

## Impact of palm date consumption on microbiota growth and large intestinal health: a randomised, controlled, cross-over, human intervention study

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### Abstract

The reported inverse association between the intake of plant-based foods and a reduction in the prevalence of colorectal cancer may be partly mediated by interactions between insoluble fibre and (poly)phenols and the intestinal microbiota. In the present study, we assessed the impact of palm date consumption, rich in both polyphenols and fibre, on the growth of colonic microbiota and markers of colon cancer risk in a randomised, controlled, cross-over human intervention study. A total of twenty-two healthy human volunteers were randomly assigned to either a control group (maltodextrin–dextrose, 37.1 g) or an intervention group (seven dates, approximately 50 g). Each arm was of 21 d duration and was separated by a 14-d washout period in a cross-over manner. Changes in the growth of microbiota were assessed by fluorescence *in situ* hybridisation analysis, whereas SCFA levels were assessed using HPLC. Further, ammonia concentrations, faecal water genotoxicity and anti-proliferation ability were also assessed using different assays, which included cell work and the Comet assay. Accordingly, dietary intakes, anthropometric measurements and bowel movement assessment were also carried out. Although the consumption of dates did not induce significant changes in the growth of select bacterial groups or SCFA, there were significant increases in bowel movements and stool frequency ( $P < 0.01$ ;  $n$  21) and significant reductions in stool ammonia concentration ( $P < 0.05$ ;  $n$  21) after consumption of dates, relative to baseline. Furthermore, date fruit intake significantly reduced genotoxicity in human faecal water relative to control ( $P < 0.01$ ;  $n$  21). Our data indicate that consumption of date fruit may reduce colon cancer risk without inducing changes in the microbiota.

**Key words:** Date palm fruit: Gut microbiota: Gastrointestinal health: DNA genotoxicity: Cancer biomarkers

Dates are considered a staple food item in the Middle East and North Africa, and are also imported in Europe, the UK and USA<sup>(1)</sup>. Dates contain relatively high levels of polyphenols and insoluble fibre<sup>(2)</sup>, both of which have been postulated to possess anti-cancer activity, especially in the gastrointestinal (GI) tract<sup>(3)</sup>. Other plant foods have been reported to selectively and positively influence the growth of the gut microbial ecology<sup>(4)</sup>, leading to specific changes in the composition and/or activity of the GI microbiota<sup>(5)</sup> and potentially an enhancement of immune responses, increased vitamin synthesis in the gut, and even a reduction in cholesterol and TAG<sup>(6)</sup>. Recently, a new approach to testing prebiotic potentials and/or functional abilities of plant foods on modulating the gut microbiota in human trials have highlighted that foods rich in dietary fibre<sup>(7)</sup>, polyphenols<sup>(8)</sup> and/or both<sup>(9)</sup> may exert such actions. For

example, cocoa<sup>(8)</sup>, wine<sup>(10)</sup>, blueberry<sup>(11)</sup>, whole grain cereals<sup>(9)</sup>, maize-derived whole grains<sup>(12)</sup>, inulin extracted from artichoke<sup>(13)</sup> and apples<sup>(14)</sup> have all been shown to induce beneficial changes in gut bacterial growth.

The saccharolytic metabolism of carbohydrates by gut microbiota results in the production of SCFA<sup>(15)</sup>, whereas exposure to (poly)phenols leads to the generation of phenolic metabolites<sup>(16)</sup>, some of which have been postulated to enhance colon health and reduce colorectal cancer (CRC)<sup>(17)</sup>. Furthermore, an enhancement of beneficial bacterial growth has been shown to improve bowel function, thus preventing constipation and reducing other factors associated with the promotion of carcinogenesis<sup>(18)</sup> and intestinal epithelial damage, such as secondary bile acids, heterocyclic amines, *N*-nitrosocompounds, cresols and the production of ammonia,

**Abbreviations:** CRC, colorectal cancer; GI, gastrointestinal.

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resulting from the bacterial metabolism of proteins<sup>(19)</sup>. Previous studies have shown strong associations between fibres and/or prebiotic intake and a reduction in many of these bowel cancer biomarkers<sup>(20)</sup>. As both polyphenols and fibres escape significant metabolism in the upper GI tract, they possess the potential to reach the large intestine where they may modify the gut ecology and limit the formation of carcinogenic by-products of bacterial and host metabolism, thus leading to a reduction in CRC risk<sup>(21)</sup>.

