



**STUDIES PERFORMED
WITH ACTIVIA®
OR *B.lactis* DN-173 010**



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***Bifidobacterium lactis* was formerly named *Bifidobacterium animalis*.**

Now, according to new taxonomy, this strain is classified as *Bifidobacterium animalis subspecies lactis* and can be commonly named *B. lactis*.

(Masco et al, Polyphasic taxonomic analysis of Bifidobacterium animalis and Bifidobacterium lactis reveals relatedness at the subspecies level: reclassification of Bifidobacterium animalis as Bifidobacterium animalis subsp. animalis subsp. nov. and Bifidobacterium lactis as Bifidobacterium animalis subsp. lactis subsp. nov.; International Journal of Systematic and Evolutionary Microbiology; 2004, 54, 1137–1143)

SURVIVAL OF *B. lactis* DN-173 010 THROUGHOUT THE GASTROINTESTINAL TRACT

1

- A** Gastric level study
- B** Intestinal level study
- C** to **G** Through the gastrointestinal tract level studies

A *Bifidobacterium* from fermented milks: survival during gastric transit (Berrada *et al*, 1991)

Study methodology:

The study was a randomised, controlled, double-blind, cross-over trial. Both in vivo survival (A) and gastric emptying rate (B) were studied.


- A) 10 healthy volunteers (20-45 yo) received 250 g of either Activia® or a control product, another commercial *Bifidobacterium* fermented milk, in one shot.
- B) 12 healthy volunteers (22-25 yo) received 250 ml of either Activia® or a control product, another commercial *Bifidobacterium* fermented milk.

Evaluation criteria:

- A) Survival of bifidobacteria strains (Samples collected using a gastric tube immediately and 30, 60 and 90 minutes after ingestion; enumeration by plate counting).
- B) Gastric emptying rate (detection of ^{99m}Tc-technetium-labeled solution of rhenium sulfur colloids incorporated in ingested fermented milk, by Scintigraphy, every 10 minutes, over 3 hours).

Results:

- A) After 90 minutes of gastric transit, *Bifidobacterium* population in Activia® decrease by less than 2 log units. *Bifidobacterium* population in the control product decreased by 4 log units. The difference between the survival of the bifidobacteria strains is significant ($p < 0.001$).
- B) There is no significant difference in gastric emptying between Activia® and the control product.

 **Conclusion:** after consumption of 250 g of Activia®, *B. lactis* DN-173 010 survives to gastric transit.

B Survival of bifidobacteria ingested via fermented milk during their passage through the human small intestine: an in vivo study using intestinal perfusion (Pochart *et al*, 1992)

Study methodology:

In this randomised, controlled, open study, 6 healthy volunteers (18-30 yo) consumed either 400 g of *B. lactis* DN-173 010 fermented milk or a monitored diet containing no bifidobacteria, in one shot.

Evaluation criteria:

Quantification of viable bifidobacteria reaching the terminal ileum (Enumeration by plate counting, in ileal perfusion, throughout the 8 hours of product ingestion).

Results:

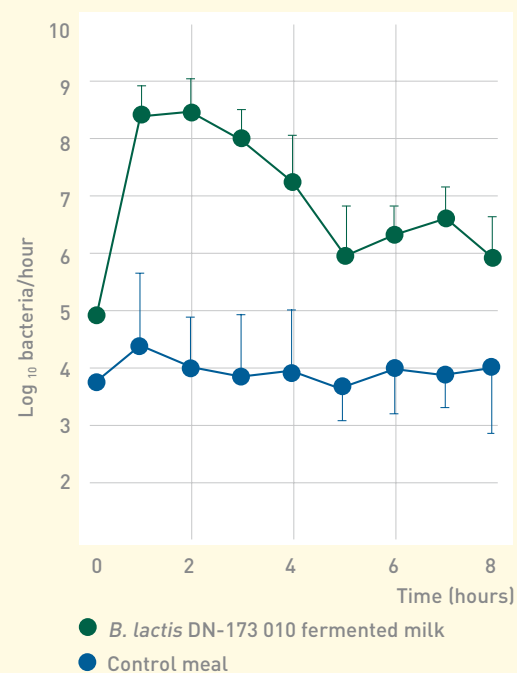
In the control group, no modification of the flow of bifidobacteria was observed, and the concentration of viable bifidobacteria throughout the experiment remained low (1.6×10^5 cfu/L).

In the *B. lactis* DN-173 010 fermented milk group, a significant increase in the ileal flow of bifidobacteria was observed within one hour in all subjects, and a peak flow (6.3×10^8 cfu/hour), corresponding to a concentration of 2.5×10^9 cfu/L in the ileal fluid, was observed, after 1.7 ± 0.4 hours.

The mean numbers of bifidobacteria recovered was significantly smaller than the amounts ingested (10^9 cfu versus 10^{10} cfu, $p < 0.02$).

Conclusion: after consumption of 400 g of *B. lactis* DN-173 010 fermented milk, ileal flow of bifidobacteria increases significantly. After 8 hours, 10^9 cfu of the strain is recovered in the terminal ileum, indicating that a large quantity of ingested bifidobacteria reaches the colon.

FLOW RATE OF BIFIDOBACTERIA THROUGH THE ILEUM AFTER INGESTION OF *B. lactis* DN-173 010 FERMENTED MILK OR CONTROL MEAL



C Identification of bifidobacteria in the faeces after prolonged ingestion milk with bifidus (Pochart *et al*, 1990)

Study methodology:

In this randomised, controlled, open, cross over study, 12 healthy volunteers (17-50 yo), ingested daily either 3 x 125 g of *B. lactis* DN-173 010 fermented milk or a standard yoghurt, over a 10 days period. The follow up period lasted 10 days.

Evaluation criteria:

Quantification of bifidobacteria (Enumeration by plate counting from faeces; *Bacillus stearothermophilus* spores were used as markers of bacterial transit, evaluation every fifth days, during 3 consecutive periods (baseline, consumption period, follow up period).

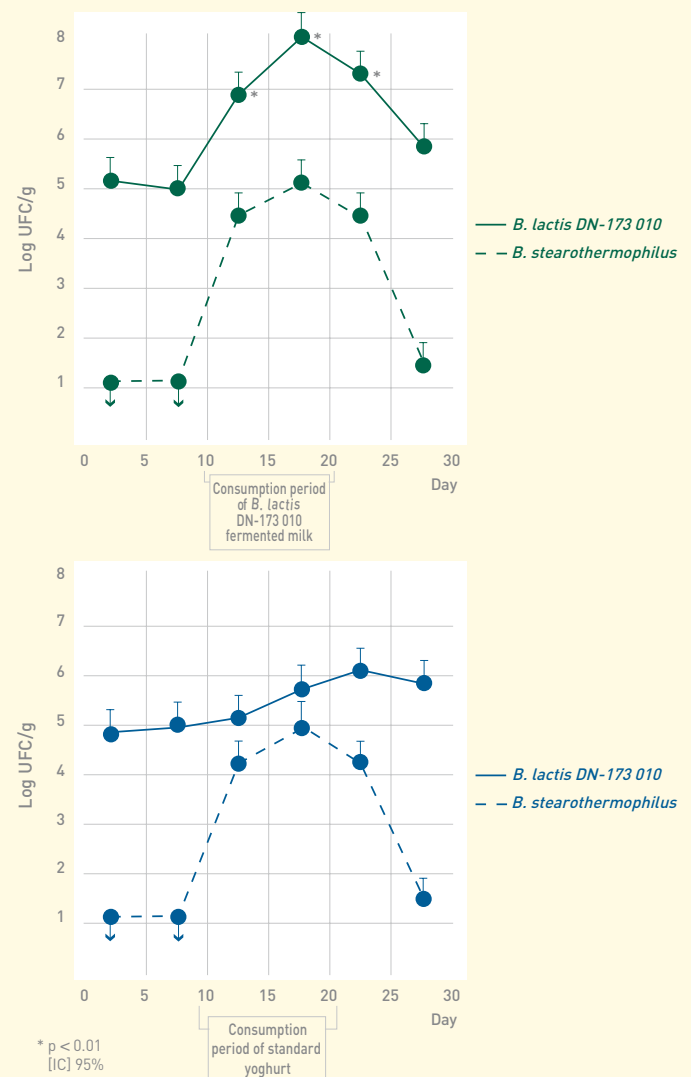
Results:

A significant ($p < 0.001$) difference in bifidobacteria concentration was observed during the product consumption period, between the *B. lactis* DN-173 010 fermented milk group (respectively 1.3×10^7 and 1.6×10^8 cfu/g) and the yoghurt group (respectively 1.3×10^5 and 8×10^5 cfu/g).

