Small intestinal brush border enzymes in cystic fibrosis

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Abstract

The study concerns the maltase, saccharase, lactase and alkaline phosphatase activity in small intestinal biopsy specimens from 61 consecutively admitted, untreated, Caucasian cystic fibrosis patients. A group of 319 age matched controls admitted during the same time period for undefined gastrointestinal or nutritional disorders acted as the controls.

In order to eliminate morphological damage as a confounding factor, the enzyme activities were studied in small intestinal biopsy specimens having both normal stereomicroscopic and histological features. It was shown that neither maltase nor saccharase activity was different in the two groups, in contrast to lactase and alkaline phophatase activity, that was significantly lower in cystic fibrosis patients. The differences could not be explained by the nutritional status as judged by the body mass index.

Lactase activity is known to be easily affected by numerous enteropathies. As the information on alkaline phosphatase activity is limited, the low activity is discussed in more detail. Taking into account the literature data, the low alkaline phophatase activity is tentatively attributed either to enhanced release from the brush border or to the faulty handling of alkaline phophatase protein in the post-golgi compartments secondary to the accumulation of incorrectly glycosylated CFTR in the same cell structures. (Acta gastroenterol. belg., **1999**, 62, **267-271**).

Key words : cystic fibrosis, intestinal alkaline phosphatase, small intestinal brush border enzymes.

Up to now, the information on the small intestinal enzyme activities in cystic fibrosis (CF) is limited and controversial. Antonowicz found increased disaccharidase activities and speculated that the decreased output of pancreatic enzymes in CF caused a decreased degradation of the small intestinal brush border enzymes (1,2). On the contrary, isolated lactase deficiency, as well as partial enzyme depletion of glycosidases and alkaline phosphatases, but not of enterokinase, have been observed in CF (3,4,5). These data prompted us to study the enzymatic activities in small intestinal biopsy specimens in CF.

Patients

61 Untreated Caucasian CF patients entered the study at the time of initial diagnostic work up.There were 26 boys and 35 girls with a median age of 16 months, ranging from 1 month to 14 years. The diagnosis of CF was made on clinical signs and symptoms and 3 positive sweat tests (Gibson-Cooke method), defined by a chloride concentration of > 60 mEq/L. The genotype was defined in 24 patients.

As intestinal biopsy sampling in normal control subjects is ethically not allowed, 319 age matched subjects served as the concurrent control group. The control subjects underwent an intestinal biopsy because of failure to thrive or unspecific gastrointestinal complaints. In none of the controls a diagnosis of well defined digestive or resorptive disorder was made.

Informed consent was obtained from the parents of patients and controls.

Methods

At the time of diagnosis, duodenal juice with alkaline pH, was obtained by use of the open paediatric Crosby capsule placed at the angle of Treitz. At the end of the collection a biopsy specimen was aspirated from the intestinal mucosa.

After collection of the specimen, stereomicroscopic examination was performed, allowing classification of the mucosal biopsies into 3 types according to Shmerling (6) : type-I : normal mucosa, type-II : partial mucosal atrophy and type-III : total atrophy.

A fragment was taken for classical histology and the remaining tissue was homogenised, after wet weighing, for determination of enzymatic activities. The glycosidases were determined according to Eggermont (7), and the intestinal alkaline phosphatases according to Garen and Levinthal (8). Lumenal chymotrypsin was determined by the Boehringer Mannheim Monotest Chymotrypsin (9) and amylase by using the Bio-Mérieux α-Amylase PNP-kit (10). Activities were respectively expressed in international units as mMoles of substrate hydrolysed per minute and gram of wet tissue or μMoles of substrate hydrolysed per minute and mL of juice.

For each patient or control subject, the quotient of the body mass index (BMI) to the 50th centile value of the BMI of a normal population, matched for gender and age, was used to compare the nutritional status of CF patients and control subjects (11).

Computer analysis of the data was performed with a Macintosh Computer using Excell-4.0®, Microsoft and Statview IV®, Abacus. A standard non-parametric statistical method was used ; the Mann-Whitney U test, to know whether the studied groups have been drawn

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from the same or a different population and the Spearman rank correlation coëfficient.

Results

Nutritional status. The height or length of CF patients and control subjects had a normal distribution around the 50th centile value of age- and gender matched normal children. Their weight distributions, however, were shifted towards the 25th centile values of the normal population (data not shown).

