

ANEMIA IMPAIRS SMALL INTESTINAL ABSORPTION MEASURED BY INTESTINAL PERMEABILITY IN CHILDREN

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SUMMARY

■ **Objective:** To determine the effect of anemia and iron status on intestinal permeability in children.

n **Design:** A routine prospective study was performed in 64 children with symptoms suggesting cow's milk allergy (CMA) (11.8±16 mo, 2-94 mo). They exhibited a negative cow's milk challenge upon the ESPGHAN criteria. Hemoglobin (Hb), mean corpuscular volume (MCV), blood iron (Fe) and ferritin (Fer) concentrations were studied in all. Permeability was measured as percent of urinary excretion of lactitol (L,%) and mannitol (M,%) (oral absorption, 0.1 g/Kg for each sugar) and determination of the L/M ratio (L/M,%).

■ **Results:** L/M was significantly higher in anemic children than in non-anemic ones, 2.45, (median), 1.92-3.43 (extremes), n=29, vs. 1.72, 1.56-2.18, n=35, p=0.03. Hb correlated negatively with L/M (p=0.0001) and L (p=0.05) and positively with M (p=0.03). Also, L/M correlated negatively with MCV (p=0.001) and Fe (p=0.04).

■ **Conclusion:** IP depends on anemia and iron status. The interpretation of IP should be taken cautiously into account in the diagnosis of CMA in case of anemia or iron deficiency.

Key-words: Intestinal permeability - Iron deficiency - Anemia - Age - Cow's milk allergy - Children.

INTRODUCTION

Intestinal permeability (IP) may be assessed using various probes among which orally absorbed non metabolizable sugars excreted in urine. Lactitol (L) which may replace lactulose, a marker of large molecule absorption and mannitol (M), a marker of small molecule absorption, allow to detect alterations of IP in different intestinal pathological conditions (1-4). In children (3), IP was shown to be a good tool for diagnosis of food allergy. In hypersensitive subjects, ingestion of the offending food decreased M urinary excretion and increased both L urinary excretion and L/M urinary ratio (3). Cow's milk allergy (CMA) modified IP during both digestive and cutaneous manifestations (3).

On the other hand, both age (4) and iron deficiency can induce in otherwise healthy infants (5) reversible alterations of IP, that might interfere with modifications of IP during pathological states. The aim of the study was to analyze to what extent anemia and iron deficiency may alter IP in children.

A routine prospective study was performed in 64 children (11.8±16 mo, 2-94 mo) with clinical manifestations

suggesting CMA: gastro-esophageal reflux (GER) (n=17, 26.5%), failure to thrive (22, 34.3%), chronic diarrhea (5, 7.8%), eczema (5, 7.8%), recurrent abdominal pain (3, 4.6%), recurrent bronchitis (3), recurrent ear-nose-throat (ENT) infections (2, 3.1%), acute diarrhea (1, 1.5%), vomiting (1), constipation (1), acute life threatening events (ALTE) and anaphylactic choc (1). All enrolled children exhibited no cow's milk allergy (CMA), as defined according to the clinical ESPGHAN criteria (6). Final diagnosis in those children was GER (n=18), failure to thrive (n=14), eczema (n=5), irritable bowel syndrome (n=14), allergic asthma (n=2), others (2 *Helicobacter pylori* gastric infections, 2 lactose intolerance, 2 diet errors, 1 allergic urticaria, 1 laryngomalacia, 1 pyloric stenosis, 1 systemic infection and 1 vagal hypertonia).

A blood cell count was performed in all, including the measurement of Hb and of the mean corpuscular volume (MCV). Anemia was defined as Hb<10 g/dl (<6 mo), <11 g/dl (6 mo-6 yr), <12 g/dl (>6 yr). Microcytosis was defined as MCV (ft) <77 (0-3 mo), <74 (3-6 mo), <70 (6 mo-2 yr), <75 (2-6 yr), <77 (6-12 yr), <78 (12-18 yr). Iron status was further explored by assaying blood iron (Fe) and ferritin (Fer) concentrations. Iron deficiency was defined as Fe <10 mM/l, and ferritin deficiency as Fer <12 mg/l.

Children with food allergy, cystic fibrosis, inflammatory bowel disease, celiac disease, digestive surgery,

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small-bowel bacterial overgrowth, parasitic or infectious gastroenteritis and diabetes mellitus were excluded.

IP was analyzed as already described (3). Briefly, on the morning following a 6h overnight fast, children were given a mixture of a 10% M and L aqueous solution at a dosage of 0.1 g/Kg of body weight for each sugar. Urine collection was maintained for the 5 following hours, using a specific adhesive urine bag in younger children. The urine volume was measured, and aliquots stored frozen.

Gas chromatography was used for urinary analysis. The results of the IP was measured as percent of urinary excretion of L (L,%) and M (M,%) and then determination of the L/M ratio (L/M,%). All studies were done with the informed consent of parents.