Previously, we have shown that whole date fruit, and polyphenols extracted from them, selectively stimulates *Bifidobacterium* spp. and *Bacteroides*, along with the total bacterial counts in faecal batch culture models, increasing SCFA production concurrently. In addition, promising changes in DNA damage were observed through the anti-proliferative actions of both polyphenol extracts and whole date extracts<sup>(22)</sup>. In the present study we aimed to confirm such actions in humans by investigating the potential for regular date consumption to alter the gut microbial ecology, to influence bowel function, to affect levels of various gastro-metabolic end products and to influence blood markers such as cholesterol, TAG and glucose. Furthermore, we also assessed whether the intake of dates reduces the pro-carcinogenic and/or genotoxic potential of human faecal water.

## Methods

### Participants

A total of twenty-two healthy volunteers (aged between 18 and 55 years; eleven males and eleven females) were recruited from Reading, Berkshire, UK. Inclusion criteria included a medical questionnaire, anthropometric and biochemical measurements including blood pressure (<140/90 mmHg), cholesterol (<5.0 mmol/l), fasting blood glucose (<5.5 mmol/l), Hb (>115 g/l: females; >140 g/l: males) and BMI (20–25 kg/m<sup>2</sup>). Exclusion criteria included abnormal blood biochemistry based on standard clinical cut-offs, smokers, pregnant women and those with a history of abnormal gut health. Sample size calculations using a significance level of 5% (one-sided), and within patient SD 0.3, indicated that, to detect a log change in bifidobacteria of 0.31 at a power of 90%, twenty-two individuals were required<sup>(23)</sup>. Eligible volunteers were informed of their inclusion in the trial by letter, which included their blood analysis results, and were asked to attend the Hugh Sinclair Unit of Human Nutrition, Food and Nutritional Sciences Department, at the University of Reading, where the trial was conducted. Volunteers were requested not to consume probiotics, prebiotics and/or dates for at least 4 weeks prior to the trial, and antibiotics and laxatives for 6 months prior to the start. Medication taken during the trial was recorded. Participants were requested not to deviate from their regular habitual diet for the duration of the study.

### Interventions

Ajwa dates at the Tamr stage of ripening were obtained commercially from Bateel. The control intervention was composed

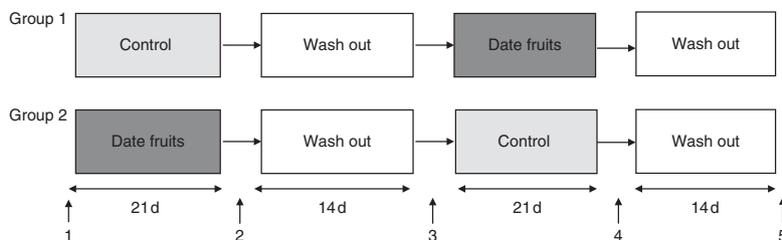
**Table 1.** Chemical composition of control and date fruit interventions

	Control	Date fruits
Energy (kJ)	620.8	669.4
Energy (kcal)	148.36	160
Total amounts (g)	37.1	50
Carbohydrates (g)	3.9 (maltodextrin) + 33.19 (dextrose)	33.19
Protein (g)	0	3.12
Fibre (g)	0	3.9
Polyphenols (mg)	0	750

of maltodextrin and dextrose, purchased from Myprotein, and was weighed and packaged at the Department of Food and Nutritional Sciences, University of Reading. Volunteers were advised to store both the control (maltodextrin–dextrose, 37.1 g) and the date intervention (seven dates, approximately 50 g) at room temperature. Both the control and the date intervention were isoenergetic and contained similar levels of mainly sugars (Table 1).

