

## EFFECTS OF ASYMPTOMATIC *GIARDIA INTESTINALIS* INFECTION ON CARBOHYDRATE ABSORPTION IN WELL-NOURISHED MEXICAN CHILDREN

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**Abstract.** The aim of this study was to evaluate carbohydrate absorption in well-nourished children with asymptomatic giardiasis. Two groups were selected based on results of stool examination of 211 children attending pre-school centers in Hermosillo, Sonora, Mexico: a control group of six non-infected children, and an infected group of seven children harboring *Giardia intestinalis*, without gastrointestinal symptoms of disease. Carbohydrate absorption was determined in the control group, before and after drug therapy in the infected group by the hydrogen breath test. Hydrogen production after lactose ingestion was higher in children with giardiasis compared with control group and after anti-parasite treatment; however, hydrogen production was not high enough to classify children as lactose malabsorbers by the cut-off criteria. Similar results were obtained for xylose absorption. None of the children had hydrogen increments high enough to be considered xylose malabsorbers. In conclusion, children asymptotically infected with *G. intestinalis* showed significantly higher hydrogen production. However, the biological relevance is questionable since they did not exceed cut-off criteria to classify them as carbohydrate malabsorbers.

### INTRODUCTION

*Giardia intestinalis* is the most common intestinal parasite identified worldwide. In industrialized countries, *G. intestinalis* infection may reach a prevalence of 2–7%, whereas in developing countries the prevalence may reach as high as 20–60%.<sup>1</sup> This parasite colonizes and proliferates in the upper part of the small intestine. In humans, the infection produced by *G. intestinalis* (giardiasis) may produce several symptoms such as diarrhea, abdominal discomfort, distension, flatulence, and vomiting.<sup>2</sup> In addition, giardiasis may present with severe damage of the small intestine with the subsequent effects on nutrient absorption.<sup>3</sup> One of the main complications of giardiasis is disaccharide (most frequently lactose) malabsorption.<sup>4,5</sup> Because of its interference with nutrient absorption, severe giardiasis may cause malnutrition and growth retardation in children.<sup>6</sup> However, many patients infected with *G. intestinalis* do not present symptoms or damage to the small intestine.<sup>7</sup> The presence or absence of symptoms has been related to the strain of *G. intestinalis*,<sup>8,9</sup> to the severity of the infection,<sup>10</sup> or to the immune status of the host.<sup>11–13</sup>

The potential consequences of asymptomatic giardiasis on nutrient absorption are particularly important in northwestern Mexico, where many children carry this parasite. However, few studies have focused on the potential effects of moderate or asymptomatic giardiasis on nutrient absorption, and thus its effects on the nutritional status of the host. Therefore, the aim of this study was to evaluate intestinal absorption of carbohydrates in well-nourished children with asymptomatic giardiasis.

### MATERIALS AND METHODS

**Subjects.** Thirteen children 3–6 years of age from different social backgrounds in the northwestern state of Sonora, Mexico participated in the study. Participants were selected based on stool examinations of 211 children attending pre-school centers located in diverse suburbs around the city of Hermosillo, Sonora, Mexico. Parents were asked to collect fresh stool samples every morning for three consecutive days. *Giardia* cysts or trophozoites and other parasites were identified

by the zinc sulfate concentration method.<sup>14</sup> One hundred twenty-nine (61.1%) of the 211 children screened were free of intestinal parasites, whereas in 82 children (38.9%) at least one parasite was identified. *Giardia intestinalis* was the most frequent infection (44 children, 20.9%) (Table 1). Twenty children (9.5%) were infected only with *G. intestinalis*.

From the screened population, two groups that met the selection criteria were selected based on the presence of *G. intestinalis*: 1) a control group composed of six non-infected children (4 girls and 2 boys) and 2) an infected group composed of seven children (3 girls and 4 boys) infected only with *G. intestinalis*. All participating children met the following selection criteria: 1) none had symptoms of gastrointestinal disease, such as abdominal discomfort, diarrhea, nausea, vomiting or flatulence; 2) none had received any antibiotic treatment in the three weeks preceding the study; and 3) all had to be adequate H<sub>2</sub> producers without a bacterial overgrowth condition, as determined by the hydrogen breath test following the ingestion of lactulose and by the urinary Indican test. The study protocol was fully explained to the parents of the children, and they then provided written informed consent for the participation of their children in the study. The study protocol was approved by the internal-external Ethics Committee of the Centro de Investigación en Alimentación y Desarrollo (CIAD) (Hermosillo, Sonora, Mexico).

