



Alimentary Tract

Intestinal permeability in patients with adverse reactions to food

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Abstract

Background. An abnormal intestinal permeability could contribute to establish an altered sensitivity to food-allergen.

Aim. To evaluate the intestinal permeability in subjects with adverse reactions to food on allergen-free diet.

Subjects. Twenty-one patients with food allergy and 20 with food hypersensitivity on allergen-free diet were enrolled and divided in four groups according to the seriousness of their referred clinical symptoms when they were on a free diet.

Methods. Intestinal permeability was evaluated by Lactulose/Mannitol ratio urinary detection determined by anion-exchange chromatography.

Results. Statistically significant different Lactulose/Mannitol ratio was evidenced in subjects with food allergy ($p = 0.003$) or hypersensitivity ($p = 0.0008$) compared to control patients. The correlation between Lactulose/Mannitol ratio and the seriousness of clinical symptoms, by using Spearman test, was statistically significant for food allergy ($p = 0.0195$) and hypersensitivity ($p = 0.005$) patients.

Conclusions. The present data demonstrate that impaired intestinal permeability, measured in our conditions, is present in all subjects with adverse reactions to food. In addition, for the first time, we report a statistically significant association between the severity of referred clinical symptoms and the increasing of Intestinal Permeability Index. These data reveal that intestinal permeability is not strictly dependent on IgE-mediated processes but could better be related to other mechanisms involved in early food sensitisation, as breast-feeding, or microbial environment that influence the development of oral tolerance in early infancy.

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1. Introduction

Intestinal permeability (I.P.) is the mucosal capacity to allow the passage of molecules from the intestinal lumen to the blood stream. Recent laboratory techniques enable to evaluate the I.P. through urinary detection of sugars probes, administrated in couples that passively cross the intestinal epithelium. Recent studies suggest the use of Lactulose (La) and Mannitol (Ma) as probes; indeed these sugars cross the mucosal epithelium, are recovered in the urine and the ratio

of their clearance is used as I.P. assessment [1,2]. Recently, it has demonstrated that the use of a highly dedicated chromatographic device permits to measure appropriately the presence of La and Ma in the urine, providing a good methodology to explore the I.P. in normal and pathological conditions [3]. Thanking to the availability of these technologies, many studies have been performed to determine the I.P. in diseases that involve the gastrointestinal tract, in which the gut integrity is altered, such as Crohn's disease [4,5] and coeliac disease [6,7]. In addition, our previous data on newborns [8] and researches on animal models [9–11] have demonstrated that, during the first months of life, when the intestinal mucosa is still immature, there is an incomplete gut integrity that

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allows the passage of large molecules to blood. Such passage of macromolecules in this phase of life could impair the early mechanisms involved in the physiological establishment of the oral tolerance, predisposing to food sensitisation.

Adverse reactions to food are divided into IgE-mediated (food allergy, FA) and non-IgE-mediated (food hypersensitivity, FH) reactions. FA is correlated to the production of food specific IgE antibodies. These antibodies, binding to high affinity FcεRI receptors on mast cells and basophiles, cause histamine release and correlated allergic symptoms. Also in FH, which in some cases clinically mimic the IgE-dependent syndromes, the histamine release occurs, even though it is not dependent on IgE binding to specific receptors on mast cells [12].

In literature, many works on I.P. in FA patients are available. In a paper, focusing on I.P. evaluation in subjects with IgE-mediated reactions [13], the authors have shown an increased I.P., compared to healthy controls, when the patients were on allergen-free diet, that augments more after food allergens ingestion. In the same patients the contemporary administration of sodium chromoglycate prevented such increase. On the contrary, very few and disaccorded data in FH patients can be found [14–16].

The lack of defined data on the I.P. in patients with FH and the absence, to our knowledge, of studies that compare the seriousness of clinical symptoms referred by the patients on a free diet with the I.P. levels, prompted us to perform the present study.

2. Patients and methods

2.1. Subjects

In this study we included 41 patients with adverse food reaction. Pregnant women and patients with gastrointestinal, immunologic, metabolic and/or neoplastic diseases were excluded from the protocol. As controls, 41 healthy volunteers, matched for age and gender, selected from the medical

staff (10 men and 31 women, mean age 29, range 21–50), with no symptoms or signs of gastrointestinal disease for at least two weeks, were included. They had no FA, cardiovascular and respiratory insufficiencies, liver cirrhosis, signs of malnutrition or rheumatic disease. None of the enrolled subjects had undergone recent gastrointestinal surgery, smoked more than two cigarettes per day, drunk more than 20 g/day of alcohol, and were not receiving corticosteroids from at least two months and antihistamines from at least two weeks, at the enrolment in this study. All the participants provided their written informed consent.

