PROVOCATIVE TESTING IN PATIENTS WITH JACKHAMMER ESOPHAGUS: EVIDENCE FOR ALTERED NEURAL CONTROL

Running head title: Altered neural control in Jackhammer Esophagus

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Abstract

Background. Jackhammer esophagus (JE) is a hypercontractile disorder, the pathogenesis of which is incompletely understood. Multiple rapid swallows (MRS) and rapid drink challenge (RDC) are complementary tests used during high resolution manometry (HRM) that evaluate inhibitory and excitatory neuromuscular function and latent obstruction respectively.

Methods&aim. Our aim was to evaluate esophageal pathophysiology using MRS and RDC in 83 JE patients (28 males; 63; 54-70 years). Twenty one healthy subjects (11 males; 28; 26-30 years) were used as a control group. All patients underwent solid state HRM with ten 5 ml single swallows (SS) and one to three 10 ml MRS; 34 patients also underwent RDC. Data are shown as median-IQ range.

Results. Abnormal motor inhibition was noted during at least one MRS in 48% of JE vs 29% of controls ($p=0.29$). Mean DCI after MRS was significantly lower than after SS 6028 (3678-9267) mmHg.cm.s vs 7514 (6238-9197) mmHg.cm.s, $p=0.02$, as was highest DCI ($p<0.0001$). Consequently, 66% of JE patients had no contraction reserve. At least one variable of obstruction during RDC (performed in 34 patients) was outside the normal range in 25 (74%) of JE. Both highest DCI after SS and pressure gradient across the esophagogastric junction during RDC were higher in patients with dysphagia vs those without ($p=0.04$ and 0.01 respectively).

Conclusions. Our data suggest altered neural control in JE patients with heterogeneity in inhibitory function. Furthermore, some patients had latent esophagogastric junction obstruction during RDC which correlated with the presence of dysphagia.

55 **New & Noteworthy**

56 • Presence of abnormal inhibition was observed during MRS in some but not in all JE patients.
57 Unlike healthy subjects, JE patients were more strongly stimulated after single swallows than
58 after MRS.

59 • An obstructive pattern was frequently observed during RDC and was related to presence of
60 dysphagia

61 • MRS and RDC during HRM are useful in order to show individual pathophysiological patterns
62 in JE and may guide optimal therapeutic strategies.

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64 **Key words**: High resolution manometry, jackhammer esophagus, multiple rapid swallows, rapid drink
65 challenge, dysphagia.

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74 INTRODUCTION

75 Jackhammer esophagus (JE) is a hypercontractile motility disorder characterized by the presence of
76 vigorous peristaltic waves that may be associated with dysphagia and/or chest pain(31). With
77 conventional manometry, a hypercontractile motor pattern characterized by the presence of high
78 amplitude (>180 mmHg) peristaltic waves in the distal esophagus was termed nutcracker esophagus
79 (34). The development of esophageal high resolution manometry (HRM) has allowed detailed spatial
80 definition of motor activity in the entire esophagus(10). With HRM, a new metric was introduced used
81 to assess vigor of esophageal smooth muscle contractility: the distal contractile integral (DCI). This
82 takes into account the amplitude, duration, and the length of the contractile segment (26), and therefore
83 allows accurate characterization of smooth muscle contractile activity in the distal esophagus. The
84 latest version of the Chicago classification of motor disorders defines JE as the presence of more than
85 20% of swallows with a DCI >8000 mmHg.s.cm(18), based on available data and consensus opinion
86 suggesting that this degree of hypercontractility is not encountered in healthy asymptomatic
87 individuals.

88 The pathophysiology of JE is incompletely understood. The prevailing hypothesis suggests that
89 exaggerated smooth muscle contraction results from an excess of cholinergic drive that leads to
90 excessive excitation or myocyte hypertrophy (12, 19). The associations between JE on the one hand,
91 and gastroesophageal reflux disease (GERD) and obstruction of esophagogastric junction (EGJ) on the
92 other, remain incompletely understood (1, 8). Impaired deglutitive inhibition has been reported in distal
93 esophageal spasm(5) and in nutcracker esophagus(33). Since imbalance between excitatory and
94 inhibitory forces in the smooth muscle esophagus has been proposed as a mechanism for exaggerated
95 contraction, provocative testing evaluating esophageal physiology could add to our understanding of
96 JE(16, 17). Multiple rapid swallows (MRS) is a provocative test performed during HRM that assesses