Both groups were visited at their pre-school centers on three non-consecutive mornings for anthropometric measurements and breath samples for determination of carbohydrate absorption. In infected children, these measurements were performed before and three weeks after treatment for *G. intestinalis* infection with 25 mg of tinidazole/kg of body weight/day for two days. One and three weeks after treatment, stool examinations were performed to verify the absence of *G. intestinalis* infection by three consecutive day copro-parasitoscopic analyses.

**Anthropometric measurements.** Weight was measured to the nearest 50 g with a mechanical scale (Accu-weigh, Model 150TKL; ME Corporation, Sunnyvale, CA). Height was measured to the nearest 0.1 cm with a portable stadiometer (Holtain, Ltd, Crymych, United Kingdom). Nutritional status was evaluated by calculating Z scores for weight-for-height,

TABLE 1

Infection with intestinal parasites among 211 children in Hermosillo, Sonora, Mexico screened on three consecutive days by the zinc sulfate concentration method

	No. of children	% Prevalence (95% confidence interval)
Non-infected	129	
Infected	82	38.9 (32.3–45.4)
<i>Giardia intestinalis</i>	20	9.5 (5.5–13.4)
<i>Giardia intestinalis</i> plus other parasites ( <i>Endolimax nana</i> , <i>Escherichia coli</i> , or <i>Hymenolepis nana</i> )	24	11.4 (7.1–5.7)
<i>Enterobius vermicularis</i>	5	2.4 (0.3–4.4)
<i>Entamoeba histolytica</i>	3	1.4 (0.0–3.0)
<i>Entamoeba coli</i>	27	12.8 (8.3–17.3)
<i>Iodamoeba butschlii</i>	3	1.4 (0.0–3.0)

weight-for-age, and height-for-age using National Center for Health Statistics (NCHS) reference values.<sup>15</sup>

**Hydrogen breath test.** The hydrogen breath test has been applied in the diagnosis of incomplete carbohydrate digestion and absorption, small bowel bacterial overgrowth, and intestinal transit time in humans.<sup>16–19</sup> This test relies on the fermentation of unabsorbed carbohydrate by intestinal bacterial flora with the release of gaseous H<sub>2</sub>, which is subsequently absorbed and exhaled.

Breath samples were taken using an anesthesia bag (5 liters) with a two-way valve (model 1400; Hans Rudolph, Kansas City, MO). Children were asked to inhale through their nose and exhale through their mouth to fill the bag. Two samples (20 ml) were taken from each bag and analyzed within 2 hr in a gas chromatograph (Clinical MicroLyzer CM-2; Quintron Instruments, Milwaukee, WI). The gas chromatograph was calibrated with H<sub>2</sub> (85 parts per million [ppm]; Quingas; Quintron Instruments). Linearity of the gas chromatograph was checked for each analysis. The minimum detection level was 1 ppm of H<sub>2</sub>.

The peak increments of breath H<sub>2</sub> concentration after an oral dose of lactulose, lactose, or xylose with respect to the basal fasting levels were taken for the carbohydrate absorption capacity.

**Lactulose absorption.** Any condition that modifies the colonic flora that ferments a non-absorbable sugar will modify hydrogen production. Without proper colonic flora, all results of breath hydrogen tests after sugar consumption will be spurious. All children were initially tested for their H<sub>2</sub> production capacity using an oral dose of 10 g of lactulose (Barre-National, Baltimore, MD) dissolved in 10 ml of water. Hydrogen increments  $\geq 10$  ppm over the baseline level 90 min after lactulose ingestion were the criterion used for classification of adequate H<sub>2</sub> production.<sup>20</sup> The presence of bacterial overgrowth was considered positive when an increment in the breath H<sub>2</sub> concentration of 20 ppm or more above the baseline level was recorded within the first 20 min after the lactulose dose.<sup>21</sup> Bacterial overgrowth was also tested by the Indican (urinary indoxyl sulfate) method.<sup>22</sup>

