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Normal values and day-to-day variability of 24-h ambulatory oesophageal impedance-pH monitoring in a Belgian–French cohort of healthy subjects

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SUMMARY

Background: Impedance-pH monitoring is the most sensitive method for detection and characterization of gastro-oesophageal reflux episodes. Normal values from European subjects are lacking.

Aim: To build a database of gastro-oesophageal reflux patterns from French and Belgian healthy subjects.

Methods: Seventy-two healthy subjects (35 men, mean age 35 years, 18–72) underwent 24-h ambulatory impedance-pH studies. Gastro-oesophageal reflux episodes were detected using impedance and characterized by pH as acid, weakly acidic, or weakly alkaline. Analysis was performed visually and effects of age, gender and intra-individual reproducibility were evaluated.

Results: The total number of gastro-oesophageal reflux episodes was 44 (25,58,75) of which 59% were acid, 28% were weakly acidic and 10% weakly alkaline. Half of gastro-oesophageal reflux episodes were mixed (liquid/gas) and 22% reached 15 cm above the lower oesophageal sphincter. The bolus clearance time was 11 s while acid was chemically cleared in 34 s. Male gender was associated with increased number and proximal extent of total and acid gastro-oesophageal reflux. Repeated studies in 27 subjects showed good reproducibility for number, acidity and air–liquid composition of reflux (Kendall's W-values = 0.72–0.85).

Conclusions: This study demonstrates good reproducibility of 24-h ambulatory impedance-pH studies and provides values of reflux patterns in healthy subjects for comparisons with European gastro-oesophageal reflux disease patients.

INTRODUCTION

The pivotal role of the acid component of the refluxate in the development of gastro-oesophageal reflux disease (GERD) has been demonstrated for many years. However, some patients with GERD are refractory to acid suppressive therapy suggesting that other factors, perhaps in the refluxate, may play an additional role in the pathogenesis of GERD. Oesophageal pH monitoring does not detect all gastro-oesophageal reflux (GER) events, particularly when little or no acid is present in the refluxate. Intraluminal electrical impedance is a recently developed technique that allows monitoring flow of liquids and/or gas within the gastrointestinal

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tract.[1] This technique, combined with pH monitoring, allows detection of GER of gas and acid or non-acid liquids. Theoretically, an increased rate or sensitivity to weakly acidic and/or gas reflux might contribute to symptoms in some patients with GERD. Initial impedance data in GERD patients suggest that weakly acidic reflux may be associated to symptoms such as regurgitation in patients on proton pump inhibitors[2] and chronic cough.[3] These promising results urge for normal biometric values and reproducibility studies of ambulatory 24-h impedance-pH monitoring for diagnostic purpose as well for assessment of therapeutic efficacy.

Normal values of ambulatory 24-h impedance-pH monitoring obtained in healthy American subjects have been recently reported[4] and a recent study in healthy subjects has shown very good reproducibility of postprandial impedance-pH monitoring in stationary conditions.[5] Normal values of 24-h impedance-pH monitoring from European healthy subjects and assessment of reproducibility of ambulatory impedance studies are lacking. The possibility of reflux variations because of cultural and dietary factors between the US and Europe led us to build a multicentre database of GER patterns in a Belgian–French cohort of healthy subjects. We also aimed to test intra-individual day-to-day variability of 24-h ambulatory oesophageal impedance-pH monitoring.

SUBJECTS AND METHODS

Subjects

Gastro-oesophageal reflux was studied using ambulatory 24-h impedance-pH measurements in healthy subjects recruited in seven university hospitals using a standard extensive questionnaire to exclude the presence of typical (heartburn, regurgitation) and atypical symptoms (increased belching, cough, asthma or wheezing, hoarseness, chest pain) suggestive or potentially related to GERD. Exclusion criteria were: history of thoracic or digestive surgery (excepted appendectomy), alcohol consumption >40 g/day, smoking >10 cig/day, nursing mothers, subjects taking medications that alter intragastric acidity or oesophageal motility, as well as those with history of diabetes, neurological disorder, or gastrointestinal disease. Written informed consent was obtained from all subjects and the protocol was approved by the *Comité de Protection des Personnes dans la Recherche Biomédicale (CCPPRB) des Pays de Loire*.

Impedance-pH equipment

Oesophageal impedance-pH monitoring was performed using a Sleuth® multi-channel intraluminal impedance ambulatory system (Sandhill Scientific, Inc.; Highland ranch, CO, USA). The system includes a portable datalogger with impedance-pH amplifiers and a catheter containing one antimony pH electrode and eight impedance electrodes at 2, 4, 6, 8, 10, 14, 16 and 18 cm from the tip of the catheter (Figure 1). Each pair of adjacent electrodes represents an impedance-measuring segment, 2 cm in length, corresponding to one recording channel. The impedance amplifier delivers AC