both deglutitive inhibition, and subsequent smooth muscle contraction(14, 32, 35), while rapid drink challenge (RDC) assesses deglutitive inhibition and evaluates for latent EGJ obstruction (2, 21). Physiologically, both MRS and RDC provoke an intense central and peripheral neural inhibition resulting in absence of contraction in the smooth muscle portion of the esophagus along with prolonged and complete relaxation of the lower esophageal sphincter (LES). The last swallow of the MRS series is followed by a powerful peristaltic sequence in the esophageal body together with a post-relaxation contraction in the LES; RDC does not always elicit a post-relaxation contraction. Thus, a normal response to MRS requires integrity of inhibitory mechanisms as well as capacity of esophageal muscle to respond to a strong stimulation at the end of the MRS(14). The ability to augment peristaltic performance following MRS is also called contraction reserve(32, 35). Using conventional manometry, motor inhibition was identified in nutcracker esophagus using 5 ml swallows at varying time intervals ranging from 5 sec to 30 sec apart(7); in contrast, motor inhibition was found to be diminished in nutcracker patients with multiple peaked waves (similar to that seen in distal esophageal spasm), using standard swallows and a sophisticated balloon sensor to measure inhibition(33). However, response to the standardized MRS (swallows of 2 mL of water at intervals of 2-3 sec for a total of 10 mL of water) and have not been reported and only limited data exist regarding RDC response in JE. (2, 22).

The primary aim of this observational cohort study was to evaluate esophageal deglutitive inhibition and post-MRS contraction using standardized provocative testing in JE patients, in comparison to that observed in healthy asymptomatic controls. Secondary aims were to better define esophageal physiology, pathophysiology, EGJ obstruction using RDC, and to determine relationships to symptomatic presentation in JE.

Methods

Adult patients referred for HRM between February 2016 and September 2017 in four Italian and one American tertiary centers (Milan, Padua, Pisa, Naples and St. Louis) were considered for this observational cohort study. The primary inclusion criterion was a manometric diagnosis of JE according to Chicago Classification v3.0 (at least 20% sequences with DCI>8000 mmHg.cm.s)(18). In addition to a standard 10-swallow HRM protocol of 5 mL water in the supine position, inclusion required at least one MRS sequence; RDC was not a requirement for study inclusion. Exclusion criteria consisted of esophageal outflow obstruction on HRM (integrated relaxation pressure, IRP>15 mmHg), eosinophilic esophagitis, known obstructive esophageal disorders, prior esophageal or gastric surgery, incomplete HRM studies and lack of at least one MRS sequence during the HRM protocol. The presence and nature of presenting symptoms were assessed by retrospectively reviewing the standardized medical interview and/or patient questionnaires specifically assessing for presence or absence of reflux symptoms, dysphagia and chest pain during patient evaluation(15, 29). HRM studies from 21 healthy subjects (mean age 28 years, range 26-30 years, 10 female) using a similar HRM protocol (single swallows, MRS) were utilized as comparative normative control data(32). This study protocol involving review of HRM studies and related patient data was approved by the respective institutional review boards at all study centers.

Esophageal high resolution manometry

HRM was performed using a 4 mm solid state probe with 36 circumferential sensors at 1-cm intervals (Medtronic, Minneapolis, MN, USA), using previously published methodology (24, 28). Manometric pressure data were visualized as topographic contour plots (Clouse plots) on a dedicated screen, and were stored for subsequent analysis using ManoView™ software (Medtronic, USA). In all cases, the esophageal manometry catheter was passed trans-nasally under topical anaesthesia (lidocaine spray or gel) after an overnight fast, and positioned with the tip in the stomach. Patients were placed in the

recumbent position and were asked to refrain from swallowing during 30 seconds of baseline recording of lower esophageal sphincter (LES) tone. Following this, the manometry protocol consisted of ten 5 mL single swallows (SS) of water at intervals of 20-30 seconds, and one to three MRS (i.e. swallows of 2 mL of water administered using a syringe at intervals of 2-3 seconds, while the operator monitored the rhythm of swallows, for a total of 10 mL of water)(24, 28). Finally, whenever possible, patients were asked to drink 100-200 mL of water as quickly as possible (rapid drink challenge, RDC) in the sitting position.

Data analysis

Single swallows

HRM studies were analyzed to extract standard HRM metrics (IRP, DCI, DL) utilized in the Chicago Classification v 3.0 using established methodology(18). The number of hypercontractile waves with DCI >8000 mmHg.sec.cm, mean DCI and highest DCI values were extracted from the HRM studies. Contractile front velocity (CFV) and distal latency (DL) of hypercontractile waves were compared to values obtained from non-hypercontractile effective sequences (DCI <8000 mmHg.sec.cm and > 450 mmHg.sec.cm).

Multiple rapid swallows and rapid drink challenge

During MRS, the 4 second IRP and motor inhibition (absence of motor activity of >3 cm length using a 30 mmHg isobaric contour in the esophageal body)(13) were evaluated. Pressurizations and contraction of striated proximal section of the esophagus were not included for the evaluation of motor inhibition. In patients with contraction during expected motor inhibition during MRS, a contractile integral was calculated using a 20 mmHg isobaric contour, using the DCI tool.

