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Effect of banana consumption on faecal microbiota: A randomised, controlled trial

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ABSTRACT

Banana is a widely consumed fruit, which contains considerable amounts of potential prebiotic indigestible carbohydrates. In our randomised, controlled trial we aimed to evaluate the *in vivo* prebiotic effect of banana consumption on faecal microbiota. Thirty-four healthy women participated in the study, having Body Mass Index (BMI) 24–30 kg/m², age 19–45 years, without history of gastrointestinal disease and no antibiotic and other medication use two months prior the initiation and during the study. All women were asked to maintain their usual dietary habits for 60 days and they were randomly assigned to consume twice a day a pre-meal snack, either one medium banana, or one cup of banana-flavoured drink or one cup of water (control group). Stool samples were collected at baseline, on days 30 and 60 of intervention for enumeration of total anaerobes, bifidobacteria and lactobacilli by plate count techniques, as well as for pH and short chain fatty acids (SCFAs) measurement. Gastrointestinal symptoms were also recorded. Mean bifidobacterial levels were increased only in the banana group both at 30 and 60 days of intervention, but this change did not reach a statistical significance. No significant overall differences in the total concentrations and molar ratios of SCFAs were detected according to dietary intervention. Analysis of the gastrointestinal symptoms records revealed significantly lower bloating levels in the banana group, compared to controls, at 26–35 days ($p = 0.009$) and 51–60 days ($p = 0.010$). Banana consumption had also no adverse effects on evacuation patterns. We concluded that daily consumption of bananas is a well-tolerated eating behaviour, which may induce bifidogenesis in healthy women experiencing body weight problems.

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1. Introduction

Dietary use of prebiotics is among the most well known food-based strategies to promote gut microflora modulation towards a health-promoting profile [1]. Prebiotics naturally occur in many plants and consumption of these natural sources is advantageous due to the lower financial cost compared to industrialised products, the better gastrointestinal tolerance and the beneficial effects highly aggregated with the associated intake of other nutrients, such as fibres, vitamins and minerals [2]. Such foods need to be tested for their prebiotic potential on a case-by-case basis by conducting human feeding trials [3].

Banana fruit can serve as a candidate whole food for potential prebiotic effects. Banana is widely consumed and has been indicated as a good source of type II (granulated) resistant starch [4] and fructooligosaccharides (FOS) [5–7], though significant

fluctuations in the fructan amounts were reported [2]. Experimental findings indicate various biological activities of banana and its constituents, including potential antimicrobial, antidiarrheic, antitumorogenic, antitumoral, antimutagenic, antihypertensive, antiatherogenic, antidiabetic and antiobesogenic effects [8–10]. Unfortunately, to the extension of our knowledge, there are no available data from long-term clinical studies concerning the prebiotic potential of banana consumption in healthy subjects. Regarding the aforementioned scientific depth, we aimed to evaluate the *in vivo* prebiotic effect of banana consumption on faecal microbiota by conducting a randomized, controlled clinical trial in human healthy volunteers.

2. Materials and methods

2.1. Subjects

Thirty-four healthy premenopausal women, aged 19–45 years and with BMI 24–30 kg/m², enrolled in the study. Exclusion criteria, such as gastrointestinal diseases, chronic diseases, a history of

Abbreviations: BMI, Body Mass Index; CFU, Colony Forming Units; FOS, Fructooligosaccharides; SCFAs, Short Chain Fatty Acids; VFAs, Volatile Fatty Acids.

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epileptic seizures, extreme dietary behaviours and the consumption of antibiotics and other medication prior 2 months and during the investigation period, have been previously described [11]. Subjects gave written informed consent and the protocol was approved by the Bioethics Committee of Harokopio University.

2.2. Feeding regime-experimental design

Dessert bananas (Cavendish cultivar) were medium sized (120g). Banana-flavoured drink contained water, banana puree (20% w/v), sugar, citric acid, ascorbic acid and banana flavourings.

Subjects were instructed and assessed properly prior to and during the intervention as previously reported [11]. Briefly, participants were instructed to preserve their usual diet and fluid intake during the study, with the exception of additional prebiotics and probiotic supplements, and were assessed for restriction of probiotic and prebiotic consumption during a period of two weeks prior the intervention.