As shown in fig. 1 the quotients of BMI values of CF as well as control subjects to the BMI of a normal population matched for gender and age were below 1 and not significantly different from each other.

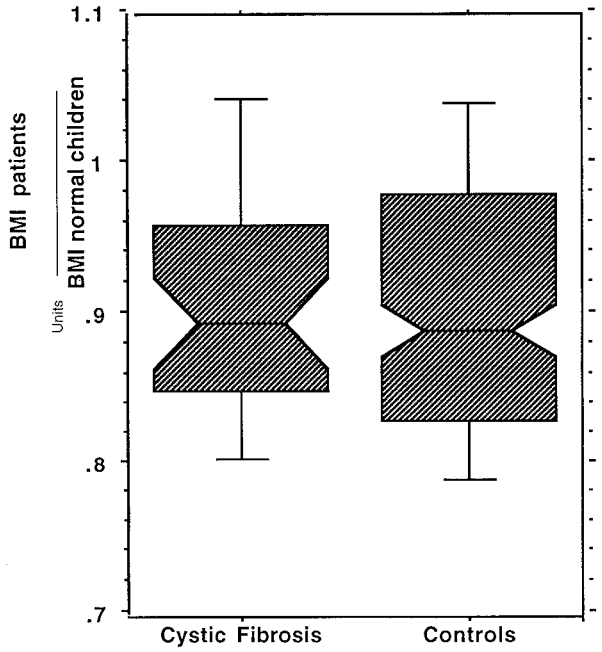

Fig. 1. — Box plots of the Quotient of body mass index (BMI) in cystic fibrosis patients (CF) and control subjects by the 50th centile value of BMI in normal children matched for age and gender.

Genotypes. Of the 24 CF patients in whom the genotype was determined, 16 (66%) were homozygous for the Δ F-508 mutation. A compound heterozygocity was noticed in the 8 (34%) others. In this small group we were unable to detect genotype-phenotype relations for the clinical condition or the enzyme activities, studied.

Pancreatic function. Although basal pancreatic enzyme activities in the duodenal juice are not an unequivocal indicator of pancreatic function, the chymotrypsin activity was significantly lower in the CF patients compared to the controls ($p < 0{,}0001$). The median and the (p25-p75) values (IU/mL) in the CF and the control subjects being 0,6 (01-1,9) and 15 (8,4-27) respectively. In agreement with the known low basal lumenal amylase activity in normal infants aged less than 6 months (12), a significantly lower basal lumenal amylase activity ($p < 0{,}01$) was only found in CF children aged 6 months or more (fig. 2).

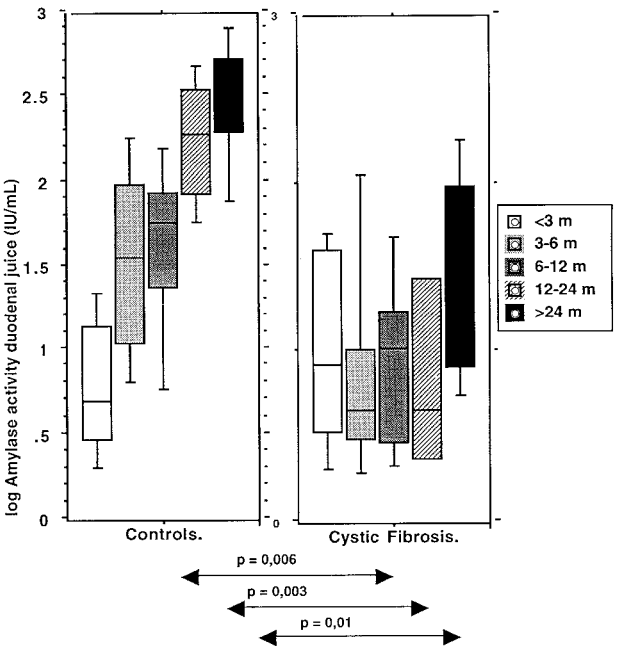

Fig. 2. — Duodenal amylase activity shown by age categories in months. From the age of 6 months on, significant differences are observed between cystic fibrosis patients and controls (M-W U-test, tied *p* values).

