: first, in a near synapse-specific manner and, second, when multiple nearby sources are active simultaneously, as a volume transmitter enabling signaling to diverse targets irrespective of anatomical connectivity [76].

**Table 5:** Sensitivity (Se), Specificity (Sp), Positive Predictive Values (PPV), Negative Predictive Values (NPV) of H<sub>2</sub>S levels>0.1ppm and NO levels>1ppm in induced belching regarding gastroduodenal voiding.

	H <sub>2</sub> S levels>0.1ppm and NO levels>1ppm in belching (46 patients)	H <sub>2</sub> S levels ≤ 0.1ppm or NO levels ≤ 1ppm in belching (99 patients)	Se Sp PPV NPV
Voiding	22 (a)	10 (c)	68.8% 78.8%
Non-voiding	24 (b)	89 (d)	47.8% 89.9%

§  $Se = a/(a+c)$ ;  $Sp = d/(b+d)$ ;  $PPV = (Se * prevalence) / (Se * prevalence + (1 - prevalence) * (1 - Sp))$ ;  
 $NPV = Sp * (1 - prevalence) / (Sp * (1 - prevalence) + prevalence * (1 - Se))$ ;  
 $prevalence = (a+c)/(a+b+c+d)$

**Table 6:** Relevant clinical and biological data related to content of NO>1ppm and H<sub>2</sub>S>0.1ppm in induced belching\*.

	H <sub>2</sub> S levels>0.1ppm and NO levels>1ppm in belching (46 patients)	H <sub>2</sub> S levels ≤ 0.1ppm or NO levels ≤ 1ppm in belching (99 patients)	P value
BMI>30	2.1%	7.1%	<0.001
Glycaemia (mmol/l)	4.9 ± 0.4	5.4 ± 0.7	<0.001
Gastroduodenal voiding	47.8%	10.1%	<0.001

\*Other parameters (gender, age, BMI, vagal syncope/arrhythmia, eosinophilia, CMV IgG+, hyaluronic acid, uric acid, alcohol intake, nodular thyroiditis, Helicobacter pylori, liver steatosis, pancreatic steatosis, jejunal hypertonia, jejunal hypotonia, Small chain fatty acids with RT<6s in exhaled breath) are no associated with NO or H<sub>2</sub>S levels in belching (p>0.01).

The ratio between the breath and the gastric levels of NO is, in order of magnitude, between 100 to 1000. This ratio suggests a massive gastric synthesis, limited diffusion through the gastric wall and no distribution to vessels, which avoids acute and prolonged vasodilatation-an undisputable effect of NO [77].

Since the involvement of nitrite reductase from bacteria has been demonstrated in the production of NO [32-36], we concluded that oral or gastric bacteria are responsible of NO production in the stomach. We also concluded that breath test are unlikely able to detect gastroparesis at any stage. By contrast, gas-analysis of belching can be fruitful as soon as dysbiosis occurs.

## H<sub>2</sub>S

H<sub>2</sub>S can be detected in breath by X-am8000\*. The ratio between the breath and the gastric levels of H<sub>2</sub>S is, in order of magnitude, between 1 to 2. This ratio suggests that the production in the stomach is low. H<sub>2</sub>S can be produced by sulfatases from mucolytic bacteria [12]. The association of NO and H<sub>2</sub>S improves the ability of the stomach to void.

## Overweight or obesity

Overweight or obesity is associated with decreased gastro-duodenal voiding either due to jejunal spasm (vagal hypertonia) or to jejunal

hypotonia [40,78]. In support, neuro modulation of vagal tone has been tested for the treatment of metabolic diseases [79]. Promising results have been published with devices able to block under-diaphragmatic vagal tone [80-82].

A decreased diversity of microbiota has been documented in patients with obesity [83,84]. For example, decreased levels of colonization by *Helicobacter pylori* or *Akkermansia multocida* is well documented in obese patients [85-87].

In this epidemiological study, NO and H<sub>2</sub>S levels from belching are reduced in the non-voiding group. This is an additional argument for a correlation between low gastric NO and H<sub>2</sub>S levels, and the paucity of the foregut bacterial diversity.

Success of bariatric surgery implies the recovery of gastric microbial diversity [88] and is associated with the recovery of NO production [46,89]. Over use of antibiotic therapy has been associated with obesity [90]. Similarly, increased atherosclerosis by antibiotics is connected to the loss of gut-microbiota diversity [91].

Detection of low levels of NO or H<sub>2</sub>S in belching could alert about the antibiotic-induced weakening of bacterial diversity. Attempts to trigger diversity (e.g. diet diversification with high nitrite vegetables) could be initiated before any occurrence of weight increase. Overweight and obesity are characterized by increased adipose tissue mass resulting in low-grade inflammation and development of T2DM and cardiovascular disease [92-94].

Since NO and H<sub>2</sub>S possess anti-oxidative properties [1-11]. Their decrease may favor oxidation and low-grade chronic inflammation.

## NLR, LMW-HA, fatty pancreas

In this epidemiological study, gastroduodenal voiding disturbance is associated with obesity, T2DM, fatty pancreas, NLR increase (inflammation) and destruction of tissue (LMW-HA increase). The association of increased LMW-HA levels with obesity supports the hypothesis that low NO and H<sub>2</sub>S levels result in the combination of increased visceral fat and tissue destruction. Increased acetic acid production, NLR and glycaemia have been documented in obesity, in association with vagal hypertonia and disturbed gastroduodenal voiding [40]. NLR is considered to be reliable marker of severe tissue destruction [95-97].

LMW-HA is known to increase endothelial permeability, stimulate receptors of cancerous stem cells and favour metastasis. The migration of stem-cells according to LMW-HA gradient has been documented [98-101]. An increase in LMW-HA levels may occur in case of non-alcoholic steatohepatitis complicated with fibrosis [102,103] or of pancreatic cancer [104]. Hyaluronan content of the pancreas governs tissue stiffness and pancreatic islet inflammation [105]. Since pancreatic steatosis characterizes the non-voiding group, we speculated that gastric NO and H<sub>2</sub>S levels may decrease only at a late stage, after inflammation has induced the release of LMW-HA.

