● G3282

NONINVASIVE ECHO-PLANAR IMAGING (EPI) MONITORING OF INTRAGASTRIC VISCOSITY, DILUTION AND EMPTYING OF VISCOUS MEALS IN NORMAL SUBJECTS. L Marciani,[1] J Wright,[2] P Manoj,[3] RJ Moore,[1] P Young,[1] D Bush,[2] S Al-Sahab,[1] A Fillery-Travis,[3] PA Gowland[1] and RC Spiller[2]. [1]Magnetic Resonance Centre, University of Nottingham,UK; [2]Queen's Medical Centre, Nottingham,UK; [3]Institute of Food Research, Norwich,UK.

EPI is a rapid form of magnetic resonance imaging which is capable of obtaining images free from motion artefacts in ≈ 130 ms and can be used to monitor gastric emptying, motility and flow. **Aims:** 1) to assess the feasibility of monitoring intra-gastric changes during the emptying of meals of different viscosity (η) noninvasively and 2) to measure meal dilution, gastric distension, and satiety feelings after meals of differing η. **Methods:** 4 polysaccharide locust bean gum (LBG) meals (η ranging from 0.02 to 12 Pas) were given randomly to 8 healthy intubated volunteers after an overnight fast. The LBG meals were labeled with a nonabsorbable Phenol red marker dye to allow digesta dilution measurements by UV spectrophotometry. Digesta samples were retrieved immediately after ingestion and 40 min later and underwent stress viscometry measurements. Transverse multi-slice single-shot MBEST EPI and spin-echo T_2 data sets were collected every 12 minutes until the meals had emptied. Satiety questionnaires were completed every 15 mins. **Results:** *In vitro* $1/T_2$ of LBG meals was linearly related to log(η) and unaltered by gastric juice or pH changes. *In vivo* viscous meals were generally well tolerated by volunteers and provided good contrast in EPI images. A marked fall in meal η was observed for all meals 40 min after ingestion ($p<0.05$), the initial η value for the most viscous meal being lowered most from 11 (9.2-14) to 0.3(0.2-1.1 Pas), $p< 0.05$. $1/T_2$ *in vivo* correlated with the measured η of aspirates, $r^2=0.93$. The UV data showed a progressive dilution for all meals (Page's test $p<0.01$), falling most for the 1.5% LGB meal from 0.8(0.4-0.8) immediately after drinking to 0.5(0.3-0.6) 40 mins later. EPI measurements of gastric volumes with time showed that increased meal η was associated with increased area under emptying curve (AUC) (p for trend <0.05). Gastric half emptying time ($T_{1/2}$) also increased, but not significantly, see Table (median(range)). The AUC for the sense of fullness increased, and hunger decreased with increasing meal η (Friedman's ANOVA, both $p<0.02$).

LBG meal	0.25%	0.5%	1.0%	1.5%
$T_{½}$ (min)	17(11-24)	18(15-22)	18(11-25)	19(10-27)
AUC Gastric vol $p < 0.05$	9.6(5.4-14.9)	11.5(7.4-15.7)	11.4(6.2-14.4)	12.1(9.4-15.8)
Fullness (trend $p < 0.05$)	369(282-432)	357(222-498)	426(348-498)	459(318-582)
Hunger (trend $p < 0.001$)	465(402-564)	477-(180-582)	435(222-516)	333(162-498)

Conclusion: Viscous meals are rapidly diluted by saliva and gastric juice with a resulting fall in viscosity. Increasing viscosity leads to a slower fall in gastric volumes with time and more prolonged satiety.
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● G3283

COLLAGENS, FIBRONECTIN AND VERSICAN IN SMOOTH MUSCLE OF OPOSSUM ESOPHAGUS. James B Martin, Siroos S Shirazi and K Schulze-Delrieu. Orthopedic and GI Research Labs, VAMC, Iowa City, IA.