**Lactose absorption.** Children fasted for at least 12 hr. Breath samples were taken (to record basal levels) and repeated after a dose of 0.5 g of lactose (LacTest, Quintron Instruments) per kilogram of body weight, and dissolved in 100 ml of purified water, every 20 min for the first 60 min, and

at 30-min intervals up to 300 min after lactose intake. Peak H<sub>2</sub> values of 20 ppm or more over the baseline levels at 90 min after the lactose dose were considered to indicate lactose malabsorption.<sup>23</sup>

**Xylose absorption.** The hydrogen breath test to determine xylose absorption was performed following the same protocol described earlier in this report. The test dose was 0.3 g of xylose/kg of body weight. In addition, urinary excretion of xylose was determined with the spectrophotometric method reported by Kerstell.<sup>24</sup>

**Urinary Indican test.** Bacterial overgrowth was also tested by the Indican (urinary indoxyl sulfate) method. This test is based on the ability of some bacteria to produce indole from tryptophan, which is further excreted in the urine as indoxyl sulfate. Indoxyl sulfate is then quantified in urine (collected after 3 hr) by using the spectrophotometric method reported by Bryan.<sup>22</sup> Values of indoxyl sulfate  $> 0.14$  mg/mg of creatinine indicate bacterial overgrowth.

**Statistical analysis.** Statistical analyses were performed using the NCSS Statistical Data System, version 6.0.3. Breath hydrogen production data are expressed as means  $\pm$  SE. The curves of breath hydrogen concentration over baseline levels of controls and infected (before and after treatment) groups in the lactose and xylose tests were analyzed by covariance analysis adjusting for the successive time intervals after 90 min. Breath hydrogen production after 90 min is the cut-off point used for this test in both cases.<sup>23</sup> Individual analysis of variance of control and infected (before and after treatment) at each time point, as well Tukey-Kramer comparison tests, were also performed to determine specific differences. Anthropometric data are expressed as means  $\pm$  SD. Z scores for height, weight, and weight-for-height were compared by groups using a non-parametric Mann-Whitney test.

## RESULTS

Table 2 shows the physical characteristics of the participants. The mean Z scores of infected and control children were greater than or very close to the median of the NCHS values, and none of the children had values  $< 2$  SD, which would classify them as malnourished, according to height-for-age, weight-for-age, and weight-for-height indicators.

**Production of H<sub>2</sub> and bacterial overgrowth.** Based upon the criterion of the H<sub>2</sub> increment over the baseline  $\geq 10$  ppm 90 min after the lactulose dose, all participants were considered to be adequate H<sub>2</sub> producers (Figure 1). In addition, even when significant differences after 40 and 60 min in the breath hydrogen increments were observed ( $P < 0.05$ ), no children presented bacterial overgrowth as defined by the cut-off criteria, that is, H<sub>2</sub> production over the baseline of less than 20 ppm within the first 20 min after lactulose intake (Figure 1). This result agreed with that of the urinary indoxyl sulfate test, in which subjects showed less than 0.14 mg of Indican/mg of creatinine excreted within a 3-hr period.

**Carbohydrate absorption.** After the lactose dose, H<sub>2</sub> values over baseline were  $3.6 \pm 0.75$  ppm in children with giardiasis,  $-0.85 \pm 0.75$  ppm in children after treatment for giardiasis, and  $0.19 \pm 0.81$  ppm in the control group (Figure 2). Based on the established cut-off points, no lactose malabsorption was found in children infected with *G. intestinalis* or after drug treatment. Although maximum levels of H<sub>2</sub> production were not high enough to indicate lactose malabsorption, mean

TABLE 2  
Morphometric analysis of the children

Group	Sex	Age (Months)	Weight (kg)	Height (cm)	Z Scores		
					H/A	W/A	W/H
Control	F	71.29	23.0	118.4	0.84	1.09	0.75
	F	72.05	19.5	110.0	-0.95	-0.01	0.65
	F	58.41	16.7	108.0	0.11	-0.41	-0.63
	F	57.13	18.0	104.0	-0.65	0.28	0.88
	M	75.73	19.0	118.0	0.02	-0.97	-1.36
	M	66.92	22.5	116.0	0.51	1.00	0.86
Mean ± SD		66.9 ± 7.8	19.14 ± 2.5	112.4 ± 5.9	-0.02* ± 0.6	0.16* ± 0.8	0.19* ± 0.9
Infected	F	33.45	14.5	95.0	0.81	0.45	0.26
	F	58.71	20.8	112.0	0.99	1.21	0.94
	F	69.98	19.4	109.6	-0.83	0.08	0.69
	M	68.50	19.4	110.7	-0.77	-0.30	0.28
	M	45.14	19.5	103.6	0.59	1.66	1.75
	M	60.48	20.9	110.0	-0.04	0.91	1.29
	M	61.08	20.6	109.7	-0.18	0.71	1.15
	Mean ± SD		56.7 ± 13.1	19.3 ± 2.2	107.2 ± 6.0	0.08* ± 0.7	0.67* ± 0.6