163 After MRS, the number of hypercontractile waves, mean and highest values of DCI were evaluated and
164 compared to those obtained during SS. CFV and DL of hypercontractile waves were compared with
165 values obtained with the normal waves. Contraction reserve was calculated as the ratio between the
166 mean MRS DCI and the mean SS DCI.

167 When RDC was performed, the ratio between the post-RDC DCI (when present) and the mean SS DCI
168 of SS was evaluated. To evaluate for EGJ obstruction, the number of panesophageal pressurizations
169 above 20 mmHg, duration of these pressurizations (as a percentage of duration of RDC), mean IRP and
170 gradient across the EGJ were measured according to methodology described by Marin et al (21).
171 Specifically, the trans-EGJ gradient was measured using software tools where mean (intrabolus)
172 pressure was measured 2 cm above and below the EGJ across the entire duration of RDC, and the
173 gradient was calculated in mmHg as the difference of the two values.

174 *Symptoms*

175 Hypercontractile variables (mean and highest DCI during SS and MRS), RDC variables (number of
176 pressurization, pressurization time and gradient across EGJ) and percentage of patients without motor
177 inhibition or contraction reserve (MRS) were compared between symptomatic (i.e. dysphagia and chest
178 pain) and asymptomatic patients. Data from patients undergoing 100 and 200 ml RDC were merged for
179 symptom analysis.

180 *Statistical analysis*

181 Data are described as median values with interquartile ranges (IQR) unless otherwise reported. The
182 Wilcoxon rank sum test was used to compare HRM variables between SS, MRS or RDC within the JE
183 cohort. Mann-Whitney or Chi-squared test as appropriate was used to compare data between the JE
184 cohort and asymptomatic controls (eight males; 28 years; 26-30)(32)and between symptomatic and

asymptomatic JE patients. Statistical analyses were performed using SPSS (version 21, IBM Corp., Armonk, NY). In all instances, a p value of <0.05 was required for statistical significance.

Results

Over the study period, 83 patients with JE (28 males; median age 63 years; IQR 54 - 70 years) fulfilled inclusion criteria for this study. Dysphagia and chest pain were the predominant presenting symptoms (59 and 52% of patients respectively); heartburn or acid regurgitation was reported by 45%. Upper GI endoscopy, performed in all patients, was normal in the majority (58%); spastic contractions were reported in 10%, whereas hiatal hernia was found in 27%. Esophagitis was rare, reported only in 5%. X-ray barium swallow was performed in 28 patients, showing tertiary contractions in three, hiatal hernia in three and normal findings in the remaining 22 patients.

Descriptive HRM parameters during SS and MRS in JE patients and asymptomatic controls are detailed in table 1. When normal sequences were compared to hypercontractile sequences with SS, neither CFV mean 3.5 (IQR 2.9-4.7) cm/sec vs 3.3 (2.7-4.2) cm/sec respectively, p=0.07 nor DL 6.6 (6-7.6) sec vs 6.9 (6.2-7.8) sec respectively, p=0.23 were different.

Inhibitory activity during MRS

The majority of JE patients (n=56, 68%) performed two, 14 patients (17%) three, and 13 (16%) one MRS. Nineteen of the 21 asymptomatic controls performed two and the remainder one MRS.

As expected, 4-sec IRP was significantly lower during MRS than SS both in JE patients and asymptomatic controls (Table 1). However, 4s IRP values following MRS reached a lower nadir in asymptomatic controls compared to JE (p=0.01); values were statistically similar during SS in both groups (p=0.12). Abnormal motor inhibition was noted in at least one MRS sequence in 40 (48%) JE

patients, and in all available MRS sequences in 19 (23%) JE patients; these were not significantly different from asymptomatic controls (24% and 15% respectively, p=0.10). In JE patients, median contractile integral of motor activity during MRS was 718 mmHg.sec.cm (IQR 391-1460 mmHg.cm.s); 44 of 64 MRS without motor inhibition (69%) had a contractile integral of motor activity >450 mmHg.sec.cm, whereas in asymptomatic controls only 3 of the 8 MRS without motor inhibition (38%) had similar contractile integral (p=0.08 vs JE), suggesting a greater impact of absence of motor inhibition in JE patients although not statistically significant. An example of absence of motor inhibition during MRS in JE is shown in figure 1.

