Effects of malnutrition on expression of lactase in children*

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This article demonstrates the successful use of RNeasy Mini Preps in retrospective clinical studies involving RNA isolation from very small amounts of embedded tissue after several years of storage.

Malnourished children often suffer from acquired lactose intolerance, making recovery difficult since they are unable to metabolize milk sugar. Most have a reduced level of lactase enzyme in their small intestine (see reference 1). Following the recent cloning of the lactase cDNA and its gene (2, 3), it is now possible to study the molecular basis of this childhood nutritional disorder, using samples collected six years earlier.

Lactase enzyme deficiency is the most common genetic enzyme disorder in human adults. In malnourished children, however, the disorder is acquired and is reversed following treatment for malnutrition. The adult form is associated with a variety of defects at the level of lactase gene transcription, translation, and posttranslational modification (see reference 1). In this report, we investigated lactase expression in malnourished children in order to test the hypothesis that its regulation differs from that of the adult disorder and occurs at the transcriptional level.

Materials and methods

The study used data from two groups of infants (average age, 8 months). The malnourished group consisted of 29 Brazilian infants with significant malnutrition. The control group consisted of 10 properly nourished Brazilian infants. A subset of each group was used for RNA studies. In addition, one control child was chosen at a later date as a positive control for genotype analysis.

Jejunal tissue specimens were obtained in 1989 from malnourished infants after they had begun recovery during a 2-week hospital stay. Tissue was obtained from the control group during hospitalization for a routine intestinal surgical procedure. A portion of the tissue was frozen at –70°C until used for enzyme assays.

Another portion of the jejunal tissue was embedded in Tissue-Tek® O.C.T. compound, frozen in liquid nitrogen, and used for RNA isolation six years later. Five frozen tissue sections (each 10 µm thick) were cut at –20°C from each embedded sample and attached to glass slides. After air-drying for 5 minutes, the tissue was scraped away from the embedding medium using a sterile razor blade. RNA was isolated from the tissue sample using the

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** Results excerpted from Nichols et al. (1997) Gastroenterology* ***112****, 742 (reference 1). Correspondence should be addressed to: Buford L. Nichols, Jr., M.D., Children's Nutrition Research Center, 1100 Bates Street, Houston, TX 77030, U.S.A. Fax: (713) 798-7078; e-mail: bnichols@bcm.tcm.edu.*

Table 1. Intestinal enzyme levels in malnourished and control children

	Control children	Malnourished children
Lactase (units/g protein)*	28.2 ± 12.7	6.5 ± 6.3
Sucrase (units/g protein)†	51.8 ± 26.3	26.9 ± 19.8

Values reported as mean ± SD

** Range of normal values: 10–92 units/g protein (see ref. 4)*
† Range of normal values: 26–191 units/g protein (see ref. 4)