### Study design

The Ethical Research Committee of the University of Reading approved this study. This study is registered in Clinical Trials.gov as NCT02288611. The study was designed and powered as a randomised, controlled, single-masked, cross-over intervention trial. A total of twenty-two volunteers were recruited, with one volunteer dropping out during the second treatment period ( $n$  21). Participants were randomly assigned to one of two groups – dates (50 g) or control (maltodextrin–dextrose (37.1 g)) – for 3 weeks (21 d). Following completion of an arm there was a 2-week (14 d) washout period prior to a volunteer's crossing over to the alternative arm (Fig. 1). Dates (seven fruits; mean weight 50 g) were provided prepackaged, whereas the control intervention consisted of maltodextrin (3.9 g) and dextrose (33.19 g) powder, mixed and packaged in the University Pilot Plant. On arrival at the clinical unit, volunteers were asked to provide a faecal sample and a fasting blood sample. Faecal and blood sampling was conducted on five separate occasions throughout the duration of the study (Fig. 1). Volunteers were asked to record daily information relating to bowel habits, including stool frequency, stool consistency (according to the Bristol chart), abdominal pain, stomach or intestinal bloating, flatulence and psychological status (subjective mood). Two fasting blood samples (30 ml venous blood) and five faecal samples (15 g) were collected following the consumption of each intervention, along with a standard breakfast. Venous blood was collected in BD Vacutainer tubes (BD). EDTA tubes were used for the analysis of fasting total cholesterol, TAG and HDL-cholesterol, whereas fluoride/oxalate tubes were used for the analysis of glucose. The blood samples were kept on ice prior to centrifugation at 1700 **g** for 10 min, and plasma collected was stored at –20°C until analysis. Anthropometric measures, including systolic and diastolic blood pressure, weight, waist, BMI and body fat percentage (body composition analyser; Tanita), were recorded during each visit. Throughout the trial the volunteers completed 4-d food intake diaries



**Fig. 1.** Study design of a randomised, controlled cross-over trial on twenty-two healthy volunteers receiving a control and/or date fruit intervention for a period of 3 weeks each, including 2-week washout periods between each treatment. At each visit, anthropometric measurements, faecal samples and blood were collected from each volunteer at five different time points (0, 21, 36, 58 and 73 d).

covering a period from each of the treatment periods (after 3 weeks of control and date intervention). To assess compliance, volunteers were required to report their adherence to the trial protocol by recording date intake and by the return of unused control/date packets.

### Blood biochemical measures

Total cholesterol, TAG and glucose concentrations were assessed using the Monarch Automatic Analyzer ILAB 600 (Instrumentation Laboratories Ltd) in conjunction with the following kits: IL test TAG, IL test cholesterol, IL test HDL-cholesterol and IL test glucose (hexokinase). In each run, included control samples, which contained high and low concentrations of each biochemical parameter, were assessed using Wako Control Serum I and Wako Control Serum II from Alpha Laboratories Ltd. Results obtained were determined to be appropriate according to the quality control values given in a specific range by the manufacturers.