Contractile activity after MRS

Interestingly, hypercontractile activity was less evident after MRS than after SS in JE patients: proportions of hypercontractile sequences were lower following MRS than with SS (34% vs 45%, p=0.01). Mean DCI after MRS was significantly lower than with SS 6028 (3678-9267) mmHg.cm.s vs 7514 (6238-9197) mmHg.cm.s, p=0.02; this difference was more pronounced when the highest DCI values during MRS and SS were compared 8884 (4585-11741) mmHg.cm.s vs 9775 (9178-12259) mmHg.cm.s, p<0.0001 (Fig. 2A). Further, when contraction reserve was analyzed, 55 out of 83 JE patients (66%) had an MRS/SS DCI ratio < 1. MRS/SS DCI ratios were significant lower than those obtained in our cohort of asymptomatic controls (0.8; 0.5-1.1 vs 1.2; 1.1-2.3 p=0.0007) (Fig. 2B). Contrary to what we have observed in our controls, peristaltic sequences after MRS were faster and more premature than those observed after SS (Table 1). The same was observed when we compared normal sequences with hypercontractile ones after MRS (CFV 4.4 cm/sec; 3.0-6.7 vs. 3.0 cm/sec; 2.5-4.2, p=0.002 and DL 5.8 sec; 4.8-6.8 vs 7.1 sec; 6.2-7.5, p=0.003).

When JE patients with normal inhibition during MRS were compared with patients with abnormal motor inhibition, mean DCI after SS was lower in those with normal inhibition median 7395 (IQR

6056-9678) mmHg.cm.s compared with those having abnormal inhibition 8214 (7528-10901) mmHg.cm.s, p=0.038. Mean DCI after MRS, however, was similar between these two subgroups 6127 (3878-9847) mmHg.cm.s vs 6357 (3208-10671) mmHg.cm.s, p=0.77. The MRS/SS DCI ratio was also similar 0.9 (0.6-1.3) vs 0.9 (0.5-1.1), p=0.26.

Rapid drink challenge

Of the 83 JE patients, 34 (41%) successfully completed RDC; 21 with 200 ml of water and 13 with 100 ml of water (Table 2). Dysphagia and chest pain were the predominant symptoms in this subset (58% and 44%) similarly to the whole cohort. Out of the remaining 49 patients, 12 did have RDC data because in one of the Centers RDC was not incorporated into routine HRM and 37 because either patients refused, did not perform it adequately or physicians considered it risky for aspiration. All 34 patients performed the RDC with a median drinking time similar to those previously reported(21). Interestingly 19 patients had at least three pressurization events, the median percentage time at 20 mmHg during 100 ml and 200 ml being 10 % and 17% respectively. Moreover there was increase in the pressure gradient across the EGJ during both 100 (0.7 mmHg;-1.8 to 4.9) and 200 ml (4.8 mmHg; -2.8 to 11.5), despite the fact that mean IRP remained within the range of normal. Nineteen patients were outside the upper limit of normal range for number of pressurizations, 20 of them for percentage of time at 20 mmHg and 10 patients for pressure gradient across EGJ. At least one of the 3 variables was out of range in 25 patients (74%). Twenty one out of 34 patients (62%) demonstrated a contraction at the end of the RDC, of which 8 (38%) were hypercontractile (four after 100 ml and four after 200 ml). The RDC/SS ratio was <1 in 13 of the 21 patients (62%).

Relationship between motor function and presenting symptoms

The highest DCI after SS was higher in patients with dysphagia compared with those without 12385 (10577-19670) mmHg.sec.cm vs 11192 (9500-14278) mmHg.sec.cm, p =0.04. Furthermore, the gradient across the EGJ during RDC was also higher in the dysphagia group vs those without dysphagia 5 (2.4-9.8) mmHg vs -2 (-4.4 to 0) mmHg, p=0.01. No relationships were observed between motor variables and chest pain.

Discussion

In this study evaluating esophageal physiology and pathophysiology in JE using provocative tests, we demonstrate that there is abnormal inhibition during MRS and RDC, but not in all JE patients. More interestingly, JE patients appear to be more strongly stimulated after SS than after MRS, with no significant augmentation of contraction following MRS and RDC, fulfilling criteria for lack of contraction reserve despite presence of smooth muscle contraction following provocative testing. Our findings suggest that esophageal motor physiology is abnormal in JE patients, with inappropriately exaggerated excitatory influences in the majority, combined with abnormal inhibitory function in some JE patients. Finally, despite relaxation of the LES (as evidenced by normal IRP), an obstructive pattern was noted with RDC in some JE patients.