Semiquantitative RT-PCR of Lactase and Sucrase RNA

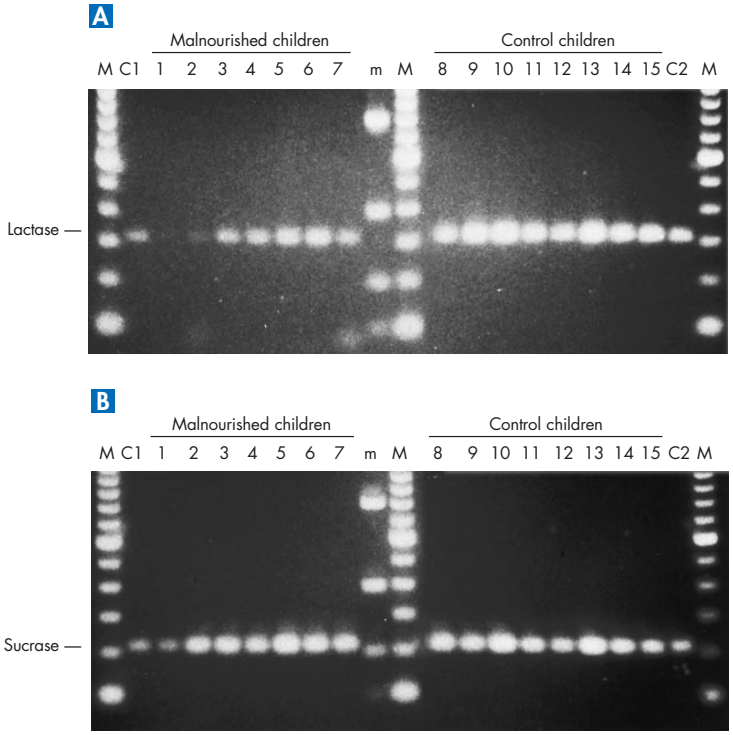

Figure 1 *RT-PCR of RNA from intestinal tissue. Total intestinal RNA (100 ng) from malnourished (**1–7**) or control children (**8–15**) was reverse-transcribed with random primers. A portion (6%) of the reaction was then amplified with gene-specific primers for **A** lactase or **B** sucrase. **C1, C2**: 20 and 100 ng control RNA, respectively; **M**: 100-bp ladder; **m**: DNA mass ladder with 10, 20, 40, and 80 ng of DNA per band, respectively, from bottom to top.*

RNeasy® Mini Kit from QIAGEN, following the protocol for isolation of total RNA from animal tissues.

RT-PCR, HPLC quantitation, and RFLP (restriction fragment length polymorphism) analysis were performed as described in reference 1.

Results and discussion

Reduced lactase, compared to sucrase activity

Many malnourished children have reduced amounts of lactase enzyme in their small intestine (see reference 1), but the level of regulation has not been previously determined. The simplest explanation would be that reduced lactase levels are due to loss of the epithelial tissues of the intestine that normally produce lactase. Children with extreme malnutrition frequently have atrophy of these tissues.

To determine the effect of tissue atrophy, we compared sucrase and lactase enzyme activity. Sucrase is produced by the same tissues as lactase and was also reduced during malnutrition. Mean sucrase enzyme levels, however, were only reduced by 48% whereas lactase enzyme levels were reduced by 77% (Table 1). This differential reduction of the two enzymes suggests that, while sucrase deficiency may be due merely to tissue atrophy, the greater reduction of lactase expression involves an additional control mechanism.

mRNA levels of lactase and sucrase

Using RT-PCR, the levels of sucrase and lactase mRNA were estimated. Figure 1 shows the levels of lactase and sucrase mRNA in malnourished and control children as investigated by RT-PCR using

equal amounts of RNA. These RT-PCR products were then quantified by high-performance liquid chromatography (HPLC) and normalized to β-actin mRNA levels from the same RNA samples (Table 2). Levels of both sucrase and lactase mRNA were reduced, but the reduction was much more dramatic for lactase. Note, for example, the reduction of lactase mRNA levels in the severely lactase-deficient infants, whose sucrase mRNA levels are much less affected (Figure 1). Overall, mean lactase mRNA abundance was reduced by 68% and sucrase mRNA by 39% relative to control subjects (Table 2). Values normalized to β-actin were similar. These results emphasize that the reduction of lactase is regulated at the mRNA level.

Table 2. HPLC quantitation of RT-PCR amplimers from Figure 1

	Control children	Malnourished children
Lactase mRNA amplimer (ng)	1766 ± 403	566 ± 368
Sucrase mRNA amplimer (ng)	1844 ± 517	1131 ± 358
β-actin mRNA amplimer (ng)	4910 ± 1105	4438 ± 619
Lactase/β-actin ratio	0.36 ± 0.03	0.13 ± 0.10
Sucrase/β-actin ratio	0.38 ± 0.09	0.26 ± 0.10