Since increased NLR and serum LMW-HA levels are markers of tissues destruction, they could rather be consequences than causes of gastroparesis.

## Uric acid levels

Increased uric acid level is common in patients with overweight/obesity and T2DM [106]. Uric acid may itself be the cause of obesity and T2DM [107-110]. However, a direct implication of high serum uric acid levels in the occurrence of gastroparesis has never been reported.

In China or in Europe, central obesity, T2DM and fatty liver disease are independent risk factors of fatty pancreas [111-113] which can therefore be considered as a sign of obesity. Uric acid possesses strong antioxidant properties [114]. In support, low uric acid levels are associated with neurodegenerative diseases [115-119] or depression [120-122].

However, high uric acid levels induce vascular or articular inflammation [114,123]. Uric acid levels should therefore be maintained into a restrictive range in order to avoid oxidation. A link between nitrate, nitrite and NO has been mentioned above. In healthy non-sedentary young men, oral nitrate increases serum uric acid or nitrite levels, and the total antioxidant capacity of the saliva [124,125]. Oral nitrate supplementation, at least on a short term basis may improve physical performance or even global health [126].

It is likely that oral nitrate/nitrite on one hand may favour gaseous/bacterial NO synthesis in saliva or in gastric fluid and on the other hand may increase serum uric blood levels which display either beneficial or detrimental effects according to its concentration. Nevertheless, even in patients with high uric acid levels, obesity or T2DM, nitrate intake improves exercise tolerance [127-130].

Interestingly, oral nitrate supplementation does not prevent metabolic syndrome development in mice [131], which suggests, a key role of the salivary nitrate-transforming microbiota rather than of nitrate itself.

### Excessive alcohol intake

Alcohol has been associated with alterations in gastric motility. Chronic alcohol consumption alters the myenteric nitrenergic system resulting in impaired gastrointestinal motor function, and inhibits the release of several neurotransmitters, including acetylcholine [132,133]. In general, beverages with high alcohol concentrations (i.e., above 15 percent) appear to inhibit gastric motility and low alcohol doses (wine and beer) accelerate gastric emptying. Acute administration of ethanol inhibits the gastric emptying and the small bowel transit, while chronic administration of a large dose of alcohol accelerates gastric motility and the small bowel transit [134].

### CMV infection

Except for CMV IgG+, the number of patients with cancer or precancerous lesions, mild-COVID-19, herpetic flares, or HPV infection was similar in the two groups. Th1-immunosuppression is therefore probably not involved in the occurrence of gastroparesis and decreased in NO or H<sub>2</sub>S levels of belching.

Ongoing CMV infection compromise gastroduodenal voiding and is associated with cancer or increased LMW-HA levels [135]. However, CMV IgG+ should be rather considered as a surrogate marker of tissue inflammation [136,137] or of senescence [138,139] rather than a marker of viral recurrences since firstly CMV is rarely implicated in infectious gastroparesis and secondly viral gastroparesis have usually self-limiting duration in non-severely-immunocompromised patients [140,141]. However, liver transplant recipients frequently present with CMV-induced altered gastric emptying [142].

In general, vagal hypotonia could explain the occurrence of arrhythmia [143-145] as well as of gastroparesis [146,147]. In contrast, vagal hypertonia which is present in obese patients, who exclusively belong to the non-voiding, may explain the scarcity of arrhythmia or of osteopenia occurring in this study.

### Limitations of the study

Analyses of salivary or gastric flora were not concomitantly performed. They are time consuming or expensive, and they do not belong to systematic ambulatory practice. In addition, gastric tubing is invasive and appears quite inappropriate in the pandemic period of SARS-COV-2 infection. We were rather looking for non-invasive and inexpensive examinations which could be correlated with gastric voiding. Microbiota diversity was therefore not the key issue of the study.

It is difficult to certify that collected gases are not spoiled by exhaled breath. However, in 112 patients, NO or H<sub>2</sub>S levels were higher in belching than in breath. The gastric origin is therefore immediately confirmed.

All the 33 remaining patients present with gastroparesis. Consequently, the conclusion regarding the gastric emptying is not impaired by a hypothetical spoiling of belching with breath. When in doubt, a second belching test can immediately validate or not the results.

### Application of this new knowledge for routine practice

This epidemiological study demonstrates that the new device X-am8000\* may detect NO and H<sub>2</sub>S levels in belching, and is discriminant enough to diagnose impaired gastric voiding. Since the major part of gastric NO is expected to be produced by local bacteria, X-am8000\* may help to detect decreased gastric microbiota diversity which is associated with obesity, T2DM or hyperuricemia.

We suggest employing this belching test an device in association with transabdominal ultrasound examination of the liver, the pancreas, the stomach and the small gut in all patients presenting with obesity/overweight, increased glycaemia (>5.0mmol/l), increased uric acid level (>250µg/l), positive CMV serology, increased LMW-HA (>30 µmol/l), increased NLR (>1.5), excessive alcohol intake or with a clinical history of fatty liver/fatty pancreas.

In addition, such a test may help to follow the recovery of gastric voiding after the application of hygiene-dietetic advices, physical training or electric vagal stimulation.

### Conclusion

X-am8000\* can detect NO and H<sub>2</sub>S in induced belching. NO levels >1 and H<sub>2</sub>S levels >0.1 are associated with adequate gastroduodenal voiding and may represent a conserved bacterial diversity and a preserved automatic innervations in the stomach. The device and the method may help to follow these latter parameters which are known to be key markers in obesity/overweight, T2DM, or excessive alcohol intake.

### Acknowledgment(S) and Conflicts of Interest

No conflict of interest to disclose.