### Faecal sample preparation and analysis

The samples were collected on site on the day of the experiment and processed immediately as follows: a 1:10 (w/v) dilution of faecal samples in anaerobic phosphate buffer (0.1 M; pH 7.4) was homogenised in a stomacher (Seward; Thetford) for 2 min. Then, 1:10 (w/v) faecal slurries were processed for analysis of bacterial populations using fluorescence *in situ* hybridisation (FISH) analysis, as previously described<sup>(22,24)</sup>. FISH was conducted using six different bacterial probes, which have been covered previously<sup>(9,22)</sup>: Bif 164 for *Bifidobacterium*, Lab 158 for *Lactobacillus/Enterococcus*, ATO for the *Atopobium-Coriobacterium* group, Erec 482 for *Clostridium coccoides-Eubacterium rectale*, Chis 150 for the *Clostridium* subgroup *Histolyticum*, Bac 303 for *Bacteroides-Prevotella*, Rrec 584 for *Roseburia + Eubacterium rectale*, RFO probe (a combination of two probes, Rbro 730 for *Ruminococcus bromii* and Rfla 729 for *Ruminococcus flavefaciens*) and EUB338-mix for total bacteria. The number of bacterial cells was assessed using fluorescence microscopy (Nikon Eclipse E400; Nikon), fitted with appropriate filters for the DAPI stain (excited at 359 nm and emitting at 461 nm) and Cy3 dye (excited at 550 nm and emitting at 565 nm). A total of fifteen to twenty fields were counted on six-well slides (Tekdon Incorporated).

### SCFA

Faecal samples were centrifuged at 13 000 g for 5 min to remove particulate matter, and supernatants were filtered through a 0.2 µm acrodisc filter prior to injection (20 µl) onto the HPLC system (Merck Millipore) equipped with refractive index detection. Separation of compounds was achieved using an ion-exclusion REZEX-ROA organic acid column (Phenomenex) maintained at 85°C. The mobile phase was aqueous H<sub>2</sub>SO<sub>4</sub> (0.0025 mmol/l) with a flow rate of 0.5 ml/min. Quantification of samples was performed using calibration curves constructed using authentic lactic, acetic, propionic, butyric and valeric acids, based on retention time and spectral mapping.

### Faecal water genotoxicity and proliferation

Faecal slurries at 1:1 (w/v) were prepared in PBS and homogenised in a stomacher for 2 min. Then, 10 ml aliquots were taken from each sample and centrifuged at 64 000 g for 2 h at 4°C. Supernatants were filtered using 0.22 µm acrodisc filters and stored at -80°C until analysis. Genotoxicity was tested using the Comet assay and HT29 proliferation assays. The Comet assay (single-cell gel electrophoresis) employed was the alkaline Comet assay in which cells were lysed at pH 10 and electrophoresed at pH 13 to detect DNA damage and DNA breaks in faecal water samples<sup>(25)</sup>. Tail intensity was measured as light intensity to determine damage as compared with PBS and H<sub>2</sub>O<sub>2</sub><sup>(26)</sup>. In all, twenty-two faecal water samples before treatment and after treatment (with control and date fruits) were tested with respect to HT29 cell lines, and then single-cell gel electrophoresis was performed. Slides stained with fluorescent dye ethidium bromide were examined under a microscope to examine the comet shape, wherein the undamaged DNA represents the head of the comet and the damaged DNA represents the tail. In each experiment, cell numbers and viability were assessed using Trypan blue staining. Proliferation was tested using the Sulforhodamine B assay. The colorimetric assay was used to determine the anti-inhibitory effects of treatments by measuring the 'cellular protein content' that determines the cell density<sup>(27)</sup>, which was explained in a previous work<sup>(22)</sup>. Cell density was determined using SRB (500 µl of 0.4 % SRB; 0.5 h) (Sigma Aldrich). Dye incorporation, reflecting cell biomass, was measured at 492 nm, using a GENios microplate reader (Tecan).

### Faecal ammonia levels

Faecal ammonia concentrations were determined as previously detailed<sup>(28)</sup>, using faecal water samples collected before treatment and after intervention. Ammonia levels in the samples were then detected using a GENios Pro microplate reader set at an absorbance of 570 nm (Tecan). The concentration of ammonia was measured using a standard curve, using ammonium chloride expressed as micromoles per millilitre.

### Diet diary analysis

Diet diaries were analysed using Dietplan 6 software, using the Nutrition Data System and USF databases combined with the USDA flavonoid database.