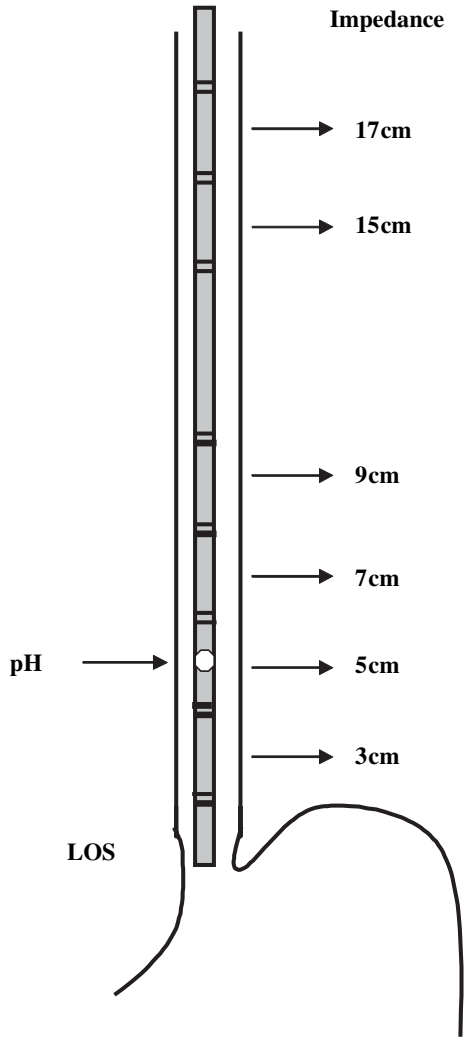

Figure 1. Schematic representation of combined impedance-pH catheter, which consists of one antimony pH electrode, located 5 cm above the lower oesophageal sphincter (LOS) and six pairs of impedance electrodes set at 2-cm intervals allowing impedance measurements at 3, 5, 7, 9, 15 and 17 cm above LOS.

voltage in a range of 1–2 kHz with resulting current flow variations in response to intraluminal impedance changes. The six impedance and pH signals were recorded at 50 Hz on a 128 MB CompactFlash card for further analysis.

Study protocol

The studies were performed on an out-patient basis after an overnight fast. Before the start of the recordings, the pH recorder was calibrated using pH 4.0 and pH 7.0 buffer solutions.

After lower oesophageal sphincter (LOS) location by oesophageal manometry, the impedance-pH catheter was passed transnasally under topical anaesthesia and positioned in the oesophageal body to record pH at 5 cm and impedance at 3, 5, 7, 9, 15 and 17 cm proximal to the LOS.

Subjects were discharged and were encouraged to maintain normal activities, sleep schedule, and eat their usual meals at their normal times. They were asked to remain upright during the day, and lie down only during their usual bedtime. Event markers on the data-logger recorded meal times and posture changes. Subjects were provided with a diary card to record with precision their food intake (nature and quantity). Between meals, volunteers abstained from frequent snacks, beverages with a pH < 5, and were asked to avoid gum chewing.

For reproducibility testing, each centre included two subjects that had a second 24-h impedance-pH recording separated by 4–10 days and two subjects for whom the recording prolonged for an additional 24-h period (total 48-h recording). Subjects were instructed to reproduce as far as possible similar levels of activity and food intake between the first and second 24-h impedance-pH recordings.

Data analysis

The data stored on the CompactFlash card were downloaded onto a personal computer and visually analysed with the assistance of dedicated software (Bioview Analysis®, version 5.0.9; Sandhill Scientific, Inc.). Prior to the study, investigators from each centre participated in a series of workshops aimed at training on visual characterization of GER by oesophageal impedance-pH. These training sessions were organized in order to reduce inter-observer and improve the interpretation of impedance-pH tracings.

Analysis included identification, enumeration and characterization of individual reflux events, measure of clearance times (bolus and pH clearance) and finally of oesophageal exposure to volume and acid.

Liquid reflux was defined as a retrograde 50% drop in impedance starting distally (at the level of the LOS) and propagating to at least the next two more proximal impedance measuring segments. Only liquid reflux lasting at least 3 s were taken into account. Gas reflux was defined as a rapid (3 kΩ/s) increase in impedance >5000 Ω, occurring simultaneously at least in two oesophageal measuring segments, in the absence of swallowing. Mixed liquid–gas reflux was defined as gas reflux occurring immediately before or during a liquid reflux.

Reflux episodes were characterized by pHmetry as acid, weakly acidic, or weakly alkaline according to a recently published consensus report on detection and definitions of GER (Figure 2):[6] (i) *Acid reflux*: refluxed gastric juice with a pH <4 which can either reduce the pH of the oesophagus to below 4 or occur when oesophageal pH is already below 4; (ii) *Weakly acidic reflux*: reflux events that result in an oesophageal pH between 4 and 6.5; (iii) *Weakly alkaline reflux*: reflux episodes during which nadir oesophageal pH does not drop below 6.5. A slight modification of the Porto Consensus definitions was used to differentiate weakly acidic from weakly alkaline reflux, considering pH 6.5 instead of pH 7 as a limit between them.

Gas reflux events without liquid (belches) were considered separately and were not characterized by pH.

