



Revisión

Intestinal permeability measurements: general aspects and possible pitfalls

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Abstract

Introduction: Disturbances of the gut barrier function have been related to a variety of diseases, including intestinal and extra-intestinal diseases. The intestinal permeability tests are considered useful tools for evaluating disease severity and to follow-up patients after a therapeutic intervention and indirectly assess barrier function.

Objective: The aims of this review were to highlight the possible factors underlying higher intestinal permeability and the clinical conditions that have been associated with this in different age range; and also provide some insight into methodological aspects.

Results and discussion: Abnormal regulation of tight junction function is the main cause of altered intestinal barrier. The impaired barrier function results in higher permeation rates of administered probes through the intestinal mucosa. Lactulose and mannitol are one of the most commonly used probes. The innocuousness and easiness of intestinal permeability tests can be explored to expand the knowledge about the clinical situations in which intestinal barrier dysfunction can be an important feature. Many factors may influence the results of the test. Researchers and healthcare professionals should try to circumvent the possible pitfalls of the intestinal permeability tests to produce consistent evidences. The use of others markers of intestinal physiology may also contribute to understand the role of barrier function in different diseases.

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Key words: *Intestinal permeability. Gut barrier. Lactulose. Mannitol.*

MEDICIONES DE PERMEABILIDAD INTESTINAL: ASPECTOS GENERALES Y POSIBLES RIESGOS

Resumen

Introducción: Alteraciones funcionales de la barrera intestinal se han relacionado con una variedad de enfermedades intestinales y también con enfermedades no intestinales. Las pruebas de permeabilidad intestinal son consideradas herramientas útiles para evaluar la gravedad de la enfermedad para el posterior seguimiento de los pacientes después de una intervención terapéutica.

Objetivo: El objeto de esta revisión ha sido destacar los posibles factores que pueden estar asociados a una mayor permeabilidad intestinal y revisar condiciones clínicas que han sido asociadas en individuos de diferentes edades. También revisar ciertos aspectos metodológicos de las pruebas de permeabilidad intestinal.

Resultados y discusión: Las uniones estrechas entre los enterocitos son las principales estructuras encargadas de la regulación de la barrera intestinal. Una alteración de éstas, resulta en una deficiencia en la permeabilidad intestinal y una mayor penetración de las sustancias marcadoras de permeabilidad intestinal. La lactulosa y el manitol son las sustancias marcadoras más utilizadas. La inocuidad y facilidad de los test de permeabilidad han sido de ayuda para explorar y ampliar el conocimiento de muchas condiciones clínicas en las que la disfunción de la barrera intestinal ha sido un sello distintivo. Muchos factores pueden influir en los resultados de los test de permeabilidad. Sin embargo, los investigadores y los clínicos han de tratar de eludir los posibles inconvenientes de las pruebas de permeabilidad intestinal para poder producir evidencias más consistentes. El uso de otras sustancias marcadoras de la fisiología intestinal también puede contribuir a comprender mejor el papel de la barrera intestinal en diferentes enfermedades.

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Palabras clave: *Permeabilidad intestinal. Barrera intestinal. Lactulosa. Manitol. Fisiología intestinal.*

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Abbreviations

BMI: body mass index.
 IP: intestinal permeability.
 GFR: glomerular filtration rate.
 L/M: lactulose/mannitol ratio.
 TJ: tight junctions.

Introduction

The gastrointestinal tract has the complex task of absorbing nutrients while excluding the uptake of dietary antigens, luminal microbes and their products. The in-

testinal mucosa exhibit a selectively permeable barrier property, which supports this task. The histological organization of the gastrointestinal tract mucosa and the interaction between cellular (polarized epithelial cell membrane, tight junctions (TJ), lymphocytes) and extracellular components (mucin, unstirred layer of fluid)¹⁻⁴ are essential for the gut barrier function. Homeostasis of gut barrier function is critical for the ability of gastrointestinal tract to articulate aggressive reactions against enteric microbes while developing oral tolerance for food antigens and commensal bacteria⁵.

Disturbances of the gut barrier function have been related to a variety of clinical conditions in different age range (Tables I and II)^{2,6}. The investigation of gut

Table I
Intestinal permeability markers for healthy and diseased infants, children and adolescents

