Effects of Consumption of a Milk Fermented by the Probiotic Strain *Bifidobacterium animalis* DN-173 010 on Colonic Transit Times in Healthy Humans

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Objectives: The aim of our study was to ascertain whether the specific *Bifidobacterium animalis* DN-173 010 fermented milk could modulate colonic transit time in humans. Bifidobacteria are a major component of the gut microflora and may interact with gut transit. Methods: The trial compared in a parallel double blind study in seventy two healthy volunteers the effect of a *Bifidobacterium animalis* fermented milk containing $2.6 \times 10^8$ CFU/g living bifidobacteria versus heat-treated *Bifidobacterium* fermented milk on colonic transit times. The main marker was the total colonic transit time (CTT) measured with radio-opaque pellets. Segmental colonic transit times were also calculated. Results: A 11-day-consumption of this *Bifidobacterium animalis* DN-173 010 fermented milk significantly reduced the total CTT (−20.6%) comparatively to the initial CTT and to the control group where no significant change were recorded. The effect was more pronounced in women than in men. Conclusion: Our study demonstrated that the consumption of the fermented milk containing living *Bifidobacterium animalis* DN-173 010 was able to improve CTT in humans.

Key words: probiotic; bifidobacteria; digestive transit; fermented milk

INTRODUCTION

Metchnikoff (21) was the first scientist to pinpoint the potential beneficial effects of lactic acid bacteria on the human intestinal flora and their consequences on health. Since that time, a lot of works have been published mainly on the effect of probiotics on digestibility, immunity, protection against diarrhea and it is still a matter of scientific interest at the end of the 20th century (6, 10, 15) even on gastrointestinal diseases (17).

Most of the human trial on probiotics involved fermented dairy products containing various living microorganisms, including bifidobacteria (9, 19). IDF defined probiotics as “A living microorganism which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition” (27).

In order to be active on the intestinal microflora, bifidobacteria have to survive the gastrointestinal environments: aerophilic conditions, acidity of the stomach, bile acids and digestive enzymes aggression. We selected among the Danone collection a resistant strain surviving the passage through the human gastrointestinal tract. The survival of *Bifidobacterium animalis* DN-173 010 in high quantity has been well-documented (4, 13, 22). In the study of Berrada et al. (4), product number 1 was BIO® containing *Bifidobacterium* strain DN-173 010 and in the study of Pochart et al. (22), the *Bifidobacterium* sp. BB was the strain DN-173 010 since then renamed. Considering the multiple effects of the bifidobacteria (26), we chose to focus our study on colonic transit time, which is a relevant functional marker. It could be also a marker for gut flora interaction as germ-free animals have a decreased intestinal motility and a slower transit time than conventional animals (16, 24) and bifidobacteria have been reported to improve bowel movement of bedridden elderly (30).

This has led us to investigate the effect of a bifidus (*Bifidobacterium* DN-173 010) fermented milk on the colonic transit time in healthy adults as measured by Chaussade’s method (8).

MATERIALS AND METHODS

Subjects: Seventy-two healthy volunteers (36 males and 36 females), with a mean age of 30 ± 8 years (range, 21–42 years) and a mean weight of 67 ± 11 kg (range, 46–88 kg) were enrolled in this study. All subjects were considered healthy including a normal gut function after a medical examination. They had not taken any medication for at least four weeks before the beginning of the study and they maintained their usual diet all along the study. Written consent was obtained from

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each volunteer. It was a double blind, placebo controlled study with two parallel groups. The protocol was approved by the Ethical Committee of the Faculty of Medicine, University de Marseille II, France.

Test products. Danone (Danone Vitapole, Le Plessis Robinson, France) prepared the test products. The "product A" (Bifidus) was a milk fermented by one culture: *Bifidobacterium animalis* DN-173 010 (strain used in the commercial BIO product). The fermentation lasted 18 hr at 40°C. The "product B" (Control) was prepared as the product A first, then heat-treated at 72°C for 45 sec. Product A contained 2.6 × 10⁸ ± 0.5 CFU/g of *Bifidobacterium animalis* DN-173 010 (mean value of two counts). The numeration was performed before consumption by the volunteers in MRSn agar complemented with 0.3 g/l cysteine and 0.5 mg/l dicloxacilline after a 5 day incubation of the plates under anaerobic conditions. Product B contained no viable detectable *Bifidobacterium* (< 10 CFU/g). Both products were identical in the terms of color, taste and external appearance and nutrient content, including lactose. Each subject was randomly allocated to group A or B.