All reflux events were analysed during both upright and supine positions and the following parameters were obtained from the impedance and pH recordings: bolus exposure [reflux time (min) and reflux per cent time], proximal extent (reflux reaching 15-cm impedance site) and median bolus clearance time. Meals were excluded for the analysis.

Total 24-h oesophageal acid exposure (%) was defined as the total time at pH below 4 divided by the time of monitoring. This parameter included all pH drops either related or not to bolus reflux detected by impedance, i.e. slow pH drifts or pH drops because of acidic beverages. Reflux-related acid exposure (%) was analysed separately and only included pH drops associated with bolus reflux detected by impedance.

Asymptomatic subjects with total oesophageal acid exposure higher than the 95th percentile of normal values previously published were excluded from the

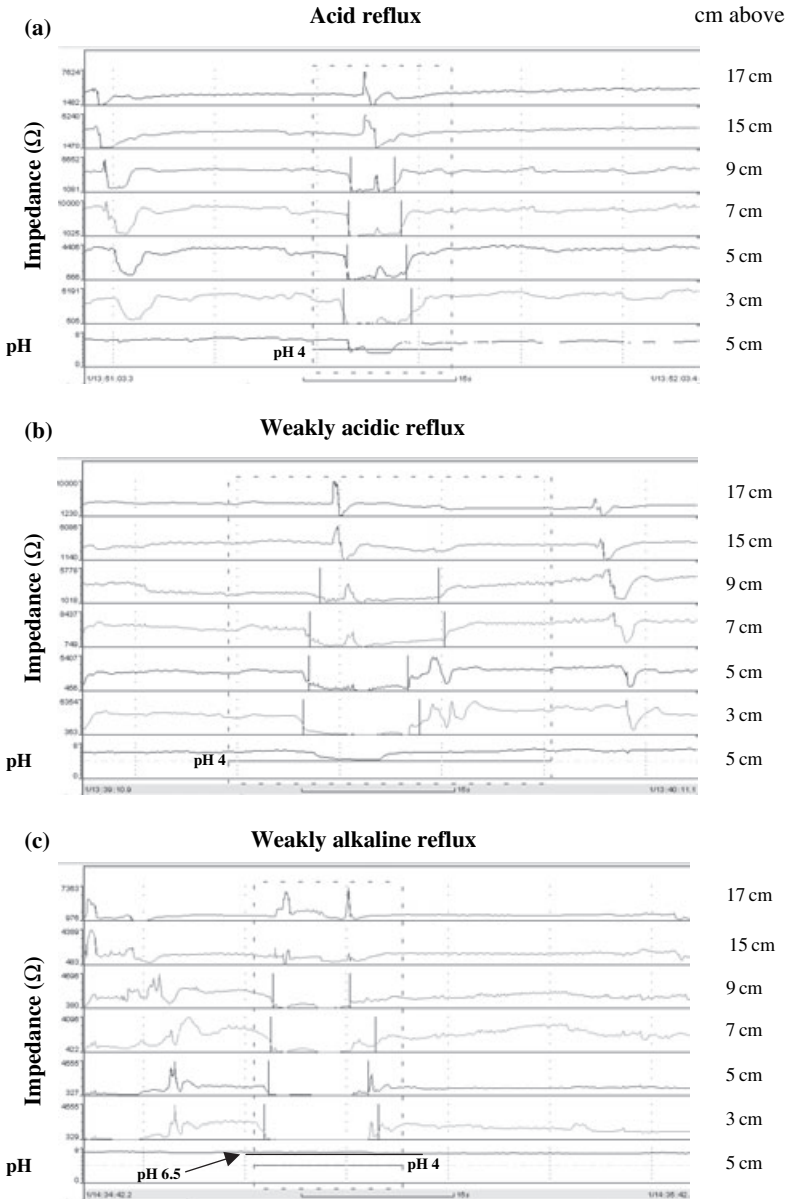

Figure 2. Combined impedance-pH recordings showing examples of the three types of reflux. Vertical bars on impedance channels indicate the beginning and the end of bolus reflux. Horizontal bars on the pH channel indicate pH 4 and 6.5 values. (a) Liquid acid reflux: retrograde drop in impedance starting distally and propagated retrogradely up to 9 cm above lower oesophageal sphincter (LOS), together with a pH fall below 4. (b) Liquid weakly acidic reflux: retrograde drop in impedance starting distally and propagated retrogradely up to 9 cm above LOS, together with a pH fall below 6.5 but above 4. (c) Liquid weakly alkaline reflux: retrograde drop in impedance starting distally and propagated retrogradely up to 9 cm above LOS with pH values above 6.5.

final analysis (before 45 year: upright 7.3%, supine 3.6% and after 45 year: upright 16%, supine 6.7%).[7]

Statistical analysis

As data were not normally distributed, they were expressed as median and percentile values (25th, 75th, 95th percentile). Comparisons of median values were made using the Mann–Whitney *U*-test. For reproducibility assessment, data were presented in Bland–Altman plots.[8] In these plots, the differences between the first and second measurement (*y*-axis) are plotted against the mean values of the two measurements (*x*-axis). If the difference between the two measurements is small, data points are scattered closely to the *x*-axis. Reproducibility is considered to be adequate when all data points are comprised within the 2DS limits. Symmetrical scattering around the *x*-axis indicates that there is no trend towards a difference of the measurements of the second day compared with the measurements of the first day and that the difference between the two measurements occurs in a random fashion. As another expression of reproducibility, Kendall's coefficients of concordance (*W*-value) were calculated using the mean values from

individual recordings and tested for significance. An error probability of $P \leq 0.05$ was considered statistically significant.