Calculation of mean, median, SD and extremes of all quantitative parameters were done using the StatView System (Abacus®, CA, USA). Since all the parameters exhibited large distribution results (not belonging to the normal law), statistical evaluation of those results was made using non parametric tests as the Anova test for continuous variables. Correlation between different parameters was assessed using the simple regression correlation. A *p* value < 0.05 was considered statistically significant.

Correlation between L, %, M, %, L/M, % ratios and Fe, Fer, Hb and MCV, in anemic and non anemic children are summarized in table I. Considering the entire population, (n=64), Hb correlated negatively with L/M (*r*:-0.53, *p*=0.0001) and L (*r*:-0.24, *p*=0.05) and positively with M (*r*:0.25, *p*=0.03), table I. L/M was higher in children with Fer deficiency (3.52, 3.01-3.85, n=49, vs. 1.95, 1.72-2.05, n=15, *p*=0.003) and correlated negatively with MCV (*r*:-0.38, *p*=0.001) and Fe (*r*:-0.24, *p*=0.04).

Among the 64 enrolled children, there were 29 anemic children and 34 non anemic. The L/M ratio was higher in anemic children, n=29, 2.45 (median), 1.92-3.43 (extremes), than in non anemic ones, n=35, 1.72, 1.56-2.18, *p*=0.03.

The present study confirms that IP varies according to anemia and iron status and that the L/M ratio is raised in anemic as compared to non anemic children. The role of iron deficiency in mucosal intestinal structural abnormalities was suggested in the alterations of IP already described by Berant et al (5). We confirm an inverse correlation between Fe and L/M ratio, found by Goto et al (4) and also show an inverse correlation of L/M with Hb and MCV, thus further assessing that adequate iron stores are needed to maintain normal IP (4).

The reason why iron deficiency may alter IP remains difficult to assess. Iron deficiency in infants alters small intestinal function and impairs absorption of fat, vitamin A and xylose (5), through a mechanism that was envisioned as a decreased synthesis of brush border enzymes (5). Lactitol and mannitol absorption does not depend on brush border enzymes. Permeability probably reflects both the ability of the epithelium to be crossed by molecules and the ability of the blood flow to wash out the molecules accumulating in the subepithelial medium (7). Therefore, the L/M ratio could depend both on a normal functioning of villus epithelial cells in metabolite absorption and on a normal villus blood flow (8), at least partially responsible for the water transepithelial passage or solvent drag. Considering the role of Hb and Fe in the blood flow supply in normal villus epithelial cells, one may postulate that in case of iron deficiency anemia, alterations in the vascular counter-current lead to a decrease in the solvent drag and secondly in the passage of mannitol and of other micro-nutrients. The role of iron deficiency, even if

	Fe	Fer	Hb	MCV	
L, %	NS	NS	<i>r</i> : -0.24, <i>p</i> =0.05	NS	All the enrolled children n=64
M, %	NS	NS	<i>r</i> : 0.25, <i>p</i> =0.03	NS	
L/M, %	<i>r</i> : -0.24, <i>p</i> =0.04	NS	<i>r</i> : -0.53, <i>p</i> =0.0001	<i>r</i> : -0.38, <i>p</i> =0.001	
L, %	NS	NS	NS	NS	All children with anemia: n=29
M, %	NS	NS	NS	NS	
L/M, %	NS	NS	<i>r</i> : -0.41, <i>p</i> =0.007	NS	
L, %	NS	<i>r</i> : 0.47, <i>p</i> =0.0041	NS	<i>r</i> : 0.30, <i>p</i> =0.01	All children without anemia: n=35
M, %	<i>r</i> : 0.28, <i>p</i> =0.02	NS	NS	NS	
L/M, %	NS	NS	NS	NS	

Table 1: Correlation between L, %, M, %, L/M% and Fe, Fer, Hb & MCV. Correlation is significant, *p*<0.05.

anemia is not present, might be similar, leading to limited alterations of permeability parameters.

The role of CMA in disrupting IP was already described by us (3) and by others (4). The reason why CMA alters IP has already been widely discussed and might imply the local release, following the immunologic conflict, of cytokines able to disrupt the intestinal wall, such as gamma interferon and TNF alpha (9). Our data show that these alterations of IP are sufficient to disrupt almost all the correlations observed between iron status and permeability in control children.

Iron deficiency is a feature of CMA (10). We have shown that children with CMA exhibit iron deficiency in 28 % of cases despite normal daily iron intakes (11). Anemia obviously limits the reliability of IP tests. Our data imply that in the purpose of using permeability for diagnosis of CMA, analysis criteria might first require the verification of normal iron status parameters.

In conclusion, IP depends on anemia and iron status and the interpretation of IP should be taken cautiously into account in case of iron deficiency or anemia.

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