Ref	Sample	Volume, sugar and osmolarity Urine collection (hours) and method	% Excretion (mean ± SD or median [range])		
34	6 term (fed human milk) 21 preterm infants (4 fed human milk and 17 fed formula milk) L/M: Term human milk: 0.18 ± 0.19	300 mg Lac and 60 mg MA dissolved in liquid diet or water 5h and GC vs HPLC	L/M: Term human milk: 0.18 ± 0.19 Pre term human milk: 0.20 ± 0.16 Pre term formula: 0.32 ± 0.31		
94	12 CMPSE (6m-2y) 28 AD (6m-15y) 39 H	10% MA and 65% Lac 0.1 g/kg BW for each sugar; 1,001 mosm/L 5 h and GC	Control Lac: 0.37 ± 0.18 MA: 15.6 ± 5.98 L/M: 2.45 ± 1.01	CMPSE Lac: 0.39 ± 0.14 MA: 15.07 ± 5.67 L/M: 2.88 ± 1.5	AD Lac: 0.52 ± 0.51 [†] MA: 15.5 ± 8.9 L/M: 3.6 ± 3.31 [†]
95	77 underweight (44M and 33F, mean 13.1m) 17 H (11M and 6 F; mean 13.2m)	400 mg Lac and 100 mg MA/3 ml Dose 3 ml/kg BW 5 h and enzymatic	Control Lac: 0.44 (0.34-0.71) MA: 5 (3.87-8.71) L/M: 0.09 (0.05-0.12)	Underweight Lac: 0.55 (0.35-0.88) MA: 3.89 (2.14-5.69) [†] L/M: 0.15 (0.09-0.26) [†]	
50	28 H (12M and 16F; mean 9y) 28 GSE (10M and 18F; mean 10y)	0.55 mL/kg 18.2 g LAC/100 mL and 18.2 g MA/100 mL 1,500 mosmol/L 5 h and GC	Control Lac: 0.28 ± 0.04% MA: 15.61 ± 5.8% L/M: 0.022 ± 0.007 (all <0.035)	GSE Lac: 0.73 ± 0.5% [†] MA: 8.72 ± 3.5% [†] L/M: 0.084 ± 0.054 [†] (all >0.035)	
96	49 infected (helminthiasis) (mean 7.2y) 95 H (mean 7.2y)	2 mL/kg 5 g/100 mL Lac and 2 g/100 mL MA 5 h and enzymatic	Control L/M: 0.031 ± 0.023 L/M: 0.042 ± 0.018 [†]	Infected L/M: 0.042 ± 0.018 [†]	
37	30 H (13M and 17F; mean 7.4 y) 10 ileocolitis Crohn s (mean 14.7y) 10 Celiac (mean 5.8y) with severe or active phase	50-100 mL 5 or 10 g Lac and 2 or 5 g MA (younger than 12 y had the lower dose) 6 h and HPLC	Control Lac: 0.33 ± 0.13% MA: 14.1 ± 6.6% L/M: 0.024 ± 0.006	Crohn's Lac: 2.25 ± 2.1% [†] MA: 11.91 ± 7.95% L/M: 0.2 ± 0.08	
54	15 H (no diarrhea episode in last 2 wk) 15 Diarrhea (3 or more liquid stools in the last 24h) Both groups age < 5y of both genders	2 mL/kg 200 mg/mL Lac and 50 mg/mL MA 5 h and HPLC	Control Lac 0.1183 ± 0.0855% L/M ratio: 0.0394 ± 0.0235	Diarrhea Lac: 0.3029 ± 0.2846% [†] L/M ratio: 0.1404 ± 0.1206 [†]	
97	52 H (13 M and 39 F; 8.2 y) 93 FAB/IBS (28 M and 65 F; 8.5 y) Participants 7-10 y	125 mL 5 g/dL Lac; 1g/dL MA; 10 g/dL S; 1 g/dL SU + 240 mL water 3 h and HPLC	Control Lac: 0.09 ± 0.06 MA: 7.6 ± 4.7 S: 0.02 ± 0.03 SU: 0.42 ± 0.32 L/M: 0.07 ± 0.03 S/L: 0.36 ± 0.26 SU/L: 0.81 ± 0.43	FAB/IBS L: 0.10 ± 0.08 MA: 7.6 ± 5.5 S: 0.02 ± 0.03 SU: 0.44 ± 0.42 L/M: 0.06 ± 0.03 S/L: 0.59 ± 0.50 [†] SU/L: 1.01 ± 0.67 [†]	

M: men; F: female; H: healthy (control); AD: atopic dermatitis; BW: body weight; CMPSE: cow s milk-sensitive enteropathy; FAB/IBS: functional abdominal pain and irritable bowel syndrome; GC: gas chromatography; HPLC: high-performance liquid chromatography; Lac: Lactulose; LGSE: gluten sensitive enteropathy; L/M: lactulose/mannitol ratio; MA: mannitol; S: sucrose; SU: sucralose; S/L: sucrose/lactulose ratio; SU/L: sucralose/lactulose ratio. [†]p < 0.05 compared to the control, [‡]p = 0.05 compared to the control.