During the follow-up period, a significant ($p < 0.001$) difference in bifidobacteria concentration between *B. lactis* DN-173 010 milk group (8×10^7 cfu/g) and yoghurt group (2×10^6 cfu/g) was observed. No more significant difference was detected after 10 days follow-up.

Conclusion: after the consumption of 3 x 125 g of *B. lactis* DN-173 010 fermented milk over 10 days, the count of bifidobacteria increases up to a mean concentration of 10^8 cfu/g. This increase is transient: after discontinuation of fermented milk ingestion, the level of bifidobacteria in faeces returned to the baseline value (10^5 to 10^6 cfu/g). *B. lactis* DN-173 010 does not colonize the gastrointestinal tract.

B. lactis DN-173 010 & *BACILLUS stearothermophilus* SPORES COUNTS IN FECES BEFORE, DURING AND AFTER INGESTION OF *B. lactis* DN-173 010 FERMENTED MILK OR STANDARD YOGHURT



Pochart P, Marteau P, Bisetti N, Goderet I, Bourlioux P, Rambaud JC. Isolement des bifidobactéries dans les selles après ingestion prolongée de lait au bifidus (LB). *Médecine et Maladies Infectieuses* 1990; 20: 75-78 - Publication in French

D A colony-immunoblotting method for quantitative detection of a *Bifidobacterium animalis* probiotic strain in human faeces (Duez *et al*, 2000)

Study methodology:

In this non randomised, non controlled, open study, 5 healthy women, (20-48 yo), consumed daily 3 x 125 g servings of Activia® over 7 days.

Evaluation criteria:

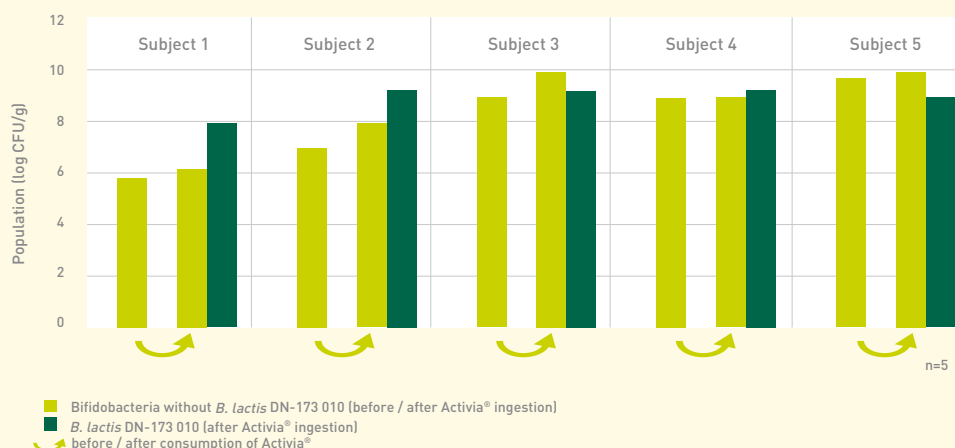
Quantification of *B. lactis* (Enumeration by plate counting, Immunodetection methods specific to *B. animalis lactis* species, evaluation at baseline and after 7 days of product consumption).

Results:

No colonies of *B. lactis* were detected in any of the faecal samples before ingestion of Activia®. After 7 days of Activia® consumption, *B. lactis* count varied amongst subjects, between 10⁸ and 10⁹ cfu/g.

Conclusion : after consumption of 3 x 125 g servings of Activia® during one week, *B. lactis* DN-173 010 is found viable, in large quantities ($\geq 10^8$ cfu/g), in faeces.

ENUMERATION OF THE BIFIDOBACTERIA AND THE *B. lactis* POPULATION IN FAECAL SAMPLES OF FIVE HUMAN VOLUNTEERS BEFORE AND AFTER ONE WEEK'S INGESTION OF ACTIVIA®



Duez H, Pelletier C, Cools S, Aissi E, Cayuela C, Gavini F, Bouquelet S, Neut C and Mengaud J. A colony-immunoblotting method for quantitative detection of a *Bifidobacterium animalis* probiotic strain in human faeces. *Journal of Applied Microbiology*, 2000; 88:1019-27

E Molecular detection of *Bifidobacterium animalis* DN-173 010 in human faeces during fermented milk administration (Collado *et al*, 2006)

Study methodology:

In this non randomised, non controlled, open trial including 12 healthy volunteers (25-40 yo), 10 subjects consumed daily 250 ml of Activia® (n = 10), over 4 weeks, 1 subject ingested any product (negative control) and 1 subject continuously ingested Activia®, for 3 months, prior study (positive control). The follow up period lasted 4 weeks.

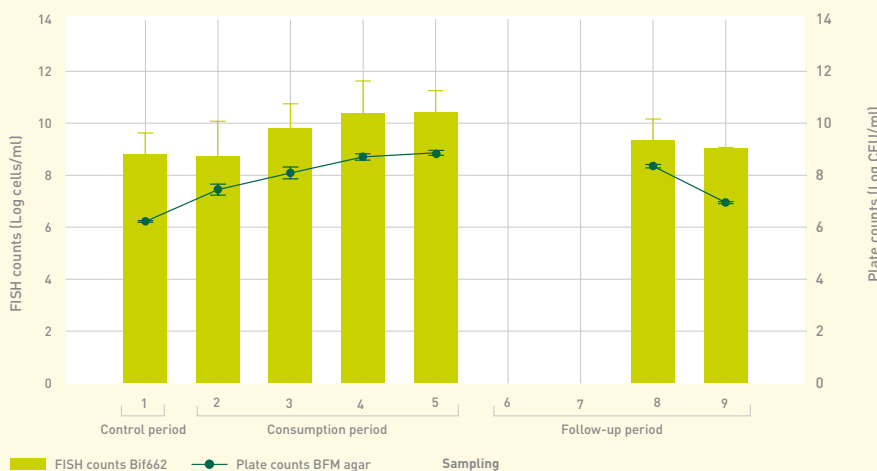
Evaluation criteria:

- Detection of bifidobacteria (Enumeration by plate counting and Fluorescent In Situ Hybridization (FISH); evaluation at baseline, and each week of product consumption and follow up periods).
- Identification of *B. animalis lactis* (Polymerase Chain Reaction (PCR))
- Identification of *B. animalis sp. lactis* DN-173 010 profile (Amplified Ribosomal DNA Restriction - PCR (ADRA-PCR); evaluation at baseline, and each week of product consumption and follow up periods).