### Statistical analysis

For studying the changes in bacterial counts ( $\log_{10}$ ), SCFA (mM), ammonia concentrations ( $\mu\text{mol}$ ), DNA tail percentage and HT29 growth inhibition between different treatment groups and across time points (before treatment and after control intervention, before treatment and after date intervention, and between control and date interventions), a general linear model, with a two-way repeated measures ANOVA was used. Significant differences between visits were detected using the least significant difference test, and represented by \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ . For analysing dietary intakes and bowel frequency during the treatment period, the paired (two-tailed)  $t$  test was applied. All statistical analyses were performed with SPSS software, version 18.0 (SPSS Inc.). All data were checked for normality and log transformed when necessary before statistical analyses.

## Results

### Anthropometry, bowel function and food intake

Consumption of dates for 21 d led to a significant increase in stool frequency ( $P < 0.01$ ;  $n$  21), relative to the control, with stool types recorded between 3 and 4 on the Bristol chart with no signs of diarrhoea. There were no significant differences between the control and date interventions with respect to stool type (Table 2). There were no other significant changes in GI

tract function in response to any of the treatments (Table 2). Furthermore, there were no significant differences in any of the biochemical or anthropometric measures (Table 3). Date consumption resulted in a significant increase in polyphenol intake ( $P < 0.01$ ;  $n$  21), whereas none of the other nutrient measures showed a change in response to the interventions (Table 4).

### Growth of faecal microbiota and increase in metabolic products

The regular intake of dates for 21 d resulted in no significant alterations in the growth of the faecal microbiota, relative to control (Table 5). Furthermore, lactic acid, acetic acid, valeric acid, butyric acid and propionic acid levels were unaffected by either date or control intake (Table 6). However, the 21-d intake of dates resulted in a significant reduction in faecal ammonia levels in comparison with pretreatment levels ( $P < 0.05$ ;  $n$  21) (Fig. 2).

### Cellular DNA damage and HT29 proliferation

Consumption of dates for 21 d led to a significant reduction in the genotoxicity of faecal water, as evidenced by a significant decrease in Comet DNA tail length, compared with that observed after the control intake ( $P < 0.01$ ;  $n$  21) (Fig. 3). Furthermore, faecal water collected following date consumption also showed an inhibition of cancer cell proliferation (6.76% inhibition) in comparison with faecal water harvested after control intake (Fig. 4), although this change was not statistically significant ( $P = 0.1$ ).

## Discussion

Epidemiological studies have suggested that the regular intake of fruit and vegetables may help reduce CRC risk<sup>(29)</sup>, although the precise bioactive components responsible for these actions remain unclear<sup>(21)</sup>. The microbial fermentation of certain dietary components potentially acting as a bioactive compound, such as dietary fibres<sup>(7)</sup> and polyphenols<sup>(8)</sup> or both<sup>(9)</sup>, and the resultant effects on the growth of microbiota and the bioactive actions of any metabolic products may represent one mechanism by which such foods enhance colon health. We show that, although the consumption of dates for 21 d does not induce selective changes in the growth of selected bacterial groups (or SCFA concentration), beneficial changes with respect to stool frequency, ammonia concentrations and genotoxicity of faecal water were observed.

Previously, we have observed that date fruit extracts significantly increase the growth of bifidobacteria and SCFA *in vitro*<sup>(22)</sup>. However, 21 d of intervention failed to lead to increases in the selective growth of microbiota, despite the actions of dietary fibres<sup>(7)</sup> and polyphenols<sup>(8)</sup> or both<sup>(9)</sup>. One reason for the absence of bacterial growth alterations in the current study may be the lower content of soluble and insoluble fibres in our intervention (3.4–4.0 g of insoluble fibres and 0.5–1.0 g of soluble fibres) relative to doses tested previously in other whole foods<sup>(9)</sup>. Furthermore, polyphenol levels were also

**Table 2.** Summary of bowel habits and gastrointestinal symptoms recorded after the 3-week control or date fruit intervention (Mean values and standard deviations;  $n$  21)

	Control		Date fruits	
	Mean	SD	Mean	SD
Stool frequency	1.21	0.5	1.40**	0.71
Stool type	3.49	1.43	3.61	1.44
Abdominal pain	0.25	0.32	0.22	0.29
Bloating	0.32	0.41	0.34	0.40
Flatulence	0.49	0.60	0.76	0.73

\*\* Mean value was significantly different from that at baseline ( $P < 0.01$ ; paired (two-tailed)  $t$  test).