Both excitatory and inhibitory influences need to be present in a balanced fashion for normal esophageal function during swallowing. The most obvious consequences of abnormal inhibition consist of abnormal sequencing of esophageal body peristalsis (resulting in premature sequences) and abnormal LES relaxation after SS. However, abnormal inhibition has also been described when esophageal contraction demonstrates prolonged duration and multiple contraction peaks(33). Using MRS we found abnormality of inhibitory function in some, but not all our JE patients and, interestingly, DCI after SSs was higher in these patients compared to those having normal inhibitory

function. This finding, in agreement with previous data in a broader spectrum of hypercontractile motility patients(28), may suggest two different groups of JE from a pathophysiological perspective. On the whole the difference in prevalence of defective inhibition between our JE patients and our cohort of asymptomatic controls, i.e 48% vs 24%, did not reach statistical significance, possibly because of the low number of controls, although difference in contractile integral was statistically significant. Furthermore another control series in the literature has shown that defective inhibition was present in 5% only of healthy controls (13). The finding of abnormal inhibition even in healthy control suggests that there is inherent variation in refractoriness of the esophageal muscle to contraction during repetitive swallowing. Despite this, the frequency of abnormal inhibition was higher in patients with JE. However we have to point out that normative values are based on small cohorts of healthy subjects and therefore no clear thresholds of motor inhibition are available in literature. Regardless, the inhibitory abnormality in JE is heterogenous and variable; more abnormal and homogeneous inhibitory dysfunction would likely shift the diagnosis towards more severe motor disorders, i.e. type 3 achalasia, diffuse esophageal spasm or abnormal LES relaxation.

Abnormally increased excitation has been described in patients with ‘nutcracker’ esophagus(9) in some of the early HRM studies as merging of the two smooth muscle contraction segments, leading to a single exaggerated contraction in the distal esophageal body. Korsapati et al has shown that presence of muscular asynergy (peak longitudinal muscle contraction occuring earlier than peak circular muscle contraction) during SSs in similar ‘nutcracker’ patients was due to a hypercholinergic state since, with high-frequency EUS, it was observed to revert after infusion of atropine(19). Chicago Classification criteria for JE identify the most extreme end of the hypercontractile spectrum, such that many patients with nutcracker esophagus do not fulfil criteria for JE(25). Our findings support the concept that the esophagus is strongly stimulated with SSs in JE. Physiologically, esophageal smooth muscle when

provoked, is able to increase contraction vigor. This is the so called contraction reserve. However, in JE, the muscle is not able to increase contraction vigor even when provoked with MRS, because it is strongly stimulated with just SS (Figure 2B), even though the vigor of smooth muscle contraction following MRS in JE is higher than that seen in normal controls. We believe that the lack of contraction reserve could suggest a primary motor hyperstimulatory mechanism rather than an obstructive secondary response where further increase in esophageal contraction could be possible. However, this is speculative, as we excluded patients with identified secondary obstructive processes on esophageal testing. Furthermore, the observation that hypercontractility was less common and latencies were shorter after MRS than after SS in JE confirms that regulation of timing and strength of contraction after MRS is different from that triggered by SS.

It is well known that there is swallow to swallow variation in esophageal motor metrics. This is most profound in motor disorders that are not well developed, in contrast to profound and well-developed motor disorders such as achalasia types 1 and 2, and absent contractility. In fact, these well-developed motor disorders demonstrate a remarkable consistency and reproducibility in response both to SSs and provocative testing, especially MRS(28). In contrast, less developed motor disorders demonstrate variation among SSs, requiring finite criteria for diagnosis (e.g. 20% premature with DES, 20% hypercontractile with JE). In this setting, response to provocative testing is useful, as the contractile response appears less variable, even though motor inhibition remains heterogeneous (28).

Our study provides important information about the behavior of JE patients during RDC and relationship between motor abnormalities and symptoms. Our results have shown that a considerable proportion of patients who underwent RDC had alterations in the esophageal body and/or a high EGJ pressure gradient suggesting latent obstruction. Previous data in the literature in smaller groups of patients are in line with our findings. Marin et al described the response to RDC in 14 JE patients

318 concluding that they had a certain degree of pressurization associated with a minimal increment of the
319 EGJ pressure gradient and a valid LES relaxation(22). Ang et al have shown pressurization in 64.7%
320 of 17 patients with either JE or esophageal spasm during RDC (3); the presence of esophageal
321 pressurization suggests the presence of an EGJ pressure gradient even though this was not reported.
322 While IRP remains a robust metric during single swallows, its performance has not been specifically
323 tested during provocative testing; on the contrary, trans-EGJ pressure gradient is reported often as an
324 outcome measure for RDC, either indirectly in terms of pressurization(3, 22), or directly as the trans-
325 EGJ pressure gradient(21, 22). It is interesting to note that, in our series, dysphagia was present in those
326 patients with higher DCIs after SS, and particularly those with higher values of EGJ pressure gradient,
327 giving a clinical perspective to our findings. This latter subgroup of patients may have an advanced
328 motor disorder and the increase of resistance to outflow may be related to a primary motor obstruction
329 (i.e achalasia) that is not completely expressed phenotypically. Indeed previous studies have shown that
330 increase of peripheral resistance may lead to increase of peristaltic vigour(6, 11). However early
331 identification of patients that may progress to achalasia could be difficult with HRM given that IRP is
332 normal both during SS and RDC. This data have been also highlighted in the paper by Ang et al where
333 IRP was increased during RDC in only 1/17 (6%) of JE patients. Therefore use of EGJ pressure
334 gradient could be of better value in this regard. The observation that some patients have latent
335 obstruction on RDC may explain why performing a POEM procedure without involving the LES has
336 resulted in incomplete relief of dysphagia, whereas if the LES was also included in the POEM,
337 symptom relief was more consistent(4).