Results:

A significant increase ($p < 0.05$) of bifidobacteria in faeces was observed during product consumption versus baseline. Count decrease to baseline level 4 weeks after discontinuation of the product. In contrast, the number of bifidobacteria in the positive and negative controls remained stable, over the studied period. 30% of bifidobacteria were identified as *B. lactis* by PCR after 2 weeks of product consumption (90% after 4 weeks). Subspecies were no more detectable after 4 weeks follow-up period. 40% of bifidobacteria were identified as DN-173 010 strain by ARDRA-PCR, after 2 weeks of product consumption (90% after 4 weeks). No more detection was observed after 4 weeks follow-up period.

Conclusion: after daily consumption of 250 ml of Activia® over 4 weeks, *B. lactis* DN-173 010 survives passage through the gastrointestinal tract.



TOTAL BIFIDOBACTERIA COUNTS IN THE FECES OF 10 HEALTHY VOLUNTEERS AFTER CONSUMPTION OF ACTIVIA®

Sample 1 = control period
 Sample 2-5 = administration period and
 Sample 6-9 = post-administration period
 (bars: counts using genus-specific probe Bif 662; line: counts using plate count method).

Collado M.C., Y. Moreno, J.M. Cobo, J.A. Mateos, M. Hernandez. Molecular detection of *Bifidobacterium animalis* DN-173 010 in human faeces during fermented milk administration. *Food Research International*, 2006; 39: 530-535

F Effect of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects (He *et al*, 2007)

Study methodology:

In this non randomised, non controlled study, 11 lactose-intolerant Chinese adults (23-54 yo), consumed daily 3 x 125 g servings of Activia® and 3 x 3 Bifina® capsules (encapsulated *B. longum*), over 2 weeks. The follow up period lasted 1 week.

Evaluation criteria:


- Quantification and composition of the faecal microbiota (Fluorescent In Situ Hybridization (FISH), Polymerase Chain Reaction- Denaturing Gradient Gel Electrophoresis (PCR-DGGE), evaluation at baseline, after 2 weeks of product consumption, and after one week of follow up period).
- Faecal β-galactosidase activity (evaluation one day before and one day after the supplementation period).
- Lactose Digestion Index (LDI – evaluation one day before and one day after the supplementation period, in blood samples collected before, 45 and 60 minutes after lactose ingestion).
- Symptoms scores (SSC - after a lactose challenge test of 25 g of lactose in water, evaluation one day before and one day after the supplementation period).
- Oro-Caecal Transit Time (OCTT - evaluation by hydrogen breath, 1 day before and 1 day after the supplementation period).

Results:

A significant increase in the total number of cells ($p = 0.05$), total bacteria ($p = 0.03$) and *E. rectale/C. coccoides* group ($p = 0.04$) was observed in faeces, during product consumption, compared to baseline. The number of *Bifidobacterium* tended to increase during and after supplementation ($p = 0.07$), compared to baseline.

According to PCR-DGGE results, a band appear at the same level as the band from *B. lactis* during supplementation, in 10 out of the 11 subjects. This band disappears after the supplementation stops.

β-galactosidase activity significantly increased ($p = 0.02$) compared to baseline, during the supplementation period. LDI and OCTT did not change during product consumption, whereas SSC significantly decreased after supplementation ($p = 0.01$), compared to baseline.

 **Conclusion:** after consumption of 3 x 125 g / day servings of Activia®, during 2 weeks. *B. lactis* DN-173 010 survives through the gastro-intestinal tract.

NUMBERS OF TOTAL CELLS, TOTAL BACTERIA AND PREDOMINANT BACTERIAL GROUPS IN THE FAECES OF LACTOSE-INTOLERANT SUBJECTS BEFORE, DURING AND AFTER CONSUMPTION OF ACTIVIA® AND BIFINA® CAPSULES

Stain or probes	Targeted groups	Baseline period		Supplementation period		Follow-up period	
		Cells (10 ¹⁰)*	% Total bacteria †	Cells (10 ¹⁰)*	% Total bacteria †	Cells (10 ¹⁰)*	% Total bacteria †
DAPI	Total cells	14.5 ± 6.3		20.6 ± 4.9 [§]		17.4 ± 8.5	
Eub338	Bacteria	12.9 ± 4.9		19.5 ± 5.5 [¶]		17.6 ± 7.9	
Bac303	Bacteroides/Prevotella	3.4 ± 1.7	27.7 ± 12.3	5.3 ± 2.9	26.5 ± 12.0	4.6 ± 3.3	27.1 ± 18.2
Erec482	Eubacterium rectale/ Clostridium coccoides group	2.5 ± 1.4	19.6 ± 7.1	4.2 ± 1.3 ^{**}	22.2 ± 6.9	3.3 ± 1.8	20.2 ± 9.0
Elgc01	Eubacterium low G+C2	0.8 ± 0.5	6.4 ± 3.1	1.2 ± 0.6	6.1 ± 2.7	1.5 ± 0.8	8.9 ± 4.4
Rbro729/Rfla730	Ruminococcus group	1.1 ± 1.1	7.7 ± 6.5	0.6 ± 0.5	3.6 ± 3.2	1.3 ± 0.7	8.6 ± 7.5
Bif164y	Bifidobacterium	0.1 ± 0.1	0.8 ± 1.0	0.2 ± 0.3	1.2 ± 1.4	0.3 ± 0.5	2.2 ± 3.6

*Values are means ± SD or %, n = 11 (baseline period and during) or 10 (after). - * Per g faeces, dry weight. - † Percentage of bacteria [Eub338].

§ P = 0.05 compared with baseline period. - ¶ P = 0.03 compared with baseline period. - ** P = 0.04 compared with baseline period.

He T, M G Priebe, Y Zhong, C Huang, HJM Harmsen, GC Raangs, JM Antoine, GW Welling and RJ Vonk. Effect of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects. *Journal of Applied Microbiology*, 2007; 104(2):595-604.

G Survival of *Bifidobacterium animalis* DN-173 010 in the faecal microbiota after administration in lyophilised form or in fermented product – A randomised study in healthy adults (Rochet *et al*, 2008)

Study methodology:

In this randomised, open, parallel study, 12 healthy subjects (24-46 yo) consumed daily either 3 x 125 g servings (6.6.10¹⁰ CFU / day) of Activia® (n = 6) or 1 g (2.1.10¹¹ CFU / day) of freeze-dried powder of *B. lactis* DN-173 010 (n = 6), over 7 days. The follow up period lasted 10 days.

Evaluation criteria:


- Survival of *B. animalis sp. lactis* in faeces (Colony immunoblotting, Fluorescent In Situ Hybridization (FISH) and Polymerase Chain Reaction (PCR)-Temporal Temperature Gradient Gel Electrophoresis (PCR-TTGE), evaluation at baseline, at the end of the product consumption and at the end of the follow-up period
- Determination of faecal enzyme activities and metabolites (evaluation at baseline, at the end of product consumption and at the end of the follow-up period).

Results:

According to the quantification by the immunoblotting method, the mean number of *B. animalis sp. lactis* was $\geq 10^8$ cfu/g of faeces, in 5 out of 6 subjects, in both group, after 7 days of product consumption.

With PCR-TTGE, *B. lactis* DN-173 010 pattern were detected for 11 out of 12 subjects.

No major modification were observed in either the dominant members of the faecal microbiota or their activities.

 **Conclusion:** after the daily consumption of 3 x 125 g servings of Activia® or 1 g of freeze-dried powder during 10 days, *B. lactis* DN-173 010 survives gastrointestinal transit.