### References

- Xue X, Bian JS (2015) Neuroprotective Effects of Hydrogen Sulfide in Parkinson's Disease Animal Models: Methods and Protocols. *Methods Enzymol* 554: 169-186.
- Shefa U, Yeo SG, Kim MS, Song IO, Jung J, et al. (2017) Role of Gasotransmitters in Oxidative Stresses, Neuroinflammation, and Neuronal Repair. *Biomed Res Int* 2017: 1689341.
- Farrugia G, Szurszewski JH (2014) Carbon Monoxide, Hydrogen Sulfide, and Nitric Oxide as Signaling Molecules in the Gastrointestinal Tract. *Gastroenterology* 147: 303-313.

4. Cakmak YO (2017) Rotorua, Hydrogen Sulphide and Parkinson's Disease-A Possible Beneficial Link? *N Z Med J* 130: 123-125.
5. Kanagy NL, Szabo C, Papapetropoulos A (2017) Vascular Biology of Hydrogen Sulfide. *Am J Physiol Cell Physiol* 312: C537-C549.
6. Ruan Z, Liang M, Deng X, Lai M, Shang L, et al. (2019) Exogenous Hydrogen Sulfide Protects Fatty Liver Against Ischemia-Reperfusion Injury by Regulating Endoplasmic Reticulum Stress-Induced Autophagy in Macrophage through Mediating the Class A Scavenger Receptor Pathway in Rats. *Cell Biol Int*.
7. Sun HJ, Wu ZY, Nie XW, Wang XY, Bian JS (2020) Implications of Hydrogen Sulfide in Liver Pathophysiology: Mechanistic Insights and Therapeutic Potential. *J Adv Res* 27: 127-135.
8. Wang Y, Xing QQ, Tu JK, Tang WB, Yuan XN, et al. (2019) Involvement of Hydrogen Sulfide in the Progression of Renal Fibrosis. *Chin Med J (Engl)* 132: 2872-2880.
9. Ge X, Sun J, Fei A, Gao C, Pan S, et al. (2019) Hydrogen Sulfide Treatment Alleviated Ventilator-Induced Lung Injury through Regulation of Autophagy and Endoplasmic Reticulum Stress. *Int J Biol Sci* 15: 2872-2884.
10. Luo W, Gui DD, Yan BJ, Ren Z, Peng LJ, et al. (2020) Hydrogen Sulfide Switch Phenomenon Regulating Autophagy in Cardiovascular Diseases. *Cardiovasc Drugs Ther* 34: 113-121.
11. Wallace JL, Blackler RW, Chan MV, Da Silva GJ, Elsheikh W, et al. (2015) Anti-Inflammatory and Cytoprotective Actions of Hydrogen Sulfide: Translation to Therapeutics. *Antioxid Redox Signal* 22: 398-410.
12. Kushkevych I, Cejnar J, Tremel J, Dordević D, Kollar P, et al (2020) Recent Advances in Metabolic Pathways of Sulfate Reduction in Intestinal Bacteria. *Cells* 9: 698.
13. Banez MJ, Geluz MI, Chandra A, Hamdan T, Biswas OS, et al. (2020) A Systemic Review on the Antioxidant and Anti-Inflammatory Effects of Resveratrol, Curcumin, and Dietary Nitric Oxide Supplementation on Human Cardiovascular Health. *Nutr Res* 78: 11-26.
14. Król M, Kepinska M (2020) Human Nitric Oxide Synthase-Its Functions, Polymorphisms, and Inhibitors in the Context of Inflammation, Diabetes and Cardiovascular Diseases. *Int J Mol Sci* 22: 56.
15. Mabrouk N, Ghione S, Laurens V, Plenchette S, Bettaieb A, et al. (2020) Senescence and Cancer: Role of Nitric Oxide (NO) in SASP. *Cancers (Basel)* 12: 1145.
16. Ally A, Powell I, Ally MM, Chaitoff K, Nauli SM (2020) Role of Neuronal Nitric Oxide Synthase on Cardiovascular Functions in Physiological and Pathophysiological States. *Nitric Oxide* 102: 52-73.
17. Wallace JL (2019) Nitric Oxide in the Gastrointestinal Tract: Opportunities for Drug Development. *Br J Pharmacol* 176: 147-154.
18. Han T, Tang Y, Li J, Xue B, Gong L, et al. (2017) Nitric Oxide Donor Protects Against Acetic Acid-Induced Gastric Ulcer in Rats *via* S-Nitrosylation of TRPV1 on Vagus Nerve. *Sci Rep* 7: 2063.
19. Stancic A, Jankovic A, Korac A, Buzadzic B, Otasevic V, et al. (2018) The Role of Nitric Oxide in Diabetic Skin (Patho) Physiology. *Mech Ageing Dev* 172: 21-29.
