software to determine the nerve volume density of PGP9.5 positive cell bodies and nerve fibers as a percentage of total tissue area (μm^2). The nerve volume densities were significantly decreased in the jejunum (23.7 vs. 31.3; $p < 0.001$), ileum (29.6 vs. 34.5; $p < 0.001$) and colon (31.8 vs. 34.3; $p < 0.05$) of GF as compared to SPF mice. Similar to our observations, there were no significant differences between the nerve volume densities of the germ-free and control stomach (33.5 vs. 33.4; $p > 0.05$) and duodenum (30.1 vs. 28.8; $p > 0.05$). These findings suggest that endogenous microbiota are necessary for the normal patterning of the ENS in the early postnatal period, particularly in the mid to distal small intestine and colon. Further studies are needed to determine whether the mechanisms by which the endogenous microbiota act on the ENS involve a direct effect on neural cells.

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Developmental Regulation of Sam Pointed Domain Ets Factor (SPDEF) in Rat Ileum Cultured With Bile Acids

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Background: Age-based differences in the regulation of components of the intestinal epithelial barrier may have implications in the pathogenesis of necrotizing enterocolitis (NEC). Mucins, produced by goblet cells, protect the epithelial surface of the GI tract by forming a semi-permeable layer between the lumen and the intestinal epithelium. In premature infants, a deficiency in the mucous layer has been suggested to contribute to intestinal injury in NEC. We have previously reported that ileal MUC2, the predominant secreted mucin produced by goblet cells, is significantly decreased in neonatal rats with NEC. Recently, we showed that bile acids (BAs), which are elevated in the ileum in NEC, play a role in the decreased MUC2 observed in experimental NEC, and also decrease MUC2 + cells in ex vivo ileal cultures from neonatal (up to post-natal day 6) but not older rats. **Objectives:** To determine if expression of SAM pointed domain ETS factor (SPDEF), a transcription factor that regulates the maturation of goblet cells in the intestinal epithelium, is regulated by BAs and if there are differences in BA-mediated expression of SPDEF in ileum from neonatal rats. **Methods:** Ileal tissue from fasted 3, 6 and 17 day old rats ($n = 6$ for each age group) was removed and cut into sections. Each section was opened longitudinally on sterile Metclic Membrane Filters and cultured in media alone (control) or media with 0.25 μM chenodeoxycholic acid (CDCA) for 2.5 hrs. RNA was extracted from these ileal explant cultures and single-stranded cDNA was reverse-transcribed. Real-time PCR amplification was performed using Assay By Design (Applied Biosystems, Foster City, CA) sequenced primers and probes for SPDEF. A separate piece of tissue from each animal was fixed in 70% ethanol, paraffin embedded and sectioned for immunohistological evaluation of MUC2 + cells. **Results:** While there were no significant differences in levels of SPDEF mRNA between control ileal explants from 3, 6 or 17 day old groups (1.0, 1.1 and 0.9, respectively), SPDEF mRNA was significantly lower in ileal explants from 3 day old rats cultured with CDCA compared to explants from both 6 and 17 day old rats cultured with CDCA. Interestingly, SPDEF expression after exposure to CDCA increased on post-natal day 6, the same day when BAs' ability to decrease MUC2 ends. **Conclusion:** These data suggest that the inability of neonatal ileum to up-regulate SPDEF in response to exposure to BAs may be a mechanism by which BAs decrease MUC2 in neonatal intestine. NEC is a disease of premature infants; in experimental NEC, ileal BAs are elevated and MUC2 is decreased. The data also suggest that developmental differences in the regulation of the intestinal barrier may play a role in NEC. Supported by RO1 HD054485.