QUANTIFICATION OF *B. lactis* DN-173 010 BY IMMUNODETECTION

Lyophilised form				Fermented product			
Subject	Day 0	Day 7	Day 17	Subject	Day 0	Day 7	Day 17
1 LF	UD	→ 10 ⁹	UD	2 FP	UD	4.7x10 ⁸	UD
4 LF	UD	8.2x10 ⁸	UD	3 FP	UD	8.3 x10 ⁸	UD
5 LF	UD	4.5 x10 ⁸	UD	6 FP	UD	1.9 x10 ⁸	UD
8 LF	UD	UD	UD	7 FP	UD	2.1 x10 ⁷	6.4x10 ⁵
10 LF	UD	2.3 x10 ⁹	UD	9 FP	UD	UD	UD
11 LF	UD	1.0 x10 ⁷	10 ⁵	12 FP	UD	1.0 x10 ⁷	1.0x10 ⁷
Mean		3.9 x10 ⁸		Mean		1.1 x10 ⁸	

Data are presented as cfu/g of faeces. UD: Under Detection limit (<10⁴ cfu/g faeces).

Rochet V, L Rigottier-Gois, A Ledaire, C Andrieux, M Sutren, S Rabot, A Mogenet, J-L Bresson, S Cools, C Picard, N Goupil-Feuillerat and J Doré. Survival of *Bifidobacterium animalis* DN-173 010 in the faecal microbiota after administration in lyophilised form or in fermented product - a randomised study in healthy adults. *Journal of Molecular Microbiology and Biotechnology*, 2008;14: 128-136.

STUDIES PERFORMED WITH ACTIVIA® OR *B. lactis* DN-173 010

EFFECTS OF ACTIVIA® OR *B. lactis* DN-173 010 FERMENTED MILK ON TRANSIT TIME AND BOWEL FUNCTIONS

2

A to **E** Transit time studies

F Bowel functions study

A Effect of consumption of a milk fermented by the probiotic strain *Bifidobacterium animalis* DN-173 010 on colonic transit time in healthy humans (Bouvier *et al*, 2001)

Study methodology:

In this randomised, controlled, double-blind, parallel study, 72 healthy volunteers (21-42 yo) consumed daily 3 x 125 g of either *B. lactis* DN-173 010 fermented milk (n = 36) or heat-treated *B. lactis* DN-173 010 fermented milk with no viable bacteria (n = 36), over 11 days.

Evaluation criteria:

Colonic transit times : total, right, left and sigmoid (Radio-opaque marker method, evaluation at baseline and after 11 days of product consumption).

Results:

Total transit time and sigmoid transit time were significantly reduced by 20.6% (p = 0.013) and 38.9% (p = 0.02)

respectively, vs baseline, after consumption of *B. lactis* DN-173 010 fermented milk. The difference between initial and final colonic transit times was significantly greater in *B. lactis* DN-173 010 fermented group than in the control group (p < 0.05).

In the *B. lactis* DN-173 010 fermented milk group, analysis by gender demonstrated a significant reduction of colonic transit time in men (p < 0.03) and women (p < 0.05) compared to baseline.

Conclusion: the daily consumption of 3 x 125 g of *B. lactis* DN-173 010 fermented milk during 11 days, significantly reduces colonic and sigmoid transit times. This study demonstrates that live *B. lactis* DN-173 010 is required to reduce transit time.

COLONIC AND SIGMOID TRANSIT TIME BEFORE AND AFTER PRODUCTS CONSUMPTION

Transit Time (h)	<i>B. lactis</i> DN-173 010 fermented milk (n = 36)			Control product (n = 36)		
	Baseline	After consumption	Delta	Baseline	After consumption	Delta
Colonic	33.0 ± 16.1	26.2 ± 14.7*	- 6.8 ^a [- 20.6%]	30.1 ± 16.4	30.6 ± 17.4	+ 0.5 [+ 1.6%]
Sigmoid colon	9.5 ± 8.6	5.8 ± 7.7*	- 3.7 [- 38.9%]	7.9 ± 6.3	7.1 ± 8.7	- 0.8 [- 10.1%]

* p < 0.05: significantly different from baseline (Wilcoxon test).

^a p < 0.05: significantly different between groups (Mann-Whitney test).

Bouvier M, Méance S, Bouley C, Berta JL and Grimaud JC. Effect of consumption of a milk fermented by the probiotic strain *Bifidobacterium animalis* DN-173 010 on colonic transit time in healthy humans. *Bioscience Microflora*. 2001; 20 (2): 43-48

B A fermented milk with a *Bifidobacterium* probiotic strain DN-173 010 shortened oro-faecal gut transit time in elderly (Meance *et al*, 2001)

Study methodology:

In this randomised, open, parallel study, 90 elderly subjects (60-75 yo), with a baseline transit time < 40 hours (n = 44) or ≥ 40 hours (n = 44), consumed either 2 or 3 x 125 g servings of Activia®, over 2 weeks.

Evaluation criteria:

Oro-faecal transit time (Coloured marker method, evaluation at baseline and after 2 weeks of product consumption).

Results:

In the subgroup of subjects with a transit time < 40h, significant reductions by 1.7h (6.9%, p < 0.001) after consumption

of 2 servings, and by 2.9h (11.8%, p < 0.001) after consumption of 3 servings were observed, versus baseline.

In the subgroup of subjects with a transit time ≥ 40h, significant reductions by 24.6h (38.6%, p < 0.001) after consumption of 2 servings, and by 28.6h (46.4%, p < 0.001), after consumption of 3 servings were observed, versus baseline. The effect of 3 servings was significantly greater than 2 servings (p < 0.05).

Conclusion: daily consumption of 2 or 3 x 125 g servings of Activia® during 2 weeks shortens oro-faecal transit time, in elderly subjects, especially those with a slow transit time.

ORO-FAECAL TRANSIT TIME BEFORE AND AFTER 2 OR 3 SERVINGS OF ACTIVIA® CONSUMPTION

	Transit time < 40 hours		Transit time ≥ 40 hours	
	3 servings (n = 19)	2 servings (n = 27)	3 servings (n = 20)	2 servings (n = 24)
Before consumption	24.6 ± 1.3	24.3 ± 1.5	61.6 ± 13.0	63.7 ± 12.1
After consumption	21.7 ± 1.0 ^{ab}	22.8 ± 1.4 ^a	32.9 ± 8.5 ^{ab}	39.1 ± 10.2 ^a
Change	2.9 ± 1.1 ^a	1.7 ± 0.6	28.6 ± 5.8 ^a	24.6 ± 4.4

Values: mean ± SD

^a p < 0.001: significantly different from baseline values (Wilcoxon's test).

^b p < 0.05: 3 servings significantly different from 2 servings (Mann-Whitney test).

Meance S, Cayuela C, Turchet P, Raimondi A, Lucas C and Antoine JM. A fermented milk with a *Bifidobacterium* probiotic strain DN-173 010 shortened oro-faecal gut transit time in elderly. *Microbial Ecology in Health and Disease*. 2001; 13: 217-222

C *Bifidobacterium lactis* strain DN-173 010 shortens the colonic transit time in healthy women: a double blind, randomized, controlled study (Marteau *et al*, 2002)

Study methodology:

In this randomised, controlled, double-blind, cross-over study, 32 healthy women (18-45 yo) were divided in two groups, and consumed daily 3 x 125 g servings of Activia® then 3x125g servings of a yogurt, the first group starting with Activia® (n = 17) and the second one with the yogurt (n = 15). Each ingestion period lasted 10 days, with a 10 days interval period between ingestion of each product.

Evaluation criteria:


- Colonic transit times : total right, left and sigmoid. (Colour marker method, evaluation at baseline and after each product ingestion period).
- Faecal bile salts, pH, microbial mass and weight (Evaluation at the end of each ingestion period).

Results:

In the whole population, both colonic and sigmoid transit times were significantly ($p < 0.05$) shortened in the Activia® group, versus the control group, but no difference was observed vs baseline.

In the subgroup of subjects with an initial transit time > 40 hours, both colonic and sigmoid transit times were significantly ($p < 0.05$) shortened in the Activia® group versus the control group and vs baseline.