RESULTS

Subjects

Eighty-six subjects were recruited in this study (45 men, mean age 34.2 years). The overall tolerance of the recordings was good. After completing impedance-pH studies, a preliminary analysis was performed and tracings were classified according to their quality (spontaneous pH and impedance drifts, error in pH calibration, probe migration with pH sensor into the stomach, dysfunction of one or more impedance channels) and only those tracings without artefacts were included in the final analysis. Fourteen subjects were excluded (2 ± 0.6/centre) (mean age 39.8 years) because of one or more technical artefacts detected during the preliminary analysis. In 10 of these subjects, artefacts were responsible for abnormally high oesophageal acid exposure: the mean upright and supine oesophageal acid exposure of excluded subjects was 12.6% (range1.3%–26.9%) and 7.9% (0–18.2%), respectively. Therefore, a total of 72 subjects represent the final cohort included for the definition of normal parameters (35 men, mean age 35 years, range 18–72). The median body mass index (BMI) of our subjects was 22 (range 16–36) and did not differ between males (22, range 19–28) and females (22, range 16–36).

Impedance data

The median number of reflux events in 24 h was 44 (25, 58, 75) of which 59% (42, 73, 83) were acid, 28% (17, 44, 63) were weakly acidic and 10% (3, 18, 33) weakly alkaline (Table 1). Gas–liquid and pure liquid refluxates were more frequent than pure gas reflux (belches) (Table 2). Whereas almost half of the events [48% (30, 60, 81)] were mixed, consisted of gas and liquid, pure gas reflux (belches) occurred at a median rate of 10 (3, 17, 30)/24 h.

Only 22% (12, 39, 64) of reflux events reached 15 cm above LOS. Oesophageal volume clearance time (measured by impedance) was significantly faster than oesophageal chemical clearance (measured by pH). The median bolus clearance time (volume clearance of individual reflux events) was 11 s compared with a median of 34 s for acid clearance (Table 3).

Table 1. Gastro-oesophageal reflux events detected during 24-h ambulatory impedance-pH recordings in 72 healthy subjects

	All reflux	Acid reflux	Weakly acidic reflux	Weakly alkaline reflux
Total				
Median (25th, 75th)	44 (25, 58)	22 (10, 35)	11 (5, 18)	3 (1, 7)
95th percentile	75	50	33	15
Upright				
Median (25th, 75th)	40 (23, 52)	20 (10, 31)	10 (4, 15)	3 (1, 6)
95th percentile	64	45	31	14
Supine				
Median (25th, 75th)	3 (1, 6)	1 (0, 4)	1 (0, 2)	0 (0, 1)
95th percentile	14	8	5	3

Table 2. Gastro-oesophageal reflux content determined by ambulatory 24-h impedance-pH recordings in 72 healthy subjects

	Liquid reflux	Mixed reflux	Gas
Total			
Median (25th, 75th)	20 (10, 32)	17 (11, 26)	10 (3, 17)
95th percentile	55	42	30
Upright			
Median (25th, 75th)	17 (8, 27)	17 (10, 25)	9 (3, 16)
95th percentile	46	41	29
Supine			
Median (25th, 75th)	2 (0, 5)	0 (0, 1)	0 (0, 0)
95th percentile	13	3	1

Table 3. Parameters of gastro-oesophageal reflux detected by impedance (bolus reflux)

	24-h bolus exposure		Proximal extent		Bolus clearance time/event (s)
	Time (min)	Per cent time (%)	Number	Per cent of reflux (%)	
Total					
Median (25th, 75th)	11 (6, 16)	0.8 (0.4, 1.2)	9 (4, 17)	22 (12, 39)	11 (8, 13)
95th percentile	27	2.0	30	64	20
Upright					
Median (25th, 75th)	10 (5, 14)	1.0 (0.6, 1.8)	8 (3, 15)	21 (10, 38)	11 (8, 13)
95th percentile	21	2.7	27	64	22
Supine					
Median (25th, 75th)	1 (0, 1)	0.1 (0.0, 0.3)	1 (0, 2)	11 (0, 50)	10 (4, 13)
95th percentile	5	0.9	3	100	35