MUC2+ cells and SPDEF mRNA in CDCA-cultured rat ileal explants

Post-Natal Day	MUC2 + cells ¹	SPDEF mRNA ²
3	0.68*	0.78 $\pm$ 0.22
6	0.94	2.21 $\pm$ 0.42**
17	1.20	3.42 $\pm$ 1.07**

¹ % change CDCA-cultured ileal explants versus control. ² Mean mRNA for each age point's control group was assigned a value of 1.0; mean mRNA levels for the corresponding CDCA group was determined relative to this number. Data are shown as mean $\pm$ SD. * $P \leq 0.05$ versus 6 and 17 day old, ** $P \leq 0.05$ versus 3 day old

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Intestinal Overexpression of Tis7 Increases Survival and Fat Absorption in Mice Fed a High Fat Diet Following 50% Small Bowel Resection

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Following loss of functional small bowel surface area, the intestinal epithelium exhibits an adaptive response characterized by increased crypt cell proliferation, epithelial hyperplasia and enhanced nutrient absorption. Expression of the immediate early gene Tis7, a transcriptional co-regulator, is increased in adaptive enterocytes within the first 48 hours following 50% small bowel resection. Transgenic mice that overexpress Tis7 in intestinal enterocytes have increased adiposity and intestinal triglyceride absorption despite shorter bowel length and decreased villus absorptive area. These mice have a higher rate of weight gain than their wild type littermates when fed a high fat diet. Herein we addressed the hypothesis that overexpression of Tis7 induces an earlier and more robust increase in fat absorption and functional adaptation following 50% small bowel resection. **Methods:** Intestinal Tis7 transgenic (TG) mice (FVB/N) and wild type (WT) FVB/N mice were subjected to 50% small bowel resections. Mice were fed liquid high saturated fat (42% energy) or isocaloric control (equivalent to standard laboratory chow) diets for 21 days. Body weights and food consumption were measured daily. Stool was collected over the last 3 days for fecal fat absorption analyses. **Results:** As expected, post-resection survival was significantly reduced in WT mice fed the high fat diet ($n=18$) compared to WT mice fed the control diet ($n=6$) (44% vs. 67% $p=0.04$ by Chi square). In contrast, Tis7TG mice fed the high fat diet ($n=11$) had increased survival compared to Tis7TG mice fed the control diet ($n=9$) (73% vs.

44% $p=0.05$). When fed the high fat diet, Tis7TG mice also had a survival advantage over WT littermates (73% vs. 44% $p=0.05$, at d21). All mice lost weight immediately following resection (10-14% of initial BW). By d17 all of the mice had regained the lost weight. On either diet, the WT mice continued to gain weight between d17 and 21; whereas only the Tis7TG mice fed the high fat diet continued to gain weight beyond d17. Fat absorption was higher in Tis7TG mice fed the high fat diet compared to high fat-fed WT littermates (0.8% increase, $p=0.04$ Student's t-test). **Conclusions:** Tis7 overexpression improves fat absorption and results in a survival advantage post resection when a high fat diet is provided. Tis7 overexpression may affect absorption of other nutrients, as suggested by reduced weight gain and higher mortality in Tis7TG mice fed the control vs. high fat diet. Our studies suggest that identification of downstream targets of Tis7 overexpression may provide novel therapeutic approaches to optimize nutrient absorption and functional adaptation in patients with intestinal failure and short bowel syndrome.

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SPDEF Functions Downstream of Notch-ATOH1 to Regulate Cell Proliferation and Differentiation in the Intestine