During the intervention, subjects were randomly assigned to three groups according to feeding regime (banana group, banana drink group, control group) and consumed, respectively, twice a day a pre-meal snack, consisting of either one medium banana (120g), or one cup of banana-flavoured drink (170 ml) or one cup of water (170 ml), for 60 days. The pre-meal snack was consumed half an hour before lunch and dinner meal. Banana and banana-flavoured drink snacks were equal in terms of energy. Faecal samples were obtained prior to the intervention (Day 0) and on one month (Day 30) and two months (Day 60) of treatment period.

2.3. Gastrointestinal symptoms

Gastrointestinal side effects were evaluated for three time intervals during the treatment period (Day 1–10, Day 26–35 and Day 51–60), as a 10-d symptom score, using a daily questionnaire in which symptoms (i.e. abdominal pain, bloating, flatulence) were graded from 0 (no symptoms) to 3 (severe symptoms), as previously described [11]. Frequency and consistency of evacuations were also noted.

2.4. Sample collection and bacterial enumeration

Faecal sampling and bacterial enumeration were performed as previously reported [11]. Briefly, faecal specimens were collected and transferred under anaerobic conditions for microbiological analysis. Approximately 1 g of the specimen was serially 10-fold diluted anaerobically. Columbia blood agar, Rogosa agar and Beerens' agar were used for the enumeration of the total mesophilic anaerobic microflora, *Lactobacillus* spp. and *Bifidobacterium* spp., respectively. Colony counts were expressed as a log₁₀ of the colony forming units (CFUs)/g fresh faeces.

2.5. Measurement of SCFAs and pH

Faecal SCFAs concentrations were determined by capillary gas chromatography according to Mountzouris et al. (2006) [12]. Faecal sample (1.5g) was immediately diluted 1:3 with 0.9% saline and stored at –20 °C until analysis. One millilitre of the faecal saline suspension was centrifuged at 10,000g for 15 min at 4 °C and 300 µl of the supernatant were subsequently centrifuged at 13,000g for 15 min at 4 °C. Eighty-five microliters of the supernatant were mixed with 10 µl 2-ethyl-butyrate (20 mM, internal standard) and 5 µl hydrochloric acid. Samples of 1 µl were injected into a gas chromatographer (Agilent 6890 GC System, Agilent Technologies). The concentrations of SCFAs were computed based on instrument calibration with SCFA standard mix (Supelco, Sigma–Aldrich C., USA). Total volatile fatty acids (VFA) concentrations were expressed as µmol/g (wet weight) faeces and molar ratios of acetate, propionate, butyrate, branched-chain SCFAs (iso-butyrate, iso-valerate, iso-caproic acid) and other SCFAs (valerate, caproic acid and heptanoic acid) were also calculated.

For pH measurement of faecal samples, 1g of faeces was immediately diluted with 10 ml of deionised water and dispersed by vortexing. Measurement of pH was conducted in laboratory pHmeter (Inolab pH720, WTW) by a protein-resistant electrode (Sentix SP, WTW) at room temperature.

2.6. Statistics

Comparisons among study groups were performed by Repeated Measures ANOVA (RM-ANOVA) for parametric and Friedman test for non-parametric data, after Bonferroni's adjustment for multiplicity. Intragroup analysis was conducted by paired-samples T test for parametric and Wilcoxon signed ranks test for non-parametric data. The statistical analysis of the results was performed by the software program SPSS® for Windows Release 11.5 and the significance threshold was set at 5% ($p < 0.05$).

3. Results

Thirty-one subjects completed the study (mean age: 31 years, mean BMI: 27 kg/m²). Dropout was due to antibiotic consumption. Participants of the three experimental groups did not differ in their baseline characteristics, including sociodemographic factors, anthropometric indices, physical activity levels and dietary intake, in terms of nutrient and food group intake (data not shown).

3.1. Bacterial populations

Total anaerobes were estimated in comparable densities in the three study groups during the entire research period (Table 1). *Lactobacilli* levels demonstrated a trend for overall significant differences among the study groups and banana-flavoured drink

Table 1

Faecal bacterial counts^a (log₁₀ CFU/g faeces) in 3 feeding groups of healthy volunteers ($n = 31$) during a 60-d consumption of bananas ($n = 12$), banana-flavoured drink ($n = 9$) or water (control group, $n = 10$).