Table II
Intestinal permeability markers for healthy and diseased adults

Ref	Sample	Volume, sugar and osmolarity Urine or blood* collection (hours) and method	% Excretion (mean ± SD or median [range])		
			Control	Normal biopsy	Abnormal biopsy
33	10 H (7M and 3F) 28 investigation for GSE (16F and 12M)	300 mL; 10 g Lac and 5 g MA 696 mmol/kg 5 h and HPLC	Control Lac: 0.15 ± 0.09 MA: 11.8 ± 6.2 L/M: 0.02 ± 0.014	Normal biopsy Lac: 0.27 ± 0.13 MA: 12.6 ± 4.6 L/M: 0.021 ± 0.013	Abnormal biopsy Lac: 0.65 ± 0.26 MA: 9.0 ± 3.4 L/M: 0.146 ± 0.10 [†]
98	41 H (10M and 31 F; mean 29y) 20 FH (4M and 16F; mean 29y) 21 FA (6M and 15F; mean 29y)	200 mL; 5 g Lac and 2 g MA 5 h and HPAEC-PAD	Control L/M: 1.85 ± 0.8	FH L/M: 5.34 ± 4.26 [†]	FA L/M: 6.17 ± 6.07 [†]
99	30 mild pancreatitis 15 severe pancreatitis 26 H	50 mL; 10g Lac and 5 g MA 5 h and enzymatic 50 mL; 10g Lac and 5 g MA	Control L/M: 0.016 ± 0.014	Pancreatitis Mild L/M: 0.029 ± 0.027 [†]	Pancreatitis Severe L/M: 0.20 ± 0.18 [†]
35	12H (6M and 6F) 26 for PN (13 depleted and 10 non-depleted)	5 g X 6 h and GLC	Control Lac: 0.5 ± 0.1 MA: 19.2 ± 2.6 X: 29.9 ± 1.8	Depleted Lac: 2 ± 0.5 [†] MA: 12.9 ± 3.5 [†] X: 20.6 ± 3.4 [†]	Non-depleted Lac: 0.9 ± 0.3 [†] MA: 11.5 ± 1.6 [†] X: 18.1 ± 4.2 [†]
100	15 F (27-60y) Before and after pelvic external radiation	100 mL; 18.2 g Lac and 18.2g MA 1,500 mosm/l; 0.55 ml/kg BW 5 h and GC	Before Lac: 0.4 ± 0.3 MA: 14.5 ± 4.8 L/M: 0.03 ± 0.019	After Lac: 0.7 ± 0.6 [†] MA: 11.8 ± 4.4 L/M: 0.064 ± 0.062 [†]	
101	46 type I diabetic (28 M and 18F; mean 15.8y) 23 H (11 M and 12 F; mean 27.9y)	150 mL; 5 g Lac and 2 g MA 375 mOsm/L 5 h and HPAEC-PAD 5 h and HPAEC-PAD	Control Lac: 0.26 (0.07-1.14) MA: 18.8 (5.0-47.5) L/M: 0.014 (0.004-0.027)	Diabetic Lac: 0.55 (0.03-5.52) [†] MA: 17.3 (0.85-86.9) L/M: 0.038 (0.005-0.176) [†]	
102	36 type I diabetic 56 relatives of diabetic 43 H	150 mL; 5 g Lac and 2 g MA 5 h and HPAEC-PAD 150 mL; 5g Lac and 2 g MA	Control Lac: 0.48 ± 0.12 MA: 23.2 ± 3.36 L/M: 0.017 ± 0.0018	Diabetic Lac: 0.79 ± 0.11 [†] MA: 21.2 ± 2.22 L/M: 0.037 ± 0.003 [†]	Relatives Lac: 0.63 ± 0.14 [†] MA: 24.7 ± 3.2 L/M: 0.025 ± 0.01 [†]
103	22 H (11M and 11F; 62y) 22 CHF (18M and 4F; 67y)	100 mL water; 5 g SU; 10 g Lac; 5 g MA and 20 g S 5 h and HPLC	Control L/M: 0.017 ± 0.001 SU: 0.20 ± 0.06 X: 37.4 ± 1.4	CHF L/M: 0.023 ± 0.001 [†] SU: 0.62 ± 0.17 [†] X: 26.7 ± 3.0 [†]	
104	57 H (mean 40y) 40 FM (8M and 32 F; 48y) 17 CRPS (4M and 13 F; 43y)	100 mL; 20 g S; 10 g Lac and 5 g MA 5 h and HPLC	Control S: 0.19 ± 0.075 L/M: 0.0155 ± 0.006	FM S: 0.22 ± 0.2 [†] L/M: 0.025 ± 0.012 [†]	CRPS S: 0.29 ± 0.27 [†] L/M: 0.026 ± 0.020 [†]
105	20 H (control I) 10 nonalcoholic (control II) 10 alcoholic NLD 10 alcoholic LD 10 nonalcoholic LD	150 mL; 7.5 g Lac; 2g MA and 40 g S 5 h and GC	Control I Lac: 0.17 (0.03-0.49) MA: 16 (3-72) S: 0.03 (0.005-0.09)	Alcoholic NLD Lac: 0.17 (0.05-0.55) MA: 12 (7-27) S: 0.11 (0.02-0.4)	Non-alcoholic LD Lac: 0.17 (0.05-0.8) MA: 13 (2-34) S: 0.05 (0.01-0.15)
			Control II Lac: 0.08 (0.02-0.02) MA: 4 (0.6-14) S: 0.02 (0.006-0.05) [†]	Alcoholic LD Lac: 3.8 (0.03-10) [†] MA: 5 (2-9.5) S: 1 (0.04-2.1) [†]	
68	12 H (4M and 8F) 6 steatosis (3M and 3F) 10 NASH (6M and 4F)	1 g SU; 7.5g Lac; 40 g S and 2 g MA 5 h and CG	Control Lac: 0.07 ± 0.05 MA: 10.7 ± 9.1 L/M: 0.007 ± 0.003 SU: 2.49 ± 1.34	Steatosis Lac: 0.23 ± 0.15 MA: 15.0 ± 4.9 L/M: 0.015 ± 0.008 SU: 3.07 ± 0.87	NASH Lac: 0.14 ± 0.12 MA: 18.5 ± 12.1 L/M: 0.020 ± 0.035 SU: 2.79 ± 1.55
106	134 H (40 M and 94 F) 43 chronic hepatitis 40 cirrhosis	150 mL 5 g Lac and 2 g MA 5 h and HPAEC	Control L/M: 0.016 ± 0.014	CLD Hepatitis: L/M: 0.037 ± 0.04 [†] Cirrhotics: L/M: 0.056 ± 0.08 [†]	
107	11 H (7M and 4 F) 32 cirrhosis + SAI (26 M and 8F)	100 mL 10 g Lac and 5 g MA 6 h and HPLC	Control Lac: 0.001 ± 0.0001 MA: 0.0838 ± 0.007 L/M: 0.0209 ± 0.0009	Cirrhosis Lac: 0.007 ± 0.0004 [†] MA: 0.074 ± 0.004 L/M: 0.1003 ± 0.003 [†]	