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Background: Notch signaling activity determines cell fate and regulates proliferation in the intestine. Notch mediates these effects mainly through repression of Atoh1 to regulate cell cycle exit and secretory cell differentiation. We have recently reported that Spdef (SAM Pointed domain ETS Transcription Factor, also called PDEF) is expressed downstream of Atoh1 and its expression is also repressed by Notch activity. Spdef plays a role in cell cycle exit and maturation of goblet and Paneth cells. Furthermore, our preliminary data indicate that SPDEF expression is often silenced in human colon cancer where it functions as a tumor suppressor. SPDEF and ATOH1 silencing in colon cancer are tightly correlated, suggesting SPDEF as a mediator of ATOH1's function in both intestinal homeostasis and tumorigenesis. We hypothesize that Spdef is a key effector of intestinal Notch-Atoh1 effects. Here, we assess the sufficiency of Spdef in Atoh1-null intestinal epithelia and tumors, and extend the analysis to elucidate the requirement of Spdef for Atoh1 functions to promote cell cycle exit and goblet cell differentiation in the intestine. **Method:** All analysis was performed in transgenic mice. The sufficiency of Spdef was tested by re-expressing Spdef in epithelial cells that lack Atoh1. The requirement of Spdef was tested by inducing Atoh1 expression through inhibition of the Notch activity in cells that lack Spdef. In order to assess the sufficiency of Spdef in tumors that lack Atoh1, Spdef was re-expressed in tumors that lack Atoh1. Progenitor cell proliferation and differentiation were immunohistologically assessed. **Results:** Cell proliferation was significantly reduced and goblet cell markers were induced in SPDEF-expressing Atoh1-null progenitor cells compared to Atoh1-null cells which lack SPDEF. In Atoh1-null tumors, proliferation was significantly reduced upon expression of SPDEF without induction of goblet cell markers. In response to Atoh1 induction by Notch inhibitor treatment, cell proliferation was inhibited and goblet cell differentiation was induced in the intestine; this response was inhibited in Spdef^{-/-} mice. **Conclusions:** SPDEF mediates the effect of the Notch inhibitors on cell proliferation and goblet cell maturation by functioning downstream of Atoh1 in intestinal epithelia and tumors. The sufficiency and requirement of Spdef on promoting cell cycle exit indicated in this study suggest Spdef as a potential therapeutic target for colon cancer. Future studies will investigate the requirement of Spdef for the Notch-Atoh1 effects in intestinal tumors, and Notch-Atoh1 independent mechanisms to activate expression of SPDEF, which can be utilized as a colon cancer therapy.

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Short-Time Maternal Separation in Early Neonate Rats Markedly Increases Intestinal Permeability, Induces Bacterial Translocation and Impacts Gene Expression in the Liver

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Background and aims: Intestinal permeability (IP) is high at birth for maturation of gut barrier function and liver development. In neonate rats, it is well established that chronic maternal deprivation for 12-14 days induces long-term defect in IP in adulthood. In human newborns, transient maternal separation (MS) is common practice, mainly when medical care is required (e.g. incubator care), but whether a short episode of MS affects gut-liver axis in neonates has not been explored. Our aim was to investigate whether a short-time single MS during postnatal development affects IP and liver gene expression. **Methods:** Randomized female rat pups were separated from their dams for 4h or left undisturbed (controls) at postnatal day (PND) 10 or PND20. First, total IP was determined through mucosal-to-blood flux of FITC-4kD dextran orally administered to female rats immediately after MS i) at PND10 or PND20 in comparison with controls, ii) at PND10 after pretreatment with selective inhibitor of intestinal myosin light-chain (MLC) kinase (ML7: 1mg/kg i.p.) before MS. Second, colonic paracellular permeability (CPP) to FITC-dextran and transcellular permeability to horseradish peroxidase (HRP 40kD) were measured in Ussing chambers at PND10 with or without MS. Bacterial translocation (BT) was assessed in liver and spleen at PND10 and PND20 with or without MS. Hepatic transcriptome was obtained using Agilent Whole Rat Genome microarrays (4x44k v3). **Results:** Single short-time MS significantly increased total IP (+90%, $P<0.001$) at PND10 compared to controls, but not at PND20. In PND10 pups, pretreatment with ML7 blocked MS-induced raise of IP (-47% compared to vehicle-MS controls, $p<0.05$). In the colon of PND10 pups, MS markedly increased CPP to dextran compared to controls (1.7 $\pm$ 0.3 vs 0.6 $\pm$ 0.1 nmol.cm⁻².h⁻¹, $p<0.05$), and transcellular permeability (2.4 $\pm$ 0.3 vs 0.8 $\pm$ 0.5 pmol.cm⁻².h⁻¹). At PND10, control pups showed no BT to extraintestinal sites, while MS induced BT in the liver (2.2 log CFU/ml counted in 60% of MS rats) and spleen (1.5 log CFU/ml in 80% of MS rats). In PND20 pups, no BT has been detected. A significant impact of MS at PND10 was observed on liver transcriptome. More specifically, several genes involved in cell cycle were downregulated while genes involved in response to hormonal stimuli (growth hormone, insulin, glucocorticoids) or in lipid catabolism were upregulated. **Conclusion:** Our study shows that a single episode of short-time MS in very early life (PND10), but not later at PND20, exacerbated total IP of