338 Some methodological points and limitations need further discussion. Firstly, the number of MRS
339 performed: most of our patients underwent two MRS. In a recent paper from our group, we suggested
340 that three MRS is the most optimal number in order to obtain reliable data regarding contraction

341 reserve(24); however those results were obtained in a different setting (i.e. IEM and normal motility
342 patients) who have higher variability among MRS series, whereas reproducibility with two MRS series
343 has been previously shown adequate in hypermotility patients(28). Regarding reliable evaluation of
344 motor inhibition we have previously shown that one MRS only is sufficient(23). Secondly, we did not
345 perform RDC in all our JE patients, and we did not have a control group of our own for RDC.
346 Therefore data about relationship between symptoms and obstructive parameters during RDC should be
347 interpreted with caution. However, on one hand it needs to be acknowledged that performance of RDC
348 cannot be expected in all patients because of patients' refusal, inability to do it adequately or safety
349 especially in the elderly, nevertheless our RDC subgroup had similar clinical presentation to the one of
350 the whole cohort and a size which was still the biggest of the literature. On the other hand, the
351 reference values that we have used have been obtained with the same protocol and equipment used in
352 our study, with a similar cohort size as our patients' group[22]. Therefore, we feel our findings are
353 representative of esophageal physiology in JE despite these limitations.

354 Finally, data about opioid medication use was not available in our series: it is known from the literature
355 that opioids alter esophageal motility by decreasing inhibitory function at the level of LES(27);
356 hypercontractile effect in the esophageal body is less evident(20, 30) and therefore we do not feel this
357 detracts from our overall conclusion of pathophysiological mechanisms in JE.

358

359 In conclusion, our data with MRS and RDC suggest altered neural control in JE patients with
360 heterogeneity in inhibitory function. Furthermore, some patients had latent EGJ obstruction during
361 RDC which correlates with dysphagia. Performing RDC during HRM studies, therefore, may also
362 guide optimal therapeutic strategies.

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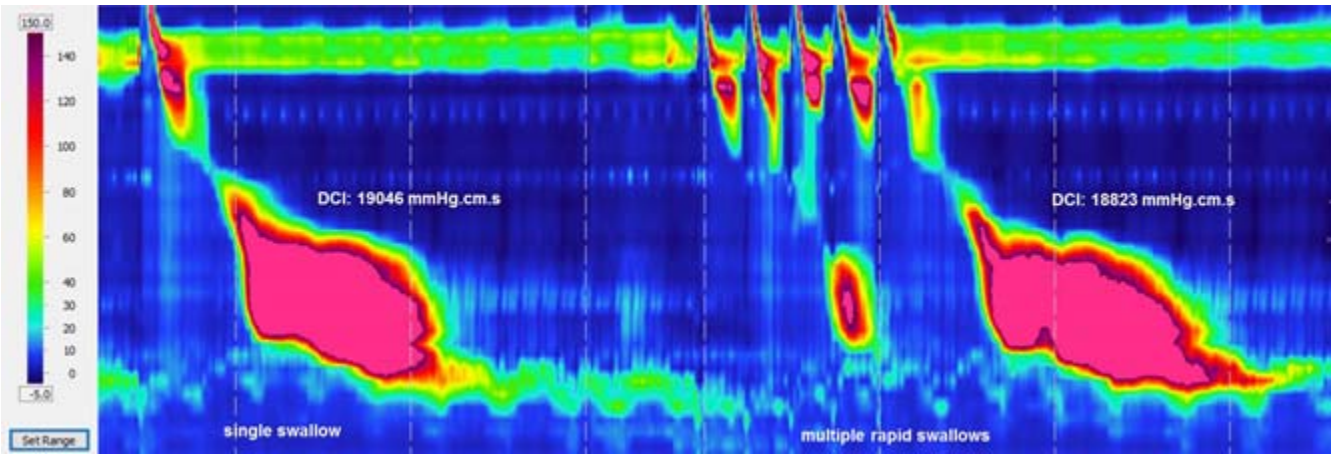
Figure 1 Single swallow and multiple rapid swallows from a patient with Jackhammer Esophagus. There is a short segment of contraction during multiple rapid swallows, indicating abnormal inhibition. Further, esophageal smooth muscle is maximally stimulated during single swallows, and there is no further reserve for augmentation of contraction following multiple rapid swallows.. These findings demonstrate an imbalance in esophageal inhibition and contraction in Jackhammer Esophagus.