Table 4. Parameters of acid gastro-oesophageal reflux

	24-h acid exposure		Reflux-related acid exposure		Acid clearance time/event (s)
	Time (min)	Per cent time (%)	Time (min)	Per cent of reflux (%)	
Total					
Median (25th, 75th)	21 (7, 36)	1.6 (0.5, 2.6)	12 (5, 25)	0.9 (0.4, 1.9)	34 (18, 51)
95th percentile	69	5.0	50	3.7	87
Upright					
Median (25th, 75th)	13 (6, 31)	1.5 (0.8, 3.6)	8 (3, 18)	1.1 (0.4, 2.5)	30 (16, 45)
95th percentile	51	6.2	35	4.3	69
Supine					
Median (25th, 75th)	0 (0, 6)	0.1 (0.0, 1.2)	0 (0, 3)	0.0 (0.0, 0.5)	42 (12, 149)
95th percentile	30	5.3	18	3.1	212

The oesophageal mucosa was exposed to reflux volume (detected by impedance) during 0.8% (0.4, 1.2, 2.0) of the 24 h. Reflux-related acid exposure (pH drops associated with reflux detected by impedance) was 0.9% (0.4, 1.9, 3.7) whereas total oesophageal acid exposure (all pH drops) was 1.6% (0.5, 2.6, 5.0) (Table 4).

Effect of position

All types of reflux were very rare in supine position. During this period, most reflux events were pure liquid reflux: only 18.6% were mixed reflux and virtually no pure gas reflux (belch) occurred (median value 0 and 95th percentile value 1). The proportion of reflux that reached 15 cm above lower oesophageal sphincter was twofold higher in upright position (21%) when compared with supine position (11%) (Table 3). Position did not influence volume clearance, i.e. the median bolus clearance time was similar in upright (11 s) and supine (10 s) positions. However, acid clearance time was longer in supine than in upright (Table 4): 95th value of acid clearance time was three times higher in supine (212 s) than in upright position (69 s).

Effects of age and gender

As shown in Table 5, although there was a trend for a higher total number and weakly acidic reflux events in older subjects, there was no significant difference between subjects aged less and more than 45 years. This also holds true for subjects over 60 years ($n = 9$) as compared with subjects younger than 45 (data not shown).

The total number of reflux events was significantly higher in men when compared with women (48 vs. 36, $P = 0.02$) (Table 6). This was mainly related to an increased number of acid reflux ($P = 0.03$), although there was also a non-significant trend for increased weakly acidic and weakly alkaline reflux events.

Table 5. Characteristics of gastro-oesophageal reflux detected by ambulatory 24-h impedance-pH recordings according to age [median (25th–75th–95th percentiles)]

	Age ≤ 45 years ($n = 50$)	Age >45 years ($n = 22$)	P
All bolus reflux (n)	41 [23–53–77]	51 [32–63–99]	0.09
Acid reflux (n)	20 [10–35–50]	27 [13–45–54]	0.29
Weakly acidic reflux (n)	10 [5–15–34]	15 [8–20–39]	0.07
Weakly alkaline reflux (n)	3 [1–8–15]	4 [0–10–24]	0.81
Acid reflux (%)	60 [38–73–83]	50 [43–70–90]	0.92
Weakly acidic reflux (%)	25 [18–46–62]	30 [22–47–67]	0.49
Weakly alkaline reflux (%)	14 [4–19–46]	6 [0–17–34]	0.27
Mixed reflux (n)	47 [27–60–80]	54 [35–59–90]	0.23
Proximal extent (n)	24 [11–40–67]	19 [13–35–55]	0.92
Median bolus clearance (s)	10 [8–13–19]	11 [9–18–27]	0.16
Acid clearance (s)	34 [18–47–100]	42 [17–56–90]	0.58
Acid exposure (%)	1 [0–3–5]	2 [1–4–6]	0.11

Table 6. Characteristics of gastro-oesophageal reflux detected by ambulatory 24-h impedance-pH recordings according to gender [median (25th–75th–95th percentiles)]

	Male ($n = 35$)	Female ($n = 37$)	P
All bolus reflux (n)	48 [37–62–97]	36 [22–52–73]	0.02
Acid reflux (n)	29 [18–39–51]	18 [9–33–50]	0.03
Weakly acidic reflux (n)	13 [6–19–37]	10 [5–15–26]	0.19
Weakly alkaline reflux (n)	6 [3–9–15]	3 [1–7–25]	0.06
Acid reflux (%)	62 [45–73–85]	55 [35–69–83]	0.19
Weakly acidic reflux (%)	25 [18–41–56]	31 [18–48–67]	0.22
Weakly alkaline reflux (%)	12 [6–16–33]	8 [1–22–46]	0.74
Mixed reflux (n)	48 [37–65–84]	49 [26–57–75]	0.40
Proximal extent (n)	35 [18–44–70]	15 [9–31–61]	0.001
Median bolus clearance (s)	11 [8–14–22]	10 [8–12–20]	0.35
Acid clearance	36 [18–50–79]	34 [18–53–124]	1.00
Acid exposure (%)	2 [1–4–5]	1 [0–2–5]	0.11

Moreover, the number of reflux events that reached 15 cm above LOS was significantly increased in male when compared with female subjects.