Strictures, diverticula and distention/hypertrophy must affect the connective tissue matrix of the esophagus, but unfortunately, little is known about its normal composition and distribution. Here we used immunohistochemistry to study the anatomical distribution of collagens, of fibronectin, (a cell interactive glycoprotein which can form fibrils), and of versican, (a chondroitin-sulfate proteoglycan with the capacity to swell) in esophageal smooth muscle of 9 American opossums. Sections were embedded in cryoform, and following exposure to hyaluronidase exposed to antibodies to collagens, versican (12C5, antiversican-monoclonal hybridoma supernatant from developmental studies Hybridoma Bank, NIH) or fibronectin. They were stained using secondary antibody conjugates of rhodamine or isothiocyanate, and intensity of staining was scored by two observers on a four grade scale. We found that collagen I was present in all layers but particularly prominent in the submucosa and in the muscular septa; collagen III formed fibrillar meshes in the lamina propria and the submucosa but was virtually absent from the epithelial and muscular layers; collagen IV was restricted to the function of the epithelium with the lamina propria; collagen V was distributed in a pattern virtually reverse from that of collagen III, being prominent in the epithelium and the muscularis mucosae and fairly sparse in the muscular septa and submucosa. Fibronectin distribution closely resembled collagen III, forming an apparently continuous framework through all layers of the wall, with prominent strands of fibronectin along the organ axis in the lamina propria and submucosa in continuity with fibronectin inside the septa of muscle bundles and inside the space between individual cells of the circular, mucosal and longitudinal muscle layers. Versican distribution resembled more that of collagen V, being prominent in the septa which surrounded bundles of muscle; the largest septa connected to thick sheets of versican at the boundaries between submucosa and circular muscle and circular /longitudinal muscle; versican was sparsely distributed between individual muscle cells and throughout the submucosa. We conclude that matrix composition differs within the various connective tissue spaces of the smooth muscle esophagus. The connective tissues of lamina propria and submucosa are similar with regard to fiber orientation but lamina propria contains relatively more collagen III (small fibril) and submucosa comparatively more collagen I (large fibril). Collagen V and versican are particularly prominent on the boundaries between contracting muscle tissue and connective tissue framework.
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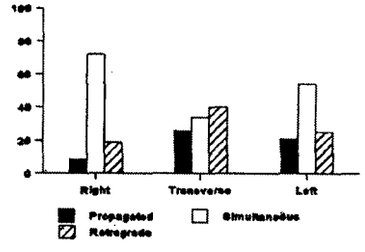
G3284

24-HOUR PANCOLONIC MOTOR ACTIVITY IN HEALTHY SUBJECTS RECORDED WITH A PORTABLE DEVICE. L Marzio, L Grossi, AF Ciccaglione, F Carmosino, A Castellano, M Falcucci, MG Malatesta. School of Gastroenterology, G.D' Annunzio University, Pierangeli Clinic, Pescara, Italy.