Figure 2. A) Mean and highest DCI was lower after MRS than after SS in Jackhammer esophagus patients B) MRS/SS DCI ratio was lower in Jackhammer esophagus patients than in healthy subjects

522 Figure 1



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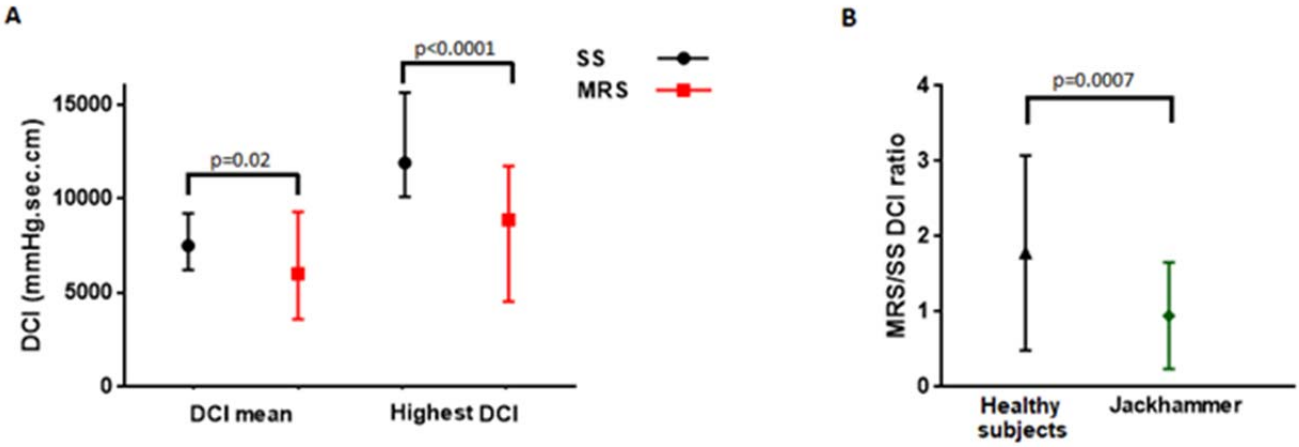
527 Figure 2



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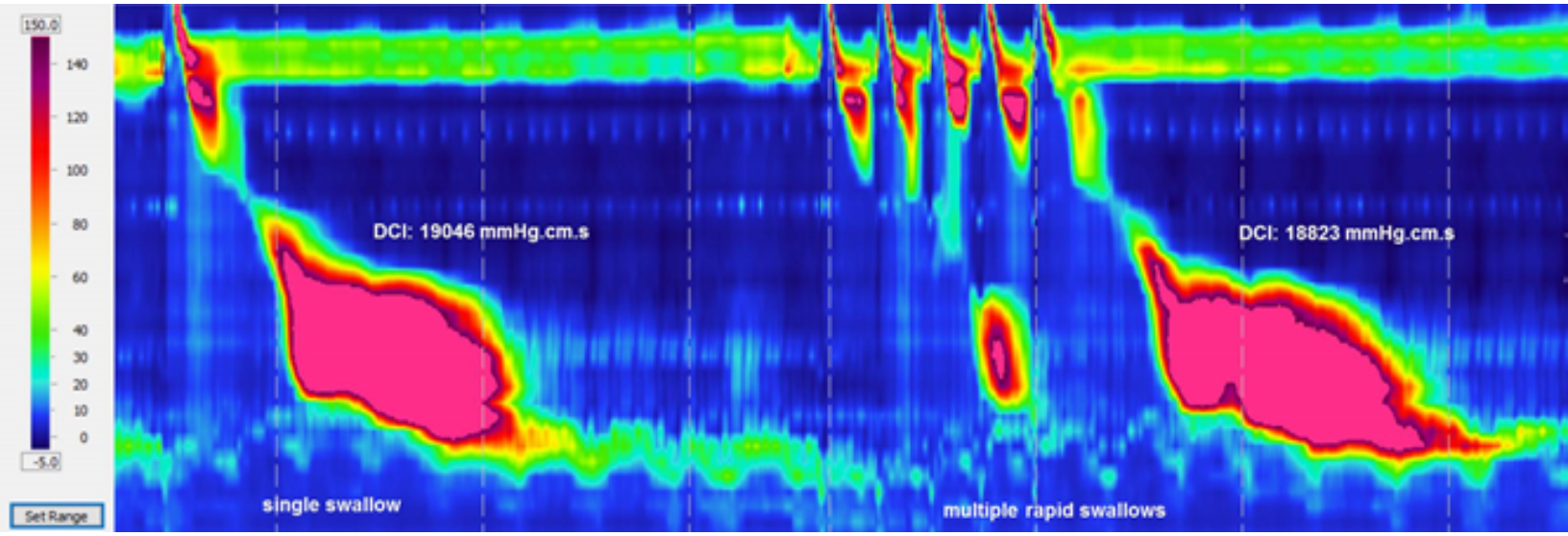