Table II (cont.)
Intestinal permeability markers for healthy and diseased adults

Ref	Sample	Volume, sugar and osmolarity Urine or blood* collection (hours) and method	% Excretion (mean ± SD or median [range])		
33	10 H (7 M and 3 F) 28 investigation for GSE (16 F and 12 M)	300 mL; 10 g Lac and 5 g MA 696 mmol/kg 5 h and HPLC	Control Lac: 0.15 ± 0.09 MA: 11.8 ± 6.2 L/M: 0.02 ± 0.014	Normal biopsy Lac: 0.27 ± 0.13 MA: 12.6 ± 4.6 L/M: 0.021 ± 0.013	Abnormal biopsy Lac: 0.65 ± 0.26 MA: 9.0 ± 3.4 L/M: 0.146 ± 0.10 [†]
108	54 diarrhea-IBS 22 H	100 mL 5 g Lac and 2 g MA; 24 h	Control All had L/M < 0.07	IBS 39% had L/M ≥ 0.07	
32	6 (3 M, 3 F) H 6 (2 M, 4 F) Celiac	50 mL 10 g Lac and 2.5 g MA 1070 mOsm 30, 60, 90, 120* and HPLC	Control Lac (1h): 0.125 (0.11-0.15) MA (1h): 0.156 (0.15-0.19) L/M: 0.039 (0.028-0.043)	Celiac Lac (1h): 0.56 (0.29-0.94) [†] MA (1h): 0.06 (0.018-0.9) [†] L/M: 0.42 (0.15-8.3) [†]	
109	30 H (13 M, 17 F, mean 37 y) 18 Dermatitis herpetiformis (9 M, 9 F, mean 38 y) 30 Celiac (12 M, 18 F, mean 36 y)	450 mL 5 g Lac and 2 g MA 5 h and HPLC	Control L/M: 0.017 ± 0.0007	Celiac L/M: 0.073 ± 0.017 [†]	Dermatitis L/M: 0.082 ± 0.013 [†]
110	11 H 22 Celiac (11 M and 11 F; mean 41 y) (1 y after a gluten free diet)	120 mL 6 g Lac and 3 g MA 6 h and HPLC	Control Lac: 2.75 ± 1.71 MA: 22.56 ± 3.32 L/M: 0.12 ± 0.07	Celiac AGA+ Lac: 10.27 ± 3.37 [†] MA: 10.18 ± 3.82 [†] L/M: 1.02 ± 0.46 [†]	Celiac AGA- Lac: 3.79 ± 1.46 [†] MA: 11.12 ± 5.64 [†] L/M: 0.39 ± 0.11 [†]
21	15 H (8 M, 7 F; mean 36 y) 22 Celiac > 1 y GD (11 M and 11 F; mean 41 y) 31 Crohn (18 M and 20 F; mean 37 y)	120 mL 6 g Lac and 3 g MA 6 h and HPLC	Control Lac: 0.07 (0.05-0.28) MA: 21 (18.3-28) L/M: 0.003 (0.002-0.013)	Celiac Lac: 0.15 (0.04-0.85) [†] MA: 10.9 (3.3-19.5) [†] L/M: 0.013 (0.005-0.07) [†]	Crohn Lac: 0.42 (0.15-0.99) [†] MA: 21 (13.5-29.5) L/M: 0.021 (0.07-0.046) [†]
111	64 H (31 M and 33 F; mean 40 y) 23 Crohn s disease (13 M and 10 F; 43 y) and 28 H first degree relatives of Crohn s patients (14 M and 14 F; 62 y)	50 mL 10 g Lac and 5 g MA 1300 mOsm/L 6 h and enzymatic	Controls Lac: 0.313 (0.047-1.240) MA: 26.83 (16.9-50)	Crohn Lac: 0.418 (0.03-1.5) [†] MA: 8.27 (4.1-36) [†]	First degree relatives Lac: 0.27 (0.012-3.56) [†] MA: 9.54 (3.2-28) [†]
112	22 H 125 Crohn (66 M and 59 F; median 36 y)	100 mL 5 g Lac; 2 g MA and 5 g X 6 h and enzymatic	Control Lac: 0.293 (0.0089-0.665) MA: 14.2 (4.95-30.8) L/M: 0.0164 (0.0018-0.0548) X: 1.89 (0.8-4.73)	Crohn Lac: 0.326 (0.0204-2.76) [†] MA: 12.5 (1.43-43.75) L/M: 0.027 (0.0029-0.279) [†] X: 1.45 (0.32-4.5) [†]	
61	20 HF 20 OB F	120 mL 6.25 g Lac and 3 g MA 5 h, GC	Control Lac: 0.247 ± 0.087 MA: 17.32 ± 7.31 L/M: 0.0144 ± 0.006	Obese Lac: 0.418 ± 0.267 [†] MA: 21.86 ± 7.77 L/M: 0.018 ± 0.008	

M: men; F: female; H: healthy (control); Lac: Lactulose; MA: mannitol; L/M: lactulose/mannitol ratio; S: sucrose; SU: sucralose; X: xylose; S/M: sucrose/mannitol ratio; BW: body weight; GC: gas chromatography; HPLC: high-performance liquid chromatography; HPAEC-PAD: High-performance anion exchange chromatography coupled with pulsed amperometric detection; CCGC: capillary column gas chromatography; PCGC: packed column gas chromatography; AGA: anti-gliadin antibody; CRPS: complex regional pain syndrome; CHF: Chronic heart failure; FA: food-allergy IgE-mediated; FH: food hypersensitivity non-IgE mediated; FM: fibromyalgia; GSE: gluten sensitive enteropathy; IBS: Irritable Bowel Syndrome; LD: with liver disease; NASH: nonalcoholic steatohepatitis; NLD: with no liver disease; OB: obese; PN: parenteral nutrition; SAI: spontaneous ascitic fluid infection. [†]p < 0.05 disease vs healthy; [‡]p < 0.025 controls vs relatives.

barrier dysfunction and other intestinal abnormalities (such as polyps, tumors) can be done through methods such as collection of a biopsy sample using surgical and/or endoscopic procedures. However, these procedures are invasive, often inconvenient to the patient and usually imply high healthcare costs⁷. This has led to the development of alternative methods to assess gut barrier function while preventing patients from undergoing such kind of invasive methods.

Intestinal permeability (IP) tests represent one alternative method. The concept of intestinal epithelial barrier function is tightly related to the concept of perme-

ability, which is the property of the membrane to allow non-mediated solute diffusion^{8,9}. When the barrier is intact, the permeability of substances is highly selective and controlled. Disturbances in gut barrier function can affect the control of permeating substances^{9,10}. Based on these principles the oral administration of specific probes has been commonly used to indirectly assess gut barrier dysfunction and measure IP. These probes are subsequently quantified in blood or more frequently in urine¹¹. In a simplistic way, injuries in the intestinal mucosa can impair its barrier function. The impaired barrier function results in higher permeation rate

of probes and intact proteins through the intestinal mucosa^{12,13}.

Intestinal permeability tests are not widely used in clinical practice. Their use has been usually restricted for scientific purposes. However, evaluation of IP can be a useful tool in screening for small intestinal disease, in assessing the response in the follow-up period after a therapeutic intervention and in predicting the prognosis, especially in celiac disease^{14,15}. The majority of probes used have been shown to be non-toxic to patients and relatively easy to quantify. These characteristics can be explored by medical professionals to expand the knowledge about the clinical situations in which intestinal barrier dysfunction can be an important feature.

In this context, the aims of this review were to highlight the possible factors underlying higher IP and the clinical conditions that have been associated with this in different age range; and also provide some insight into methodological aspects to be considered in future studies.

Methods

Medline/Pubmed, Scielo and Lilacs were used to search for articles accomplishing the following terms (alone or associated): intestinal or gut permeability, intestinal or gut barrier, lactulose, mannitol, tight junctions. Review and original articles were selected and read critically.