Introduction: although widely studied the motor pattern of the human colon is still not completely known. Therefore it remains difficult to establish a direct relationship, if present between clinical features and motor abnormalities of the large bowel. **Aim:** to identify by means of a 24-hour manometric recording the main motor patterns at the level of right transverse and left segments of the colon in healthy subjects. **Materials and methods:** 8 healthy subjects underwent a total colonoscopy. A biopsy forceps was passed through the operative channel of the endoscope to anchor the tip of a manometric probe with six electronic recording sites, 15 cm apart (SME; Solothurn, Switzerland) connected to a portable data logger (Gastroscan, SME) able to transfer data to a personal computer with a dedicated software for their analysis (Scan4, SME). Once the caecum was reached, the forceps was opened and the probe left inside. According to the exact placement of the recording sites for each subject, fluoroscopically checked at the beginning and at the end of the recording period, data were referred to the three segments of the colon (right, transverse and left colon). During the 24 hours the subjects were allowed to move freely and ate 2 standard meals of 850 Kcal at 12.00 and 7.00 p.m. **Results:** during the 24 hours no significant difference was found in the total number, amplitude and duration of motor waves among the various segments of the colon. (Number: right 730 ± 95.8, transv. 721 ± 88.7, left 724 ± 47.3; Amplitude: right 37.1 ± 4.7 mbar, transv. 32.4 ± 2.6, left 35 ± 1.8; duration: right 5.5 ± 0.4 sec., transv. 4.6 ± 0.2, left 5.3 ± 0.2, mean ± SEM, all n.s.). The right and left colon showed a greater percentage of simultaneous waves in comparison with retrograde and propagated ones (figure, * $p<0.01$). Propagated, simultaneous and retrograde waves were found in similar percentage in the transverse colon (figure). A mean of 6.2 ± 1.2 high amplitude propagated contractions (HAPC)/subject/24h. was detected, starting from the right and propagating toward the left colon. After meals there was a significant increase in the number and amplitude of motor waves and a trend toward an increased propagated motor activity only at the right and left colon, whereas the transverse remained unchanged. **Conclusion:** In normal subjects the whole colon shows a continuous motor activity with an increase in mealrelated propagated waves in the right and left colon. With our portable device a normal 24-hour colonic motor pattern has been identified. This pattern could be useful for a future "positive" diagnosis of motor-related colonic diseases.



● G3285

DELAYED LIQUID AND SOLID GASTRIC EMPTYING IN GENE KNOCKOUT MICE LACKING NEURONAL NITRIC OXIDE SYNTHASE. H. Mashimo, A.P. Kjellin, E.J. Ashegh, and R.K. Goyal, VAMC, W. Roxbury, MA, and Mass. General Hosp., Boston, MA.

Background/Aims: Nitric oxide is a major inhibitory neurotransmitter in the gut. The purpose of this study was to identify the role of nitric oxide derived from the neuronal constitutive nitric oxide synthase (nNOS) in both liquid and solid gastric emptying using mutant (nNOS(-)) mice with targeted disruption of this gene. The pylorus was evaluated to test whether hypertrophic pyloric stenosis mimicking the human condition was responsible for any dysfunction.

Methods: nNOS(-) mice were compared with normal wild-type mice for (1) differences in liquid and solid gastric emptying, (2) radiographic appearance of the pylorus after barium gavage, and (3) histology of the pyloric sphincter after treatment with isoproterenol (10^{-5} M). For emptying tests, 10 fasted mice were gavaged with thirty 0.8 mm beads 2 hrs. before sacrificing, and gavaged with 0.3 ml of solution containing 1.5%

methylcellulose and 0.05% phenol red 20 min. before sacrificing. For baseline, 4 mice were gavaged with the phenol red solution immediately before sacrifice. Gastric emptying of liquids was assessed by measuring the OD560 of the homogenized alkaline-treated stomach after acidifying in 2% acetoacetic acid and was expressed as: [1 - (OD of test sample/ OD of baseline)] x 100%. Solid emptying was expressed as the percent of beads that have passed the stomach after two hours.

Results: There was marked delay for emptying of both liquids (wild-type = 39.7% ± 22.2% cf. nNOS(-) = 19.3% ± 22.2%; P=.06) and solids (wild-type = 96.0% ± 7.0% cf. nNOS(-) = 29.8% ± 32.1; P<0.01) in nNOS(-) mice. On fluoroscopic exam, nNOS(-) adult mice showed gastric dilation and narrowed pylorus after barium gavage which was partly reversed by amyl nitrate inhalation. There was also delayed emptying of barium-impregnated food from the stomach. However, histology of the longitudinal section of the adult nNOS(-) pyloric sphincter showed minimal smooth muscle thickening and no evidence of stenosis after treatment with isoproterenol compared with the wild-type sphincter.