The study protocol was approved by the Ethical Committee of the Policlinico Hospital, University of Bari, Italy. When the analyses were carried out, all patients were on an allergen free diet since six months. Moreover, the medical history of adverse reactions, the correlation with the consumption of a specific food, the quantity of food ingested, and the gap between ingestion and symptoms' onset were examined.

As stated by the European Academy of Allergology and Clinical Immunology position paper on FA, the diagnosis of FA should be confirmed by positive skin prick tests (SPT) [17], radioallergosorbent tests (RAST) [18] and double-blind placebo-controlled food challenges (DBPCFCs) [19–21]. According to the revised nomenclature for allergy, FH is defined as a reaction with appearance of symptoms at a dose tolerated by normal subjects, without an IgE-mediated mechanism and, consequently, with negative SPT and RAST [22], positive to DBPCFCs.

On the basis of these diagnostic tools, we divided all patients into two classes: 21 patients with FA and 20 patients with FH.

All patients with FA exhibited SPT positive reaction and for 10 subjects (48%), the diagnosis was further confirmed by RAST positivity. Concerning patients with FH, with negative SPT and RAST, the DBPCFCs confirmed the diagnosis on a clinical basis for at least one of the suspected food. In the control group, SPT and RAST resulted negative.

Table 1 shows the number of patients reacting to specific foods at DBPCFCs for each group. Hazelnut, Peanuts, Celery

Table 1
Reactivity of patients (FA and FH) to specific foods determined by DBPCFCs as reported in Section 2

	Patients with FA <i>n</i> = 21		Patients with FH <i>n</i> = 20	
	Patients reacting to the specific food	Percentage	Patients reacting to the specific food	Percentage
Hazelnut	15	71.4	2	10
Peanuts	13	62	1	5
Celery	9	42.8	2	10
Soy	8	38	0	0
Codfish	7	33.3	2	10
Fruit (banana, peach)	7	33.3	8	40
Flour	6	28.6	2	10
Rice	6	28.6	0	0
Maize	5	23.8	0	0
Tomato	3	14.3	4	20
Milk	1	4.8	5	25
Egg white	1	4.8	0	0

Table 2
Characteristics of the patients

	FH	FA
Number of patients	20	21
Mean age (years) (range)	29 (14–53)	29 (16–50)
Male/female	4/16	6/15
Clinical symptoms		
Grade 1: pruritus	4 (20%)	2 (9.5%)
Grade 2: pruritus and urticaria and/or rhinitis	7 (35%)	5 (23.8%)
Grade 3: pruritus, generalized urticaria and diarrhoea and/or asthma	7 (35%)	8 (38.1%)
Grade 4: angioedema and/or anaphylaxis	2 (10%)	6 (28.6%)

Both groups (FH and FA) are homogeneously distributed for gender, age and severity of clinical symptoms as referred at the enrolment in the study.

and Soy are the most involved foods for patients with IgE-mediated reactions, instead fruits (peach, banana), milk and tomato are the most involved for patients with FH.

Both groups of patients (FA and FH) were divided in four subgroups according to the intensity of their referred clinical manifestations, onset when the subjects were not on allergen free-diet and graded as follows:

Grade 1 pruritus

Grade 2 pruritus and urticaria and/or rhinitis

Grade 3 pruritus, generalized urticaria and diarrhoea and/or asthma

Grade 4 angioedema and/or anaphylaxis.

Table 2 summarizes patients characteristics.

2.2. Allergological tests

2.2.1. SPT

A positive control (histamine, 10 mg/l) and a negative control (glycerinated saline) with a standard panel of food allergens (hazelnut, peanuts, celery, soy, codfish, fruits: banana, peach, flour, rice, maize, tomato, milk, egg white) (Bayer, Milan, Italy) were tested and interpreted according to the European Academy of Allergology and Clinical Immunology-Subcommittee on Allergen Standardization and Skin Tests [17]. If the diameter of the reaction was more than 3 mm, bigger than that provoked by the negative control, the response was considered positive.

2.2.2. RAST

Specific serum IgE antibodies to food allergens (hazelnut, peanuts, celery, soy, codfish, fruits (banana, peach), flour, rice, maize, tomato, milk, egg white) were measured according to the manufacturer's instructions as well as the CAP System (Pharmacia, Uppsala, Sweden) [18]. Results are reported in kU/l and values ≥ 0.35 kU/l were considered positive.

