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Bifidobacterium animalis *strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study*

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SUMMARY

Background: A previous study has suggested that *Bifidobacterium animalis* DN-173 010 shortens the colonic transit time in women.

Aim: To confirm this effect and to determine whether modifications of the faecal bacterial mass and/or faecal secondary bile salts may be the explanation.

Methods: A double-blind, cross-over study was performed. Thirty-six healthy women were studied in four consecutive 10-day periods. During periods 2 and 4, they ingested three 125 g cups per day of a fermented milk which was either a product containing *B. animalis* DN-173 010 or a control without bifidobacteria. Periods 1 and 3 were run-in and washout periods, respectively. The total and segmental colonic transit times were assessed using a pellet method. In 12 subjects, all stools were collected and analysed for pH, faecal weight, bacterial mass and bile acids.

Results: The total and sigmoid transit times were significantly shorter during dosing with *B. animalis* compared to the control period. The other transit times, faecal weight, pH, bacterial mass and bile acids were not significantly affected.

Conclusions: *B. animalis* DN-173 010 shortens the colonic transit time in healthy women. This effect is not explained by modifications of the faecal bacterial mass or secondary bile acids.

INTRODUCTION

There is a growing interest in functional food which can 'beneficially affect one or more target functions in the body in a way that is relevant either to promoting the state of well-being and health and/or reducing the risk of disease'. Probiotics belong to these functional foods and have been defined as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host'.[1, 2] Bifidobacteria represent about 20% of the cultivable faecal bacteria in adults and up to 80% in infants. Some strains which are used in fermented milks have a high survival capacity in the gastrointestinal tract and exhibit probiotic properties in the colon.[3–9]

Colonic transit disturbances (constipation and irritable bowel syndrome) are frequent and represent an important target for functional food, including probiotics.[1] Probiotics are believed to influence intestinal transit; however, the evidence is poor as randomized controlled studies are lacking.[2] An open study has suggested that the ingestion of propionibacteria may slow down the

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transit time in the left colon in healthy men.[10] We observed, in a previous study, that a milk fermented by *Bifidobacterium animalis* strain DN-173 010 shortened the colonic transit time in women, particularly those with a long transit time.[11] The effect on faecal weight and stool frequency was not assessed and the mechanism is unknown. We hypothesized that an increase in the faecal bacterial mass and/or in the secondary bile salts in the colon may be responsible for this effect. We speculated that the probiotic bifidobacteria which survive to the faecal stage may significantly increase the faecal bacterial mass and/or stimulate colonic motility by enhancing colonic concentrations of secondary bile salts. Indeed, some bifidobacteria may directly metabolize bile acids[12, 13] and some probiotics influence bile salt metabolism by endogenous flora.[14]

This study aimed to confirm, in a double-blind, randomized, controlled trial, that *B. animalis* strain DN-173 010 shortens the colonic transit time in women, and to establish whether this effect is due to an increased faecal bacterial mass or an increase in secondary faecal bile acids.

SUBJECTS AND METHODS

Subjects

This randomized, double-blind, cross-over study was conducted in three centres in France (Clermont-Ferrand, Marseille, Paris). Inclusion criteria were: healthy female volunteers, aged 18–45 years; absence of pregnancy; body mass index < 25; absence of a past history of digestive disease; absence of constipation; and absence of symptoms of irritable bowel syndrome. Each volunteer provided written consent to the protocol which had been approved by the Ethics Committee of the Factulté de Médecine, Université Marseille II. Thirty-six women were included (12 in each centre). Four were excluded because the study endpoint could not be assessed (errors in the timing of pellet ingestion). The 32 volunteers in whom the study endpoints were measured had a mean age of 27 ± 7 years, a body weight of 55 ± 5 kg and a height of 164 ± 6 cm.