rat pups through a MLCK-dependent pathway, and induced bacterial translocation to the liver and spleen. Our data reveal that early neonatal period represents a critical window for a short-time MS, increasing the risk of pathogen uptake toward the remaining organism and altering gene expression in the maturing liver.

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The Human Lactase Persistence-Associated SNP -13910*T Enables *In Vivo* Functional Transgene Persistence Upon Maturation in Mice
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Background: Lactase is the intestinal enzyme responsible for digestion of the milk sugar lactose. Lactase gene expression declines dramatically upon weaning in mammals and during early childhood in humans (lactase nonpersistence). In various ethnic groups, however, lactase persists in high levels throughout adulthood (lactase persistence). Genetic association studies have identified that lactase persistence in Northern Europeans is strongly associated with a single nucleotide polymorphism (SNP) located 14 kb upstream of the lactase gene: -13910*C/T. **Aim:** To determine whether the -13910*T SNP can function *In Vivo* to mediate lactase persistence. **Methods:** Transgenic mice were generated harboring human DNA fragments with the -13910*T SNP or the ancestral -13910*C SNP cloned upstream of a 2 kb rat lactase gene promoter in a luciferase reporter construct and assayed for intestinal luciferase expression in preweaned and adult mice. **Results:** We previously reported that the 2 kb rat lactase promoter directs a post-weaning decline of luciferase transgene expression similar to that of the endogenous lactase gene. In the present study, the post-weaning decline directed by the rat lactase promoter is impeded by addition of the -13910*T SNP human DNA fragment, but not by addition of the -13910*C ancestral SNP fragment. **Conclusion:** Persistence of transgene expression associated with the -13910*T SNP represents the first *In Vivo* data in support of a functional role for the -13910*T SNP in mediating the human lactase persistence phenotype.

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Postprandial Suppression of Reflux by a Raft Forming Alginate (Gaviscon Advance) Compared to a Simple Antacid: Technical Assessment of pH-Impedance Monitoring and Clinical Feasibility Study in Gastro-Esophageal Reflux Disease (GERD) Patients
 Rami Sweis, Angela Anggiansah, Terry Wong, Mark R. Fox

Proton Pump Inhibitors (PPI) reduce acid reflux but not the frequency or proximal extent of reflux events that are a cause for persistent symptoms on PPI. Alginate preparations containing bicarbonate are effective for short-term control of reflux symptoms. The mechanism of effect includes both reflux suppression by formation of a viscous raft above the meal and acid neutralization. Previous mechanistic studies using pH-impedance have found equivocal effects of alginates on reflux suppression. It is unclear whether this was due to lack of effect, study power or technical issues. **Aim:** (i) technical assessment of standard pH-impedance equipment (Sandhill, Highlands Ranch, CO) for clinical measurement of patients taking acid and reflux suppressants (ii) *In-Vivo* assessment of mechanistic effects of Gaviscon Advance (GA; Reckitt Benckiser, UK) and Milk of Magnesia (MM; Boots, UK) on postprandial reflux. **Method:** (i) To assess effects on signal detection by pH and impedance sensors 10 patients took 10ml GA or MM followed by repeated, single 10ml swallows of orange juice (pH 4) until chemical and volume clearance was detected (ii) A randomized, controlled, double-blind, cross-over clinical study in 20 patients (9 male:11female; median age 40 (range 25-63)) referred for investigation of reflux symptoms. On subsequent days at the beginning and end of a 24hr monitoring period, patients were randomized to receive either 10ml GA or MM (both mint flavoured) after a mixed test meal (600 kcal). Postprandial distal and proximal reflux events (acid and non-acid) were documented over 4 hours by pH-impedance with the patient in the upright, seated position. **Results:** (i) Technical assessment: After intake of 10ml GA or MM the pH and impedance signal fully recovered after median 6 (2-12) and 4 (2-10) swallows of orange juice. (ii) Clinical assessment: During the 4 hour postprandial observation acid exposure time (mean 2.3% (SD 3.3%) vs. 3.4% (4.2%), p=0.296) and number of distal reflux events (20.5 (13.6) vs. 22.5 (9.4), p=0.500) was similar after ingestion of GA and MM. There was a trend to less proximal reflux events with the alginate compared to the antacid (10.5 (8.9) vs. 13.9 (8.3), p=0.070). No difference in the number of symptoms (5.0 (6.0) vs. 4.2 (8.3), p=0.701) and reflux related symptoms (2.5 (4.0) vs. 2.8 (5.6), p=0.988) was reported. **Conclusion:** Standard pH-impedance monitoring is suitable for clinical studies of reflux suppression in GERD patients. The clinical study documented similar frequency of distal reflux but a trend to suppression of proximal reflux by GA compared to MM. This feasibility data indicates that trials will require a minimum of 70 GERD patients to demonstrate effects (power 90%, p<0.05) on proximal reflux suppression after meals by alginates. More prolonged studies are required to assess effects on symptom control.