Bacterial group	Pre-treatment samples Day 0			Treatment samples Day 30			Treatment samples Day 60			p- group
	control	banana drink	banana	control	banana drink	banana	control	banana drink	banana	
Total anaerobes	10.16 ± 0.18	10.09 ± 0.16	10.01 ± 0.50	10.22 ± 0.22	10.16 ± 0.50	10.05 ± 0.51	10.01 ± 0.49	9.84 ± 0.63	10.07 ± 0.65	0.809
<i>Lactobacillus</i> spp.	6.19 ± 1.53	5.58 ± 1.28	6.22 ± 1.12	6.63 ± 1.18	4.97 ± 1.04 ^c	6.44 ± 0.87 ^d	6.02 ± 1.45	5.55 ± 1.18	6.13 ± 1.35	0.079
<i>Bifidobacterium</i> spp. ^b	9.62 ± 0.40	8.82 ± 2.31	9.32 ± 1.17	9.67 ± 0.26	8.95 ± 1.90	9.68 ± 0.42	9.43 ± 0.36	8.69 ± 1.82	9.81 ± 0.20 ^e	0.274

^a All values are mean ± S.D; CFU, colony forming units.

^b For banana group $n = 10$ and for banana-flavoured drink group $n = 8$.

^c Significantly different from control group: $p = 0.002$.

^d Significantly different from banana-flavoured drink group: $p = 0.003$.

^e Significantly different from banana-flavoured drink group: $p = 0.026$.

group was characterised by significantly lower counts of lactobacilli compared to control group and banana group at 30 days of intervention (Table 1).

Banana group demonstrated higher viable counts of bifidobacteria at Day 60 compared to banana-flavoured drink group (Table 1). Mean bifidobacterial levels were increased only in the banana group both at 30 and 60 days of intervention, but this change did not reach a statistical significance. Based on enumeration data, at the end of intervention 60% of subjects in the banana group exhibited an increase in their initial bifidobacterial levels ($0.96 \pm 1.14 \log_{10}$ CFU/g faeces), compared to 25% of subjects in the banana-flavoured drink group ($0.82 \pm 0.49 \log_{10}$ CFU/g faeces) and 30% in the control group ($0.34 \pm 0.23 \log_{10}$ CFU/g faeces). Three volunteers (two in banana group and one in the banana-flavoured drink group) had no detectable bifidobacterial levels throughout the entire study.

Group-specific analysis revealed no significant intragroup differences in the tested bacterial viable counts.

3.2. Measurement of SCFAs and pH

Overall, no significant differences in total Volatile Fatty Acids (VFAs) levels and molar ratios of the tested SCFAs were detected according to dietary intervention, though marginally in the case of branched-chain SCFAs (Table 2). Significantly elevated total VFAs concentration was detected in the controls compared to banana drink at 30 days of intervention. At 60 days, banana group exhibited higher total VFAs levels compared to control and banana drink group. Higher molar ratio of propionate in banana compared to banana drink group was reported at 30ds. Group-specific analysis indicated significantly higher levels of total VFAs levels in banana group at 60ds compared to 30ds and significantly decreased branched-chain SCFAs ratio in banana drink group at 60ds compared to baseline.

Overall, no significant differences were observed in faecal pH levels according to feeding regime (Table 2).

3.3. Gastrointestinal symptoms

Overall, significant differences were observed for number of evacuations and bloating levels during the dietary intervention, with no other overall significant influence of feeding regime in the

tested gastrointestinal symptoms and characteristics of evacuations (Table 3). Banana consumption was related with significantly higher frequency of evacuations at Days 1–10 and Days 26–35 compared to banana drink group. Intragroup comparisons indicated no significant changes in evacuation patterns into each experimental group. Subjects consuming banana reported significantly lower bloating levels at 26–35 days and 51–60 days compared to controls. Group-specific analysis exhibited a drop of initially (Day 1–10) reported bloating levels in banana group after one month of ingestion, which reached significance at the last 10 days of intervention ($p = 0.021$).

Banana group reported significantly lower scores of abdominal pain at Day 1–10 compared to banana drink ingestion. Group-specific analysis indicated that sum of symptoms were significantly decreased in banana-flavoured drink group at 26–35 days ($p = 0.028$) and 51–60 days ($p = 0.039$) compared to 1–10 days.