Diet and experimental design. From the first day (D.0) until the end of the study (D.21), usual fermented milks and yogurt consumption were excluded from the diet of volunteers. Products A and B (3 × 125 g) were added to the diet during D.10–D.21 period. Volunteers kept a daily record of their diet including product A or B and nutritional intakes were assessed by a computer program. No differences during the period preceding the intervention period (D.0 to D.10) were found between Bifidus and Control groups for energy (1707 vs. 1788 kcal/day respectively), proteins (75.6 vs. 74.7 g/day), lipids (67 vs. 76 g/day), carbohydrates (201 vs. 201 g/day) and fibers intakes (17.9 vs. 17.1 g/day). The intervention with either Bifidus or Control did not lead to nutritional differences between both groups (D.10 to D.21, energy: 1830 vs. 1978 kcal/day, proteins: 88.4 vs. 91.0 g/day, lipids: 77.5 vs. 85.3 g/day, carbohydrates: 195 vs. 211 g/day and fibres: 15.9 vs. 15.3 g/day). Thus, the fiber content of the diet was stable during the whole study. Ingestion of tested product increased energy intake by 5 to 10%. Timing of radio-opaque markers ingestion were also registered. The subjects ingested twenty identical radio-opaque pellets at 9:00 a.m. for the initial period on D.7, D.8 and D.9 and for the end period on D.18, D.19, and D.20 in accordance with the protocol described by Chaussade et al. (8). The abdomen was X-rayed on D.10 and D.21, and the radio-opaque markers were counted in each segment of the colon (right, left and sigmoid) by an independent radiologist unaware of the product ingested by the volunteers.

Statistical analyses and calculations. Colonic transit times (CTT) were calculated using the Chaussade’s formula (8):

$$\text{CTT} = 1.2 \times \sum n_i$$

where $i$ is from 1 to 3, $n_i$ being the number of pellets present in each segment of the colon (right, left and sigmoid colon). CTT were calculated for the entire, the right, the left and the sigmoid parts of the colon.

The results are expressed as means ± SEM. Differences between the initial (D.10) and final transit times (D.21) within the same group were assessed by a Wilcoxon test. Differences between the two groups of Bifidus and Control were assessed by a Mann-Whitney test.

RESULTS

The two groups were homogenous for the sex ratio, age (34.7 vs. 32.0 year), weight (66.9 vs. 67.2 kg), height (1.73 m in both groups). Initial colonic transit time (33.0 vs. 30.1 hr) were not statistically different ($p > 0.40$).

Within the Bifidus group, total colonic transit time was reduced by 20.6% ($p = 0.013$) and sigmoid transit time was reduced by 38.9% ($p = 0.02$) between D.10 and D.21, whereas right and left colonic transit times tend to be shorter, but not enough to reach statistically significant differences (Fig. 1). Within the Control group there were no significant variation of transit times during the eleven days of the test (D.10–D.21) whatever the colon location (Fig. 1).

The difference between initial and final total colonic transit time was significantly ($p < 0.05$) greater in the Bifidus group (−6.8 hr) than in the Control group (+ 0.5 hr), whereas the difference of sigmoid transit time between both groups did not reach the statistical significance ($p > 0.15$) (Table 1).

In the whole population of Bifidus and Control groups, initial total and sigmoid transit times tended to be longer for the females (33.3 and 10.2 hr) than for males (29.9 and 7.2 hr) but this was not statistically significant ($p = 0.3$ and $p = 0.09$).

The effect of Bifidus fermented milk intake on the reduction of total colonic transit time was statistically significant in the male group ($p < 0.03$) as well as in the female group ($p < 0.05$) (Table 2). The total benefit on male colonic transit time was the sum of non-statistically significant reductions of transit times of all the segments of their colon (right, left and sigmoid). In females the difference of sigmoid transit time was statis-
Fig. 1. Segmental colonic transit times after consumption of either Bifidus or Control milk. Values are mean for 72 data. Standard errors are shown by vertical bars. *Significantly different from initial, \( p < 0.05 \) (Wilcoxon test).

Table 1. Colonic transit times for Bifidus and Control group (hr) before (D.10) and after (D.21) ingestion of Bifidus or Control milk.

<table>
<thead>
<tr>
<th></th>
<th>Bifidus (n = 36)</th>
<th>Control (n = 36)</th>
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<tbody>
<tr>
<td></td>
<td>D.10</td>
<td>D.21</td>
</tr>
<tr>
<td>Total colonic transit time (hr)</td>
<td>33.0 ± 16.1</td>
<td>26.2 ± 14.7*</td>
</tr>
<tr>
<td>Sigmoid transit time (hr)</td>
<td>9.5 ± 8.6</td>
<td>5.8 ± 7.7*</td>
</tr>
</tbody>
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*Significantly different from initial (D.10) (Wilcoxon test; \( p < 0.05 \)).