Factors underlying increased intestinal permeability

The intestinal epithelium is a single layer of columnar epithelial cells that separates the intestinal lumen from the underlying lamina propria. It is believed that there are two routes for substances permeation through the intestinal epithelial cells: transcellular (across the cells, both by active and passive processes), and paracellular (between adjacent cells, by a passive process)^{16,17}. The epithelial cells are tightly bound together by intercellular junctional complexes. They are formed by TJ, gap junctions, adherens junctions and desmosomes. The space between cells is called paracellular space. The permeability of molecules through this space is under control of the junctional complexes, which are crucial for the integrity of the epithelial barrier¹⁷.

Tight junctions are complex structures comprising over 50 types of proteins (claudin, occludin, zonulin, junctional adhesion molecules). They form a continuous, circumferential seal around cells through the interaction with the perijunctional acto-myosin ring of epithelial cells¹⁷. It has been observed that TJ have a central role in processes that regulate epithelial proliferation and differentiation¹⁸.

Regulation of the assembly, disassembly and maintenance of TJ structure is influenced by various physio-

logical and pathological stimuli. The knowledge of how TJ are modified in response to signals that alter their functional properties is of great importance in the context of diseases associated with altered IP^{16,19-21}. Experimental studies using animal and cell culture models or human studies have shown that deregulated TJ are the main cause of altered intestinal barrier. This alteration can be induced by endogenous and exogenous factors (Table III).

Recently, it has been demonstrated that increased IP can occur due to discontinuities in the epithelial cell layer in the gut. These discontinuities are called gaps and have been identified in the mouse and humans. They are formed when epithelial cells leave the epithelium. These gaps have the diameter of an epithelial cell and are devoid of cellular contents, but filled with an unknown substance that maintains local barrier function. The rate at which cells leave may have implications for the permeability of the epithelium as a unit. The processes that control the rate of cell egress have not been well defined. This mechanism of increased permeability may be important in human diseases^{22,23}.

As summarized by Teshima and Meddings²² "simply measuring an increase in permeability provides no information to the physician about the mechanisms underlying the abnormality. However, an understanding of these mechanisms may prove valuable in designing interventions". Thus the main causes of increased IP that should guide the development of efficacious intervention are: genetic alterations of TJ proteins, abnormal microbiota, abnormal regulation of TJ function (increased zonulin release), mucosal inflammation and abnormal epithelial dynamics²².

General aspects of intestinal permeability tests

Intestinal permeability tests are based on probes of different molecular weight, which determines the route of permeation (Table IV). Smaller molecules usually permeate through membrane pores. They are expected to be present in urine in higher proportion (10 to 30% of an orally ingested dose)²⁴. Less than 1% of higher molecular weight molecules are expected to be recovered in urine after an oral dose²⁵. These molecules need to cross the barrier through the paracellular route, which is more tightly regulated by protein complexes.

The choice of probes depends on the intention of what part of intestine is meant to be assessed. Usually, recovery of sucrose in the urine reflects gastroduodenal permeability²⁶, since sucrose is rapidly hydrolyzed by sucrase-isomaltase upon entering the duodenum and reflects absorption only in the most proximal portion of the gut²⁷. Lactulose and mannitol, which are one of the most commonly used probes, are destroyed in the caecum and provide information regarding the small intestinal epithelium¹⁶. Sucralose is an artificial sweetener with similar molecular weight of lactulose and is resis-

Table III
Factors that influence tight junctions assembly

<i>Endogenous or exogenous factors</i>	<i>Evidences from human, animal or cell culture models</i>
Genetic susceptibility	10-25% of first-degree relatives of inflammatory bowel disease patients have increased IP in the absence of clinical symptoms ⁴⁵⁻⁴⁷ . Divergent study can be found ¹¹¹ .
Gender	Oestrogen receptors are expressed in intestinal epithelial cells. Oestradiol regulates epithelium formation, occludin and junctional adhesion molecule expression ¹¹³ . Female rats are more resistant to intestinal injury induced by hypoxia and/or acidosis. The administration of estradiol or blockade of the testosterone receptor in male rats mitigates the gender differences found for histomorphological changes ¹¹⁴ . It was found differences in the recovery of sugar probes with aging just in females ³⁰ .
Cytokines (TNF-α and interferon-γ)	Inflammatory cytokines disrupt TJ structure through inductions of changes on lipid composition and fatty acyl substitutions of phospholipids in membrane microdomains of TJ ¹¹⁵ . They also modulate myosin II regulatory light chain (MLC) phosphorylation through MLC kinase upregulation ¹¹⁶ , which is involved in barrier function. TNF- α caused occludin depletion in Caco-2 intestinal epithelial monolayers through a progressive decrease in occludin mRNA level ¹¹⁷ .
Recruitment of immune cells	Th2 cell responses contribute to gastrointestinal inflammation and dysfunction. Intestinal mastocytosis predispose to increased IP and food allergy ¹¹⁸ .
Microbial-host interaction	Small intestinal bacterial overgrowth has been detected in diseases related to altered IP. ¹¹⁹ Probiotic bacteria can reduce IP ¹²⁰ ; they increase TJ resistance and reduce cellular permeability ¹²¹⁻¹²² through influence on cytoskeleton organization ¹²³ and cytokine production ¹²⁴ .
Alcohol consumption	Acetaldehyde accumulation and induction of nitric oxide production contributes to increased tyrosine phosphorylation of TJ and adherens junction proteins and damaged microtubules cytoskeleton, which in turn increase IP ⁴⁰ .
Non-steroidal anti-inflammatory drugs	Exert detergent properties on phospholipids membrane causing direct damage on epithelial surface; uncoupling of mitochondrial oxidative phosphorylation reduce ATP availability, which is necessary for actin-miosin ATP-dependent complexes of intercellular junctions ³⁸ .
Enteric pathogens	<i>Clostridium difficile</i> , enteropathogenic <i>Escherichia coli</i> ; <i>Bacteroides fragilis</i> , <i>Clostridium perfringens</i> , <i>Vibrio cholera</i> may activate inflammatory cascade in epithelial cells; directly modify TJ proteins and perijunctional actomyosin ring; induce fluid and electrolyte secretion ^{49,125} .
Nutrients	<i>Retinoic acid</i> : Metabolic depletion of retinoic acid in cells, alters expression of genes related to TJ modulation ¹²⁶ . <i>Zinc</i> : Supplementation reduces lactulose excretion ^{127,128} . Activation of the zinc finger transcription factor (Hepatocyte nuclear factor-4 α) is essential for enterocyte differentiation and regulation of TJ proteins ¹²⁹ . <i>Polyunsaturated fatty acids</i> (particularly ω -3): Stimulate intestinal cells differentiation and maturation, improves TJ formation through their proteins redistribution and reduction of TNF- α effect ^{130,131} . <i>Vitamin D</i> : Critical for preserving junctional complexes integrity and renew epithelial ability ¹³² . <i>Magnesium</i> : its deficiency has been shown to reduce cecal content of bifidobacteria and to lower expression of TJ proteins (occludin and zonulin) ¹³³ .
Stress	Modify and redistribute TJ transmembrane protein occludin and the plaque protein zonula occludens-1 ¹³⁴ and alter epithelial cell turn-over ¹³⁵ .
High fat diet	It reduces TJ protein expression in the small intestine ¹³⁶ . It may alter the bile acid metabolism, which in turn would increase IP ¹³⁷ .
Polyamines	Spermine may loosen the TJ of the epithelium increasing the intestinal absorption of drugs via a paracellular route ¹³⁸ .

