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Intestinal Enzymes During Malnutrition & Infection In Rabbits

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Intestinal enzymes during malnutrition & infection in rabbits


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Background & objectives: Malnutrition plays an important role in the intestinal absorption of nutrients. However, reports are not consistent whether intestinal enzymes are decreased in the presence of malnutrition. It is also not clear whether simultaneous presence of malnutrition and infection adds to the problem of malabsorption of nutrients. The aim of the present study was to determine intestinal functions in terms of concentrations of disaccharidase enzymes during diarrhoea and protein energy malnutrition.

Methods: Concentrations of three disaccharidase enzymes, namely maltase, sucrase and lactase were measured in nine energy-restricted and five control rabbits during diarrhoea induced by rabbit diarrhoeagenic Escherichia coli (RDEC-1). Malnutrition was achieved in the rabbit model by feeding the animals for 30 days with half the amount of food fed to well-nourished control rabbits. Both the energy-restricted and the control groups were challenged by RDEC-1. Diarrhoea occurred on day 1-7 after administration of the strain. After onset of diarrhoea, both groups of rabbits were sacrificed and their intestinal mucosa was examined to determine the concentration of lactase, maltase and sucrase.

Results: The energy-restricted animals and controls did not differ significantly for concentrations (units/mg proteins) of lactase (0.65 ± 0.28 vs 0.56 ± 0.17 ), maltase (6.20 ± 2.70 vs 6.47 ± 1.90) and sucrase (5.42 ± 2.30 vs 5.13 ± 1.40) measured during acute infectious diarrhoea.

Interpretation & conclusion: The results suggested that the enzymatic functions of the intestinal brush border were not statistically different during diarrhoea among malnourished rabbits compared with their well-nourished counterparts.

Key words Diarrhoea - enzymes - malnutrition - rabbit model
Infectious diseases, coupled with malnutrition, account for 70 per cent of all childhood deaths in developing countries\(^1\). Over two million children die each year in developing countries from diarrhoeal diseases. It is well known that malnourished children are more susceptible to diarrhoea and other infections, and infection may invite malnutrition, thus making a vicious cycle\(^2,3\). However, several issues still remain unclear as to how infection precipitates malnutrition. Malnourished children may have had altered permeability because of more frequent prior infections and prolonged intestinal injury thereafter. Also, aberrations of intestinal permeability may be associated with malabsorption of nutrients, thereby causing secondary malnutrition.

The gastrointestinal tract has many characteristics that make it particularly susceptible to the effect of nutrient deprivation, for example, it is lined by highly differentiated epithelial cells with a high turnover rate, it handles large volumes of fluids and nutrients, and it must act as a barrier against intrusion of foreign molecules. Malnutrition may affect all these characteristics at the local and systemic levels\(^4\).

Several studies have observed decrease in the villus heights in malnutrition due to significant decrease in the number of cells, reduced proliferation of enterocytes, and slower migration of crypt cells along the crypt-villus axis\(^4,6\). Studies have shown a direct correlation between severity of malnutrition and magnitude of decrease in activities of lactase, sucrase and maltase\(^7\). Also, there are evidences supporting a positive correlation between villus height and mucosal disaccharidase activities\(^8-10\). However, evidences are not consistent about enzyme activities and histological changes in intestinal villi during malnutrition\(^11-17\). It is also not clear whether malnutrition is an added risk factor for malabsorption of nutrients during infection. We, therefore, evaluated intestinal enzyme functions in rabbit model with diarrhoea and malnutrition through a controlled experimental design.

**Material & Methods**

New Zealand white rabbits at the age of six weeks were used in this study. The study was conducted at International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Centre for Health and Population Research, Dhaka, Bangladesh from February to May, 2001. Appropriate facilities for animal care exist at ICDDR,B. The protocol was approved by the Centre’s Ethical Committee.

