Revisión
Intestinal permeability measurements: general aspects and possible pitfalls

Tatiana Fiche Salles Teixeira¹, Ana Paula Boroni Moreira¹, Nilian Carla Silva Souza¹, Rafael Frias³ and Maria do Carmo Gouveia Peluzio¹


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.

Correspondence: Tatiana Fiche Salles Teixeira.
Departamento de Nutrición e Saúde.
Universidade Federal de Viçosa.
Av. Ph. Rolfs. Campus Universitário.
36570-000 Viçosa. Minas Gerais. Brasil.
E-mail: tatifichee@hotmail.com

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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 gastrointestinales 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 estas, 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 inocuosidad y facilidad de los test de permeabilidad pueden ser exploradas para ampliar el conocimiento sobre las condiciones clínicas en las que la disfunción de la barrera intestinal puede ser una característica distintiva. Muchos factores pueden influir en los resultados de los test de permeabilidad. Sin embargo, los investigadores y los clínicos han de tratar de evitar 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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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 intestinal 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) 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 bacteria.

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

<table>
<thead>
<tr>
<th>Table I</th>
<th>Intestinal permeability markers for healthy and diseased infants, children and adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref</td>
<td>Sample</td>
</tr>
<tr>
<td>34</td>
<td>6 term (fed human milk) 21 preterm infants (4 fed human milk and 17 fed formula milk)</td>
</tr>
<tr>
<td>94</td>
<td>12 CMPSE (6m-2y) 28 AD (6m-15y) 39 H</td>
</tr>
<tr>
<td>95</td>
<td>77 underweight (44M and 33F, mean 13.1m) 17 H (11M and 6 F, mean 13.2m)</td>
</tr>
<tr>
<td>50</td>
<td>28 H (12M and 16F; mean 9y) 28 GSE (10M and 18F; mean 10y)</td>
</tr>
<tr>
<td>96</td>
<td>49 infected (hemmophilia) (mean 7.2y) 95 H (mean 7.2y)</td>
</tr>
<tr>
<td>37</td>
<td>30 H (13M and 17F; mean 7.4y) 10 ileocolitis Crohn's (mean 14.7y) 10 Celiac (mean 5.8y)</td>
</tr>
<tr>
<td>54</td>
<td>15 H (no diarrhea episode in last 2 wk) 15 Diarrhea (3 or more liquid stools in the last 24h)</td>
</tr>
<tr>
<td>97</td>
<td>52 H (13 M and 39 F; 8.2 y) 93 FAB/IBS (28 M and 65 F; 8.5 y)</td>
</tr>
</tbody>
</table>