Conclusion: nNOS-deficient mice have delayed gastric emptying to both solids and liquids. Although nNOS-deficient mice demonstrate a narrowed pyloric sphincter, this is not a fixed stenosis as evidenced by response to amyl nitrate (*in vivo*) or isoproterenol (*ex vivo*). Moreover, there is minimal circular smooth muscular thickening in these mice, distinguishing this animal model from the human condition of hypertrophic pyloric stenosis.

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● **G3286**

PYLORIC DYSFUNCTION OF INHIBITORY NEUROTRANSMISSION IN KNOCKOUT MICE LACKING NEURONAL NITRIC OXIDE SYNTHASE. H. Mashimo, X.-D. He, R.K. Goyal, VAMC, West Roxbury, MA, and Mass. General Hosp., Boston, MA.

Background/Aims: Nitric oxide derived from different isoforms of nitric oxide synthase serves distinct biological roles. The purpose of the present studies was to identify the role of neuronal constitutive isoform (nNOS) in inhibitory neurotransmission of the pyloric sphincter using mutant (nNOS(-)) mice with targeted disruption of this gene.

Methods: Pyloric muscle strips from nNOS(-) and normal wild-type mice were compared using intracellular membrane potential recordings of circular smooth muscle cells to study differences in inhibitory junction potential (IJP) response to electrical field stimulation.

Results: Electrical field stimulation of nonadrenergic noncholinergic nerves in the pyloric muscle strips in wild-type mice produced IJPs with overlapping fast, slow, and very slow components. Both the slow and very slow components were sensitive to the nitric oxide synthase inhibitor N^{ω}-nitro L-nitroarginine (L-NA), whereas the fast component was sensitive to apamin. In nNOS(-) mice, the IJP response consisted of only the fast component which was not affected by L-NA but was sensitive to apamin. This apamin-sensitive IJP in nNOS(-) mice resembled the fast IJP seen after L-NA treatment in wild-type mice. The fast IJP in both the wild-type and nNOS(-) tissues was blocked after tachyphylaxis with methylene adenosine 5'-triphosphate, a stable analogue of ATP, suggesting that it is mediated by ATP. The fast IJP is also blocked by reactive blue 2, suggesting that ATP acts on P_2 receptor to produce this hyperpolarization. Apamin sensitivity of the fast IJP suggests that this hyperpolarization represents opening of apamin-sensitive potassium channels.

Conclusion: nNOS(-) mice have selective loss of the slow IJP in the pyloric smooth muscle. The fast IJP is mediated by ATP and is preserved in these mice, but cannot fully compensate for the absent slow IJPs. The impaired pyloric inhibition in these animals may be responsible for gastric stasis and dilation observed in these mutant mice.

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● **G3287**

EFFECT OF INTRAGASTRIC PRESSURE ON BELCH-ASSOCIATED ESOPHAGEAL COMMON CAVITY REFLUX EVENTS. B.T. Massey, J. M. Gorny, C. Hofmann, RC Arndorfer. MCW Dysphagia Institute, Medical College of Wisconsin, Milwaukee, Wisconsin.

BACKGROUND: Belching is associated with transient lower esophageal sphincter relaxations (TLESRs) and esophageal common cavity pressure waves due to gastroesophageal reflux. Previous studies in man examining the effect of gastric distension on TLESRs and/or belching have either employed fixed volumes of air where the gastric pressure was not held constant or bag enclosed air that could not be refluxed. We studied the effects of constant pressure gastric air distension on TLESRs and belching. **METHODS:** 8 healthy volunteers (5M/3F, age 19-54yr) were studied while seated, using esophageal manometry with a sleeve device to record LES pressure. Distal esophageal pH and abdominal wall EMG were also recorded in some subjects. An electronic barostat (G&J Electronics) was used to distend the stomach with air at constant pressures of 10, 12.5, 15.0 and 17.5 mmHg via a tube placed 5cm below the bottom of the LES. Distension pressures were maintained up to 30 minutes. Belch-associated common cavity events, air volumes infused, and amplitude of common cavity pressure waves were also recorded. **RESULTS:** Common cavity events were usually accompanied by belching, and were associated with TLESRs or swallow-related LESRs. Belches were equally prevalent throughout the 30 minute period. Subjects typically felt fuller with higher pressures. Higher gastric distension pressures were associated with more common cavity events and greater volumes of air infused to maintain the pressure.