Non-fasted animals were randomly selected from different breeding stocks. A total of 18 animals were divided into two groups of nine each, designated as malnourished or energy-restricted animals (the test group) and well-nourished animals (the control group). The control group of animals was fed maintenance stuff *ad libitum* (Table). The food was prepared at the laboratory of ICDDR, B based on the

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Constituents (%)</th>
<th>Additives (quantity/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>Protein 15-17</td>
<td>Vitamin A 6000 μl</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Fat 2.5 - 3.5</td>
<td>Vitamin D3 1500 μl</td>
</tr>
<tr>
<td>Oat</td>
<td>Fibre 8.0 - 10.0</td>
<td>Vitamin E 30 mg</td>
</tr>
<tr>
<td>Green gram (whole) mug</td>
<td>Crude ash 6</td>
<td>Copper 30 mg</td>
</tr>
<tr>
<td>Gram (Chola)</td>
<td>Gross energy 4.0 kcal/g</td>
<td></td>
</tr>
<tr>
<td>Soybean meal-44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Til oil cake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molasses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: Composition of rabbit’s diet
Certified Laboratory Chows Diets, Purina Lab Chows-12T (Ralston Purina Company, St. Louis, MO, 1980), and was served in the form of pellets. Details of the diet are mentioned elsewhere\textsuperscript{18}. The energy-restricted animals were given only half the amount of the diet consumed by the control rabbits. The amount of diet to be given to the test group was determined based on an earlier study which showed that 50 per cent of the diet consumed by the control rabbits could produce significant weight loss and malnutrition in the experimental group of rabbits\textsuperscript{19}. Water was provided in a heavy bowl and changed daily. Both the groups received the scheduled diet for 30 days. Body weights of the two groups were obtained at baseline and every morning by using a weighing scale (precision ± 1 g). As expected, the test group of animals became more malnourished compared to controls. At day 30, both the groups of rabbits were challenged with rabbit diarrhoeagenic Escherichia coli (RDEC-1), which causes natural infections in rabbits\textsuperscript{20,21}. RDEC-1 was obtained from the Department of Gastroenterology, Walter Reed Army Institute of Research, Washington, D.C. Diarrhoea occurred in most cases within 1-7 days after administration of the strain.

After producing diarrhoea, the rabbits were sacrificed at different days depending on production of diarrhoea and their intestinal mucosa was scraped off with a piece of glass. The distal part of ileum mucosa was examined to determine the concentration of lactase, maltase and sucrase by methods described by Dahlqvist\textsuperscript{22}.

Statistical analysis: Data were analysed using SPSS for windows, version 14.0 (SPSS Inc., Chicago, IL). Descriptive statistics were done for major variables of interest, including composition of diet, concentrations of intestinal enzymes, and body weight. Animals in the test group and the control group were compared in terms of intestinal enzymes and changes in body weight using Student’s t-test. A probability level of .05 was considered statistically significant.

\begin{center}
\textbf{Fig.} Per cent weight change among the energy-restricted rabbits (test) and well-nourished rabbits (control) before and after infection with RDEC-1.
\end{center}
Results

After challenging with RDEC-1 infection, 4 controls died leaving 5 controls and 9 test rabbits for the entire analysis. The mean calorie intake per day was significantly higher in the control group compared to the test group of rabbits (591.7 ± 34.4 kcal vs. 294.2 ± 13.6 kcal, \( P < 0.001 \)). The control group of rabbits gained 46 per cent of body weight whereas the test group (energy-restricted) gained about 14 per cent of body weight after 30 days of controlled feeding (\( P < 0.001 \)) (Fig.). After challenge with RDEC-1 the control group lost 10 per cent of weight and the energy-restricted group lost 2 per cent of weight at the end of 42 days (\( P < 0.001 \)). The absolute body weights (mean ± SD) between the control and the test groups did not differ at baseline (860.6 ± 47.9 g vs 835.1 ± 60.8 g); however, the control group had significantly higher body weights compared to the test group after 30 days of feeding (1256.4 ± 69.9g vs 952.0 ± 69.3 g, \( P = 0.001 \)).

Concentrations of lactase, maltase and sucrase in the energy-restricted group were 0.65 ± 0.28, 6.20 ± 2.7 and 5.42 ± 2.3 IU/mg protein respectively, and those in the control group were 0.56 ± 0.17, 6.47 ± 1.9, and 5.13 ± 1.4 IU/mg protein respectively. There were no significant differences in the concentration of any of these enzymes during diarrhoea between the two groups.