20. Del Rosso JQ, Kircik LH (2017) Spotlight on the Use of Nitric Oxide in Dermatology: What Is It? What Does It Do? Can It Become an Important Addition to the Therapeutic Armamentarium for Skin Disease? *J Drugs Dermatol* 16: s4-s10.
21. Sevgili AM, Balkanci DZ, Erdem A (2017) Potential Excitatory Role of Nitric Oxide on 2-Deoxy-d-glucose-Induced Gastric Motility in Rats. *Clin Exp Pharmacol Physiol* 44: 693-699.
22. Beckett EAH, Sanders KM, Ward SM (2017) Inhibitory Responses Mediated by Vagal Nerve Stimulation are Diminished in Stomachs of Mice with Reduced Intramuscular Interstitial Cells of Cajal. *Sci Rep* 7: 44759.
23. Sivarao DV, Mashimo H, Goyal RK (2008) Pyloric Sphincter Dysfunction in nNOS-/- and W/Wv Mutant Mice: Animal Models of Gastroparesis and Duodenogastric Reflux. *Gastroenterology* 135: 1258-1266.
24. Page AJ, O'Donnell TA, Cooper NJ, Young RL, Blackshaw LA (2009) Nitric Oxide as an Endogenous Peripheral Modulator of Visceral Sensory Neuronal Function. *The J Neurosci* 29: 7246-7255.
25. Lucinda N, Figueiredo MM, Pessoa NL, da Silva Santos BSA, Lima GK, et al. (2017) Dendritic Cells, Macrophages, NK and CD8+ T Lymphocytes Play Pivotal Roles in Controlling HSV-1 in the Trigeminal Ganglia by Producing IL1-beta, iNOS and Granzyme B. *Virology* 14: 37.
26. Zolini GP, Lima GK, Lucinda N, Silva MA, Dias MF, et al. (2014) Defense against HSV-1 in a Murine Model is Mediated by iNOS and Orchestrated by the Activation of TLR2 and TLR9 in Trigeminal Ganglia. *J Neuroinflammation* 11: 20.
27. Wu B, Geng S, Bi Y, Liu H, Hu Y, et al. (2015) *Herpes Simplex Virus 1* Suppresses the Function of Lung Dendritic Cells *via* Caveolin-1. *Clin Vaccine Immunol* 22: 883-895.
28. Flowerdew SE, Wick D, Himmelein S, Horn AK, Sinicina I, et al. (2013) Characterization of Neuronal Populations in the Human Trigeminal Ganglion and their Association with Latent *Herpes Simplex Virus-1* Infection. *PLoS One* 8: e83603.
29. Mokry RL, Schumacher ML, Hogg N, Terhune SS (2020) Nitric Oxide Circumvents Virus-mediated Metabolic Regulation during Human *Cytomegalovirus* Infection. *mBio*.
30. Förstermann U, Sessa WC (2012) Nitric Oxide Synthases: Regulation and Function. *Eur Heart J* 33: 829-837.
31. Gantner BN, LaFond KM, Bonini MG (2020) Nitric Oxide in Cellular Adaptation and Disease. *Redox Biol* 34: 101550.
32. Ivy JL (2019) Inorganic Nitrate Supplementation for Cardiovascular Health. *Methodist Debakey Cardiovasc J* 15: 200-206.
33. Koch CD, Gladwin MT, Freeman BA, Lundberg JO, Weitzberg E, et al. (2017) Enterosalivary Nitrate Metabolism and the Microbiome: Intersection of Microbial Metabolism, Nitric Oxide and Diet in Cardiac and Pulmonary Vascular Health. *Free Radic Biol Med* 105: 48-67.
34. Qu XM, Wu ZF, Pang BX, Jin LY, Qin LZ, et al. (2016) From Nitrate to Nitric Oxide: The Role of Salivary Glands and Oral Bacteria. *J Dent Res* 95: 1452-1456.
35. Hezel MP, Weitzberg E (2015) The Oral Microbiome and Nitric Oxide Homeostasis. *Oral Dis* 21: 7-16.
36. Rocha BS, Laranjinha J (2020) Nitrate from Diet Might Fuel Gut Microbiota Metabolism: Minding the Gap between Redox Signaling and Inter-kingdom Communication. *Free Radic Biol Med* 149: 37-43.
37. Bharucha AE, Kudva YC, Prichard DO (2019) Diabetic Gastroparesis. *Endocr Rev* 40: 1318-1352.
38. Buddam A, Hoilat GJ, Dacha S (2020) Gastric Stasis. In: *StatPearls*. Treasure Island (FL): Stat Pearls Publishing.