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; LGE: 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**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Sample</th>
<th>Volume, sugar and osmolarity</th>
<th>% Excretion (mean ± SD or median [range])</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>10 (7M and 3F) 28 investigation for GSE (10F and 12M)</td>
<td>300 mL; 10 g Lac and 5 g MA 696 mmol/kg; 5 h and HPLC</td>
<td>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: 0.9 ± 3.4 L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>98</td>
<td>41 (10M and 31 F; mean 29y) 20 FH (4M and 16F; mean 29y) 21 FA (6M and 15F; mean 29y)</td>
<td>200 mL; 5 g Lac and 2 g MA 5 h and HPAEC-PAD</td>
<td>Control L/M: 1.85 ± 0.8 Normal biopsy FH: 5.3 ± 4.26 Severe L/M: 6.17 ± 6.07</td>
</tr>
<tr>
<td>99</td>
<td>30 mild pancreatitis 15 severe pancreatitis 26 H</td>
<td>50 mL; 10 g Lac and 5 g MA 5 h and enzymatic 50 mL; 10 g Lac and 5 g MA</td>
<td>Control L/M: 0.02 ± 0.014 Normal biopsy L/M: 0.024 ± 0.013 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>35</td>
<td>12H (6M and 6F) 26 for FN (13 depleted and 10 non-depleted)</td>
<td>5 g X 6 h and GLC</td>
<td>Control L/M: 0.02 ± 0.014 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>100</td>
<td>15 F (27-60y) Before and after pelvic external radiation</td>
<td>100 mL; 18.2 g Lac and 18.2 g MA; 1,500 mosm/l; 0.55 ml/kg BW 5 g and GC</td>
<td>Control L/M: 0.02 ± 0.014 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>101</td>
<td>46 type I diabetic (28 M and 18F; mean 15.8y)</td>
<td>150 mL; 5 g Lac and 2 g MA 375 mmol/L 5 h and HPAEC-PAD</td>
<td>Control L/M: 0.016 ± 0.014 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>102</td>
<td>36 type I diabetic 56 relatives of diabetic 43 H</td>
<td>150 mL; 5 g Lac and 2 g MA 5 h and HPAEC-PAD</td>
<td>Control L/M: 0.017 ± 0.001 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>103</td>
<td>22 H (11M and 11F; 62y)</td>
<td>100 mL water; 5 g SU; 10 g Lac; 5 g MA and 20 g S 5 h and HPLC</td>
<td>Control L/M: 0.017 ± 0.001 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>104</td>
<td>57 H (mean 40y) 40 FM (8M and 32 F; 48y) 17 CRPS (4M and 13; 43y)</td>
<td>100 mL; 20 g S; 10 g Lac and 5 g MA 5 h and HPLC</td>
<td>Control S: 0.19 ± 0.075 Normal biopsy S: 0.22 ± 0.2 L/M: 0.0155 ± 0.006 Abnormal biopsy S: 0.29 ± 0.27 L/M: 0.02 ± 0.02</td>
</tr>
<tr>
<td>105</td>
<td>20 H (control I) 10 nonalcoholic (control II) 10 alcoholic NLD 10 alcoholic LD 10 nonalcoholic LD</td>
<td>150 mL; 7.5 g Lac; 2 g MA and 40 g S 5 h and GC</td>
<td>Control I L/M: 0.017 ± 0.001 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>68</td>
<td>12 H (4M and 8F) 6 steatosis (3M and 3F) 10 NASH (6M and 4F)</td>
<td>1 g SU; 7.5 g Lac; 40 g S and 2 g MA 5 h and CG</td>
<td>Control L/M: 0.017 ± 0.001 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>106</td>
<td>134 H (40 M and 94 F) 43 chronic hepatitis 40 cirrhosis</td>
<td>150 mL; 5 g Lac and 2 g MA 5 h and HPAEC-PAD</td>
<td>Control L/M: 0.016 ± 0.014 Normal biopsy L/M: 0.02 ± 0.014 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
<tr>
<td>107</td>
<td>11 H (7M and 4 F) 32 cirrhosis + 5A (26 M and 8F)</td>
<td>100 mL; 10 g Lac and 5 g MA 6 h and HPLC</td>
<td>Control L/M: 0.02 ± 0.014 Normal biopsy L/M: 0.037 ± 0.04 Abnormal biopsy L/M: 0.146 ± 0.10</td>
</tr>
</tbody>
</table>
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 costs. 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 permeability, which is the property of the membrane to allow non-mediated solute diffusion. 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. 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 urine. In a simplistic way, injuries in the intestinal mucosa can impair its barrier function. The impaired barrier function results in higher permeation rates.

### Table II (cont.)

<table>
<thead>
<tr>
<th>Ref</th>
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<th>Volume, sugar and osmolarity</th>
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<td>33</td>
<td>10 H (7 M and 3 F)</td>
<td>300 mL; 10 g Lac and 5 g MA</td>
<td>Control Lac: 0.15 ± 0.09; MA: 0.27 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>28 investigation for GSE (16 F and 12 M)</td>
<td>696 mmol/L g</td>
<td>Normal biopsy Lac: 11.8 ± 6.2; MA: 2.6 ± 4.6</td>
</tr>
<tr>
<td>108</td>
<td>54 diarrhea-IBS 22 H</td>
<td>100 mL</td>
<td>Abnormal biopsy L/M: 0.02 ± 0.04; L/M: 0.021 ± 0.013</td>
</tr>
<tr>
<td>32</td>
<td>6 (3 M, 3 F) 6 (2 M, 4 F) Celiac</td>
<td>50 mL</td>
<td>Control Celiac Lac (1h): 0.125 (0.11-0.15)</td>
</tr>
<tr>
<td></td>
<td>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)</td>
<td>450 mL</td>
<td>Normal biopsy Lac (1h): 0.136 (0.15-0.19)</td>
</tr>
<tr>
<td>110</td>
<td>11 H 22 Celiac (11 M and 11 F; mean 41 y) (1y after a gluten free diet)</td>
<td>120 mL</td>
<td>Abnormal biopsy MA (1h): 0.06 (0.038-0.09)</td>
</tr>
<tr>
<td>21</td>
<td>15 H (8 M, 7 F, mean 36 y) 22 Celiac &gt; 1 y GD (11 M and 11 F; mean 41 y) 31 Crohn (18 M and 20 F; mean 37 y)</td>
<td>120 mL</td>
<td>Control Celiac Lac (0.017 ± 0.0007) MA: 0.073 ± 0.017†</td>
</tr>
<tr>
<td>111</td>
<td>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)</td>
<td>50 mL</td>
<td>Control Celiac Lac: 2.75 ± 1.71</td>
</tr>
<tr>
<td>112</td>
<td>22 H 125 Crohn (66 M and 59 F; median 56 y)</td>
<td>100 mL</td>
<td>Celiac AGA+ Lac: 10.18 ± 3.82†</td>
</tr>
<tr>
<td></td>
<td>61 H F 20 OB F</td>
<td>120 mL</td>
<td>Celiac AGA– Lac: 10.18 ± 3.82†</td>
</tr>
</tbody>
</table>