Discussion

Results of our study confirmed that food deprivation using the energy-restricted diet could produce a malnourished rabbit model for the study, and concentrations of small intestinal brush border enzymes did not differ between the energy-restricted and control group of rabbits.

In this study, animals were challenged with RDEC-1 to produce diarrhoea. The RDEC-1 is a serogroup 015 Escherichia coli that has pili and surface polysaccharide. The bacterium is not invasive and does not synthesize enterotoxins. Both pili and surface polysaccharide make the bacteria adhere to the membraneous (M) cells of lymphoid follicle epithelium of ileal Peyer’s patches within a few hours of inoculation of rabbits. By 3–6 days, microcolonies of the bacteria adhere to the ileal, cecal, and colonic mucosa and diarrhoea occurs.

The gastrointestinal flora is altered in malnourished state, and protein energy malnutrition is associated with various degrees of intestinal malabsorption. These changes are believed to predispose such individuals to repeated enteric infections. Stanfield and coworkers observed severe alterations, either flat or short and thick villi in the majority of children with kwashiorkor. In these children, no significant improvement in the mucosal architecture was noted even at a follow up one year later.

In protein energy malnutrition, intestinal villus heights are typically decreased because of the significant decrease in the number of cells as well as decrease in enterocyte proliferation and migration rates along the crypt-villus axis. This led to reductions in total surface area and mucosal mass. Malnutrition is typically associated with significant reductions of body weight, mucosal mass, intestinal protein content, and total intestinal surface area. These effects are closely associated with the loss of luminal nutrition.

Reductions in the activities of intestinal disaccharidases have been well documented during malnutrition and infection both in animals and in humans. An earlier study observed reduction in the disaccharidases activities in jejunal biopsies of 24 malnourished children with gastrointestinal symptoms. In a clinical trial, Mehra and coworkers have described significant reduction in the lactase activity in subjects with 3rd and 4th degree of malnutrition (\( P < 0.05 \) and \( P < 0.005 \), respectively), but maltase activity was significantly reduced only in subjects with 4th degree of malnutrition (\( P < 0.01 \)).
To our knowledge, ours is probably the first report that compared intestinal disaccharidase concentrations in malnourished and well-nourished rabbits infected with RDEC-1. It is not possible to develop an experimental malnutrition model which would exactly represent human beings with different grades of malnutrition. However, in human studies it is difficult to demonstrate the effects of malnutrition per se because a number of other factors (including food intolerance, other diseases and infections, antibiotic use, etc.) could alter the intestinal morphology and thus confound the results. The rabbit provides an excellent animal model for the study of the human cases of diarrhoea due to enteropathogenic serogroup of E. coli, in which the human gut histopathology is similar to that of strain RDEC-1 diarrhoea in the rabbit 21.

An earlier study observed similar activity of intestinal mucosal disaccharidases in growth retarded (malnourished) and control rats but without infection 26, while another had observed reduced specific activities of lactase, sucrase and maltase in prenatally malnourished rats 27. But the activities of these enzymes were higher in other malnourished groups (postnatal, post-weaning and adulthood) as compared to corresponding controls 27. However, none of these studies examined the role of malnutrition during infection.

Our study had some limitations. In our study, RDEC-1 was injected on day 30 of the study, and rabbits were sacrificed after they developed diarrhoea. It would have been better to have a standard time period after administration of RDEC-1 infection before sacrifice and determination of disaccharidase activity. The variability in time of estimation of disaccharidase activity may be one reason why a significant difference was not found between the two groups. However, we should also recognize the fact that the time of bacterial colonization and appearance of diarrhoea is variable. Another weakness of the study was relatively small sample size. The study could have been more powerful if it included a second control group of animals that did not have RDEC-1 infection, in order to know the enzyme activity in the normal animals.

In conclusion, our study suggests that the intestinal enzyme concentrations (lactase, maltase, and sucrase) remain similar during diarrhoea in energy-restricted animals and controls. Further controlled studies with larger sample sizes are needed to demonstrate the effect of malnutrition and infection on intestinal enzyme functions.

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References


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