TNF: Tumor necrose factor; IP: intestinal permeability; TJ: tight junctions.

tant to bacterial fermentation²⁸. It spends most of a 24 hour exposure period in the large intestine¹⁶. Therefore, sucralose has been suggested as better suitable sugar for whole gut permeability assessment²⁹.

An inconvenience of IP tests is the prolonged period of urine collection, usually 5 to 6 hours. The introduction of sucralose into permeability measurements might extend the test period up to 24 hours,

Table IV
Frequently used probed for assessment
of intestinal permeability

Lower molecular weight (Molecular weight < 200 Da)	Higher molecular weight (Molecular weight > 300 Da)
D-mannitol	Lactulose
L-rhamnose	Lactose
L-arabinose	Sucrose
	Cellobiose
	Sucralose
	PEGs (polyethylene glycols)
	Raffinose
	⁵¹ CrEDTA (51)Cr-labelled ethylenediaminetetraacetic acid)
	⁹⁹ Tc-DTPA (99m Tc diethylenetriamine pentaacetate)
	Iohexol
	Other contrast media (iodixanol, etc.)

Source: Travis and Menzies⁴⁸, Frias et al³⁹ and Andersen et al⁴⁰.

making it less convenient in clinical practice. McOmber and co-workers recommend re-examining the usual 5 to 6 hours collection times to compare healthy individuals to those with abnormal permeability, because this period of time might not include the point of maximal urinary recovery. They studied the recovery of sucrose, lactulose, mannitol and sucralose over a 24 hours period in healthy adults and children³⁰. It was suggested that by using different collection periods greater differences may be seen between groups with less inter-individual variation: 4 to 6 hours for sucrose, 13 to 15 hours for lactulose, mannitol and sucralose. If sucralose/lactulose ratio is to be measured, collection time might be extended to 16 to 18 hours³⁰. However, Akram and co-workers³¹ have compared different urine times collection and their results suggest that the use of Lactulose/Mannitol (L/M) ratio to assess IP could be simplified by shortening the time of urine collection³¹. The reduction of the time can also be achieved by measuring the probes in blood 60-90 min post-ingestion of solution^{32,33}. More studies are needed to confirm that prolonged time collection is not needed.

The calculation of the ratio between sugar probes used (such as L/M) is considered a good marker of

small intestinal permeation⁹. It is meant to circumvent confounding factors as inter-individual variation of gastric emptying, intestinal transit and transport, blood distribution and renal clearance³⁴.

In general, the integrity of intestinal barrier function is dependent on healthy epithelial cells and on the proper functioning of the paracellular route⁹. Theoretically, an increase in the sugar probes ratio –for example L/M ratio– would indicate altered IP. This alteration may reflect a decrease in smaller probes (e.g. mannitol) absorption and/or an increase in the absorption of higher weight probes (e.g. lactulose). Decreased small weight probes absorption can be the result of a diminished absorptive area. Increased permeation of higher weight probes may be due to a facilitated diffusion of this marker into the crypt region as a consequence of decreased villous height or TJ loosening³⁵.

The results of IP tests are usually expressed as percentage of excretion of probes (Table V). Other units can be also found (mg/mL, mmol/L, mg)^{11,31,32,36,37}.

Possible pitfalls in intestinal permeability tests

Many factors may influence the results of the test, as shown in table III. Thus, possible pitfalls for the IP tests may be circumvent by researchers or healthcare professionals when considering some details.

Previous orientation of individuals to avoid –few days before the test– the use of non-steroidal inflammatory drug^{38,39}, acute alcohol ingestion^{32,40,41}, psychological and physical stressful situations⁴²⁻⁴⁴ should be given as part of the protocol. Considering that some genetic background may exert negative influence on barrier function, family history of inflammatory bowel diseases should be considered before inclusion of patients in a study. Regarding the personal medical history some clinical factors influencing IP such as food allergy, human immunodeficiency virus, diabetes, starvation, iron deficiency, diarrhea, viral gastroenteritis, smoking⁴⁵⁻⁴⁸ should be an exclusion criteria, except if this is the topic under investigation. Additionally, search for evidence of endoparasite infection in the stools should be ideally performed before inclusion of individuals in the study⁴⁹.