4. Discussion

In the present study we explored the prebiotic potential of banana fruit as a conventional whole food, which can become part of a regular diet. Banana ingestion induced exclusively a non significant increase in baseline bifidobacterial levels during the entire study period, with a higher and wider magnitude (mean increase of $0.96 \log_{10}$ CFU/g faeces in 60% of colonised subjects) compared to other study groups. It has been proposed that a 0.5 – $1.0 \log_{10}$ numerical increase in bifidobacterial levels constitutes a major shift in the gut microbiota towards a healthier composition [13]. Lack of statistical significance of our results may be attributed to the rather limited number of volunteers participating in our trial. Furthermore, the stage of banana ripeness is determinant for the FOS and resistant starch content of the fruit [4,7]. In our study, bananas were provided in the volunteers in the form of bunches on a weekly basis and were consumed from yellow with green tips (unripe) till yellow with black patches (ripe). This fact could influence the amount of provided FOS and resistant starch and the potential bifidogenic effect of banana consumption. Banana –flavoured drink contained a small portion of banana puree, which probably contributed limited to the total daily intake of FOS [6] and resistant starch in the diet compared to banana

Table 2
Faecal total VFAs concentrations ($\mu\text{mol/g}$ wet faeces), molar ratios (%) of SCFAs and pH values in 3 feeding groups of healthy volunteers ($n = 31$) during a 60-d consumption of bananas ($n = 12$), banana-flavoured drink ($n = 9$) or water (control group, $n = 10$)^a

	Pre-treatment samples Day 0			Treatment samples Day 30			Treatment samples Day 60			p- group
	control	banana drink	banana	control	banana drink	banana	control	banana drink	banana	
Total VFAs	52.78 \pm 20.68	48.80 \pm 18.36	60.43 \pm 22.66	74.69 \pm 28.98	48.40 \pm 20.03 ^d	58.36 \pm 15.89	55.80 \pm 18.47	48.21 \pm 22.77	70.93 \pm 10.32 ^{e,f,j}	0.087
Molar ratios										
Acetate	47.40 \pm 3.44	46.21 \pm 6.99	45.47 \pm 7.30	44.20 \pm 4.22	47.35 \pm 6.09	47.09 \pm 4.58	45.47 \pm 4.03	45.53 \pm 5.43	47.54 \pm 5.77	0.842
Propionate	13.94 \pm 1.70	14.08 \pm 2.23	14.73 \pm 2.67	14.24 \pm 2.62	13.35 \pm 2.15	15.91 \pm 2.72 ^g	14.53 \pm 2.61	15.58 \pm 2.60	14.60 \pm 2.93	0.552
Butyrate	26.26 \pm 4.46	25.91 \pm 4.28	29.25 \pm 8.29	29.53 \pm 5.15	26.69 \pm 7.61	27.08 \pm 5.63	28.61 \pm 4.99	28.03 \pm 6.67	28.59 \pm 4.06	0.740
Branched-chain SCFAs ^b	6.69 \pm 3.25	8.06 \pm 3.16	5.37 \pm 3.15	5.92 \pm 3.25	7.57 \pm 3.60	4.97 \pm 2.18	6.26 \pm 2.74	6.32 \pm 3.77 ^k	4.20 \pm 2.42	0.066
Other SCFAs ^c	5.71 \pm 0.71	5.75 \pm 3.35	5.18 \pm 1.88	6.11 \pm 2.26	5.04 \pm 1.25	4.94 \pm 1.04	5.13 \pm 0.91	4.55 \pm 1.24	5.06 \pm 1.39	0.462
pH	7.33 \pm 0.28	7.58 \pm 0.41	7.08 \pm 0.62 ^h	6.93 \pm 0.59	7.44 \pm 0.37 ⁱ	7.29 \pm 0.46	7.09 \pm 0.53	7.14 \pm 0.43	7.00 \pm 0.48	0.127

^a All values are mean \pm S.D; VFAs: Volatile Fatty Acids; SCFAs: Short Chain Fatty Acids.

^b Iso-butyrate, iso-valerate, iso-caproic acid.

^c Valerate, caproic acid, heptanoic acid.

^d Significantly different from control group: $p = 0.018$.

^e Significantly different from control group: $p = 0.048$.

^f Significantly different from banana-flavoured drink group: $p = 0.007$.

^g Significantly different from banana-flavoured drink group: $p = 0.036$.

^h Significantly different from banana-flavoured drink group: $p = 0.035$.

ⁱ Significantly different from control group: $p = 0.043$.

^j Significantly different from 30 ds ($p = 0.040$).

^k Significantly different from baseline ($p = 0.024$).