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.
of probes and intact proteins through the intestinal mu-
cosal.13,15

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 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 know-
ledge about the clinical situations in which intestinal ba-
dary dysfunction can be an important feature.

In this context, the aims of this review were to high-
light 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 se-
arch for articles accomplishing the following terms
(alone or associated): intestinal or gut permeability, in-
testinal or gut barrier, lactulose, mannitol, tight junc-
tions. Review and original articles were selected and
read critically.

Factors underlying increased intestinal
permeability

The intestinal epithelium is a single layer of column-
ar 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 para-
cellular (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 desmoso-
mes. 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 barrier.17

Tight junctions are complex structures comprising
over 50 types of proteins (claudin, occludin, zonulin,
junctional adhesion molecules). They form a con-
tinuous, circumferential seal around cells through the
interaction with the perijunctional acto-myosin ring of
epithelial cells.18 It has been observed that TJ have a
central role in processes that regulate epithelial prolife-
ration and differentiation.18

Regulation of the assembly, disassembly and main-
tenance 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.19,20 Ex-
perimental studies using animal and cell culture mo-
dels or human studies have shown that deregulated TJ
are the main cause of altered intestinal barrier. This al-
teration 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 hu-
mans. They are formed when epithelial cells leave the
epithelium. These gaps have the diameter of an epit-
helial cell and are devoid of cellular contents, but fi-
led with an unknown substance that maintains local
barrier function. The rate at which cells leave may ha-
ve 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.20,21

As summarized by Teshima and Meddings22 simply
measuring an increase in permeability provides no in-
formation to the physician about the mechanisms un-
derlying the abnormality. However, an understanding
of these mechanisms may prove valuable in designing
interventions.23 Thus the main causes of increased IP
that should guide the development of efficacious inter-
vention are: genetic alterations of TJ proteins, abnor-
mal microbiota, abnormal regulation of TJ function
(increased zonulin release), mucosal inflammation and
abnormal epithelial dynamics.24

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 mo-
lecular weight molecules are expected to be recovered
in urine after an oral dose.25 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
permeability, since sucrose is rapidly hydrolyzed by
sucrase-isomaltase upon entering the duodenum and
reflects absorption only in the most proximal portion
of the gut.26 Lactulose and mannitol, which are one of
the most commonly used probes, are destroyed in the cae-
cum and provide information regarding the small intes-
tinal epithelium.27 Sucrose is an artificial sweetener
with similar molecular weight of lactulose and is resis-
It is spent most of a 24 hour exposure period in the large intestine. Therefore, sucralose has been suggested as better suitable sugar for whole gut permeability assessment.

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.
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 children.30 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 hours.30 However, Akram and co-workers 31 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 collection. The reduction of the time can also be achieved by measuring the probes in blood 60-90 min post-ingestion of solution32,33. 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 permeation. It is meant to circumvent confounding factors as inter-individual variation of gastric emptying, intestinal transit and transport, blood distribution and renal clearance.

In general, the integrity of intestinal barrier function is dependent on healthy epithelial cells and on the proper functioning of the paracellular route. 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 loosening.

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, acute alcohol ingestion, psychological and physical stressful situations 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, smoking 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 study.