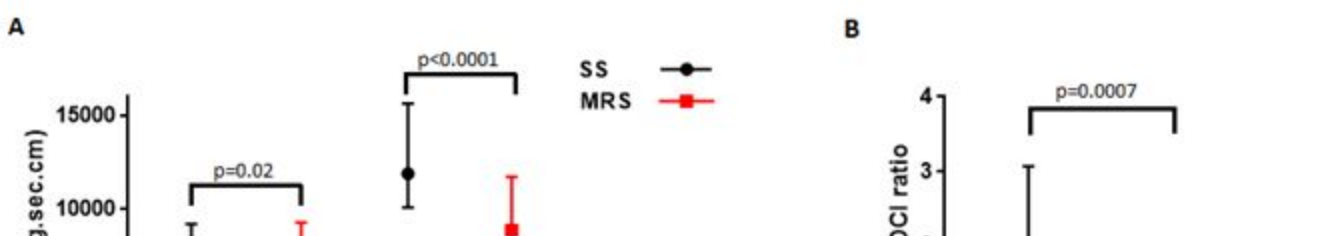

Table 1 HRM variables during single and multiple rapid swallows in Jackhammer esophagus (JE) patients and healthy controls. Data expressed as Median (IQR); full range.

	JE patients	Healthy controls
Single Swallows		
4-sec IRP, mmHg	8.1 (8.4); 0-20.8*	5.9 (5.9);1.2-13.7**
IBP, mmHg	14 (10.6); 1-40°	11.2 (7.7); 2.5-18.6**
DL, sec	6.7 (1.3); 4.7-10°	7.6 (1.4); 5.5-9.9
CFV, cm/sec	3.4 (1.8); 1.7-12*	3.3 (0.9); 2.1-5.1†
Multiple rapid swallows		
4-sec IRP, mmHg	5.5 (6.3); -0.6 to 24.5	2.2 (4); 0-13.2
IBP, mmHg	16.5 (12); 1-40	13 (4.3); 6.8-17.4
DL, sec	6.4 (2); 1.2-14.1	8.4 (2.8); 3.5-11
CFV, cm/sec	4.2 (3.3); 1.3-22	2.7 (1.1); 2-4.6

SS: single swallows; MRS, multiple rapid swallows; IRP: integrated relaxation pressure; IBP: intrabolus pressure; DL: distal latency; CFV: contraction front velocity. *$p<0.005$ vs JE MRS; °$p<0.05$ vs JE_MRS; **$p<0.01$ vs healthy controls MRS; †$p=0.01$ vs healthy controls MRS.

Table 2 HRM variables during and after RDC with either 100 ml (13 patients) or 200 ml (21 patients) of water in JE patients.

	RDC parameters	100 mL RDC		200 mL RDC	
		JE patients N=13 *Median (IQR); full range*	Healthy controls[21] N=17 5th-95th CI	JE patients N=21 *Median (IQR); full range*	Healthy controls[21] N=73 5th-95th CI
RDC performance	Time, sec	11 (3); 8-17	8-26	29 (14); 14-64	12-47
Pressure response during RDC	Pressurization at 20 mmHg, n	1 (2); 0-7*	0-0	2 (3); 0-9*	0-2
	Time at 20 mmHg, percentage	10 (58); 6-67*	0-0	17 (28); 0-66*	0-8
	Mean IRP, mmHg	5.8 (6); 0-17.1	-3 to 8	6.7 (7); 2.9-19.3	-2 to 12
	Gradient across EGJ, mmHg	0.7 (6.8); -3.6 to 14.8*	-10 to 1	4.8 (14); -8 to 35.2*	-6 to 4
Activity after RDC†	CFV, cm/sec	4.1 (2.5); 1.1-18.2	1-18	5.3 (6); 0.9-21.4	1-14
	DL, sec	8 (5.3); 3.4-14	n/a	5 (3); 1.8-7.5	n/a
	DCI, mmHg.sec.cm	5680 (9813); 448-26271*	61-3877	3903 (7975); 1376-64904*	206-6557
	RDC/SS DCI ratio	0.7 (1.5); 0.1-2.9	0.05-2.68	0.6 (0.9); 0.1-9.2*	0.1-2.9

†in nine out 13 patients after 100 ml and 12 out 21 patients after 200 ml

*RDC variables outside the normative range as reported by Marin et al[21] (95th CI for all variables except for RDC/SS DCI ratio where the 5th CI was considered)