Reproducibility studies

Reproducibility of impedance-pH measurements was assessed in 27 subjects (15 men, mean age 32 years, range 21–63). Fourteen subjects had recordings on two consecutive days while 13 had their recordings on two separate occasions (4–10 days interval).

Figures 3 and 4 show Bland–Altman plots for all types of reflux events. The data points are scattered relatively closely around the x-axis within the limits of 2 DS, indicating a relatively small difference between the two measurements. Moreover, the symmetrical scattering of data points around the x-axis indicates that the differences between the first and the second study were randomly distributed (no 'order' effect).

As shown in Table 7, concordance between the two recordings could be considered as satisfactory as Kendall's W-values varied from 0.72 to 0.85. The concordance was statistically significant for all parameters excepted acid exposure (0.72, $P = 0.07$) and number of gas reflux (0.73, $P = 0.06$).

DISCUSSION

Combined oesophageal impedance and pH monitoring allow the detection of GER of gas and acid or non-acid liquids and are now considered to be the best technique to detect and characterize GER.[6] Ambulatory impedance-pH devices are now commercially available and increasing use of this technique is expected.

Normal values of ambulatory 24-h impedance-pH monitoring obtained in healthy American subjects have been recently reported[4] and a recent study in healthy subjects has shown very good reproducibility of postprandial impedance-pH monitoring in stationary conditions.[5]

The possibility of reflux variations because of cultural and dietary factors between the US and Europe led us to

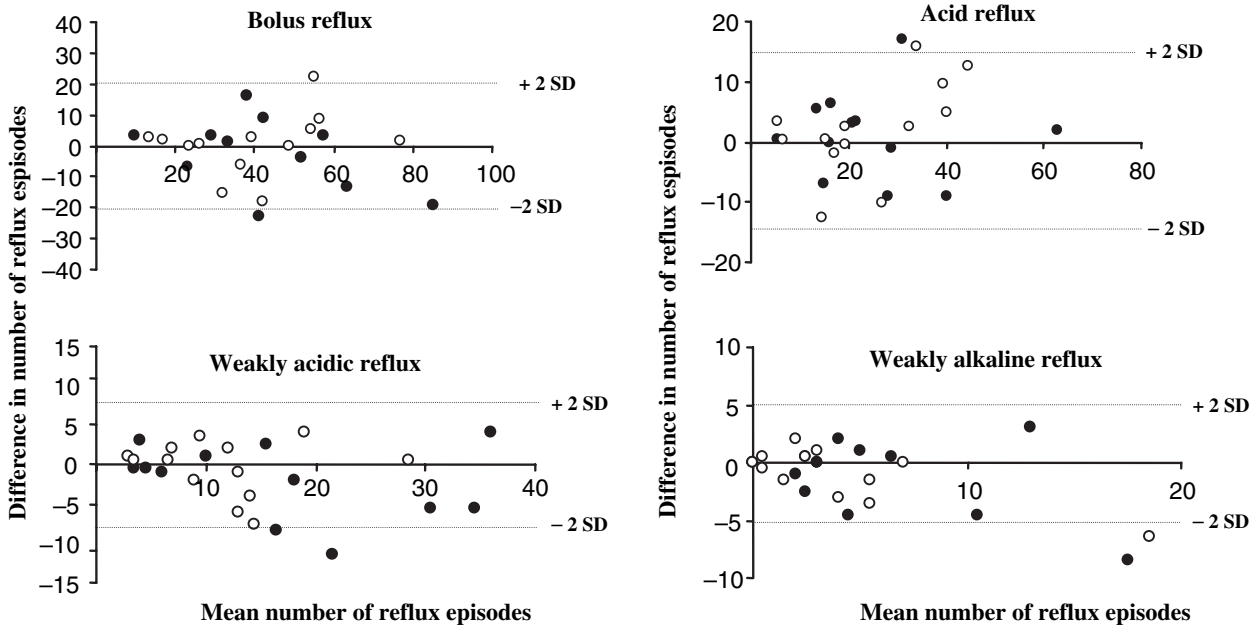

Figure 3. Reproducibility of different types of gastro-oesophageal reflux detected by ambulatory 24-h impedance-pH recording in 27 healthy subjects (Bland–Altman plots) (black dots: non-consecutive recordings; white dots: 48-h recordings).

build a multicentre database of GER patterns in a Belgian–French cohort of healthy subjects. We also tested intra-individual day-to-day variability of 24-h ambulatory oesophageal impedance-pH monitoring.