2.2.3. DBPCFCs

The DBPCFCs represent the gold standard for the diagnosis of FA and FH [23]. The test is administered in the

fasting state using the most common food allergens (Lofarma S.p.A., Milan, Italy) in our population and placebo. The dose of food is doubled every 30 min. If the challenge is negative it is necessary to carry out an open feeding under observation to exclude the possibility of a false-negative challenge. It is important to eliminate suspected foods for 7–14 day before challenge.

2.3. I.P. test

All subjects were fasted overnight and, between 9 am and 10 am, they ingested 200 ml of tap water containing 2 g of Ma and 5 g of La. Urine samples were collected before ingestion of the probes in order to ensure the absence of endogenous sugars. After ingestion of the markers, urine was collected for a period of 5 h. The volume of urine collected was measured and 5 ml samples were stored at -80°C until the chromatographic analysis of the sugars was performed as described by Generoso et al. [3]. Briefly, anion-exchange chromatography coupled with pulsed amperometric detection was performed on a Dionex Model DX-500 with an eluent degassing device module, a gradient pump module GP 40 an ED 40 detector with a gold working electrode (DIONEX Corp., Sunnyvale, CA, USA). Sample injection was via an auto sampler module AS-50 Dionex equipped with a 25- μl peek sample loop with a rheodyne valve. Carbohydrate separation was carried out by a Carbowac PA-100 pellicular anion-exchange resin connected to a Carbowac PA-100 guard column at room temperature. The calibration curve for each sugar was linear to 40 mM as previously described [3]. For each sample the percentage of ingested La and Ma in urine was evaluated and their ratio (La/Ma) was calculated. Each La/Ma value is expressed multiplied by 100 (detected value $\times 100$). Each sample was analysed three times.

2.4. Statistical analysis

Student's *t*-test for unpaired values was used to compare the data obtained from control subjects, FA and FH patients. The correlation between the seriousness of the symptoms and the I.P. (La/Ma) was evaluated by Spearman test. A $p < 0.05$ was considered statistically significant.

3. Results

The analysis of the urinary recovery of the two probes used to evaluate the IP in our experimental conditions shows, in normal subjects, a percentage of urinary recovery of La equal to 0.22. This datum confirms the previous data referred in literature [2,3]. Higher percentages of La recovery are registered for FA and FH patients (0.66 and 0.77, respectively). Also Ma urinary percentage recovery from controls reflects the one present in literature (11.80). Differing from what observed for La urinary recovery, Ma urinary percentage recovery in FA (12.56) and FH (12.28) patients

Table 3

I.P. Index in normal subjects and in patients with FA and FH

Groups (number of subjects)	La/Ma (mean $\pm$ S.D.)	<i>p</i> (vs. control)
Controls (41)	1.85 $\pm$ 0.81	
FH (20)	5.34 $\pm$ 4.26	0.0008
FA (21)	6.17 $\pm$ 6.07	0.003

I.P. Index is statistically significantly higher in FA and FH patients compared to controls.

does not show any difference, compared to control subjects value.

Table 3 reports La/Ma ratio (mean $\pm$ S.D.) in the two groups of patients. The analysis of the data shows a statistically significant increase both in FA (La/Ma = 6.17 $\pm$ 6.07; $p < 0.003$) and in FH patients (La/Ma = 5.34 $\pm$ 4.26; $p < 0.0008$), compared to controls (La/Ma = 1.85 $\pm$ 0.81).

Figs. 1 and 2 report La/Ma ratio (mean $\pm$ S.D.) detected in the FA and FH subjects divided in the four clinical subgroups as reported in Table 2.

For FA subjects (Fig. 1) the results clearly demonstrate a La/Ma ratio that progressively increases, in respect to normal subjects value, from the subgroup of patients referring pruritus after food assumption (Grade 1) to patients referring angioedema and/or anaphylaxis (Grade 4) after food assumption that mirrors the severity of clinical symptoms.

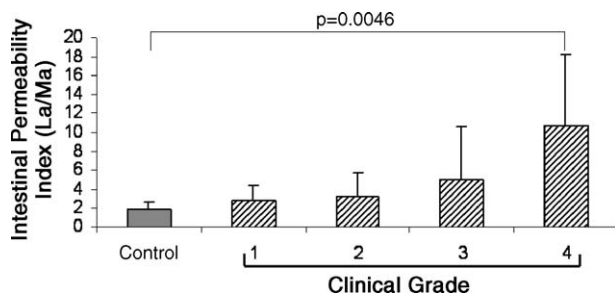


Fig. 1. I.P. Index of FA patients assembled as considered in Table 2. A gradual increase of La/Ma with the increase of severity of clinical symptoms is evident, that becomes statistically different, compared to controls, in Grade 4 patients ($p = 0.0046$).