Test products

The two test products were prepared by Danone (Danone Vitapole, Le Plessis Robinson, France) 15 days before the beginning of the ingestion periods. They had similar colour, taste, texture and lactose content. The Bifidus product (Bifidus) was the commercial product 'BIO', i.e. a semi-skimmed milk fermented by yoghurt cultures and *B. animalis* strain DN-173 010. The control product was the same fermented milk without bifidobacteria (i.e. fermented only with yoghurt cultures). The control and Bifidus products contained at least 10^8 colony-forming units/g (CFU/g) of yoghurt cultures on the first day of each ingestion period. The Bifidus product contained additionally between 5×10^7 and 10^8 CFU/g of *B. animalis* strain DN-173 010.

Experimental design

The study comprised four consecutive periods, i.e. a 10-day run-in period and two 10-day ingestion periods with an interval of 10 days between them. Subjects were asked to retain their normal stable diet, except that milk, sauces and fermented dairy products, including yoghurt and soft cheeses, were excluded throughout the study. During the ingestion periods, the subjects were given, in a randomized, double-blind, cross-over fashion, three 125 g cups per day of either control or Bifidus. Half of the subjects received the control before Bifidus (and were referred to as group A), and half received the Bifidus before the control (group B). The subjects recorded the number of bowel movements in the last week of each period. In the last 3 days of the run-in and each test period, the subjects ingested every day 20 identical radio-opaque pellets at 09.00 h, and an abdominal X-ray was taken on the last day. Total and segmental colonic transit times were calculated according to the distribution of the markers in the different segments of the bowel, using the formula:

$$\text{transit time} \quad 1.2 \times \sum n_i$$

where n_i is the number of pellets present in each segment (right, left and sigmoid colon).[15]

Stool analysis

A stool analysis was performed in the 12 volunteers followed in Paris. All stools in the last 3 days of each study period were collected, and brought to the metabolic ward within 12 h in closed boxes carried in a larger box which was filled with ice. Faecal pH was measured using a pH-meter (Radiometer, Copenhagen, Denmark). Samples were then frozen and kept at

– 80 °C until analysis. Faecal bacterial mass was determined on an aliquot according to the fractionation procedure of Stephen and Cummings.[16] To determine the faecal bile acids, 70% isopropanol was added to a faecal aliquot to inhibit bacterial degradation of bile acids. These samples were immediately freeze-dried, and the wet and dry weights were measured before and after freeze-drying, respectively. Further steps included heating with ethanol (three reflux steps during 2 h at 90 °C), centrifugation, purification of bile acids with ethyl acetate, extraction through a Bond Elut cartridge, methylation and sialylation. Separation of bile acids was performed by gas chromatography (Hewlett Packard 5890 series II gas chromatograph equipped with an HP-5 MS capillary column), and identification and quantification by mass spectrometry (HP 5971A mass spectrometer). The analysis conditions have been described previously by Setchell *et al.*[17]

Statistical analysis

Study endpoints. The major endpoint was the comparison of the total colonic transit time after consumption of Bifidus vs. control. Secondary endpoints were the segmental colonic transit times (the right, left and sigmoid colon), stool frequency, faecal weight, faecal bacterial mass, faecal pH and faecal bile salts (individual bile salts, pooled primary and pooled secondary bile salts). Comparisons between each product period and run-in period were also performed. In our previous study using the same method, the mean total colonic transit time in healthy volunteers was 36 ± 16 h (mean ± s.d.). To test the hypothesis of a difference of 12 h with an α value of 0.05 and a β value of 0.15, we calculated that 32 subjects were needed, and decided to include 36. Results are expressed as means with their s.d.

Comparisons. The homogeneity of groups A and B was checked with the non-parametric Mann–Whitney test. The analysis of product effect and period effect was performed using analysis of variance (ANOVA) or Kruskall–Wallis test. Comparisons between products were also performed using Student's test for paired data.

RESULTS

Groups A and B did not differ significantly at the end of the run-in period for any transit time or faecal parameter, except for pH (Table 1).

Colonic transit time and number of bowel movements

The total colonic transit time and the sigmoid transit time were significantly shorter after Bifidus than after control consumption (Table 2). They did not differ significantly from those measured at the end of the run-in period with any of the treatments (Table 2). Individual results are shown in Figures 1 and 2. In the sub-group of subjects with an initial transit time above 40 h ($n = 21$), a significant decrease in the total colonic transit time was observed during the Bifidus consumption period in comparison with the run-in period and the control period (Table 2). In this sub-group, a significant decrease in the sigmoid transit time was also observed during the Bifidus consumption period in comparison with the run-in period and the control period (Table 2). The number of stools per week did not significantly differ between the Bifidus, control and run-in periods (7.9 ± 2.7, 7.6 ± 2.8 and 7.0 ± 1.6 stools/week, respectively).