Considering total number of reflux events detected by impedance, our results are very similar to those previously reported in the US study. Indeed, although we observed a higher median number of reflux events (44 vs. 30), the 95th values were virtually identical (75 vs. 73). The same holds true for the number of acid and weakly acidic reflux but in our study, the number of weakly alkaline reflux was significantly higher because of a slight modification of the definition proposed by the 'Porto consensus'.[6] Indeed, we considered pH 6.5 instead of pH 7 as a limit between weakly alkaline and weakly acidic as reflux with pH above 7 are very rare and that basal pH values are usually between 6.5 and 7. It is to note that the above-mentioned experts did not reach a consensus agreement on this point.[6] In the ambulatory study of Sifrim *et al.*[9] in 28 healthy subjects, the median number of bolus reflux events (39.5) was also similar to our study but the proportion of traditional acid reflux was lower (33% vs. 56%). This may be related to different definitions of acid reflux events as we included in this category all reflux events detected by impedance whatever the duration of pH fall below 4. Moreover, our subjects received the instruction to eat their usual meals while in the previous study the subjects received standardized liquid meals. This further confirms that recording conditions play a major role and we believe that non-standardized meals are probably more relevant to elaborate normal values that will be used for diagnosis in patients with suspected GERD.

In our study, the GER events detected by impedance resulted in a median oesophageal 'bolus' exposure of 0.8% (95th value of 2.0%). Median clearance time of these reflux events was 11 s while median acid clearance time was threefold higher (34 s). These results are very similar to those reported by Shay *et al.* in the American database[4] and confirm that volume clearance and chemical clearance relate to two different mechanisms: volume clearance is achieved by primary and secondary peristalsis while chemical clearance requires neutralization by saliva of residual acid into the oesophagus.[10] This probably explains why we observed that volume clearance was not affected by position (secondary peristalsis induced by reflux), while acid clearance time was much longer in supine than in upright position (virtually no neutralizing swallowed saliva during sleep periods).

We found significant differences between total oesophageal acid exposure and impedance reflux-related oesophageal acid exposure. This suggests that a significant part of oesophageal acid exposure is not directly

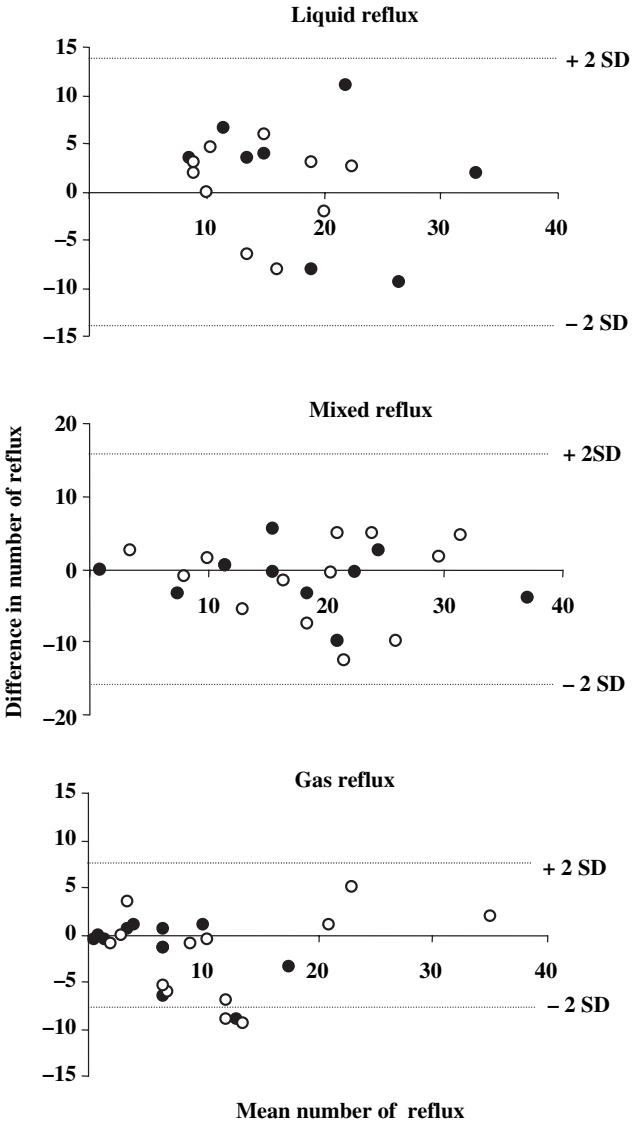

Figure 4. Reproducibility of different types of gastro-oesophageal reflux content detected by ambulatory 24-h impedance-pH recording in 27 healthy subjects (Bland–Altman plots) (black dots: non-consecutive recordings; white dots: 48-h recordings).

related to GER, at least detected by impedance. In the American study, pH drops independent from reflux are reported to be very rare (95th value of three) but no information is given concerning the resulting oesophageal acid exposure.[4] These pH drops may be related either to ingestion of acid beverages between meal periods (not indicated by the subjects in the diary) or to reflux not detected by impedance. In spite of this difference, the majority of our normal subjects had a median total oesophageal acid exposure within accepted normal values (1.6%, 3.7%–5.0%).

In contrast, 10 asymptomatic subjects were excluded because of a much larger dissociation between total oesophageal acid exposure and impedance reflux-related oesophageal acid exposure. In these subjects, the pHmetry showed abnormally high acid exposure (12.6% and 7.9% in upright and supine position, respectively) without evidence of reflux as determined by impedance. Indeed, while pH values remained below 4 for a very long time, impedance showed complete volume clearance and normal baseline values suggesting absence of acidic refluxate. Therefore, the analysis of impedance data helped us consider these very long reflux events as artefacts (impaction of acidic food?).

