

Aims: To characterize and compare the symptomatic response to lactose- and fructose loads during breath hydrogen tests (BHT) with a validated, test-specific symptom questionnaire.

Methods: Patients with functional abdominal disorders (age $\pm$ SEM: 45 ± 15 years; 39% male) swallowed 25 g fructose ($n = 422$) or 50 g lactose ($n = 466$). Symptoms during BHT were ascertained regularly up to 9 hours on a 100 mm VAS-scale. An increase of breath-hydrogen concentration ≥ 20 ppm over baseline was considered as malabsorption. Intolerance was defined as increase of at least one symptom ≥ 20 mm over baseline in the VAS scale. Significant symptom increase over baseline ($P < 0.01$) is reported, duration of symptoms after lactose and fructose are compared by student's *t*-test ($P < 0.05$), NS = not significant.

Results: 148/422 patients were fructose malabsorbers, 202 were intolerant to fructose (of whom 96 (48%) malabsorbed fructose). 199/466 patients were lactose malabsorbers, 298 were intolerant to lactose (of whom 159 (53%) malabsorbed lactose). Hydrogen peaks occurred significantly earlier after fructose (median: minute 60) than after lactose ingestion (minute 90) ($P < 0.001$). Pain increased significantly (vs. baseline, $P < 0.01$) in fructose intolerant patients from minute 30 to minute 120, pain resolved thereafter and did not recur. In lactose intolerant patients, pain lasted from minute 30 to 9 hours ($P = 0.05$ vs. fructose). Meteorism lasted from 30 minutes to 9 hours (fructose) and 60 minutes to 9 hours (lactose) respectively ($P = \text{NS}$). Flatulence: 60 minutes to 9 hours (fructose) vs. 120 minutes to 9 hours (lactose) ($P = 0.003$). Diarrhea: 6 to 9 hours (fructose) vs. 120 minutes to 9 hours (lactose) ($P = 0.04$). Significant increase in nausea symptom scores was only induced by fructose ingestion from 30 to 120 minutes but not by lactose ingestion ($P = \text{NS}$).

Conclusion: There are distinct differences in symptom quality and timelines of symptoms after fructose and lactose ingestion in intolerant patients. The adult carbohydrate perception questionnaire (aCPQ) is a valuable tool for an unbiased symptom assessment.

63 | Colonic dysmotility associated with high fat diet-induced obesity: Role of glial adenosine A_{2B} receptors

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Background/Objectives: The contribution of adenosine A_{2B} receptors (A_{2B}Rs) to the modulation of enteric glia (EG) activity, and thereby their involvement in intestinal dysmotility occurring in obese patients is largely unknown. We evaluated the role of A_{2B}Rs on EG activity in the colonic neuromuscular compartment of obese mice.

Methods: C57BL/6 mice were fed with standard (SD) or high fat diet (HFD) for 8 weeks. Colonic longitudinal muscle strips (LMS) were set up in organ baths with Krebs solution containing guanethidine,

L-NAME and atropine to record contractile responses driven primarily by tachykinins. BAY 60-6583 (BAY; A_{2B}Rs agonist), MRS1754 (MRS; A_{2B}Rs antagonist) and the gliotoxin fluorocitrate (FC) were tested on colonic contractions. A_{2B}Rs, glial fibrillary acid protein (GFAP) and SP distribution were examined by immunohistochemistry. To mimic a HFD-induced obesity scenario, in vitro experiments were performed exposing EG cells to palmitate (PA) and/or LPS, with or without A_{2B}R ligands. Glial cell-derived neurotrophic factor (GDNF), interleukin-1 β (IL-1 β) and SP levels were assayed in the supernatants of EGC culture. Toll-like receptor (TLR)-4 expression was assessed by western blot analysis.

Results: MRS enhanced the electrically induced NK₁-mediated tachykinergic contractions in LMS from HFD mice. BAY decreased the ES-evoked colonic tachykinergic contractions, acting with higher efficacy in preparations from HFD mice. The effects of A_{2B}R ligands on colonic contractions were blunted upon incubation with FC. HFD mice showed an increased expression of colonic A_{2B}Rs, GFAP and SP as compared with SD mice. In cultured EG, upon pre-exposure to PA, LPS increased TLR-4 expression as well as IL-1 β , GDNF and SP release. BAY reduced TLR-4 expression as well as IL-1 β , GDNF and SP release. Such effects were blunted by MRS.

Conclusions: A_{2B}Rs, expressed on EG, modulate the enteric inflammation and abnormal tachykinergic responses associated with obesity. In vitro experiments showed a modulatory action of A_{2B}Rs on SP release from EG via GDNF pathway.

64 | Enteric α -synuclein accumulation impairs intestinal epithelial barrier through inflammasome activation before the onset of brain pathology in a transgenic mouse model of Parkinson's disease

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Background/Objectives: Enteric α -synuclein inclusions, impaired intestinal epithelial barrier (IEB) and bowel inflammation could represent early events in Parkinson's disease (PD) and contribute to bowel motor dysfunctions. This study examined the correlation among enteric α -synuclein accumulation, altered IEB and enteric inflammation in a transgenic model of PD.