Faecal mass, pH, bacterial mass and bile acids were not significantly modified.

 **Conclusion:** the daily consumption of 3 x 125 g servings of Activia® significantly reduces colonic transit time in women, this effect being more pronounced in women with initial slow transit time.

COLONIC AND SIGMOID TRANSIT TIME IN WHOLE POPULATION AND WOMEN WITH COLONIC TRANSIT TIME > 40 H

Transit Time (h)	Whole population (n = 32)			Subjects with colonic transit time → 40 h (n = 21)		
	Baseline	Activia®	Control	Baseline	Activia®	Control
Colonic	55.2 ± 28.0 ^{ab}	51.5 ± 30.2 ^a	60.7 ± 27.1 ^b	70.4 ± 21.8 ^a	62.4 ± 29.8 ^b	71.9 ± 26.5 ^a
Sigmoid	25.2 ± 18.9 ^{ab}	21.6 ± 14.9 ^a	26.8 ± 14.2 ^b	32.8 ± 18.3 ^a	27.1 ± 14.9 ^b	32.1 ± 13.1 ^a

Values: mean ± SD
Two values having different letters within a single line are statistically significantly different ($p < 0.05$).

Marteau P, Cuillerier E, Méance S, Gerhardt MF, Myara A, Bouvier M, Bouley C, Tondou F, Bommelaer G, Grimaud JC. *Bifidobacterium animalis* strain DN-173 010 shortens the colonic transit time in healthy women : a double blind, randomized, controlled study. *Alimentary Pharmacology and Therapeutics*, 2002; 16: 587-593

D Recent advances in the use of functional foods: effects of the commercial fermented milk with *Bifidobacterium lactis* strain DN-173 010 and yoghurt strains on gut transit time in the elderly (Meance *et al*, 2003)

Study methodology:

In this randomised, open, parallel study, 159 elderly volunteers (50-75 yo), with a baseline transit time between 40 and 50 hours (n = 81) or > 50 hours (n = 78), consumed daily either 1 or 2 x 125 g servings of Activia®, over 2 weeks. This consumption period was followed by a 6 weeks period without product ingestion.

Evaluation criteria:

Oro-faecal transit time (Coloured marker method, evaluation at baseline, after 2 weeks of product consumption, and every 2 weeks of the follow up period).

Results:

In the subgroup of subjects with a baseline transit time between 40 and 50 hours, significant reductions of total transit time by 21% after consumption of 1 serving (p < 0.05) and by 42% after consumption of 2 servings (p < 0.05) were observed, versus baseline.

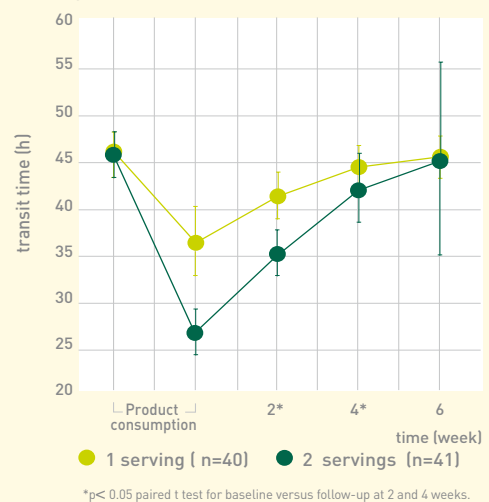
The effect of product consumption was still significant after 4 weeks of follow up for both groups, versus baseline. In the subgroup of subjects with a baseline transit time > 50 hours, significant reductions of transit time by 27% after consumption of 1 serving (p < 0.05), and by 38% after consumption of 2 servings (p < 0.05) were observed, versus baseline.

The effect of the product was still significant after 2 weeks of follow up for the group consuming 1 serving, and after 6 weeks of follow up for the group consuming 2 servings, vs baseline.

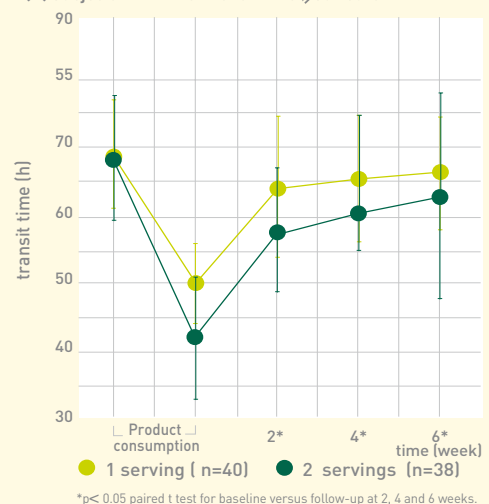
Conclusion: daily consumption of 1 or 2 x 125 g servings of Activia® during 2 weeks, shortens total transit time in elderly subjects, especially those with a slow transit time.

ORO-FAECAL TRANSIT TIME BEFORE AND AFTER 1 OR 2 SERVINGS OF ACTIVIA® CONSUMPTION

(A) Subjects with initial transit time between 40 and 50 hours



(B) Subjects with initial transit time > 50 hours



Méance S, Cayuela C, Raimondi A, Turchet P, Lucas C and Antoine JM. Recent advances in the use of functional foods: effects of the commercial fermented milk with *Bifidobacterium lactis* strain DN-173 010 and yoghurt strains on gut transit time in the elderly. *Microbial Ecology in Health and Disease* 2003; 15: 15-22

E Effect of *Bifidobacterium lactis* DN-173 010 on the intestinal transit time, the condition of defecation and intestinal microflora: A randomized, double-blind, placebo-controlled, cross-over study among healthy Japanese women (Nishida *et al*, 2008)

Study methodology:

In this randomised, double blind, placebo-controlled, cross-over study, 50 healthy Japanese women (mean age 19.43 ± 1.62 yo) were divided in 2 groups, and consume daily 2 x 185 g servings of Activia®, then 2 x 85 g servings of a control product without *B. lactis* DN-173 010. The first group starting with Activia® (n=25) and the second one with the control product (n=25). Each ingestion period lasted 2 weeks with a 6 weeks interval period between ingestion of each products.

Evaluation criteria:

- Oro-faecal transit time (Colored marker method, evaluation at baseline and after 2 weeks of product consumption).
- Stool frequency, quantity, color and shape; (stool frequency expressed as number per week; color and shape defined according to scale and model respectively, daily evaluation recorded in a diary).
- Faecal microflora analysis (Enumeration by plate counting, evaluation at baseline, and after 2 weeks of product consumption).

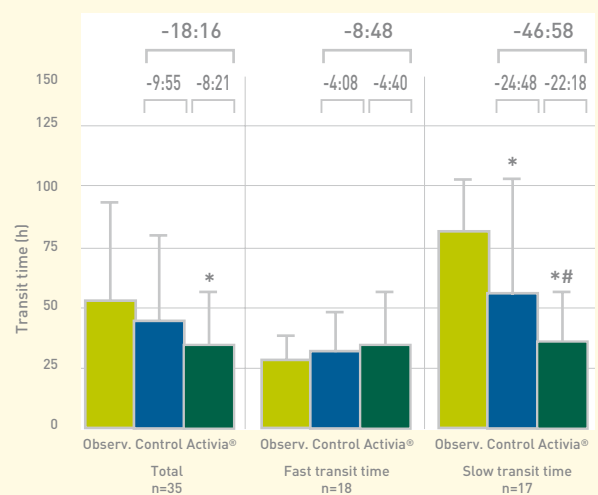
Results:

Analyses on intestinal transit time were performed on 35 subjects. In the overall population, intestinal transit time was significantly reduced after Activia® consumption ($p < 0.05$) as compared with baseline. In subjects with slow transit time (> 40 hours), the intestinal transit time tended to be reduced compared to the control group ($p = 0.055$). Analyses on stool frequency were performed on 43 subjects. Stool frequency significantly increased after Activia® consumption, as compared with control ($p < 0.05$). Analyses

on intestinal microflora were performed on 14 subjects. The *Bifidobacterium* cell count and occupation ratio significantly increased during the Activia® consumption ($p < 0.05$). With regard to the quantity, colour, shape and characteristics of faeces, no significant differences were observed between the two groups.