Table 1. Comparison between groups A and B at the end of the run-in period (mean ± s.d.)

	Group A ($n = 17$)	Group B ($n = 15$)	P value*
Total colonic transit time (h)	57.2 ± 28.0	52.9 ± 28.8	0.584
Right colonic transit time (h)	17.8 ± 13.6	12.6 ± 9.1	0.344
Left colonic transit time (h)	15.0 ± 11.0	14.2 ± 12.4	0.791
Sigmoid colonic transit time (h)	24.4 ± 14.2	26.1 ± 23.5	0.719
Faecal pH†	7.2 ± 0.3	6.7 ± 0.4	0.045
Faecal bacterial mass (g/day)†	12.8 ± 5.7	9.3 ± 4.6	0.584
Faecal secondary bile salts (mmol/g dry weight)†	1468 ± 1370	1256 ± 1012	0.584

Group A, consumption of control then Bifidus. Group B, consumption of Bifidus then control.
*Mann–Whitney test.
†$n = 6$ for both groups A and B.

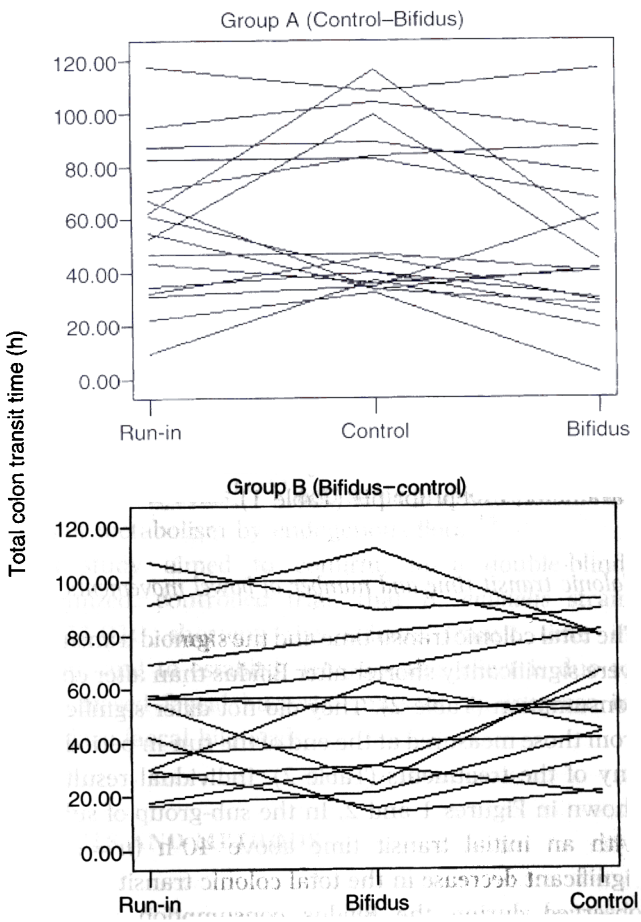

Figure 1. Total colonic transit time for each individual volunteer in both groups during the run-in, Bifidus and control periods.