\* There were no statistically significant differences between the groups ( $P > 0.05$ ).

peak  $H_2$  increments in control and post-treatment children were lower than those in children before treatment of giardiasis ( $P < 0.05$ ).

As shown in Figure 3, breath hydrogen values over baseline in children after drug therapy were statistically lower than those observed in the infected and control groups after xylose ingestion ( $-4.16 \pm 0.69$  ppm of  $H_2$ ,  $2.2 \pm 0.69$  ppm of  $H_2$ , and  $1.13 \pm 0.74$  ppm of  $H_2$ , respectively;  $P < 0.05$ ). However, these values were below the cut-off for xylose malabsorption. In agreement with this result, there was no statistically significant difference in the percentages of xylose urinary excretion in children before and after treatment for giardiasis ( $34 \pm 3\%$  versus  $46 \pm 11\%$ ), suggesting adequate absorption of xylose.

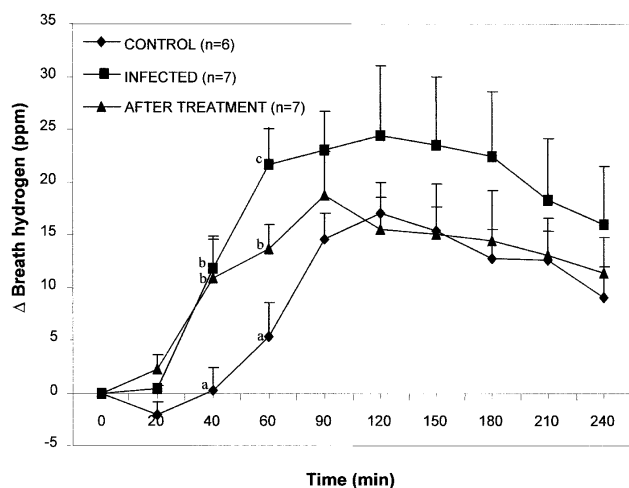


FIGURE 1. Mean  $\pm$  SE changes in breath hydrogen concentration over baseline levels at various intervals after a dose of 10 g of lactulose in control and infected children (before and after drug treatment). Hydrogen production increments  $\geq 10$  ppm 90 min after the lactulose dose indicate that the children are adequate  $H_2$  producers. Hydrogen production increments  $\leq 20$  ppm within 20 min after the lactulose dose indicate that the children do not show bacterial overgrowth. The overall plots were not significantly different ( $P > 0.05$ ). Only the mean points at 40 and 60 min were significantly different ( $P < 0.05$ ), as indicated by the different letters.

## DISCUSSION

Infection with *G. intestinalis* leads to acute/chronic diarrhea in some individuals but remains asymptomatic in others. Several studies have indicated that severe or symptomatic giardiasis may cause malabsorption of nutrients and affect the nutritional status of the host. However, few studies have reported the potential effects of moderate or asymptomatic giardiasis on nutrient absorption, and thus the effects on the nutritional status of the host.<sup>4,25-27</sup> For instance, de Moraes and others found that asymptomatic giardiasis did not affect the intestinal absorption of iron in children with iron deficiency.<sup>25</sup> We have previously found that mild *G. intestinalis* infection did not affect energy metabolism (energy intake, basal metabolic rate, and total energy expenditure) in children.<sup>26</sup> The results in the present study suggest that asymptomatic *G. intestinalis* infection does not interfere with ab-