Conclusion: the daily consumption of 2 x 85 g servings of Activia® during 2 weeks, improves the intestinal transit time and increases the stool frequency, especially in women with a slow transit time.

ORO-FAECAL TRANSIT TIME BEFORE AND AFTER ACTIVIA® OR CONTROL CONSUMPTION



Values represent the mean \pm S.D. *Significantly different compared with Observ. ($p < 0.05$). # $p = 0.055$ compared with control.

Nishida S., Ishikawa Y., Iino H. Effect of *Bifidobacterium lactis* DN-173 010 on the Intestinal Transit Time, the Condition of Defecation and Intestinal Microflora: A randomized, Double-blind, Placebo-controlled, Cross-over Study among Healthy Japanese Women. *Pharmacometrics* 2008;74 (5/6) : 99-106

F Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women (Yang *et al*, 2008)

Study methodology:

In this randomised, double-blind, placebo-controlled, parallel group study, 135 healthy Chinese women with constipation (25-65 yo), consumed daily 100 g of either Activia® (n = 67) or an acidified milk containing non-living bacteria (n = 68), over 2 weeks.

Evaluation criteria:

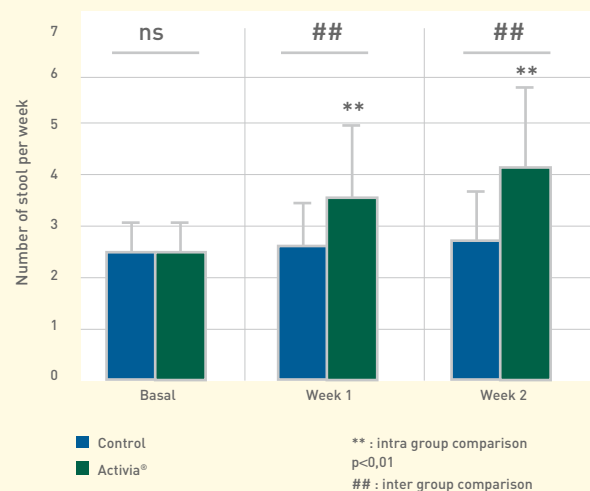
- Stool frequency (expressed as number/week; evaluation at baseline, and after 1 and 2 weeks of product consumption)
- Stool consistency (Bristol stool scale; evaluation at baseline, and after 1 and 2 weeks of product consumption).
- Defecation condition (4 grade score, evaluation at baseline, and after 1 and 2 weeks of product consumption).

Results:

After 1 and 2 weeks of product consumption, stool frequency significantly increased ($p < 0.01$) and defecation conditions and stool consistency significantly improved ($p < 0.01$) in the Activia® group, in comparison to the control group.

Conclusion: daily consumption of 100 g of Activia® during 2 weeks improves stool frequency and consistency as well as defecation conditions, in women with constipation.

STOOL FREQUENCY AFTER ONE OR TWO WEEKS OF ACTIVIA® OR CONTROL CONSUMPTION



STUDIES PERFORMED WITH ACTIVIA® OR *B.lactis* DN-173 010

EFFECTS OF ACTIVIA® ON DIGESTIVE COMFORT

3

A Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care. A multicentre, randomised, double-blind, controlled trial (Guyonnet *et al*, 2007)

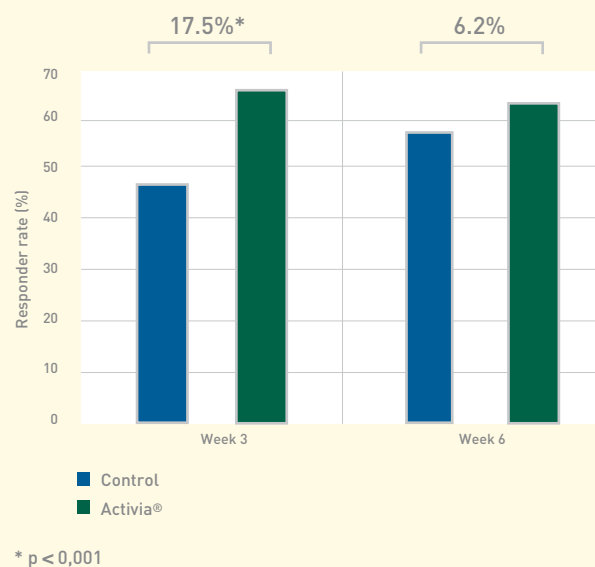
Study methodology:

In this randomised, controlled, double-blind, parallel study, 267 subjects (18-65yo) with Irritable Bowel Syndrome with predominant constipation (IBS-C), consumed daily, 2x125 g servings of either Activia® (n = 135) or a heat-treated yogurt (n = 132), over 6 weeks.

Evaluation criteria:

- Digestive discomfort dimension of the Functional Digestive Disorder Quality of Life questionnaire : FDDQL (Evaluation after 3 and 6 weeks of product consumption).
- Rate of responders for the discomfort dimension of FDDQL questionnaire (defined as subjects having an improvement $\geq 10\%$ of digestive discomfort score; evaluation after 3 and 6 weeks of product consumption).
- Global digestive symptoms (7-points Likert scale; evaluation after 3 and 6 weeks of product consumption).
- Bloating and abdominal pain intensity (6-points Likert scale; evaluation at baseline and at weeks 3 and 6 of product consumption).
- Stool frequency (expressed as number of bowel movement/week, daily evaluation).
- Stool consistency (Bristol Stool Scale, daily evaluation).

RATE OF RESPONDERS FOR DIGESTIVE COMFORT DIMENSION AFTER 3 AND 6 WEEKS CONSUMPTION



Guyonnet D, O. Chassany, P. Ducrotte, C. Picard, M. Mouret, C.H. Mercier and C. Matuchansky. Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome adults in primary care. A multicentre, randomised, double-blind, controlled trial. *Alimentary Pharmacology and Therapeutics*, 2007; 26 (3): 475-486

3 • EFFECTS OF ACTIVIA® ON DIGESTIVE COMFORT

Results:

The FDDQL digestive discomfort score improved ($p < 0.001$) in both groups at week 3 (Activia®: 10.7 ± 14.5 ; control: 7.5 ± 16.5) and week 6 (Activia®: 12.2 ± 16.2 ; control: 13.5 ± 19.3).

The responder rate for the discomfort dimension of the FDDQL questionnaire was significantly higher at week 3 in the Activia® group (65.2%) versus the control group (47.7%) ($p < 0.01$).

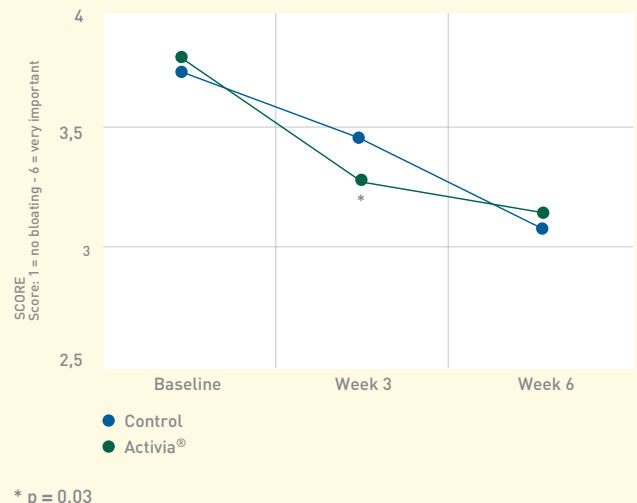
The evolution of global digestive symptoms scores did not differ between groups at weeks 3 and 6.