Usually, all tests are performed under overnight fast (8 to 10 hours). Few authors mention the instruction of indi-

Table V
Calculation of percentage of sugar probes excretion (e.g.: lactulose and mannitol)

% Lactulose excretion	% Mannitol excretion	Lactulose/Mannitol ratio
Lactulose excreted (mg) = mg/L lactulose × L urine	Mannitol excreted (mg) = mg/L mannitol × L urine	L/M = % of lactulose excretion / % of mannitol excretion
% of lactulose excretion = (mg lactulose excreted/ mg lactulose consumed) × 100	% of mannitol excretion = (mg mannitol excreted/ mg mannitol consumed) × 100	

Table VI
Possible dietary sources of the main sugar probes (lactulose, mannitol and sucralose)

<i>% Lactulose (4-O-b-D-galactopyranosyl-D-fructose)</i>	<i>Mannitol</i>	<i>Sucralose</i>
Prebiotic food additive (infant formulas and healthy foods) ⁴¹ . Lactulose is not present as such in nature but it is produced from lactose during heat treatment, and may be naturally present in considerable amounts in heat-processed dairy (UHT milk, yogurt, soymilk) ⁴² .	The most abundant polyol in nature. Some fungi, and brown seaweeds. Celery; Reduced-calorie sweetener ⁴³ . Parsley, carrot, coconut, cauliflower, cabbage, pineapple, lettuce, watermelon, pumpkin, squash, cassava, manioc, pea, asparagus, olive, coffee ⁴⁴ . Berries ⁴⁵ , chewing gum.	Sweetener and diet/light products ⁴⁶ .

viduals to follow a diet free of the sugars used as probes in the test at least 24 hours before it^{13,32,50}. Lactulose, mannitol and sucralose are commonly used in IP tests and can be present in some common foods (Table VI). An important issue mentioned in some protocols to circumvent the possible influence of the intake of the same sugars that will be used in the IP test is the collection of a urine sample before the administration of the sugar probes. The amount of sugar quantified in this sample should be subtracted from the results in the urine collected after the ingestion of the probes^{13,28,33,50}. Avoidance of some foods should be also advised when they contain other sugars that can imply in methodological difficulties to properly quantify the probes. Farhadi and co-workers recommend subjects to avoid consumption of dairy products on the previous day of the test since lactose peak tend to overlap that of lactulose⁵¹. During the IP test, in some studies it is mentioned that subjects are encouraged to drink water and/or to have a snack after 1 to 2 hours of probes administration^{11-13,37}. It is not clear if this can affect the results. However, an important detail of this practice is to standardize the type of food and the volume of liquid offered to all individuals. Mattioli and co-workers⁵² found that the L/M ratio was significantly lower in subjects that excreted more than 500 mL of urine. The greater urine volume was associated with a higher mannitol recovery. Thus, they emphasized that urine volume may influence urinary excretion of sugar probes and intake of liquids should be carefully monitored before and during the test⁵².

It is noteworthy that Camilleri and co-workers question the concept that lactulose and mannitol in urine collected between 0 to 6 hours reflect small intestine permeability. They have investigated the administration of these probes (radiolabelled) in a liquid formulation or in a delayed-release methacrylate-coated capsule. It was showed that after 2 h of liquid formulation intake around 50% of the probes was in the colon, suggesting that sugars may not be absorbed exclusively in the small intestine. Thus, they suggest that the interpretation of the 0 to 6 hours differential two sugar urine excretion as an exclusive marker of small IP should be done cautiously²⁴.

Osmolarity of test solutions should be mentioned in every study, since stress induced by high osmolarity can stimulate intestinal motility⁵³ and change the rate of sugars permeation⁸. The amount of sugar administered

and the volume of solutions vary between studies (see Tables I and II). In addition, the volume of solution administered is fixed for all subjects. Exception is observed in some studies with children, that use body weight to calculate the volume of solution to be administered individually^{50,54}. This might have been proposed based on pharmacokinetics studies. At least for children, drugs dosages are based on body weight or body surface area since body size, proportion, organ development and function affect the pharmacokinetic behavior of many drugs⁵⁵. It should be further discussed the possibility of using weight to calculate the volume of solution to be administered also to adult subjects. The body weight or body mass index (BMI) of subjects included in the majority of studies is not mentioned. Could this make any difference for the interpretation of IP results?

A higher BMI is associated with higher filtration fraction. This means that there is a higher glomerular filtration rate (GFR) relative to effective renal plasma flow, suggesting an altered afferent/efferent balance and higher glomerular pressure⁵⁶. In obese subjects, the values for GFR exceeded by 61% the values for GFR of the control group and by 32% the value of renal plasma flow, suggestive of glomerular hyperfiltration. The obesity-related glomerular hyperfiltration ameliorates after weight loss⁵⁷. It is a possible pitfall when subjects with excess of weight are included in studies: could a higher amount of excreted sugar be a consequence of higher intestinal absorption (due to higher IP) or of a higher glomerular hyperfiltration? This has not been investigated in humans. Whenever overweight and obese subjects are submitted to IP test it should be investigated if they present normal renal function (impaired renal function should be adopted as exclusion criteria).

Choosing the best method to assess renal function should consider population characteristics such as age and BMI. Serum creatinine levels, anthropometric and clinical characteristics of patients are often used to estimate GFR. Body weight is an imperfect reflection of creatinine generation because increased body weight is associated more commonly with an increase in body fat or body water, edematous disorders, rather than an increase in muscle mass^{58,59}. Creatinine clearance is not recommended when obese subjects are involved, but would be advised to exclude individuals that present creatinine level higher than 250 mmol/l¹⁴. A decline in

renal function (creatinine clearance) occurs with advancing aging. Interestingly, L/M ratio did not change with aging due to a parallel progressive decline in the ability to excrete both lactulose and mannitol with increasing age⁶⁰.

The use of the ratio L/M may not detect differences in IP between groups if one considers the possibility that an individual may be absorbing and excreting proportionally higher quantities of both mannitol and lactulose. Although this is only a hypothesis, obese women showed higher lactulose excretion, a tendency to higher mannitol excretion, while L/M ratio was not significantly different from lean women⁶¹. It is critical to assess the L/M ratio, as well as lactulose and mannitol recoveries separately, when interpreting test results⁶². Ferraris & Vinnakota⁶³ showed in animal model that genetic obesity is associated with increased intestinal growth, which augments absorption of all types of nutrients. Obese men with chronic hyperglycemia showed evidence of increased small intestinal enterocyte mass (higher plasma citrulline) and increased enterocyte loss (higher plasma intestinal fatty acid binding proteins, I-FABP), but IP was not assessed⁶⁴. Circulating levels of insulin which is a hormone usually increased in obese subjects⁶⁵, may also influence IP. The addition of insulin in a cell culture showed that the insulin-induced decline in transcellular resistance is receptor-mediated and that receptors are localized in the basolateral membrane. Increased mannitol flux was an observed effect paralleled to this altered paracellular permeability⁶⁶.