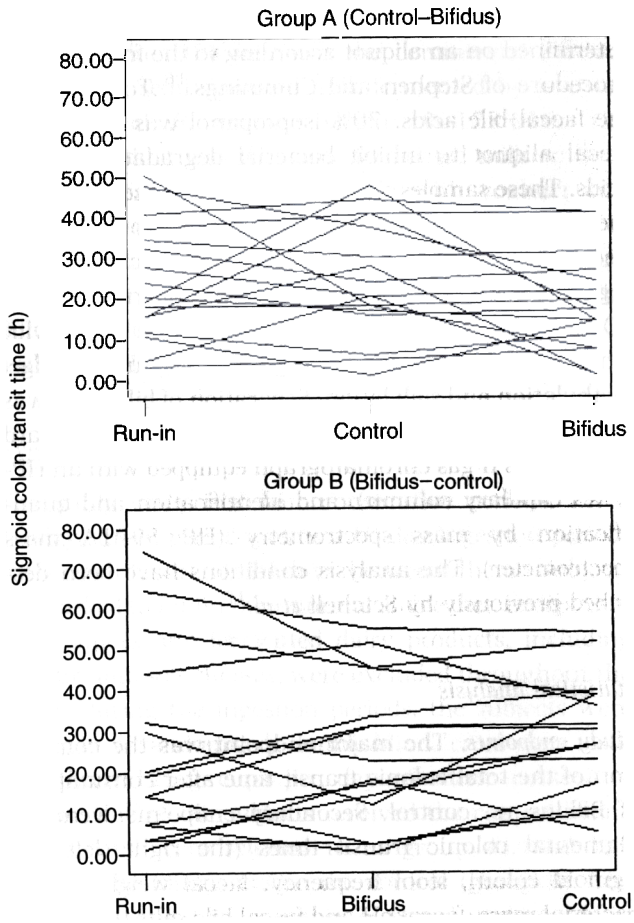

Figure 2. Transit time in the distal part of the colon for each individual volunteer in both groups during the run-in, Bifidus and control periods.

Stool analysis

In the 12 subjects analysed, faecal weight did not differ significantly between the Bifidus and control periods (96 ± 44 vs. 85 ± 36 g/day). Faecal pH was not different (6.89 ± 0.44 vs. 6.83 ± 0.28). The products had no significant effect on the faecal bacterial mass (treatment effect: $P = 0.938$), but a significant increase in the faecal bacterial mass was observed during the second consumption period in both groups A and B (period effect: $P = 0.042$) (Table 3).

Table 2. Total and segmental colonic transit times (CTT) at the run-in period and after consumption of either Bifidus or control product (mean ± s.d.)

	Whole population ($n = 32$)			Subjects with total CTT > 40 h in run-in period ($n = 21$)		
Transit time (h)	Run-in	Bifidus	Control	Run-in	Bifidus	Control
Total	55.2 ± 28.0^{ab}	51.5 ± 30.2^{a}	60.7 ± 27.1^{b}	± 21.8^{a}	52.4 ± 29.8^{b}	71.9 ± 26.5^{a}
Right colon	15.3 ± 11.8	15.5 ± 10.8	16.2 ± 10.1	± 12.7	17.0 ± 11.8	18.3 ± 10.9
Left colon	14.7 ± 11.5	14.4 ± 14.1	17.7 ± 11.8	± 11.9	18.3 ± 15.1	21.5 ± 11.6
Sigmoid colon	25.2 ± 18.9^{ab}	21.6 ± 14.9^{a}	26.8 ± 14.2^{b}	± 18.3^{a}	27.1 ± 14.9^{b}	32.1 ± 13.1^{a}

Two values having different letters within the same row in the whole population and in the subjects with a long CTT are statistically different. $P < 0.05$.

Table 3. Faecal bacterial mass (g/day) in 12 healthy women after consuming control or Bifidus product (mean ± s.d.)

	Control ($n = 12$)	Bifidus ($n = 12$)
Group A	13.3 ± 6.0	
Group B	14.9 ± 4.8	

Group A, consumption of control then Bifidus. Group B, consumption of Bifidus then control.
Treatment effect: $P = 0.938$. Period effect: $P = 0.042$.

The faecal concentrations of total secondary bile acids, deoxycholic acid and lithocholic acid were not significantly affected during any period (Table 4). There was an increase in stool concentration of primary bile acids during the Bifidus period in comparison with the control period which approached but did not achieve statistical significance (Table 4; $P = 0.079$).

DISCUSSION

This double-blind, randomized, controlled study demonstrates that healthy women have shorter total colonic and sigmoid transit times when they ingest 3 cups/day of a fermented milk containing yoghurt cultures plus *B. animalis* DN-173 010 vs. the same product without the latter strain. The faecal weight, bacterial mass and faecal excretion of secondary bile salts are not significantly influenced.