Table 3

Gastrointestinal symptoms and characteristics of evacuations in banana group ($n = 12$), banana-flavoured drink group ($n = 9$) and control group (water, $n = 10$) during the study (1–10, 26–35 and 51–60 days) ^a

Symptom score per 10 days	Day 1–10			Day 26–35			Day 51–60			<i>p</i> -group
	control	banana drink	banana	control	banana drink	banana	control	banana drink	banana	
Abdominal pain	2.90 ± 4.46	4.78 ± 5.38	0.83 ± 1.34 ^b	3.10 ± 5.24	2.89 ± 4.81	1.83 ± 4.09	3.00 ± 4.40	2.78 ± 4.66	1.08 ± 2.35	0.422
Bloating	7.40 ± 4.35	6.67 ± 5.70	3.33 ± 4.81	7.20 ± 5.45	4.67 ± 3.87	2.08 ± 3.34 ^c	6.80 ± 4.64	4.56 ± 5.08	1.67 ± 3.37 ^{d,g}	0.044
Flatulence	6.10 ± 6.26	6.11 ± 4.59	6.00 ± 5.03	7.30 ± 5.77	4.56 ± 3.94	5.33 ± 4.83	7.90 ± 6.26	4.11 ± 3.89	6.08 ± 5.62	0.616
Sum of symptom scores	16.40 ± 11.56	17.56 ± 13.84	10.17 ± 9.04	17.60 ± 13.66	12.11 ± 11.46 ^g	9.25 ± 10.37	17.70 ± 12.60	11.44 ± 11.98 ^g	8.83 ± 8.75	0.259
Number of evacuations	9.70 ± 1.42	9.22 ± 4.76	13.00 ± 4.84 ^e	10.20 ± 1.62	7.33 ± 2.50	13.00 ± 4.73 ^f	9.50 ± 2.27	9.22 ± 3.90	12.58 ± 5.73	0.029
Number of watery evacuations	0.30 ± 0.67	1.44 ± 2.96	0.33 ± 0.89	0.40 ± 0.70	1.44 ± 2.13	0.58 ± 1.38	0.30 ± 0.48	1.00 ± 2.65	0.58 ± 0.79	0.636
Days of diarrhoea	0.30 ± 0.67	1.44 ± 2.96	0.33 ± 0.89	0.40 ± 0.70	1.44 ± 2.13	0.58 ± 1.38	0.30 ± 0.48	1.00 ± 2.65	0.75 ± 0.87	0.660

Symptom intensity was graded as 0 = no symptoms, 1–10 = mild symptoms, 11–20 = moderate symptoms and 21–30 = severe symptoms. The possible range for each 10-day symptom score is 0–30 and for total symptom score 0–90. Diarrhoea was defined as ≥ 1 watery stool or > 3 stools/day.

^a All values are mean ± S.D.

^b Significantly different from banana drink group: $p = 0.030$.

^c Significantly different from control group: $p = 0.009$.

^d Significantly different from control group: $p = 0.010$.

^e Significantly different from banana drink group: $p = 0.043$.

^f Significantly different from banana drink group: $p = 0.001$.

^g Significantly different from baseline ($p < 0.05$).

consumption. The lack of alterations in bifidobacterial levels in this study group confirmed this speculation.

Significant elevations of total VFAs levels in banana and control group were observed in our study. Previous data exhibited also an elevation of total VFAs concentrations after consumption of type II resistant banana starch or fructans [14,15]. No significant changes were observed in the molar ratio of acetate, butyrate and other SCFAs after banana or banana drink ingestion. Propionate ratio was higher in banana than banana drink group at 30ds, with no group-specific significant alterations. Ingestion of fructans has been reported to increase faecal concentrations of acetate and propionate [15], while studies with resistant starches have revealed variability in concentrations and proportions of SCFAs, as a property of both the individual's microflora and type of resistant starch [14]. Decreased levels of branched-chain SCFAs after banana drink consumption in our study possibly reflect a reduction in fermentation rate of branched-chain aminoacids in the colon [16].

Analysis of digestive symptoms indicated that consumption of bananas had no adverse effects on evacuation frequency and induced a significant drop in reported bloating scores of volunteers during the intervention. Positive, but to a less extent, gastrointestinal effects were also observed in banana drink group. Our results contradict the reported perceived effect of bananas on stool consistency, which relates banana consumption with induction of constipation in apparently healthy people [17]. Furthermore, our data confirm that most healthy people would probably experience no detected increase in intestinal gas when bananas are eaten in customary amounts [18].

In conclusion, this study demonstrated that daily consumption of bananas is a well-tolerated eating behaviour, which may induce bifidogenesis in healthy women experiencing body weight problems. Larger-scale human studies are essential for the establishment of the prebiotic role of banana consumption as part of a balanced diet.

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