Barrier dysfunction may not be expressed all the time in particular conditions. It can range from mild to severe dysfunction (manifesting continuously) or intermittent dysfunction (manifesting only when the intestine is challenged). This susceptibility to barrier dysfunction can be detected using a 'challenge' test, as established by Hilsden and co-workers using aspirin⁶⁷. Accordingly, subjects are given 1300 mg of aspirin (four 325 mg tablets) the night before the test and again on the morning of ingestion of the probe mixture. The use of the aspirin challenge showed that patients with non-alcoholic steatohepatitis do not have abnormal IP all the time, but they could easily develop gut leakiness when they are exposed to intestinal barrier stressors such as aspirin⁶⁸.

Of note is the discussion presented recently by Vojdani⁶⁹ in his review entitled "For assessment of intestinal permeability, size matters". Mannitol and lactulose are considered small molecules. Their use for IP assessment will not necessarily indicate structural damage in the TJ barrier, which would in turn allow penetration of large molecules. The use of probes of higher size (polysugars of 12,000- to -15,000 Da) may be more suitable to extrapolate if IP is higher enough to allow macromolecules such as bacterial toxins (such as lipopolysaccharides) and food antigens to permeate. Small inert markers may not mimic large molecules because of the size selectivity of TJ⁶⁹.

Additional markers to indicate alteration in barrier function

There are other markers that could be associated to IP tests to improve the interpretation of dysfunctions of gut barrier. D-lactate is produced from carbohydrate fermentation by abnormal microbiota or when the number of bacteria elevates rapidly (bacterial overgrowth and short bowel syndrome)⁷⁰⁻⁷². Plasma D-lactate had the lowest false-negative rate among C-reactive protein level and leukocyte counts to diagnose appendicitis, and acute inflammatory disorder⁷³.

Circulating citrulline is an amino acid produced from glutamine by differentiated small intestinal enterocytes. Citrulline is a non-protein amino acid that seems to exert an important role in preserving gut barrier function and reducing bacterial translocation⁷⁴. The circulating levels are dependent only on de novo synthesis from intestinal metabolic activity. It reflects the functional enterocyte mass and can be used as a biological tool to quantitatively investigate epithelial integrity and follow intestinal adaptation (i.e., post-surgical) at the enterocyte level. Loss of small bowel epithelial cell mass results in declined circulating levels of citrulline, such as for short bowel syndrome, chronic villous atrophy and chemotherapy⁷⁵. Another situation in which the citrulline availability is decreased was shown to be during the course of induced endotoxemia in rats⁷⁶. There are some studies using animal models that show an association between endotoxemia and increased IP⁷⁷⁻⁷⁹. As citrulline is metabolized into arginine by kidney cells, the interpretation of its levels in patients with compromised renal function should not be reliable⁸⁰.

The quantification of claudin-3 in the urine showed that its rapid appearance in this fluid correlated with immunohistochemically visualized loss of claudin-3, which is a major sealing TJ protein. Measurement of urinary claudin-3 can be used as noninvasive marker for intestinal TJ loss⁸¹.

The assessment of urinary concentration of endogenous cytosolic enterocyte proteins such as I-FABP and liver FABP (L-FABP) are potentially useful in reflecting enterocyte damage. Pelsers and co-workers investigated the distribution of these proteins in segments of human intestine⁸². They showed similar pattern of tissue distribution along the duodenal to colonic axis, being the jejunum the segment with highest content. In each intestinal segment it is observed a more than 40-fold higher content of L-FABP than I-FABP. Elevated plasma levels of both proteins were found in patients with intestinal diseases⁸². Since FABP are small, water-soluble cytosolic proteins, the loss of enterocyte membrane integrity will lead to release of these proteins into the circulation^{71,83}. FABP are expressed in cells on the upper part of the villi. Thus, destruction of these cells can lead to increased release of these proteins to the circulation. Results from a pilot study with celiac patients showed that circulating levels of FABP are signifi-

cantly elevated in untreated patients with biopsy proven celiac disease compared with healthy controls⁸⁴.

Local inflammation is associated with increased IP. An increased migration of granulocytes into the intestinal mucosa, usually due to conditions of inflammation, might result in the degranulation of their secondary granules, resulting in an increase in their proteins in feces⁸⁵. Neutrophil derived proteins such as calprotectin, lactoferrin⁸⁵⁻⁸⁸ and elastase⁸⁹ can be present in stool and also in plasma as a marker of inflammation⁹⁰.

Finally, zonulin is a protein that exhibits the ability to reversibly modulate intercellular TJ similar to the toxin from *Vibrio cholera* known as zonula occluden toxin^{91,92}. Proteomic analyses characterized zonulin as pre-haptoglobulin-2 (pre-HP2), a multifunctional protein that contains growth factor-like repeats. In its single-chain form, zonulin has the molecular conformation required to induce TJ disassembly by indirect transactivation via proteinase-activated receptor-2⁹². Higher levels of zonulin are associated with disorders such as celiac disease and type 1 diabetes, and positive correlation between zonulin and IP has been demonstrated^{92,93}.

Conclusion

There are many clinical situations in which increased IP seems to be present. If this alteration is contributing to worsen the clinical condition of affected subjects is still a question without answer for different diseases. This field of research should be better explored. However, the possible pitfalls should be taken into account. It is important to consider the different factors that may influence IP tests result and there are open questions regarding renal function and body size that should be further tested. This could help to produce more consistent evidences. The use of larger probes may be more appropriate to affirm that macromolecules such as food antigens and bacterial derived-compounds are crossing the barrier. Besides the use of IP tests, the association with the mentioned markers would be also interesting to investigate the role of barrier function in different diseases.

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