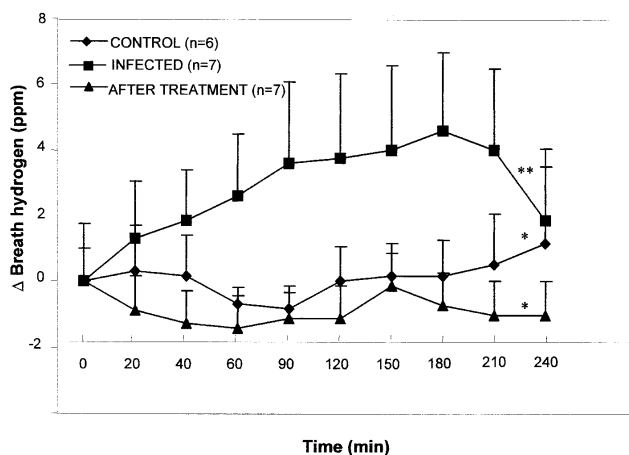


FIGURE 2. Mean  $\pm$  SE changes in breath hydrogen concentration over baseline levels at various intervals after a dose of 0.5 g/kg of body weight of lactose in control and infected children (before and after treatment). Different numbers of asterisks at the end of the plots indicate that the entire treatment was significantly different as analyzed by covariance analysis adjusting for successive time intervals after 90 min (see Statistical Analysis section).

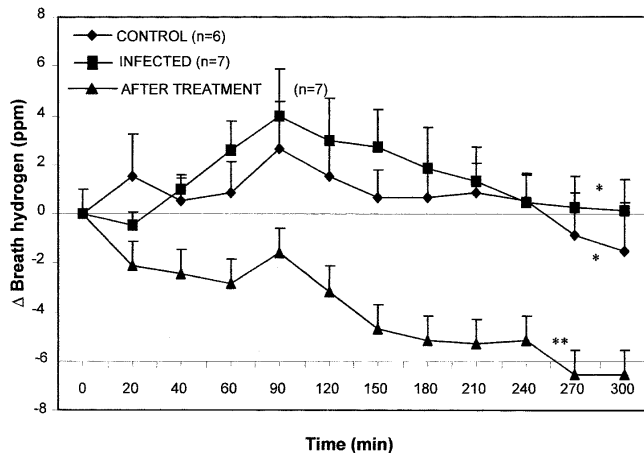


FIGURE 3. Mean  $\pm$  SE changes in breath hydrogen concentration over baseline levels at various intervals after a dose of 0.3 g/kg of body weight of xylose in control and infected children (before and after treatment). Different numbers of asterisks at the end of the plots indicate that the entire treatment was significantly different as analyzed by covariance analysis adjusting for successive time intervals after 90 minutes (see Statistical Analysis section).

sorption of carbohydrates in well-nourished children, as determined by the cut-off criteria. Similar results were found by Gendrel and others, who reported that asymptomatic giardiasis does not increase the frequency of lactose intolerance in well-nourished children.<sup>4</sup> Furthermore, Tolboom and others<sup>27</sup> found that lactose malabsorption does not correlate with the presence of giardiasis, but milk intolerance manifested as diarrhea is more common in children with giardiasis than in healthy subjects. Because of the location of the disaccharidases in the intestinal brush border, any condition that damages its morphology (such as severe diarrhea), will result in a detrimental effect on these enzymes, and thus disaccharides absorption. On the other hand, it has been proposed that infection with *G. intestinalis* interferes between the intestinal brush border enzymes and the disaccharides present in the diet. Therefore, the findings by Tolboom and others,<sup>27</sup> together with the subtle defects in breath hydrogen test results we found in asymptomatic children, suggest that lactose malabsorption may be more likely associated with the lost of lactase caused by diarrhea than the direct effects of Giardia.

Giardiasis induces proliferation of intestinal bacteria that could alter the results of absorption studies. Patients infected only with Giardia have little intestinal mucosa damage and mild lactose malabsorption, while patients with additional overgrowth have severe lactose malabsorption.<sup>28</sup> Similar observations were reported by Kerlin and others, who found that giardiasis does not affect carbohydrate absorption (from rice flour), whereas bacterial overgrowth impairs the absorption of carbohydrates as measured by the breath H<sub>2</sub> test (peak increment of 320 ppm over baseline H<sub>2</sub> production).<sup>29</sup> The children who had bacterial overgrowth as determined by the Indican test and H<sub>2</sub> production after lactulose ingestion were not included in this study. However, some strains of *G. intestinalis* may cause lesions in the intestinal mucosa and this can affect nutrient absorption in the same way as bacterial overgrowth.<sup>30,31</sup>

In conclusion, children asymptotically infected with *G. intestinalis* showed significantly higher hydrogen production.

However, the biological relevance is questionable since they did not exceed cut-off criteria to classify them as carbohydrate malabsorbers.

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