Bloating score significantly ($p < 0.001$) improved in both groups as compared to baseline, this improvement being significantly ($p = 0.03$) higher in Activia® group at week 3. Abdominal pain significantly ($p < 0.001$) improved in both groups as compared to baseline without difference between groups.

In a subgroup of subjects with less than 3 stools / week, stool frequency significantly increased over 6 weeks in the Activia® group versus the control group ($p < 0.001$).

Conclusion: the daily consumption of 2 x 125 g servings of Activia® during 6 weeks improved digestive comfort. This study suggests a beneficial effect on the health-related quality of life digestive discomfort score and bloating in subjects with IBS-C. A positive effect on stool frequency in subjects having less than 3 stools per week was also observed.

BLOATING BEFORE AND AFTER 3 AND 6 WEEKS OF ACTIVIA® OR CONTROL CONSUMPTION



B Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation (Agrawal *et al*, 2009)

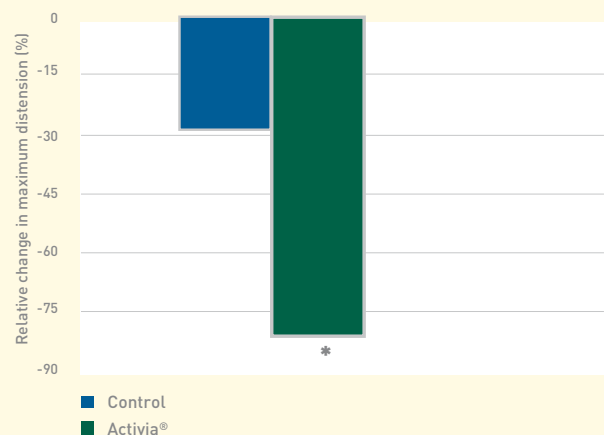
Study methodology:

In this randomised, controlled, double-blind, parallel group study, 38 women (18-70 yo) with Irritable Bowel Syndrome with predominant constipation (IBS-C), consumed daily, 2x125g servings of either Activia® (n = 18) or a non fermented dairy product with low content of lactose (n = 20), over 4 weeks.

Evaluation criteria:

- Abdominal distension (Plethysmography; 24h evaluation at baseline and after 4 weeks of product consumption).
- Colonic and small bowel transit time (Radiopaque marker and hydrogen breath test methods; evaluation at baseline and after 4 weeks of product consumption).
- Overall IBS symptoms severity and digestive symptoms severity (i.e. abdominal pain / discomfort, bloating, flatulence; daily evaluation reported on a diary).
- Stool frequency (expressed as number of bowel movement/week, daily evaluation).
- Stool consistency (Bristol Stool Scale, daily evaluation).

PERCENTAGE CHANGE IN MAXIMAL DISTENSION AFTER ACTIVIA® OR CONTROL CONSUMPTION



The data represent the median
* p < 0.05

Agrawal A., Houghton L.A., Morris J., Reilly B.,D., Guyonnet D., Goupil-Feuillerat N., Schlumberger A., Jakob S. & Whorwell P.J. Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173-010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. *Alimentary Pharmacology and Therapeutics*, 2009; 29(1): 104-114

Results:

After 4 weeks of product consumption, a trend towards a reduction in the mean distension during the day (AUC values, -1.52 cm 95% CI [3.33, 0.39], $p = 0.096$) and a significant reduction in the median percentage change in maximal distension (Activia®: -77.1% vs. control: -28.6%, $p = 0.029$) were observed in the Activia® group, in comparison with the control group.

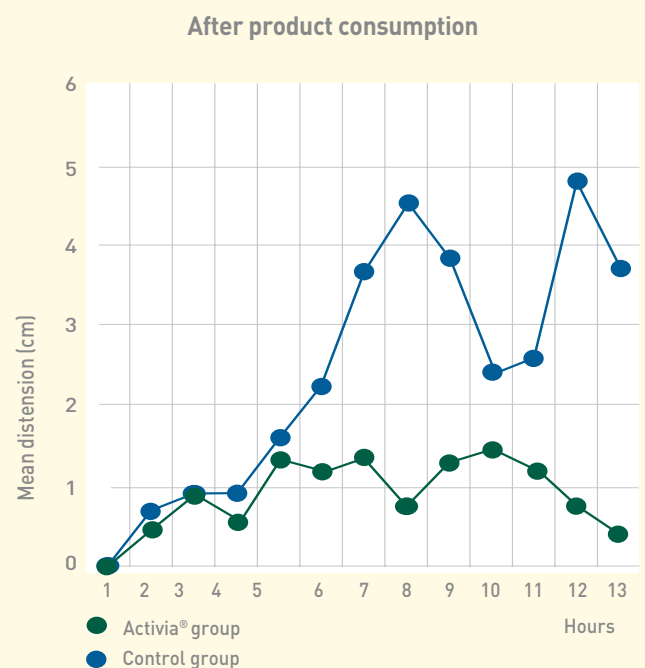
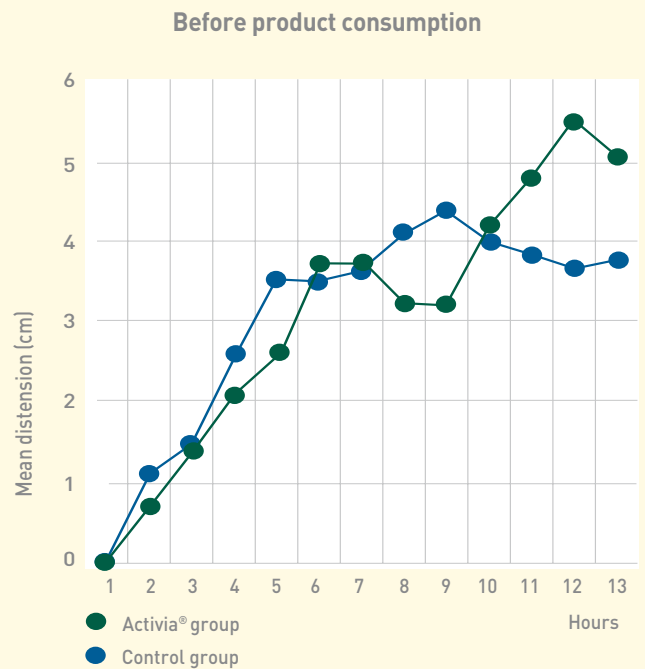
Colonic and small bowel transit times were significantly accelerated in the Activia® group, in comparison with the control group (respectively -12.2h, 95%CI [-22.8,-1.6], $p = 0.049$ and -1.2h, 95%CI [-2.3, 0.0], $p = 0.026$).

Over the 4 weeks of Activia® consumption, overall symptom severity significantly improved ($p = 0.032$), abdominal pain/discomfort significantly decreased ($p = 0.044$), and bloating ($p = 0.059$) and flatulence ($p = 0.092$) tended to reduce, compared with control group.

A trend to a normalization of stool consistency ($p = 0.06$) without modification of stool frequency was also observed.

Conclusion: the daily consumption of 2x125 g servings of Activia® over 4 weeks, significantly reduced abdominal distension in association with gastro intestinal transit IBS-C women. Activia® improves digestive comfort by reducing overall IBS symptom severity and digestive symptoms.