Combined impedance-pH monitoring not only allows detection of GER events in the distal oesophagus, but also provides additional information on their proximal extent into the oesophagus. Although the clinical relevance of the so-called 'proximal reflux' is not clearly established,[11] it is reasonable to consider that proximal reflux may play a role in typical or atypical symptoms and could contribute to the severity of the disease. The median number of reflux events detected by impedance 15 cm above LOS was very similar in our study (median 9, 95th value 30) when compared with the American data (median 8, 95th value 31)[4] and corresponded to 22% of all reflux events. The percentage of reflux reaching the proximal oesophagus was less in supine (11%) when compared with upright position (21%) but there was an important variability (interquartile range 0–50) related to the very low number of reflux events occurring in supine position. It is to note that all types of reflux were very rare in supine position. During this period, most reflux events were pure liquid reflux and virtually no belch occurred.

Comparisons according to gender demonstrated an increased total number of reflux in males ($P = 0.02$) related to an increased number of acid reflux events ($P = 0.03$) although the difference in oesophageal acid exposure did not reach the level of statistical significance ($P = 0.11$). There is no clear explanation to an increased acid reflux episodes in males, but such a difference has already been pointed out in another ambulatory 24-h pH study in healthy subjects.[12] Moreover, in a previous study in patients with GERD, it has been shown that gastric acid secretion was significantly higher in men when compared with women.[13] In our study, BMI was similar in males and females and therefore higher body weight could not account for the difference. Our results suggest that

Table 7. Intraindividual reproducibility for gastro-oesophageal reflux detected by ambulatory 24-h impedance-pH recordings in 27 healthy subjects

	Mean	s.d.	Kendall's *W*	*P*
Acid exposure (%)	1.08	0.45	0.72	0.07
All bolus reflux (*n*)	38.89	9.44	0.80	0.03
Acid reflux (*n*)	23.50	8.38	0.75	0.05
Weakly acidic reflux (*n*)	11.32	3.59	0.84	0.02
Weakly alkaline reflux (*n*)	4.07	2.32	0.78	0.04
Liquid reflux (*n*)	20.61	7.02	0.75	0.05
Mixed reflux (*n*)	18.21	5.96	0.79	0.03
Gas reflux (*n*)	11.54	5.20	0.73	0.06
Proximal extent (*n*)	8.71	5.45	0.77	0.04
Median bolus clearance (s)	10.07	1.82	0.85	0.01

separating male and female normal values should be considered in subsequent studies in patients with GERD.

We did not find any influence of age on the number and pattern of GER events in our cohort of healthy subjects. In patients with GERD studied with combined impedance-pH, Sifrim *et al.* did not observe any influence of age on the rate of acid and non-acid reflux or the air–liquid patterns of refluxate.[9] However, conflicting results have been reported so far with 24-h ambulatory oesophageal pH studies in healthy subjects regarding oesophageal acid exposure: indeed, Richter *et al.* found no significant difference related to age[12], while Smout *et al.* have reported striking differences between young (<45 year) and elderly (>45 year) subjects who experienced significantly higher oesophageal acid exposure.[7] As only nine patients were older than 60 in our study, we cannot rule out the possibility of a type 2 error and we believe that further studies should be performed to address this issue.

Testing for intraindividual reproducibility is crucial when dealing with a new technique. Reproducibility of impedance-pH recording has recently been demonstrated in healthy subjects in standardized, stationary, short-lasting postprandial studies.[5] Our study demonstrates the good reproducibility of 24-h impedance-pH recordings and can be considered as an additional important validation of this technique as ambulatory recordings appear clearly more relevant for clinical use. Both Bland–Altman plots and Kendall's *W*-values (0.72–0.85) demonstrate a good reproducibility for all relevant parameters, although the level of statistical significance was not reached for oesophageal acid exposure ($P = 0.07$) and gas reflux ($P = 0.06$). Many studies have reported that oesophageal pH monitoring was reproducible especially when oesophageal acid exposure was considered.[14, 15] Our study was not performed in standardized conditions, although subjects were asked to have similar diet and activities during the two recordings. Nevertheless, our results show that patterns of GER events can also be considered as reproducible at least in normal subjects. Neither intra- nor inter-observer reproducibilities were tested in this study. In fact, training sessions were organized prior to the study in order to decrease inter-observer variability in the interpretation of impedance-pH tracings. Therefore, inter-observer variability may be larger in conditions of clinical use.

Further validation studies of pH-impedance monitoring are necessary before its clinical application for the diagnosis of GERD. It is probably better to limit the use of this technology to reference centres in well-selected patients, e.g. refractory to PPI therapy[16, 17] or those with atypical symptoms potentially related to GERD.[3]

In summary, this study demonstrates good reproducibility of 24-h ambulatory impedance-pH studies and provides values of reflux patterns in healthy subjects for comparisons with European GERD patients.

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