Methods: A53T transgenic (Tg) mice develop a progressive PD-like neurological and motor deficiency after 9 months of age. Animals were sacrificed at 3 months of age to evaluate enteric α -synuclein inclusions, alterations of the IEB and enteric inflammation since the early phases of PD, before brain pathology. Blood was collected to evaluate circulating lipopolysaccharide (LPS) levels. Distal colon was processed for the evaluation of α -synuclein and zonulin-1 protein

expression, interleukin-1 β (IL-1 β) levels and caspase-1 activity. THP-1 cells were treated with LPS and α -synuclein, in the presence or absence of the caspase-1 inhibitor YVAD for 24 hours. Then, supernatant was collected and, after assessment of IL-1 β levels (ELISA), it was used to treat CACO-2 epithelial cells. After 72 hours, CACO-2 cells were lysed and zonulin-1 protein expression was assessed.

Results: Tg mice displayed an increase in α -synuclein expression in colonic tissues. Colonic zonulin-1 was decreased and circulating LPS levels were increased. Colonic IL-1 β levels and caspase-1 activity were elevated. Co-treatment with LPS/ α -synuclein promoted IL-1 β release in the supernatant of THP-1 cells. The THP-1-derived supernatant decreased significantly zonulin-1 expression when applied to CACO-2 cells. Such an effect was abrogated when THP-1 cells were incubated with YVAD. The incubation of LPS and/or α -synuclein did not modify zonulin-1 expression in CACO-2 cells.

Conclusion: In the early stages of PD before brain pathology, enteric α -synuclein accumulation compromises IEB through inflammasome activation. These changes could contribute to the pathogenesis of intestinal motor dysfunctions in PD patients.

65 | The additional measurement of methane during breath tests does not alter the diagnosis of carbohydrate malabsorption in a Central European paediatric population

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Introduction: The importance of methane-measurement to detect malabsorption in hydrogen non-producers is controversial. Moreover, the need for a correction factor (CO₂) is disputed. Combined detection of hydrogen with other gases increases cost and complexity of the test but is recommended by guidelines.

Aims: To evaluate the usefulness of methane and CO₂ correction in breath tests for the diagnosis of carbohydrate malabsorption.

Methods: 132 paediatric patients (59 males, 73 females; mean age $\pm$ SEM: 11.3 $\pm$ 3 years, weight: 41.2 $\pm$ 15 kg) underwent fructose- (n = 54) or lactose- (n = 78) breath tests for evaluation of carbohydrate malabsorption. 1 g fructose/kg (25 g maximum) or 2 g lactose/kg (50 g maximum) were applied. Breath samples were collected at 30-minute intervals for three hours and analysed for hydrogen, methane and CO₂. Malabsorption was diagnosed by an increase in: A) hydrogen $\geq$ 20 parts per million (ppm) over baseline; B) methane $\geq$ 12 ppm; C) hydrogen plus methane $\geq$ 15 ppm. The diagnosis was made before and after use of a correction factor (5.5% CO₂ as numerator). McNemar's test for paired proportions was calculated, *P*-values < 0.05 were considered significant.

Results: Before applying the correction factor, hydrogen detection led to a diagnosis of 58 malabsorbers/74 absorbers. None of absorbers exhaled relevant methane that would have changed diagnosis

(neither methane $\geq$ 12 ppm nor hydrogen+methane $\geq$ 15 ppm). After CO₂ correction, malabsorption (hydrogen $\geq$ 20 ppm) was diagnosed in 3 patients that would have not been diagnosed without correction; in contrast, 5 patients lost their diagnosis of malabsorption (hydrogen $\geq$ 20 ppm) after CO₂ correction (*P* = 0.7). None of absorbers exhaled relevant methane ($\geq$ 12 ppm) that would have changed diagnosis. Combination of corrected hydrogen+methane detected 2 patients who were not detected by hydrogen alone (*P* = 0.5).

Conclusion: Addition of methane measurement did not improve detection of carbohydrate malabsorbers in this Central European paediatric population. The use of CO₂ correction alters the diagnosis of malabsorption in a minority of patients, and does not significantly change the test results overall. In summary, the inclusion of the measurement of methane in breath tests does not improve the quality of malabsorption detection; the use of a correction factor might benefit individual patients.

	Hydrogen $\geq$ 20 ppm over baseline, with CO ₂ correction	
	Absorption	Malabsorption
Hydrogen $\geq$ 20 ppm over baseline, no correction		
Absorption	69	5
Malabsorption	3	55

66 | Enteric β -amyloid accumulation, bowel inflammation and intestinal dysmotility precede brain pathology in a spontaneous model of Alzheimer's disease

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Background/Objectives: Alzheimer's disease (AD) patients are characterized by β -amyloid (A β) accumulation and enteric inflammation that could contribute to intestinal dysmotility since the earliest stages of the disease. This study examined fecal A β accumulation, enteric inflammation and in vitro colonic motor activity in a spontaneous model of AD before the development of brain pathology.

Methods: Senescence-accelerated mouse prone 8 (SAMP8) mice were employed as model of AD. Animals at 4, 6 and 8 months of age underwent Morris water maze test as well as assessment of fecal output, to evaluate cognitive functions and colonic transit from the early phases of AD up to its full development. Animals were sacrificed at 6 months and colonic longitudinal muscle preparations were set up in organ baths and connected to isometric transducers. Neurogenic motor responses were evoked by electrical stimulation (ES) in Krebs solution containing guanethidine (10 μ mol/L), N^o-nitro-L-arginine