Distention Pressure mmHg	10.0	12.5	15.0	17.5
Belches/10 min	0.9 ± 0.3	2.3 ± 0.9	5.4 ± 1.8	5.1 ± 0.6
Air volume/10 min (L)	0.2 ± 0.1	0.4 ± 0.1	1.0 ± 0.2	1.4 ± 0.4

Esophageal common cavity pressure wave amplitudes were significantly higher at 17.5 mmHg gastric pressure than at 10 mmHg gastric pressure (19 ± 3 vs 12 ± 1, p=.047). Reflux events were typically preceded by a small increase in gastric pressure even when a strain event was not detected by surface EMG. **CONCLUSIONS:** Constant pressure gastric distension with air produces greater frequencies of TLESRs and reflux events over sustained periods than most previously reported methods and may be a useful technique for study of TLESRs. Higher gastric pressures produce a greater driving force for reflux. The origin of the pre-reflux rise in gastric pressure is unclear, but at times seems to occur by a mechanism other than abdominal wall contraction.

● **G3288**

NITRIC OXIDE REPLACEMENT PRODUCES A REBOUND LES CONTRACTION AFTER INITIAL RELAXATION IN ACHALASIA. B.T. Massey, J.M. Gorny, C. Hofmann. MCW Dysphagia Institute. Medical College of Wisconsin, Milwaukee WI.

BACKGROUND: Recent studies suggest that nitric oxide (NO) is an important neurotransmitter mediating LES relaxation and the "off contraction" of esophageal body circular muscle. In the opossum, studies suggest that nitric oxide causes circular smooth muscle membrane hyperpolarization, with a subsequent depolarization in the esophageal body, which is important in producing the "off" contraction. However, no such depolarization is seen in the LES. While membrane potential studies have not been performed in the human esophagus, electrical field stimulation studies of the human LES have shown inconsistent and small off contractions. We examined the effect of the rapidly acting nitric oxide donor amyl nitrite in achalasia patients, who presumably have lost intrinsic NO-mediated inhibitory innervation to the LES. **METHODS:** 15 untreated achalasia patients were studied manometrically using a sleeve device to continuously record LES pressure. Basal LES pressure was recorded, after which patients were given amyl nitrite by inhalation (4 sniffs). Subsequent LES pressure nadir was recorded, followed by the peak rebound LES pressure. In 6 of the patients, amyl nitrite effects were recorded after administration of atropine (12mcg/kg i.v.). Amyl nitrite responses in achalasia patients were compared to those in 7 healthy normal volunteers. **RESULTS:** In normal volunteers amyl nitrite caused LES relaxation that was terminated during a subsequent secondary peristaltic wave, making it difficult to distinguish any specific off response from the peristaltic reflex. Achalasia patients showed a similar LES relaxation with amyl nitrite. However, this relaxation terminated with a rebound LES contraction that was 241 ± 44% of basal pressure and was concurrent with lower amplitude esophageal body pressure waves. These rebound contractions were similar in amplitude to the after-contractions seen with deglutition (84 ± 15 vs 73 ± 7 mmHg). Atropine caused LESP to fall to 58% of baseline pressure (p<.001), but did not abolish the rebound contraction following amyl nitrite (156 ± 27% of pre-atropine baseline, 300 ± 82% of post-atropine baseline). **CONCLUSIONS:** A substantial rebound response following putative NO induced LES relaxation exists in achalasia patients, suggesting that NO may act by different mechanisms in human compared to opossum LES. Whether the response results from membrane depolarization following hyperpolarization or possibly activation by an excitatory neurotransmitter remain unclear. However, this rebound contraction is unlikely to be purely cholinergically mediated.