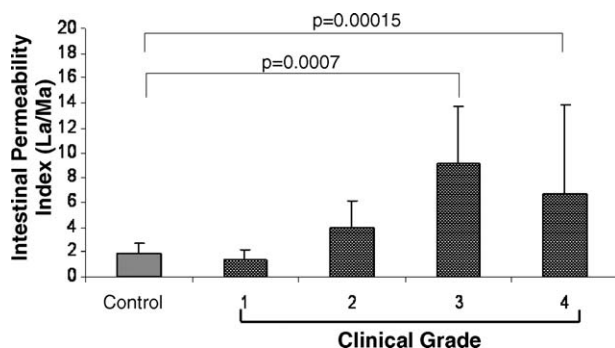


Fig. 2. I.P. Index of FH patients assembled as considered in Table 2. A gradual increase of La/Ma with the increase of severity of clinical symptoms is evident, that becomes statistically different, compared to controls, in Grade 3 ($p = 0.0007$) and Grade 4 patients ($p = 0.00015$).

This variation becomes statistically different ($p = 0.0046$), compared to control group value (La/Ma = 1.85 $\pm$ 0.81), for the subjects of Grade 4 (La/Ma = 10.71 $\pm$ 7.59).

For FH subjects (Fig. 2) La/Ma ratio (mean $\pm$ S.D.) analysis shows the same trend observed in FA patients. Particularly, the increase of I.P. Index becomes statistically significant for Grade 3 patients (referring pruritus, generalized urticaria and diarrhoea and/or asthma after food assumption; La/Ma = 9.07 $\pm$ 4.66) and Grade 4 patients (referring angioedema and/or anaphylaxis after food assumption; La/Ma = 6.70 $\pm$ 7.20), compared to control subjects ($p = 0.0007$ and $p = 0.00015$, respectively).

The rank correlation test for non-parametric variables (Spearman test), applied to our data arranged as in Figs. 1 and 2, shows a significant correlation between the severity of clinical symptoms and the I.P. Index for both groups of subjects ($\rho = 0.797$ with $p = 0.0005$ in FH; $\rho = 0.522$ with $p = 0.0195$ in FA).

4. Discussion

The data reported in the present work clearly show a similar increase of I.P. in all the subjects studied, considering them as a whole group, regardless of the type of immunogenic reaction (IgE- or non-IgE-mediated). Indeed, in all patients with adverse reactions to food, we registered an increased La/Ma ratio, compared to normal subjects, depending on an increased La urinary recovery, since no variation of Ma urinary recovery was recorded.

It is important to consider that the I.P. test was performed when the patients had been on an allergen-free diet for at least six months. No other similar evaluation has been published in literature. Moreover, the proven correlation between the I.P. value and the clinical symptoms reported by patients, determined by the Spearman test, suggests that the documented alterations in mucosal permeability could be a secondary phenomenon caused by antigen hypersensitivity in the intestinal mucosa.

Our hypothesis is that the intestinal condition of these patients could be independent of the aetiology of their disease, and could be the consequence of previous alterations of the gastrointestinal barrier, probably occurring in the first stages of development (foetal and neonatal). It has been recently reported that sensitisation to food protein may occur during lactation and is not limited to formula-fed infants [24], but may also be attributable to the proteins that are present, in small quantities, in breast milk and may reach the infant's intestinal lumen and alter the normal development of oral tolerance. In addition, previous works in both animal and human models demonstrated that during the first months of life the mucosal barrier is particularly immature, allowing a high passage of macromolecules through the intestinal barrier. It is conceivable that early events, physiological or not (exposure to allergens or pathogens, inflammation, etc.), during this phase of maturation, could influence the physiological aller-

gen recognition system, impairing the oral tolerance process and predisposing to adult pathologies.

In this paper we report that I.P., evaluated by La/Ma ingestion, is altered in patients with adverse reactions to food, not only under antigen provocation, but is a clinical situation that is present in all periods of life and could permit at least molecules of La or Ma size to pass through the intestinal mucosa.

In conclusion, our paper outlines the alterations of the I.P. observed in patients with adverse food reactions. In addition, the severity of the clinical symptoms seems to be strictly correlated with the increased I.P. Index. At the same time the I.P. is not strictly dependent on IgE-mediated processes. Other mechanisms are likely involved, such as breast-feeding or the microbial environment, in influencing the development of oral tolerance.