Mucosal morphology. On stereomicroscopic examination 68.9% (n = 42) of the CF patients displayed a normal mucosa described as type-I by Shmerling (6), 29,5% (n = 18) the Shmerling type-II and 1.6% (n = 1) the Shmerling type-III. In the controls 64,5% (n = 205) had the Shmerling type-I and 35.5% (n = 114) the Shmerling type-II mucosa. On histological examination, all patients with the Shmerling type-I mucosa presented normal microscopic findings. The patients with the type-II mucosa had microscopic abnormalities from aspecific changes to partially flattened villi. In the patient with the type-III mucosa, the microscopic examination displayed total villous atrophy. As we were interested in the effects of CFTR-mutation proteins on the alkaline phosphatase and glycosidase activity, the enzyme determinations presented in this study, both on control and CF samples, are made on stereomicroscopically normal (type-I) and histologically normal mucosae. The age distribution of the subjects with type-I mucosa is given in table I.

Table I. — **Age distribution in CF-patients and controls with normal mucosa**

Age distribution	CF-patients		Controls	
	n	%	n	%
0 - 2 Years	32	76.2	148	72.2
2 - 5 Years	5	11.9	32	15.6
> 5 Years	5	11.9	25	12.2

Small intestinal enzyme activities : Morphological damage of the small intestinal mucosa is known to result in decreased activities of the brush border enzymes (13). As we were interested in the effect of CF mutations on the brush border enzyme activities, we only report on the data obtained on structurally normal small intestinal mucosa. In the CF population with normal villi, the small intestinal maltase and saccharase activity does not differ from the controls (table II). The lactase activity on the contrary is significantly lower in the CF group ($p = 0.009$). This decrease persists if we analyse the children below the age of 2 years ($p = 0.02$). The lactase activity did not differ in CF patients having basal lumenal activities of amylase and chymotrypsin either below or above the 10th centile value of age matched controls (data not shown). Also a correlation between the nutritional status and the disaccharidase activities (maltase, saccharase and lactase) could not be demontrated (data not shown).

Table II. — **Basal lumenal amylase and chymotrypsin activities (IU/ mL duodenal juice) and small intestinal enzyme activities (IU/g wet mucosa) of cystic fibrosis patients and controls with normal mucosae. Figures are median values with p25 and p75 values between parentheses**

	Cystic fibrosis (n = 42)	Controls (n = 205)
Intralumenal enzymes		
Amylase	6 (3 - 42)	114 (32,7 - 312)
Chymotrypsin	0,6 (0,1 - 1,9)	15 (8,4 - 27)
Mucosal enzymes		
Maltase	23,5 (15,8 - 28)	24,7 (17,7 - 37,5)
Saccharase	7,1 (4,3 - 9)	7,5 (5,2 - 11)
Lactase	2,8 (1,4 - 4,7)	3,9 (2,3 - 6,7)

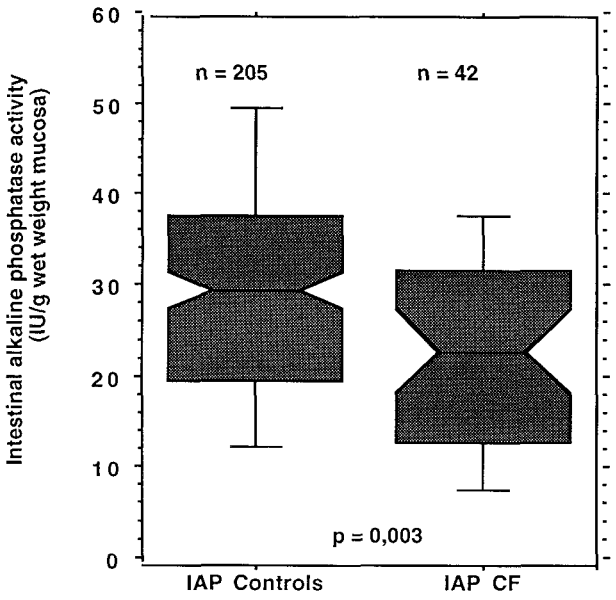

Fig. 3. — Small intestinal alkaline phosphatase activities (IAP) in controls and cystic fibrosis patients (CF).

As seen in fig. 3, the small intestinal alkaline phosphatase activity (IAP) is significantly decreased in the CF patient group ($p = 0.003$). The IAP activity is not influenced by the nutritional status (data not shown). In CF patients having a basal lumenal activity of amylase and chymotrypsin either below or above the 10th centile activity of age matched controls, the IAP activity was not different (data not shown).

