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

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Abstract

Background: Hydrogen Sulphide (H_2S) 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_2S 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 manoeuvers. 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_2S>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_2S 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_2S 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_2S: 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_2S) and Nitric Oxide (NO) are gasotransmitters with neuroprotective and anti-oxidative properties [1-5].

H_2S 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_2S 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_2S 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_2S 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]. Gastroduodenal 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_2S 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_2S 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_2S 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_2S 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 1st to 2020 June 30th.

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 $10cm^2$ 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 manoeuvers.

Jejunal hypotonia was diagnosed when jejunal diameter reaches 19.4mm. In that case, the jejunum contains few bubbles, the mucosa is thin (≥ 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).

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 › Products › Multi-Gas-Detectors]. X-am8000® detects NO or H_2S 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 fells 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_2S levels in breath or in belching are low and similar. In addition, concentrations of H_2S are not statistically different between the two groups. However, regarding belching, the percentage of patients with H_2S 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_2S levels were higher in belching than in breath.

NO and H_2S levels in induced belching are therefore highly discriminant parameters between the two groups (Table 1). In 112 patients, NO or H_2S levels were higher in belching than in breath. The 33 remaining patients have NO or H_2S 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_2S>0.1ppm in belching**	93.8%	46.9%	<0.001

*NO level in belching was detectable in 105 patients.
**H_2S levels were higher in belching than in breath in 69 patients. In 112 patients, NO or H_2S 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^3	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_2S level in breath (ppm)	0.2 ± 0.25	0.27 ± 1.4	>0.05
H_2S 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_2S levels>0.1ppm (Table 3), NO levels>1ppm (Table 4) or H_2S levels>0.1ppm plus NO levels>1ppm (Table 5) in induced belching, regarding gastroduodenal voiding.

The specificity (Sp) of theH_2S 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_2S>0.1ppm plus NO>1ppm in induced belching. All other recorded parameters do not appear to modify or to be modified by gastric H_2S and NO production in ambulatory clinical practice (Table 6).

Discussion

To our knowledge, results of NO and H_2S 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_2S 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_2S levels>0.1ppm in induced belching regarding gastroduodenal voiding.

	H_2S levels>0.1ppm (83 patients)	H_2S 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_2S 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_2S exceeds 0.1ppm simultaneously. X-am8000® is able to detect these gases in belching within a few minutes.

The combination of H_2S and NO detection in induced-belching by X-am8000® 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® 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®. 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_2S 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_2S levels>0.1ppm and NO levels>1ppm in induced belching regarding gastroduodenal voiding.

	H_2S levels>0.1ppm and NO levels>1ppm in belching (46 patients)	H_2S levels ≤ 0.1ppm or NO levels ≤ 1ppm in belching (99 patients)	Se§ Sp PPV NPV
Voiding	22 (a)	10 (c)	68.8% 78.8% 47.8% 89.9%
Non-voiding	24 (b)	89 (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 6: Relevant clinical and biological data related to content of NO>1ppm and H_2S>0.1ppm in induced belching*.

	H_2S levels>0.1ppm and NO levels>1ppm in belching (46 patients)	H_2S 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_2S levels in belching (p>0.01).

The ratio between the breath and the gastric levels of NO is, in order of magnitude, between 100 to1000. 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_2S

H_2S can be detected in breath by X-am8000®. The ratio between the breath and the gastric levels of H_2S is, in order of magnitude, between 1 to 2. This ratio suggests that the production in the stomach is low. H_2S can be produced by sulfatases from mucolytic bacteria [12]. The association of NO and H_2S 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_2S levels from belching are reduced in the non-voiding group. This is an additional argument for a correlation between low gastric NO and H_2S 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_2S 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_2S 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_2S 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_2S levels may decreases 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 nitrergic 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_2S 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_2S 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_2S 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_2S in induced belching. NO levels >1 and H_2S 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.

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