**Table 3.** Biochemical and anthropometric measurements recorded before and after the 3-week control or date fruit intervention\* (Mean values and standard deviations; *n* 21)

	Control				Date fruit			
	Pretreatment		Treatment		Pretreatment		Treatment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BMI (kg/m <sup>2</sup> )	23.20	2.59	23.28	2.47	23.28	2.27	23.34	2.45
Body fat (%)	22.41	7.49	22.52	7.35	23.29	7.04	22.69	7.06
Cholesterol (mmol/l)	4.61	0.14	4.41	0.12	4.53	0.17	4.67	0.22
TAG (mmol/l)	0.80	0.06	0.84	0.06	0.74	0.07	0.87	0.11
HDL (mmol/l)	1.59	0.03	1.50	0.05	1.54	0.05	1.55	0.06
LDL (mmol/l)	2.86	0.20	2.74	0.16	2.84	0.14	2.94	0.18
Glucose (mmol/l)	5.01	0.06	5.01	0.07	5.09	0.07	5.07	0.09

\* Mean values after treatment were compared with those at baseline, using two-way repeated-measures ANOVA and the least significant difference test.

**Table 4.** Summary of dietary intakes using diet diaries and analysed by the diet plan programme recorded after the 3-week control or date fruit intervention (Mean values and standard deviations; *n* 21)

	Control		Date fruits	
	Mean	SD	Mean	SD
Protein (g)	71.5	17.3	77.4	15.2
Fat (g)	62.7	17	75.1	35.9
CHO (mg)	211	61	232.7	96.2
Energy (kJ)	7188.1	1517.1	8101.1	2343.5
Energy (kcal)	1718	362.6	1936.2	560.1
Total sugars (mg)	91.4	52.5	107.6	91.5
Fibre (AOAC) (g)	18.2	6	18.5	7.6
Total flavonoids (mg)	154.1	139.8	229.5*	208.6

CHO, carbohydrate; AOAC, Association of Official Agricultural Chemists.

\* Mean value was significantly different from that at baseline ( $P < 0.05$ ; paired (two-tailed) *t* test).

**Table 5.** Faecal bacterial numbers in faecal samples of twenty-one volunteers in five visits over the human study period\*† (Mean values and standard deviations)

	Control				Date fruit			
	Pretreatment		Treatment		Pretreatment		Treatment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bacteria	10.69	0.19	10.76	0.36	10.68	0.31	10.78	0.24
Bifidobacteria	9.58	0.40	9.60	0.37	9.64	0.35	9.62	0.39
<i>Lactobacillus/Enterococcus</i> counts	8.79	0.30	8.84	0.31	8.71	0.46	8.66	0.62
<i>Atopobium-Coriobacterium</i> group	9.90	0.25	10.02	0.25	9.8	0.37	9.97	0.24
<i>Bacteroides</i>	10.01	0.27	10.04	0.38	10.01	0.40	10.00	0.43
<i>Clostridium</i> subgroup <i>histolyticum</i>	7.47	0.42	7.28	0.28	7.49	0.61	7.38	0.40
<i>Clostridium coccoides-Eubacterium rectale</i>	10.32	0.30	10.33	0.20	10.26	0.22	10.32	0.18
<i>Ruminococcus bromii</i> + <i>Ruminococcus flavefaciens</i>	9.99	0.26	9.75	0.77	10.08	0.39	10.04	0.41
<i>Roseburia</i> + <i>Eubacterium rectale</i>	9.94	0.29	9.99	0.35	9.85	0.33	9.94	0.33

\* Mean values for the date treatment were compared with those of the control treatment and/or baseline using two-way repeated-measures ANOVA and the least significant difference test.