Several studies have shown that probiotics may influence intestinal disturbances and, in particular, may prevent or shorten the duration of diarrhoea in infants or adults with acute gastroenteritis.[2, 18–21] The effect of probiotics on intestinal transit has been poorly studied. Bouglé *et al.* reported that the administration of propionibacteria may slow down transit in the left colon in healthy men; however, their study was open and did not use a control group.[10] The present study demonstrates, for the first time, that a probiotic strain may significantly shorten the colonic transit time. It is in accordance with our previous trial, which suggested that *B. animalis* DN-173 010 shortened the sigmoid transit time in women.[11] This strain has a high survival in the small and large intestine when it is ingested in a fermented dairy product.[6, 7, 9] Although the clinical relevance of our results is open to discussion, it has been shown that modulation of the intestinal motility can be obtained with probiotics, and a rational mechanism for some of their effects has been provided.

None of the mechanisms evaluated in the present study (increase in faecal weight, especially in faecal bacterial mass, and modifications in secondary bile salt excretion) can explain the effect of the probiotic on the colonic transit time. It is possible that some effects of probiotics on colonic bacteria may occur slowly and may remain for more than 10 days, and this may be a limitation of our cross-over study design. Other studies have shown that modifications of faecal enzymes induced by probiotic ingestion may indeed last for up to 3 weeks after cessation of ingestion.[9, 21] Future cross-over studies aimed at assessing the effects of probiotics on the colon ecosystem should include longer washout periods. In the present study, an effect of the strain on the colonic transit time was observed, while the faecal bacterial mass and bile acids were not significantly influenced.

Table 4. Individual and total secondary bile acid concentration and primary bile acid concentration (mean ± s.d.; [extreme values]) in 12 healthy women (six in group A and six in group B) after consuming control or Bifidus

Bile acid (BA) concentration (nmol/g dry weight)	Control		Bifidus		
	A	B	A	B	*P* value*
Primary BA concentration	67 ± 85 [0; 226]	7 ± 10 [0; 25]	188 ± 280 [9; 737]	22 ± 28 [0; 72]	0.079
Secondary BA concentration	1625 ± 1794 [200; 4956]	2968 ± 1550 [1041; 5232]	2413 ± 1597 [771; 5119]	3260 ± 2540 [1589; 8310]	
Deoxycholic acid concentration	904 ± 1218 [50; 3256]	1349 ± 711 [540; 2322]	1170 ± 9 42 [218; 2906]	1606 ± 1446 [645; 4488]	0.660
Lithocholic acid concentration	721 ± 595 [150; 1700]	1619 ± 878 [410; 2910]	1243 ± 703 [324; 2213]	1654 ± 1108 [880; 3822]	

Group A, consumption of control then Bifidus. Group B, consumption of Bifidus then control.
*Statistical significance of the treatment effect, i.e. control vs. Bifidus.

Some bifidobacteria can deconjugate and dehydroxylate bile salts, i.e. convert conjugated primary bile salts into free secondary bile salts which can stimulate colonic motility and secretion.[12, 13] In the present study, *B. animalis* DN-173 010 ingestion did not influence the faecal excretion of secondary bile salts. We believe that this is a good thing, as secondary bile salts may increase the risk of colon cancer.[22–24] The shortening of the colonic transit time was therefore not due to significant bile salt modifications. The concentrations of primary bile acid tended to increase after consumption of the Bifidus product and this could be due to shortening of the colonic transit time.[25] The mechanism of the effect of the tested strain on the colon motility remains unknown. One may hypothesize that a product of bacterial origin may decrease the sigmoid tonus and or stimulate the colonic motility. One of these products might be phloroglucinol which can be produced by some colonic bacteria and has strong effects on colonic motility.[26, 27] Further studies should be performed to evaluate this hypothesis.

We conclude that the regular consumption of a Bifidus fermented milk (BIO, Danone) influenced the colonic motor function. This effect is specific to the probiotic strain as it was not observed with a control fermented milk. Further studies are needed to determine the mechanism, and also to assess the clinical efficacy of the BIO product in constipated patients.

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