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Effect of PPIs on the Size, Position and Acidity of the Postprandial Acid Pocket
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AIM: Proton pump inhibitors (PPIs) reduce acid secretion in the stomach and thereby reduce the number of acid reflux episodes. Off PPIs, the risk for acidic reflux is mainly determined by the position of the highly acidic fluid of the gastric acid pocket. To what extent changes in position, size and acidity of the acid pocket contribute to the therapeutic effect of PPIs is however unknown. The aim of this study was therefore to determine the effect of PPIs on the acid pocket. **METHODS:** Thirty-four GERD patients (on PPI: n=16; off PPI: n=18) were invited (19M 15F age 55 yrs (range 20-74)) to undergo concurrent high resolution manometry/pH-impedance following a standardized meal (until 2hrs after the meal). In addition, the acid pocket was visualized using scintigraphy after intravenous administration of 350 MBq 99mTc-pertechnetate. The position of the pocket was classified as above, at

the level or below the diaphragm for each reflux event using the position of the diaphragm on HRM and radionuclide markers on the catheter as reference points. After two hours, the fluid of the acid pocket was aspirated in patients on PPI to determine the pH. Size and nuclear uptake count of the acid pocket were determined as the mean value at 10 min intervals. **RESULTS:** The number of reflux episodes was comparable on PPI and off PPI, but the rate of acid reflux was significantly reduced on PPI. (Table 1) On PPI, the acid pocket was smaller and more frequently located below the diaphragm compared to off PPI (Table 1.) Acid reflux episodes on PPI occurred primarily when the pocket was located above (16 of 29 reflux episodes [55%]) or at the level of the diaphragm (36 of 74 [49%]) compared to when the pocket was below the diaphragm (10 of 134 [7%]). When reflux episodes were acidic, the pH was significantly lower off PPI (n=139) compared to on PPI (n=62) (2.1 ± 0.1 vs 3.0 ± 0.1 p<0.001). In line with this, the pH of the acid pocket was significantly lower off PPI (n=6) compared to on PPI (n=16) (0.9 [range 0.7 to 1.2] vs. 4.0 [range 1.6 - 5.9] p<0.001). Mean nuclear uptake counts in the pocket were lower on PPI (Table 1) and correlated significantly with the pH of the acid pocket off PPI (Pearson r=-0.56 p<0.05). **CONCLUSION:** PPIs reduce the size of the acid pocket possibly leading to a change in position, i.e. more frequently located below the diaphragm. In addition, PPIs reduce the acidity of the postprandial acid pocket leading to less acidic refluxate. We further confirmed that irrespective of acid suppression the position of the acid pocket below the diaphragm is an important determinant of the acidity of the refluxate. Based on these results, we conclude that the effect of PPIs on the acid pocket may contribute to the effectiveness of these drugs in the treatment of GERD.