In this light, experiments are in progress in our department in order to evaluate the I.P. in newborns whose parents or relatives have a documented FA or FH.

Conflict of interest statement

None declared.

References

- [1] Andre F, Andre C, Feknous M, Colin L, Cavagna S. Digestive permeability to different-sized molecules and to sodium cromoglycate in food allergy. *Allergy Proc* 1991;12:293–8.
- [2] Laudat A, Arnaud P, Napoly A, Brion F. The intestinal permeability test applied to the diagnosis of food allergy in paediatrics. *West Indian Med J* 1994;43:87–8.
- [3] Generoso M, De Rosa M, De Rosa R, De Magistris L, Secondulfo M, Fiandra R, et al. Cellobiose and lactulose coupled with mannitol and determined using ion-exchange chromatography with pulsed amperometric detection, are reliable probes for investigation of intestinal permeability. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;783:349–57.
- [4] Wyatt J, Vogelsang H, Hubl W, Waldhoer T, Lochs H. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* 1993;34:1437–9.
- [5] D'inca R, Di Leo V, Corrao G, Martines D, D'Odorico A, Mestriner C, et al. Intestinal permeability test as a predictor of clinical course in Crohn's disease. *Am J Gastroenterol* 1999;94:2956–60.
- [6] Vogelsang H, Genser D, Wyatt J, Lochs H, Ferenci P, Gradiš G, et al. Screening for celiac disease: A prospective study on the value of non invasive tests. *Am J Gastroenterol* 1995;90:394–8.
- [7] Read NW, Krejs GJ, Read MG, Santa Ana CA, Morawski SG, Fordtran JS. Chronic diarrhea of unknown origin. *Gastroenterology* 1980;78:264–71.
- [8] Castellaneta SP, Masciale A, Zaccaro A, Straziuso S, Miniello V, Gatti F, et al. Intestinal permeability in healthy breast-fed infants, during the postnatal period. *J Pediatr Gastroenterol Nutr* 2005;40:632.
- [9] Strobel S, Mowat AM. Immune response to dietary antigens: oral tolerance. *Immunol Today* 1998;19:173–81.
- [10] Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology* 1993;104:622–39.
- [11] Paganelli R, Levinsky RJ, Brostoff J, Wraith DG. Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption. *Lancet* 1979;16:1270–2.
- [12] Yu LCH, Perdue MH. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol Rev* 2001;179:61–73.
- [13] André C, André F, Colin N, Cavagna S. Measurement of intestinal permeability to mannitol and lactulose as a means of diagnosing food allergy and evaluating therapeutic effectiveness of sodium cromoglycate. *Ann Allergy* 1987;59:127–30.
- [14] Buhner S, Reese I, Kuehl F, Lochs H, Zuberbier T. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. *Allergy* 2004;59:1118–23.
- [15] Arslan G, Kahrs GE, Lind R, Froyland L, Florvaag E, Berstad A. Patients with subjective food hypersensitivity: the value of analyzing intestinal permeability and inflammation markers in gut lavage fluid. *Digestion* 2004;70:26–35.
- [16] Rutgers HC, Batt RM, Hall EJ, Sorensen SH, Proud FJ. Intestinal permeability testing in dogs with diet-responsive intestinal disease. *J Small Anim Pract* 1995;36:295–301.
- [17] European Academy of Allergology and Clinical Immunology. Skin Tests used in type I allergy testing. Position paper. *Allergy* 1989;44:1–59.
- [18] Paganelli R, Ansotegui IJ, Sastre J, Lange CE, Roovers MH, de Groot H, et al. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, Uni-CAP, in six European allergy clinics. *Allergy* 1998;53:763.
- [19] Bernstein M, Day J, Welsh A. Double-blind food challenge in the diagnosis of food sensitivity in adult. *J Allergy Clin Immunol* 1982;70:205–10.
- [20] Sampson HA. Immunologically mediated food allergy: the importance of food challenge procedures. *Ann Allergy* 1988;60:262–9.
- [21] Bindslev-Jensen C. Standardization of double-blind, placebo-controlled food challenges. *Allergy* 2001;56:75–7.
- [22] Johansson SGO, Hourihane JO'B, Bousquet J, Brujinzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813–24.
- [23] Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004;113:805–19.
- [24] Heine RG, Hill DJ, Hosking CS. Primary prevention of atopic dermatitis in breast-fed infants: what is the evidence? *J Pediatr* 2004;144:564–7.