Discussion

Up to now, the information on the small intestinal brush border enzymes in CF is scarce and the data are even contradictory (14). In the present study, we deal only with untreated CF patients at the time of diagnosis. Genetic studies of the cystic fibrosis transmembrane conductance regulator (CFTR) reveal the same distribution of ΔF508 as found in other studies (15,16). As a genotype-phenotype relationship for neither the clinical condition, mucosal morphology, nor the enzyme activities studied could be detected, the data on the CF patients are presented for the whole group.

Sampling small intestinal biopsy specimen from normal healty infants and children is ethically not allowed. The control group, therefore, is made of a group of patients referred for ill-defined nutritional or gastrointestinal complaints for wich no diagnosis could be found. The selection of control subjects explains their suboptimal nutritional status (fig. 1).

In the CF patients group 42 out of 61 (69%) have normal stereomicroscopic and histological features of the small intestinal mucosa. In the control group, taking into account the same criteria, 65% have normal small intestinal morphology. Antonowicz, using different histological typing methods, found a normal small intestinal morphology in 58% of their CF patients (1,2). Cox *et al.* could show that proximal small intestinal injury in CF patients correlates with increased basal acid output (17). The low pancreatic bicarbonate output in CF, further impedes the neutralisation of the acidic duodenal lumenal content (18,19) resulting in postprandial duodenal pH values as low as 5.

Pancreatic insufficiency occurs in about 90% of the CF patients (20). Ingomar and Terslev, studying the enzyme content of duodenal juice from infants and children with chronic diarrhoea concluded that neither the fasting condition nor the oral stimulation by a test meal proved to be superior for the diagnosis of pancreatic insufficiency (21). For practical reasons, we measured the pancreatic enzyme activities only in basal conditions in the duodenal juice obtained at the time of the intestinal biopsy sampling (12,13). As can be seen in table II and fig. 2, pancreatic function is significantly lower in the CF group. For amylase, because of the low normal values during the first months of life, the difference was seen from the third month of life only (fig. 2).

Small intestinal mucosa maltase and saccharase activities do not differ in the CF patients from the controls (table II).These findings are contradictory with the data of Antonowics showing increased disaccharidase activities in the CF mucosae (1,2). She attributed the higher disaccharase activities in the CF mucosae to the absence of pancreatic enzymes, normally involved in the turnover of brush border enzymes. On the other hand, in agreement with previous studies by several authors (4,5,22,23), the lactase activity is significantly decreased (Table II). The lower lactase activity in structurally normal intestinal mucosae from CF patients, can be seen as a molecular defect of the lactase enzyme. According to Alliët, Kretchmer and Lebenthal, the finding of depressed or absent lactase activity in the presence of normal alpha-glucosidases with normal intestinal morphology is strongly suggestive of primary lactase deficiency (24). Usually the decline of lactase activity begins between 2 and 3 years and is almost complete by the age of 5 to 10 years. Both in animal and human studies, the nutritional decline in lactase activity has been attributed to transcriptional and posttranscriptional mechanisms (25). Even when only the children below the age of 2 years were evaluated, the CF patients had still a significatly lower lactase activity. It is tentative to speculate that the reduced lactase activity in our CF patients is due to an interaction of the inefficiëntly processed CFTR proteïn, along its way between endoplasmatic reticulum and Golgi apparatus (26).

In contrast to the maltase and saccharase small intestinal mucosa activity but in line with the lactase activity, the intestinal alkaline phosphatase (IAP) activity is significantly lower in the CF compared to the control population (fig. 3). The difference of about 22% is due to a shift to the left of the IAP activity in the CF patients (fig. 4).

IAP is one of three isoenzymes : intestinal, placental and germ-cell alkaline phosphatase, arisen by a series of gene duplications from the ancestral tissue-nonspecific alkaline phosphatase. The genes for intestinal, placental and germ-cell alkaline phosphatase are clustered near the top of the long arm of chromosome 2 (2q34-37) and the corresponding enzymes only differ in posttranslational modifications involving carbohydrate residues (27). IAP is deeply buried in the lipid bilayer of the brush border membrane and bound to it via glycosylphosphatidylinositol (28,29). On the contrary, the sucrase-isomaltase, the maltase- glucoamylase and the lactase- phlorizinhydrolase are lollipop-like proteins anchored via a hydrophobic segment crossing the brush border membrane once (28). In addition to the alternative anchoring of IAP, the enzyme is more easily released from the brush border, both into the intestinal lumen and into the plasma (30). The release of IAP is facilitated in bloodgroup secretors and during lipid absorption but no information is available about the release in CF (31).