39. Horowitz M, Su YC, Rayner CK, Jones KL (2001) Gastroparesis: Prevalence, Clinical Significance and Treatment. *Can J Gastroenterol* 15: 805-813.
40. Donatini B, Le Blaye I (2020) Exhaled Volatile Organic Compounds and Vagal Tone are Different in Patients with Overweight or with Obesity: Practical Consequences. *J Case Rep Stud* 8: 308.
41. Farmer AD, Bruckner-Holt C, Schwartz S, Sadler E, Kadirkamanthan S (2019) Diabetic Gastroparesis: Perspectives from a Patient and Health Care Providers. *J Patient Cent Res Rev* 6: 148-157.
42. Kurniawan AH, Suwandi BH, Kholili U (2019) Diabetic Gastroenteropathy: A Complication of Diabetes Mellitus. *Acta Med Indones* 51: 263-271.
43. Walther G, Obert P, Dutheil F, Chapier R, Lesourd B, et al. (2015) Metabolic Syndrome Individuals with and without Type 2 Diabetes Mellitus Present Generalized Vascular Dysfunction: Cross-Sectional Study. *Arterioscler Thromb Vasc Biol* 35: 1022-1029.
44. Maniscalco M, Zedda A, Faraone S, Cristiano S, Sofia M, et al. (2015) Low Alveolar and Bronchial Nitric Oxide in Severe Uncomplicated Obesity. *Obes Res Clin Pract* 9: 603-608.
45. Blum A, Ginat-Maimon L, Yehuda H, Geron N, Ami MB, et al. (2015) Inhibition of Inflammation may Enhance Nitric Oxide Availability in Patients Undergoing Bariatric Surgery for Weight Loss. *J Intern Med* 278: 401-409.
46. Jahansouz C, Serrot FJ, Frohnert BI, Foncea RE, Dorman RB, et al. (2015) Roux-en-Y Gastric Bypass Acutely Decreases Protein Carbonylation and Increases Expression of Mitochondrial Biogenesis Genes in Subcutaneous Adipose Tissue. *Obes Surg* 25: 2376-2385.
47. Hao Z, Liu Y, Liao H, Zheng D, Xiao C, et al. (2016) Atorvastatin Plus Metformin Confer Additive Benefits on Subjects with Dyslipidemia and Overweight/Obese *via* Reducing ROCK2 Concentration. *Exp Clin Endocrinol Diabetes* 124: 246-250.
48. Han X, Tao Y, Deng Y, Yu J, Sun Y, et al. (2017) Metformin Accelerates Wound Healing in Type 2 Diabetic db/db Mice. *Mol Med Rep* 16: 8691-8698.
49. Gangula PRR, Sekhar KR, Mukhopadhyay S (2011) Gender Bias in Gastroparesis: is Nitric Oxide the Answer? *Dig Dis Sci* 56: 2520-2527.
50. D'Errico F, Govere G, Dai Y, Wu W, Stakenborg M, et al. (2018) Estrogen Receptor  $\beta$  Controls Proliferation of Enteric Glia and Differentiation of Neurons in the Myenteric Plexus After Damage. *Proc Natl Acad Sci USA* 115: 5798-5803.
51. Ravella K, Al-Hendy A, Sharan C, Hale AB, Channon KM, et al. (2013) Chronic Estrogen Deficiency Causes Gastroparesis by Altering Neuronal Nitric Oxide Synthase Function. *Dig Dis Sci* 58: 1507-1515.
52. Wilson AS, Koller KR, Ramaboli MC, Nesengani LT, Ocvirk S, et al. (2020) Diet and the Human Gut Microbiome: An International Review. *Dig Dis Sci* 65: 723-740.
53. Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M (2019) Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. *Curr Obes Rep* 8: 317-332.
54. Bonaz B, Bazin T, Pellissier S (2018) The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. *Front Neurosci* 12: 49.
55. Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI (2020) Gut Microbiota and Immune System Interactions. *Microorganisms* 8.
56. Pimentel M, Kong Y, Park S (2004) IBS Subjects with Methane on Lactulose Breath Test have Lower Postprandial Serotonin Levels than Subjects with Hydrogen. *Dig Dis Sci* 49: 84-87.
57. de Lacy Costello BP, Ledochowski M, Ratcliffe NM (2013) The Importance of Methane Breath Testing: A Review. *J Breath Res* 7: 024001.
58. Ledochowski M, Widner B, Bair H, Probst T, Fuchs D (2000) Fructose- and Sorbitol-Reduced Diet Improves Mood and Gastrointestinal Disturbances in Fructose Malabsorbers. *Scand J Gastroenterol* 35: 1048-1052.
59. Donatini B (2015) Bacterial Pullulation of the Small Intestine. Interest of New Ambulatory Technologies: Respiratory Test Coupled with Hepatic Elastometry, Looking for *Herpes Virus* in Saliva or Gastrointestinal Ultrasound. Therapeutic principles. *Revue Inist Hege* 15: 92-99.
60. Saasa V, Beukes M, Lemmer Y, Mwakikunga B (2019) Blood Ketone Bodies and Breath Acetone Analysis and their Correlations in Type 2 Diabetes Mellitus. *Diagnostics (Basel)* 9: 224.
61. Méndez-Rodríguez KB, Figueroa-Vega N, Ilizaliturri-Hernandez CA, Cardona-Alvarado M, Borjas-García JA, et al. (2020) Identification of Metabolic Markers in Patients with Type 2 Diabetes by Ultrafast Gas Chromatography Coupled to Electronic Nose. A pilot study. *Biomed Chromatogr* 34: e4956.
62. Rydosz A (2018) Sensors for Enhanced Detection of Acetone as a Potential Tool for Noninvasive Diabetes Monitoring. *Sensors (Basel)* 18: 2298.
63. de la Cuesta-Zuluaga J, Mueller NT, Álvarez-Quintero R, Velásquez-Mejía EP, Sierra JA, et al. (2018) Higher Fecal Short-Chain Fatty Acid Levels are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. *Nutrients* 11: 51.
64. Cozzolino R, De Giulio B, Marena P, Martignetti A, Günther K, et al. (2017) Urinary Volatile Organic Compounds in Overweight Compared to Normal-Weight Children: Results from the Italian I. Family Cohort. *Sci Rep* 7: 15636.
65. Donatini B (2019) Interest of Abdominal Ultrasound for the Analysis of Emptying, Reflux and Gastro-duodeno-jejuno-ileal Tone. *Hegel* 3: 196-202.
66. Donatini B (2020) Dysbiose des Darms. In: Liem T, Dobler TK, Puylaert M (eds) *Leitfaden Viszerale Osteopathie*. 3<sup>rd</sup> Edition, Elsevier, München 79-95.
67. Hinata N, Hieda K, Sasaki H, Murakami G, Abe S, et al. (2014) Topohistology of Sympathetic and Parasympathetic Nerve Fibers in Branches of the Pelvic Plexus: an Immunohistochemical Study Using Donated Elderly Cadavers. *Anat Cell Biol* 47: 55-65.
68. Zhou SY, Lu YX, Yao H, Owyang C (2008) Spatial Organization of Neurons in the Dorsal Motor Nucleus of the Vagus Synapsing with Intra-gastric Cholinergic and Nitric Oxide/VIP Neurons in the Rat. *Am J Physiol Gastrointest Liver Physiol* 294: G1201-G1209.
69. Petersson J, Jädert C, Phillipson M, Borniquel S, Lundberg JO, et al. (2015) Physiological Recycling of Endogenous Nitrate by Oral Bacteria Regulates Gastric Mucus Thickness. *Free Radic Biol Med* 89: 241-247.
70. Petersson J, Phillipson M, Jansson EA, Patzak A, Lundberg JO, et al. (2007) Dietary Nitrate Increases Gastric Mucosal Blood Flow and Mucosal Defense. *Am J Physiol Gastrointest Liver Physiol* 292: G718-G724.
71. Matsunaga K, Kuwahira I, Hanaoka M, Saito J, Tsuburai T, et al. (2021) An Official JRS Statement: The Principles of Fractional Exhaled Nitric Oxide (FeNO) Measurement and Interpretation of the Results in Clinical Practice. *Respir Investig* 59: 34-52.