MEAN ABDOMINAL DISTENSION BEFORE AND AFTER ACTIVIA® ON CONTROL CONSUMPTION



C Fermented milk containing *Bifidobacterium lactis* DN-173 010 improved self-reported digestive comfort amongst a general population of adults. A randomized, open-label, controlled, pilot study (Guyonnet *et al*, 2009 a)

Study methodology:

The study was a randomised, controlled, open-label, parallel group trial. 360 healthy volunteers (18–65 yo), with self-reported digestive discomfort and a normal stool frequency, consumed daily either 1x125 g serving (n = 144) or 2x125 g servings (n = 147) of Activia®, over 14 days. The control group (n = 69) had a normal diet.

Evaluation criteria:

- Self-reported digestive comfort (one question after 14 days of product consumption: “Think back over the last 2 weeks, how has your digestive comfort changed?”, using a 5-point Likert scale; evaluation after 14 days of product consumption).
- Bother from different digestive symptoms (Self-completed, 20-item questionnaire; evaluation at baseline, and at the end of product consumption).

Results:

The number of subjects reporting an improvement of digestive comfort was significantly ($p < 0.001$) higher in the Activia® groups vs the control group (82.5% and 84.3% with 1x125g serving and 2x125g servings, respectively vs 2.9%). The between-group difference were confirmed each of the two Activia® groups differing significantly from the control group ($p < 0.001$).

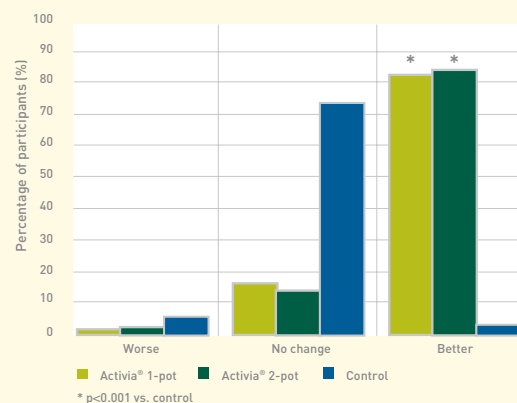
Self-reported change scores of general digestive discomfort differed significantly ($p < 0.001$) for both Activia® groups compared to the control group.

All symptoms scores (including bloated feeling, excessive

or trapped wind, swollen stomach) improved significantly ($p < 0.001$) for both Activia® groups compared to the control group.

Conclusion: the daily consumption of 1 or 2x125 g of Activia® servings during 14 days, significantly improves digestive comfort and bother from most of digestive symptoms in the general population, in real-life conditions.

PERCENTAGE OF SUBJECTS REPORTING AN IMPROVEMENT IN DIGESTIVE COMFORT



Scores are expressed as percentage of participants per category (worse, no change or better digestive comfort).

Guyonnet D., Woodcock A., Stefani B., Trevisan C. and Hall C. Fermented milk containing *Bifidobacterium lactis* DN-173 010 improved self-reported digestive comfort amongst a general population of adults. A randomized, open-label, controlled, pilot study. *Journal of Digestive Diseases*, 2009; 10: 61-70

D Fermented milk containing *Bifidobacterium lactis* DN-173 010 improves gastrointestinal well-being and digestive symptoms in women reporting minor digestive symptoms. A randomised, double-blind, parallel, controlled study. (Guyonnet *et al*, 2009 b)

Study methodology:

The study was a randomised, double-blind, controlled, parallel group trial. 197 women (18–60 yo) with minor digestive symptoms but without gastrointestinal diseases, consumed daily 2x125g servings of either Activia® (n = 100), or a non fermented dairy product with low content of lactose (n = 97), over 4 weeks. The follow up period lasted 4 weeks.

Evaluation criteria:

- Overall assessment of Gastrointestinal well-being (3-points Likert scale: worse, no change, better; and 15-points Likert scale: to precise the level of worsening or improvement, self-assessment by subjects, evaluation weekly during product consumption and follow up periods)

- Composite score and individual scores - frequency of digestive symptoms (abdominal pain/discomfort, bloating, flatulence/passage of gas, borborygmi/rumbling stomach; 5-points Likert scale, evaluation weekly throughout the study)
- Stool frequency (daily evaluation, expressed as number of bowel movement/week).
- Stool consistency (daily evaluation; Bristol Stool Scale).
- Health Related Quality of Life (self-administration of 2 questionnaires: the FBA, Food Benefit Assessment and the PGWBI, Psychological General Well-Being Index ; evaluation at baseline, after 4 weeks of product consumption and after 4 weeks of follow up period).

Guyonnet D., Schlumberger A., Mhamdi L., Jakob S., Chassany O.

Fermented milk containing *Bifidobacterium lactis* DN-173 010 improves gastrointestinal well-being and digestive symptoms in women reporting minor digestive symptoms: a randomised, double-blind, parallel, controlled study. *British Journal of Nutrition*, 2009 Jul 22:1-9.

Results:

The percentage of women reporting an improvement in their GI well-being was significantly ($p=0.006$) higher in the Activia® group vs control group (OR=1.7, [95% CI 1.17-2.45]), as well as the percentage of responders for GI well-being ($p=0.025$; 52.0% vs 36.1% respectively, OR=1.92 [95% CI 1.09-3.40]).

Over the 4 weeks of product consumption, results showed a significant more pronounced decrease in the composite score of digestive symptoms ($p=0.044$; LSmean = -0.57 [95% CI -1.12; -0.02].) and in borborygmi frequency ($p=0.016$; LSmean = -0.22 [95% CI -0.40;-0.04]) in the Activia® group compared to the control group.

The decrease in flatulence frequency was significantly higher in the Activia® group than in the control group at weeks 1, 2 and 4 ($p < 0.05$) whereas no significant differences were observed in bloating score as well as in abdominal pain/discomfort score.

A significant ($p=0.02$) improvement of the stool consistency was observed in the Activia® group vs. the control group.

Digestive comfort dimension score of the FBA, the primary HRQoL endpoint, significantly increased ($p=0.027$) after 4 weeks of product consumption in the Activia® group as compared to the control group. No difference was observed between groups for other HRQoL dimensions. PGWBI scores did not differ between groups over time.

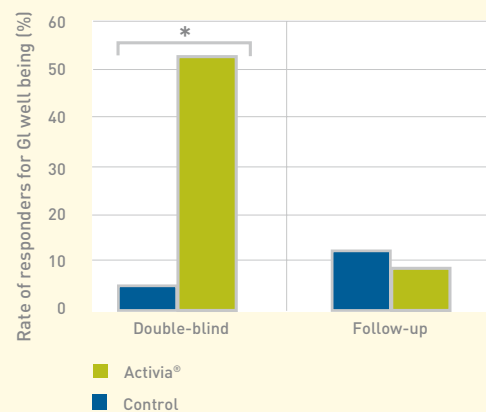
During follow-up period, the percentage of women reporting an improvement in their GI well-being didn't differ significantly between groups. The percentage of responders strongly decreased without significant differences between the Activia® and the control groups (8% vs. 11%; OR 0.68 [95%CI 0.26; 1.77]).

The composite score of digestive symptoms significantly decreased ($p < 0.05$) over the 4-weeks in the control group when comparing with the Activia® group. No significant difference in the changes of frequency for each individual symptom was observed between the groups.

No differences were observed between groups for stool frequency and score of stool consistency, as well as for all HRQoL dimensions.

Conclusion: the daily consumption of 2x125g servings of Activia® during 4 weeks significantly improves GI well-being and digestive symptoms in the general population. The beneficial effect of Activia® is not maintained once consumption is stopped.

OVERALL ASSESSMENT OF GI WELL-BEING - RATE OF RESPONDERS



A responder was defined as a subject having an improvement of their Gastrointestinal well-being at least 2 weeks among the 4-weeks; results are expressed as percentage