\(^a\) Bifidus group significantly different from Control group (Mann-Whitney test; \( p < 0.05 \)).

Table 2. Effect of Bifidus on colonic transit times in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Bifidus</th>
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<tbody>
<tr>
<td></td>
<td>Men (n = 18)</td>
<td>Women (n = 18)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total colonic transit time (hr)</td>
<td>30.0 ± 12.1</td>
<td>-6.9(^*)</td>
<td>36.3 ± 19.1</td>
<td>-7.4(^*)</td>
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<tr>
<td>Right colonic transit time (hr)</td>
<td>14.2 ± 7.5</td>
<td>-2.9</td>
<td>14.6 ± 9.2</td>
<td>-1.8</td>
<td></td>
</tr>
<tr>
<td>Left colonic transit time (hr)</td>
<td>8.7 ± 6.7</td>
<td>-2.4</td>
<td>9.9 ± 8.5</td>
<td>+0.3</td>
<td></td>
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<tr>
<td>Sigmoid transit time (hr)</td>
<td>7.1 ± 6.7</td>
<td>-1.6</td>
<td>11.8 ± 9.8</td>
<td>-5.9(^*)</td>
<td></td>
</tr>
</tbody>
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\(^*\) D.21 significantly different from initial (D.10) (Wilcoxon test; \( p < 0.05 \)).

tically significant whereas differences in the right and left segment were not (Fig. 2).

**DISCUSSION**

The purpose of this work was to determine the effect of a *Bifidobacterium* fermented milk on colonic transit time in normal volunteers. The selected strain (*Bifidobacterium* DN-173 010) was able to resist the adverse environment of the human digestive tract (4, 13, 22), and was ingested in a large number of living cultures: \( 2.6 \times 10^8 \) CFU/g, that is \( 3.2 \times 10^{10} \) bifidobacteria per serving three times a day for 11 days. The
benefit on colonic transit time was not observed with the heat-treated control, which emphasized the importance of living microorganisms for colonic transit time reduction.

The effect on transit time was not observed on all volunteers, and it was more pronounced in women than in men. In fact 5 men and 3 women did not improve their total colonic transit time during Bifidus milk ingestion, and the longer the initial transit time, the greater the reduction after *Bifidobacterium* DN-173 010 milk ingestion, but this was not statistically significant (data not shown). This suggests that the effect of *Bifidobacterium* fermented milk on colonic transit time is not an acceleration of all transits but rather a reduction of physiological long transit times, and women were a better target group than men to substantiate this effect. This reduction of the colonic transit time was always within physiological limits, which put this *Bifidobacterium* fermented milk into the functional probiotic food group (25). Another interesting group could be the elderly as they may have more often slow gut transit time (18, 20) and that improvements by means of fermented milks have been reported (28, 30).

We can only speculate on the mechanisms of action of this *Bifidobacterium* DN-173 010 fermented milk on colonic transit time as there were no attempt in this trial to measure any change of the gut flora nor of the flora functionalities because it would have increased the burden of the trial too much. Gut flora is known to interact with gut mucosa by various metabolites such as short chain fatty acids, hydrogen, methane, and many other compounds (2, 12, 23). In his review Borriello (5) listed the following potential mechanisms: increased colonization, stimulation of cholecystokinin, possible increase of prostaglandin production, decreased metabolism of primary bile acids. Modulation of the activity (3, 7, 9, 19, 29) and composition (6, 14, 29) of the gut flora by ingestion of large number of living bifidobacteria have already been documented. Similar data have been obtained with the strain *Bifidobacterium animalis* DN-173 010 on the microbial enzyme activities (1, 11) and on the fecal microflora composition (11, 13). However it is the first time that a functional benefit has been documented in normal human volunteers on total colonic transit time and on the sigmoid stasis of women.

This clinical study reported an improvement within physiological limits of colonic transit time in human volunteers after an 11-day-ingestion of a fermented milk containing living *Bifidobacterium* DN-173 010. This elicits that specific living strain to be used in some probiotic functional food targeted at the colonic transit function.

**CONCLUSION**

A milk fermented with *Bifidobacterium* DN-173 010 was able to shorten colonic transit time in humans, mainly due to improvement of sigmoid transit time. This effect was more prone in the women group, which had slower transit time than men. The selected strain was able to survive the human gut environment and was ingested in a large number of living cultures for eleven
days. Using colonic transit time as a global functional marker, this strain had demonstrated a functional benefit. It can then be used in a functional probiotic dairy food, providing that this benefit will remain in a commercially available food. Since the mechanism of action is still unknown, this food should contain a high number of living *Bifidobacterium animalis* DN-173 010 and the benefit must be documented in other human trials.

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REFERENCES