72. Chen FJ, Huang XY, Liu YL, Lin GP, Xie CM (2016) Importance of Fractional Exhaled Nitric Oxide in the Differentiation of Asthma-COPD Overlap Syndrome, Asthma, and COPD. *Int J Chron Obstruct Pulmon Dis* 11: 2385-2390.
73. Wang Z, Pianosi P, Keogh K, Zaiem F, Alsawas M, et al. (2017) The Clinical Utility of Fractional Exhaled Nitric Oxide (FeNO) in Asthma Management. Rockville (MD): Agency for Healthcare Research and Quality (US).
74. Bahadoran Z, Carlström M, Mirmiran P, Ghasemi A (2020) Nitric Oxide: To be or not to be an Endocrine Hormone? *Acta Physiol (Oxf)* 229: e13443.
75. Möller MN, Cuevasanta E, Orrico F, Lopez AC, Thomson L, et al. (2019) Diffusion and Transport of Reactive Species Across Cell Membranes. *Adv Exp Med Biol* 1127: 3-19.
76. Garthwaite J (2018) Nitric Oxide as a Multimodal Brain Transmitter. *Brain Neurosci Adv* 2: 2398212818810683.
77. Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, et al. (2018) Role of Nitric Oxide in the Cardiovascular and Renal Systems. *Int J Mol Sci* 19: 2605.
78. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, et al. (2016) Acetate Mediates a Microbiome-brain- $\beta$ -Cell Axis to Promote Metabolic Syndrome. *Nature* 9: 213-217.
79. Berthoud HR, Neuhuber WL (2019) Vagal Mechanisms as Neuromodulatory Targets for the Treatment of Metabolic Disease. *Ann N Y Acad Sci* 1454: 42-55.
80. Apovian CM, Shah SN, Wolfe BM, Ikramuddin S, Miller CJ, et al. (2017) Two-Year Outcomes of Vagal Nerve Blocking (vBloc) for the Treatment of Obesity in the ReCharge Trial. *Obes Surg* 27: 169-176.
81. Morton JM, Shah SN, Wolfe BM, Apovian CM, Miller CJ, et al. (2016) Effect of Vagal Nerve Blockade on Moderate Obesity with an Obesity-Related Comorbid Condition: the ReCharge Study. *Obes Surg* 26: 983-989.
82. Shikora SA, Toouli J, Herrera MF, Kulseng B, Brancatisano R, et al. (2016) Intermittent Vagal Nerve Block for Improvements in Obesity, Cardiovascular Risk Factors, and Glycemic Control in Patients with Type 2 Diabetes Mellitus: 2-Year Results of the VBLOC DM2 Study. *Obes Surg* 26: 1021-1028.
83. Wilson AS, Koller KR, Ramaboli MC, Nesengani LT, Ocvirk S, et al. (2020) Diet and the Human Gut Microbiome: An International Review. *Dig Dis Sci* 65: 723-740.
84. Vallianou N, Stratigou T, Christodoulatos GS, Dalamaga M (2019) Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. *Curr Obes Rep* 8: 317-332.
85. Moran-Lev H, Lubetzky R, Mandel D, Yerushalmy-Feler A, Cohen S (2017) Inverse Correlation between *Helicobacter pylori* Colonization and Pediatric Overweight: A Preliminary Study. *Child Obes* 13: 267-271.
86. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, et al. (2016) *Akkermansia muciniphila* and Improved Metabolic Health During a Dietary Intervention in Obesity: Relationship with Gut Microbiome Richness and Ecology. *Gut* 65: 426-436.
87. Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C (2019) The Role of Gut Microbiota in Obesity, Diabetes Mellitus, and Effect of Metformin: New Insights into Old Diseases. *Curr Opin Pharmacol* 49: 1-5.
88. Ciobârcă D, Cătoi AF, Copăescu C, Miere D, Crișan G (2020) Bariatric Surgery in Obesity: Effects on Gut Microbiota and Micronutrient Status. *Nutrients* 12: 235.
89. Blum A, Ginat-Maimon L, Yehuda H, Geron N, Ben Ami M, et al. (2015) Inhibition of Inflammation may Enhance Nitric Oxide Availability in Patients Undergoing Bariatric Surgery for Weight Loss. *J Intern Med* 278: 401-409.
90. Blaser MJ (2012) The Jeremiah Metzger Lecture: Global Warming Redux: the Disappearing Microbiota and Epidemic Obesity. *Trans Am Clin Climatol Assoc* 123: 230-238.
91. Kappel BA, De Angelis L, Heiser M, Ballanti M, Stoehr R, et al. (2020) Cross-omics Analysis Revealed Gut Microbiome-Related Metabolic Pathways Underlying Atherosclerosis Development after Antibiotics Treatment. *Mol Metab* 36: 100976.
92. Smith KB, Smith MS (2016) Obesity Statistics. *Prim Care* 43: 121-135.
93. Mittendorfer B (2011) Origins of Metabolic Complications in Obesity: Adipose Tissue and Free Fatty Acid Trafficking. *Curr Opin Clin Nutr Metab Care* 14: 535-541.
94. Petersen KF, Shulman GI (2006) Etiology of Insulin Resistance. *Am J Med* 119: S10-S16.
95. Bowen RC, Little N, Harmer JR, Ma J, Mirabelli LG, et al. (2017) Neutrophil-to-lymphocyte Ratio as Prognostic Indicator in Gastrointestinal Cancers: A Systematic Review and Meta-Analysis. *Oncotarget* 8: 32171-32189.
96. Mizuno H, Yuasa N, Takeuchi E, Miyake H, Nagai H, et al. (2019) Blood Cell Markers that can Predict the Long-term Outcomes of Patients with Colorectal Cancer. *PLoS One* 14: e0220579.
97. Li M, Spakowicz D, Burkart J, Patel S, Husain M, et al. (2019) Change in Neutrophil to Lymphocyte Ratio during Immunotherapy Treatment is a Non-linear Predictor of Patient Outcomes in Advanced Cancers. *J Cancer Res Clin Oncol* 145: 2541-2546.
98. Singleton PA (2014) Hyaluronan Regulation of Endothelial Barrier Function in Cancer. *Adv Cancer Res* 123: 191-209.
99. Petrey AC, de la Motte CA (2014) Hyaluronan, a Crucial Regulator of Inflammation. *Front Immunol* 5: 101.
100. Zlobec I, Terracciano L, Tornillo L, Günthert U, Vuong T, et al. (2008) Role of RHAMM within the Hierarchy of Well-Established Prognostic Factors in Colorectal Cancer. *Gut* 57: 1413-1419.
101. Wu RL, Huang L, Zhao HC, Geng XP (2017) Hyaluronic Acid in Digestive Cancers. *J Cancer Res Clin Oncol* 143: 1-16.
102. Adams LA, Chan WK (2020) Noninvasive Tests in the Assessment of NASH and NAFLD Fibrosis: Now and Into the Future. *Semin Liver Dis* 40: 331-338.
103. Valva P, Rios D, Casciato P, Gadano A, Galdame O, et al. (2018) Nonalcoholic Fatty Liver Disease: Biomarkers as Diagnostic Tools for Liver Damage Assessment in Adult Patients from Argentina. *Eur J Gastroenterol Hepatol* 30: 637-644.
104. Chen IM, Willumsen N, Dehlendorf C, Johansen AZ, Jensen BV, et al. (2020) Clinical Value of Serum Hyaluronan and Propeptide of Type III Collagen in Patients with Pancreatic Cancer. *Int J Cancer* 146: 2913-2922.
105. Nagy N, de la Zerda A, Kaber G, Johnson PY, Hu KH, et al. (2018) Hyaluronan Content Governs Tissue Stiffness in Pancreatic Islet Inflammation. *J Biol Chem* 293: 567-578.