Values reported as mean ± SD

Genetic contribution

The level of lactase regulation in lactase-deficient adults is still controversial, with proposed regulation at the transcriptional, translational, and posttranslational levels (see reference 1). However, unlike in malnourished children, the adult form of the disorder is genetically determined and irreversible. Adult lactase deficiency has been linked to heterozygosity of an *Msp*I RFLP marker in exon 17 of the lactase gene: affected individuals display the two different alleles at equal intensities (5, 6). In order to confirm that the lactase-deficient infants were not affected with the genetic disorder leading to adult lactase deficiency, we investigated the genotypes of our sample using RT-PCR followed by an *Msp*I restriction digest. Six malnourished and control infants were homozygotes and nine were heterozygotes with unequal allele intensities, but none displayed the distinctive equal-intensity heterozygosity characteristic of the adult genotype

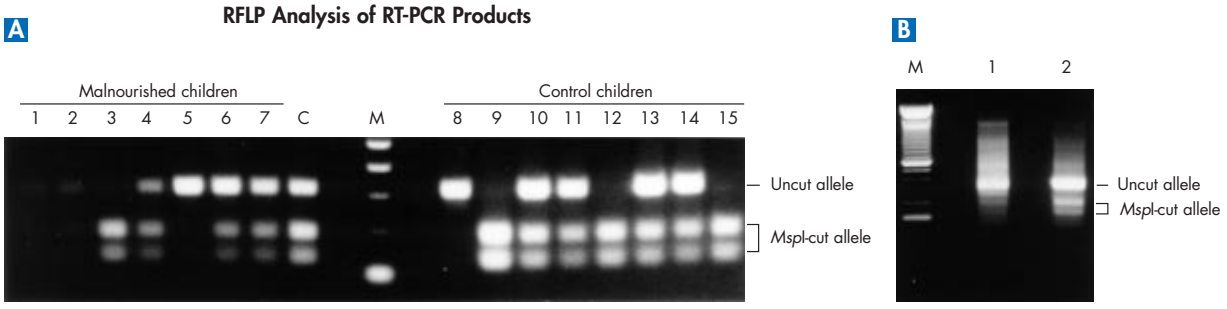

Figure 2 *RFLP analysis of lactase RT-PCR products.* ***A*** *Total intestinal RNA (100 ng) from malnourished (**1–7**) or control children (**8–15**) was reverse-transcribed with random primers then amplified with gene-specific primers for lactase, using different primers from those used in Figure 1. Following amplification, the RT-PCR products were digested with restriction enzyme MspI.* ***C****: control representing the lactase-deficient adult genotype (heterozygosity with equal allele intensities). All the children display either homozygosity or heterozygosity with unequal allele intensities.* ***B*** *RT-PCR products from children **1** and **2**, with the lowest lactase mRNA levels, were reamplified by 30 additional cycles of PCR before digestion with MspI.* ***M****: 100-bp ladder.*

(Figure 2). These results suggest that lactase deficiency in the malnourished children is due to a separate mechanism from the genetic regulation reported in adults (5, 6) and represents a unique, acquired form of lactase deficiency.

Conclusions

- Malnutrition in children is often accompanied by reduced lactase activity, making recovery difficult.
- The reduced lactase activity is regulated at the mRNA level.
- Although similar to congenital adult lactase deficiency, malnourished children do not have the same genetic determinants as adults.
- Lactase deficiency in malnourished children, unlike that in adults, is a unique, acquired, and reversible disorder. ■

Ordering Information

Product	Contents	Cat. No.
RNeasy Mini Kits	**for up to 100 µg of total RNA from animal cells or tissues, yeast, or bacteria**	
RNeasy Mini Kit (20)	20 RNeasy Mini Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74103
RNeasy Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kits	**for up to 1 mg of total RNA from animal cells or tissues, yeast, or bacteria**	
RNeasy Midi Kit (10)	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Maxi Kits	**for up to 6 mg of total RNA from animal cells or tissues, yeast, or bacteria**	
RNeasy Maxi Kit (6)	6 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75161
RNeasy Blood Kit	**for isolation of total RNA from up to 1.5 ml whole blood**	
RNeasy Blood Mini Kit (20)	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74303
QIAshredder™	**for convenient cell-lysate homogenization**	
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreparation, caps	79654

Larger kit sizes are available