Table 1.

	Off PPI (n=18)	On PPI (n=16)	P
Rate of acid reflux events (%)	51 ± 7.9	22 ± 6.1	<0.01
Acid pocket size (cm2)	15 ± 0.9	10 ± 1.3	<0.01
Acid pocket nuclear activity (counts/cm2)	188 ± 13	145 ± 12	<0.05
Location of the acid pocket:			
- Below the diaphragm (%)	40 ± 6.6	62 ± 7.1	<0.05
- At the level of the diaphragm (%)	60 ± 6.5	38 ± 7.0	<0.05
- Above the diaphragm (%)	22 ± 7.3	6.7 ± 3.3	0.06

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Effect of Baclofen on the Mechanism of Reflux Protection in Healthy Volunteers and Patients With Gastro-Esophageal Reflux Disease (GERD) Assessed by Magnetic Resonance Imaging (MRI) and High Resolution Manometry (HRM): A Randomized Controlled Study
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INTRODUCTION: Baclofen is a GABAB agonist that reduces the frequency of reflux events. The mechanism of this effect includes inhibition of transient lower esophageal sphincter relaxations (TLESR). Other effects of this agent on gastric function and the esophago-gastric junction (EGJ) that may contribute to "reflux protection" have not been well defined. **AIM:** We applied validated, minimally-invasive MRI and HRM methodology [1] to assess the effect of baclofen on postprandial gastric function and the putative "flap valve" formed by the acute insertion angle of the esophagus into the stomach in healthy volunteers (HV) and GERD patients. **METHODS:** 12 HV (6 women, age range 21-37 years, weight range 54-80kg) and 12 patients (5 women, age range 19-55 years, weight range 51-100kg) with objective evidence of GERD (esophagitis and/or acid exposure >6%, pH<4 on pH studies) entered a double blind, placebo controlled cross-over study. On 2 test days participants were randomised to receive either 40 mg of baclofen (Baclofen, Focus Pharma, UK) or identical placebo 90 minutes before ingestion of a large, high-caloric mixed solid-liquid meal (560 kcal) labelled with paramagnetic contrast agent (Dotarem, Guerbet, France). With participants in the right lateral position continuous HRM measurements were acquired by water-perfused, 21 channel HRM (AMS, Melbourne, AUS). A 1.5T whole-body MRI System (Philips, NL) acquired anatomic and cine images. Reflux events captured with HRM (common cavity) and cine MRI were independently reviewed by two observers. Proximal gastric and esophageal morphology were reconstructed in 3D and analyzed using validated algorithms [Figure 1][2]. Mixed model statistical analysis assessed differences between participant groups and treatment conditions. **RESULTS:** TLESR and reflux events were observed in 11/12 healthy volunteers and 12/12 GERD patients with concordance between observers and techniques. The number of reflux events reduced with treatment in HVs (Δ=2.5 (0 — 4.5), p=0.014) and GERD patients (Δ=2 (-3 — 7), p=0.2) [Figure 2]. 3D analysis of GEJ and proximal stomach morphology after meal ingestion revealed larger insertion angles (Δ=15° (SE=6), p<0.01) in GERD patients compared to HVs. Insertion angle was reduced in GERD patients on baclofen treatment (Δ=-22° (SE=3), p=0.02) but was unchanged in HVs (Δ=+3° (SE=2.2), p=0.18). **DISCUSSION:** GABAB agonists reduce reflux events after a meal in HVs and GERD patients by TLESR inhibition. This study demonstrates novel effects of baclofen on proximal gastric function in GERD patients that impact on EGJ function. 3D models of gastric function constructed from *In Vivo* imaging demonstrate how maintenance of an acute esophago-gastric insertion angle after meals enhances reflux protection by a flap valve mechanism. **REFERENCES:** [1] Curcic et al. Radiology 2010, [2] Roy et al. DDW 2010