106. Bellou V, Belbasis L, Tzoulaki I, Evangelou E (2018) Risk Factors for Type 2 Diabetes Mellitus: An Exposure-Wide Umbrella Review of Meta-analyses. *PLoS One* 13: e0194127.
107. King C, Lanaspas MA, Jensen T, Tolan DR, Sánchez-Lozada LG, et al. (2018) Uric Acid as a Cause of the Metabolic Syndrome. *Contrib Nephrol* 192: 88-102.
108. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, et al. (2013) Sugar, Uric Acid, and the Etiology of Diabetes and Obesity. *Diabetes* 62: 3307-3315.
109. Johnson RJ, Perez-Pozo SE, Sautin YY, Manitius J, Sanchez-Lozada LG, et al. (2009) Hypothesis: could Excessive Fructose Intake and Uric Acid cause Type 2 Diabetes? *Endocr Rev* 30: 96-116.
110. Stanhope KL (2016) Sugar Consumption, Metabolic Disease and Obesity: The State of the Controversy. *Crit Rev Clin Lab Sci* 53: 52-67.
111. Wang D, Yu XP, Xiao WM, Jiao XP, Wu J, et al. (2018) Prevalence and Clinical Characteristics of Fatty Pancreas in Yangzhou, China: A Cross-Sectional Study. *Pancreatology* 18: 263-268.
112. Wang CY, Ou HY, Chen MF, Chang TC, Chang CJ (2014) Enigmatic Ectopic Fat: Prevalence of Nonalcoholic Fatty Pancreas Disease and its Associated Factors in a Chinese Population. *J Am Heart Assoc* 3: e000297.
113. van Geenen EJM, Smits MM, Schreuder TCMA, van der Peet DL, Bloemena E, et al. (2010) Nonalcoholic Fatty Liver Disease is Related to Nonalcoholic Fatty Pancreas Disease. *Pancreas* 39: 1185-1190.
114. Bagheri B, Zargari M, Meshkini F, Dinarvand K, Mokhberi V, et al. (2016) Uric Acid and Coronary Artery Disease, Two Sides of a Single Coin: A Determinant of Antioxidant System or a Factor in Metabolic Syndrome. *J Clin Diagn Res* 10: OC27-OC31.
115. Sakuta H, Suzuki K, Miyamoto T, Miyamoto M, Numao A, et al. (2016) Serum Uric Acid Levels in Parkinson's Disease and Related Disorders. *Brain Behav* 7: e00598.
116. Abraham A, Drory VE (2014) Influence of Serum Uric Acid Levels on Prognosis and Survival in Amyotrophic Lateral Sclerosis: A Meta-Analysis. *J Neurol* 261: 1133-1138.
117. Qin XL, Zhang QS, Sun L, Hao MW, Hu ZT (2015) Lower Serum Bilirubin and Uric Acid Concentrations in Patients with Parkinson's Disease in China. *Cell Biochem Biophys* 72: 49-56.
118. Vieru E, Köksal A, Mutluay B, Dirican AC, Altunkaynak Y, et al. (2016) The Relation of Serum Uric Acid Levels with L-Dopa Treatment and Progression in Patients with Parkinson's Disease. *Neurol Sci* 37: 743-747.
119. Chen X, Guo X, Huang R, Chen Y, Zheng Z, et al. (2014) Serum Uric Acid Levels in Patients with Alzheimer's Disease: A Meta-Analysis. *PLoS One* 9: e94084.
120. Wen S, Cheng M, Wang H, Yue J, Wang H, et al. (2012) Serum Uric Acid Levels and the Clinical Characteristics of Depression. *ClinBiochem* 45: 49-53.
121. Chaudhari K, Khanzode S, Khanzode S, Dakhale G, Saoji A, et al. (2010) Clinical Correlation of Alteration of Endogenous Antioxidant-Uric Acid Level in Major Depressive Disorder. *Indian J Clin Biochem* 25: 77-81.
122. Liu T, Zhong S, Liao X, Chen J, He T, et al. (2015) A Meta-Analysis of Oxidative Stress Markers in Depression. *PLoS One* 10: e0138904.
123. Di Stolfo G, Mastroianno S, Potenza DR, De Luca G, d'Arienzo C, et al. (2015) Serum Uric Acid as a Prognostic Marker in the Setting of Advanced Vascular Disease: A Prospective Study in the Elderly. *J Geriatr Cardiol* 12: 515-520.
124. Menezes EF, Peixoto LG, Teixeira RR, Justino AB, Puga GM, et al. (2019) Potential Benefits of Nitrate Supplementation on Antioxidant Defense System and Blood Pressure Responses after Exercise Performance. *Oxid Med Cell Longev* 2019: 7218936.
125. Carriker CR, Rombach P, Stevens BM, Vaughan RA, Gibson AL (2018) Acute Dietary Nitrate Supplementation does not Attenuate Oxidative Stress or the Hemodynamic Response During Submaximal Exercise in Hypobaric Hypoxia. *Appl Physiol Nutr Metab* 43: 1268-1274.
126. Clements WT, Lee S-R, Bloomer RJ (2014) Nitrate Ingestion: A Review of the Health and Physical Performance Effects. *Nutrients* 6: 5224-5264.
127. Behrens CE Jr, Ahmed K, Ricart K, Linder B, Fernández J, et al. (2020) Acute Beetroot Juice Supplementation Improves Exercise Tolerance and Cycling Efficiency in Adults with Obesity. *Physiol Rep* 8: e14574.
128. Shaltout HA, Eggebeen J, Marsh AP, Brubaker PH, Laurienti PJ, et al. (2017) Effects of Supervised Exercise and Dietary Nitrate in Older Adults with Controlled Hypertension and/or Heart Failure with Preserved Ejection Fraction. *Nitric Oxide* 69: 78-90.
129. Ranchal-Sanchez A, Diaz-Bernier VM, De La Florida-Villagran CA, Llorente-Cantarero FJ, Campos-Perez J, et al. (2020) Acute Effects of Beetroot Juice Supplements on Resistance Training: A Randomized Double-Blind Crossover. *Nutrients* 12: 1912.
130. de Lima Bezerra AD, Costa EC, Pacheco DA, Souza DC, Farias-Junior LF, et al. (2019) Effect of Acute Dietary Nitrate Supplementation on the Post-Exercise Ambulatory Blood Pressure in Obese Males: A Randomized, Controlled, Crossover Trial. *J Sports Sci Med* 18: 118-127.
131. Matthews VB, Hollingshead R, Koch H, Croft KD, Ward NC (2018) Long-Term Dietary Nitrate Supplementation does not Prevent Development of the Metabolic Syndrome in Mice Fed a High-Fat Diet. *Int J Endocrinol* 2018: 7969750.
132. Gonzalez Z, Herlihy D, Phan C, Diaz J, Dominguez K, et al. (2020) Alcohol and Gastric Motility: Pathophysiological and Therapeutic Implications. *J Investig Med* 68: 965-971.
133. Bagyánszki M, Krecsmarik M, De Winter BY, De Man JG, Fekete E, et al. (2010) Chronic Alcohol Consumption Affects Gastrointestinal Motility and Reduces the Proportion of Neuronal NOS-Immunoreactive Myenteric Neurons in the Murine Jejunum. *Anat Rec (Hoboken)* 293: 1536-542.
134. Grad S, Abenavoli L, Dumitrascu DL (2016) The Effect of Alcohol on Gastrointestinal Motility. *Rev Recent Clin Trials* 11: 191-195.
135. Donatini B, Le Blaye I (2019) Ongoing CMV Infection (qPCR+), Nodular Thyroiditis and Periodontitis are Associated with Ileal Distension (Ileal Brake), Cancer and Increased Plasmatic Hyaluronic Acid Levels. *J Clin Case Stu* 4.
136. Chen Y, Liu S, Leng SX (2019) Chronic Low-grade Inflammatory Phenotype (CLIP) and Senescent Immune Dysregulation. *ClinTher* 41: 400-409.
137. Bauer ME, De la Fuente M (2016) The role of Oxidative and Inflammatory Stress and Persistent Viral Infections in Immunosenescence. *Mech Ageing Dev* 158: 27-37.
138. Heath JJ, Grant MD (2020) The Immune Response Against Human *Cytomegalovirus* Links Cellular to Systemic Senescence. *Cells* 9: 766.
139. Luo XH, Meng Q, Rao M, Liu Z, Paraschoudi G, et al. (2018) The Impact of Inflationary *Cytomegalovirus*-Specific Memory T Cells on Anti-tumour Immune Responses in Patients with Cancer. *Immunology* 155: 294-308.