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

### Table IV

Frequently used probed for assessment of intestinal permeability

<table>
<thead>
<tr>
<th>Lower molecular weight (Molecular weight &lt; 200 Da)</th>
<th>Higher molecular weight (Molecular weight &gt; 300 Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-mannitol</td>
<td>Lactose</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>Lactose</td>
</tr>
<tr>
<td>L-arabinose</td>
<td>Sucrose</td>
</tr>
<tr>
<td></td>
<td>Cellulbiose</td>
</tr>
<tr>
<td></td>
<td>Sucralose</td>
</tr>
<tr>
<td></td>
<td>PEGs (polyethylene glycols)</td>
</tr>
<tr>
<td></td>
<td>Raffinose</td>
</tr>
<tr>
<td></td>
<td>aCrEDTA (51)Cr-labelled ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td></td>
<td>aTc-DTPA (99mTc diethylenetriamine pentaacetate)</td>
</tr>
<tr>
<td></td>
<td>Iohexol</td>
</tr>
<tr>
<td></td>
<td>Other contrast media (iodixanol, etc.)</td>
</tr>
</tbody>
</table>

Source: Travis and Menzies, Frias et al. and Andersen et al.

### Table V

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

<table>
<thead>
<tr>
<th>% Lactulose excretion</th>
<th>% Mannitol excretion</th>
<th>Lactulose/Mannitol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactulose excreted (mg) = mg/L lactulose × L urine</td>
<td>Mannitol excreted (mg) = mg/L mannitol × L urine</td>
<td>L/M = % of lactulose excretion / % of mannitol excretion</td>
</tr>
<tr>
<td>% of lactulose excretion = (mg lactulose excreted/ mg lactulose consumed) × 100</td>
<td>% of mannitol excretion = (mg mannitol excreted/ mg mannitol consumed) × 100</td>
<td></td>
</tr>
</tbody>
</table>

Intestinal permeability measurements Nutr Hosp. 2014;29(2):269-281
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)\textsuperscript{66}. Lactulose 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\textsuperscript{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 lactulose\textsuperscript{1}. 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\textsuperscript{13,32,50}. 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-workers\textsuperscript{52} 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 test\textsuperscript{52}.

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 cautiously\textsuperscript{52}.

Osmolarity of test solutions should be mentioned in every study, since stress induced by high osmolarity can stimulate intestinal motility\textsuperscript{8} and change the rate of sugars permeation\textsuperscript{1}. 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\textsuperscript{90,91}. 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 drugs\textsuperscript{15}. 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/effenter balance and higher glomerular pressure\textsuperscript{9}. 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 loss\textsuperscript{37}. 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\textsuperscript{90,95}. 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/l\textsuperscript{94}. A decline in

### Table VI

<table>
<thead>
<tr>
<th>% Lactulose (4-O-b-D-galactopyranosyl-D-fructose)</th>
<th>Mannitol</th>
<th>Sucralose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prebiotic food additive (infant formulas and healthy foods)\textsuperscript{11}. 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)\textsuperscript{66}.</td>
<td>The most abundant polyol in nature. Some fungi, and brown seaweeds. Celery; Reduced-calorie sweetener and diet/light products \textsuperscript{146}.</td>
<td>Sweetener and diet/light products \textsuperscript{146}.</td>
</tr>
<tr>
<td>Possible dietary sources of the main sugar probes (lactulose, mannitol and sucralose)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 age.

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 women. It is critical to assess the L/M ratio, as well as lactulose and mannitol recoveries separately, when interpreting test results.

Ferraris & Vinnakota 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 assessed. Circulating levels of insulin which is a hormone usually increased in obese subjects, may also influence IP. The addition of insulin in 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 permeability.

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 aspirin. 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 aspirin.

Of note is the discussion presented recently by Vojdani 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 indicates structural damage in the TJ barrier, which would in turn allow penetration of large molecules. The use of probes of higher size (polysugars of 12,000-15,000 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 TJ.

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). Plasma D-lactate had the lowest false-negative rate among C-reactive protein level and leukocyte counts to diagnose appendicitis, and acute inflammatory disorder.

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 translocation. 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 chemotherapy. Another situation in which the citrulline availability is decreased was shown to be during the course of induced endotoxemia in rats. There some studies using animal models that show an association between endotoxemia and increased IP.

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 loss.

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 intestine. 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 diseases. Since FABP are small, watersoluble cytosolic proteins, the loss of enterocyte membrane integrity will lead to release of these proteins into the circulation. 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 controls.

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 feces. Neutrophil derived proteins such as calprotectin, lactoferrin and elastase can be present in stool and also in plasma as a marker of inflammation.

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. Proteomic analyses characterized zonulin as pre-haptoglobin-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-2. 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.

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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