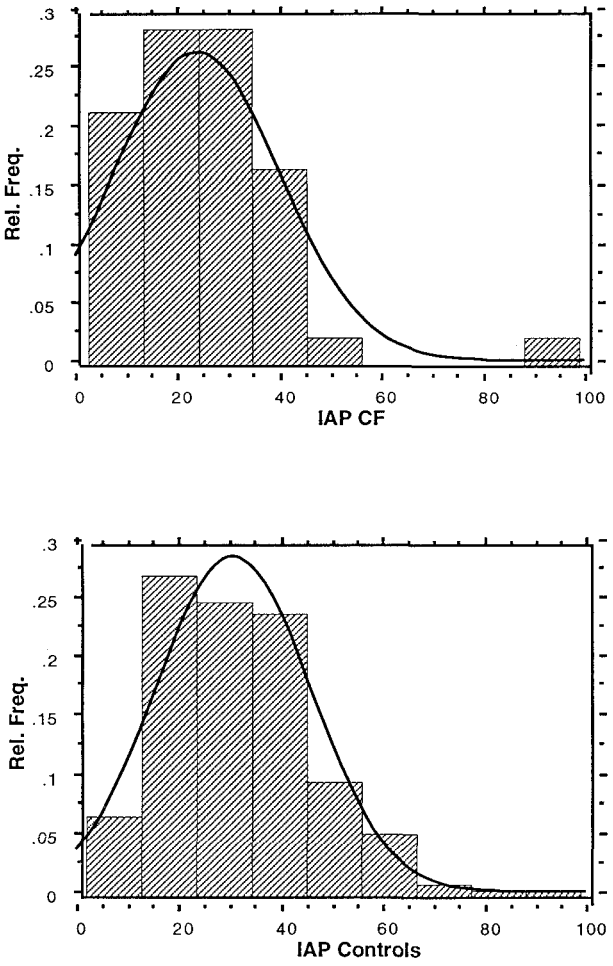

Fig. 4. — The IAP activity is significantly lower in the CF population due to a relatively higher number of CF patients with a low IAP activity.

IAP is more resistant than the disaccharidases to the action of proteases (32) but the opposite has been shown for the action by oxygen free radicals (33). Although certain CF patients have been found to be more susceptible to oxidative damage (34), there are no published data on lowered IAP in CF patients.

Finally, both the lowered IAP activity and the low lactase activity in CF mucosae could be related to the basic defect of CFTR. In a recent scanning and transmission electron study, it has been shown that in CF the absorbing cells of the villi are well preserved but that the mucus-containing sacks protude from the apical membrane of the goblet cells (35). In contrast, evident ultrastructural lesions were found in the upper portion of the crypts. in about 60% of the CF crypts examined, both secretory and immature absorbing cells show degenerative features ranging from accumulation of lysosomes in the apical portion of the cell to cytoplasmatic swelling and vacuolisation. About 70% of the known CF genes result in the absence of mature CFTR at the correct cellular location and the accumulation of incompletely glycosylated protein (26,36,37).

Therefore, it is possible that the disturbed sorting-out of maturated CFTR could influence the normal cellular processing of IAP and lactase in the enterocyte. The absence of CFTR proteins from the brush border membrane could also negatively influence both activities. Indeed, a protein-protein interaction between CFTR and the amiloride sensitive Na+ channel has been documented (38).

In conclusion, the small intestinal mucosa of CF patients shows normal stereomicroscopic and histological features in about two thirds of the patients. Enzymatic studies on morphologically normal mucosae from CF patients show normal maltase and saccharase activities but significantly decreased lactase and IAP activities. In various enteropathies, low lactase activity is a common finding. The decrease of IAP activity, however, is more surprisingly and commented in more detail. An increased release of IAP and lactase from the brush border membrane and, or a disturbed sorting-out at the Golgi-apparatus, secundary to the abnormal CFTR-proteïn handling, seem to be the most likely hypotheses for the lowered enzyme activities in CF patients.

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