140. Naftali T, Yishai R, Zangen T, Levine A (2007) Post-infectious Gastroparesis: Clinical and Electrogastrographic Aspects. *J Gastroenterol Hepatol* 22: 1423-1428.
141. Nowak TV, Goddard M, Batteiger B, Cummings OW (1999) Evolution of Acute *Cytomegalovirus* Gastritis to Chronic Gastrointestinal Dysmotility in a Nonimmunocompromised Adult. *Gastroenterology* 116: 953-958.
142. Thiel DHV, Gavaler JS, Schade RR, Chien MC, Starzl TE (1992) *Cytomegalovirus* Infection and Gastric Emptying. *Transplantation* 54: 70-73.
143. Wink J, van Delft R, Notenboom RGE, Wouters PF, DeRuiter MC, et al. (2020) Human Adult Cardiac Autonomic Innervation: Controversies in Anatomical Knowledge and Relevance for Cardiac Neuromodulation. *Auton Neurosci* 227: 102674.
144. Manolis AA, Manolis TA, Apostolopoulos EJ, Apostolaki NE, Melita H, et al. (2020) The Role of the Autonomic Nervous System in Cardiac Arrhythmias: The Neuro-cardiac Axis, more foe than Friend? *Trends Cardiovasc Med* S1050-1738: 30066-30069.
145. Linz D, Elliott AD, Hohl M, Malik V, Schotten U, et al. (2019) Role of Autonomic Nervous System in Atrial Fibrillation. *Int J Cardiol* 287: 181-188.
146. Horn CC (2014) The Medical Implications of Gastrointestinal Vagal Afferent Pathways in Nausea and Vomiting. *Curr Pharm Des* 20: 2703-2712.
147. Mohammad MK, Pepper DJ, Kedar A, Bhajjee F, Familoni B, et al. (2016) Measures of Autonomic Dysfunction in Diabetic and Idiopathic Gastroparesis. *Gastroenterology Res* 9: 65-69.