† Bacterial counts in stool samples determined by fluorescence *in situ* hybridisation expressed as mean log<sub>10</sub> cells/g faeces.

significantly lower than the levels reported to influence microbial growth in the large intestine with polyphenol-rich drinks<sup>(8)</sup>. In addition, variations in host microbial ecology influence changes in bacterial numbers, wherein some volunteers exhibit high counts at baseline of some bacteria, preventing further increases over this timeframe<sup>(30)</sup>, in comparison with volunteers at high risk, such as the elderly, malnourished populations and

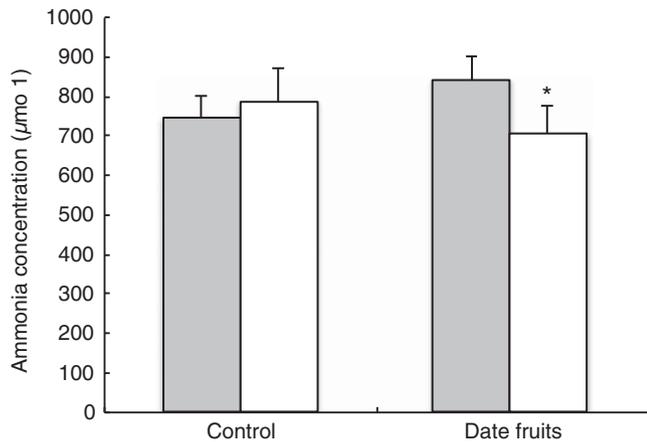
those with obesity and inflammatory conditions<sup>(31–34)</sup>. Previous studies had suggested that the intake of 100 g of date fruits for 28 d significantly reduced TAG levels and oxidative stress in human plasma<sup>(35)</sup>. However, we were unable to detect such changes following our intervention, which is in agreement with similar studies in healthy individuals<sup>(9)</sup>. In light of this information we extended our investigation by looking at the reasons

**Table 6.** SCFA concentrations in faecal samples of twenty-one volunteers in five visits over the human study period\*† (Mean values and standard deviations)

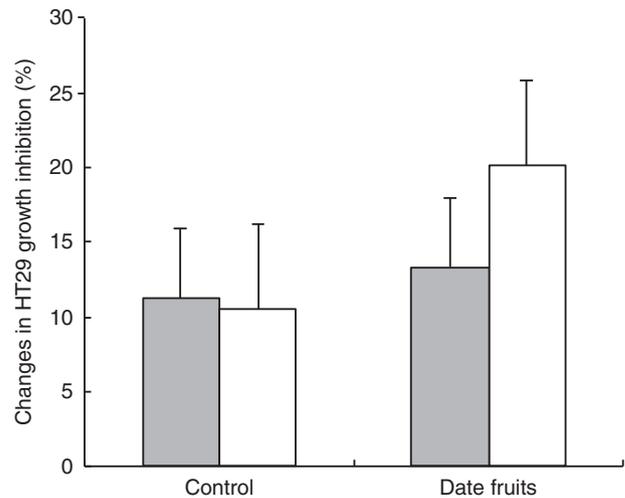
	Control				Date fruit			
	Pretreatment		Treatment		Pretreatment		Treatment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acetate (mM)	12.53	0.85	14.03	1.36	13.97	1.39	12.96	1.11
Propionate (mM)	5.90	0.44	7.23	0.91	7.41	0.83	6.57	0.78
Butyrate (mM)	3.83	0.46	5.28	0.57	4.43	0.63	4.18	0.36

\* Mean values for the date treatment were compared with those of the of control treatment and/or baseline using two-way repeated-measures ANOVA and the least significant difference test.

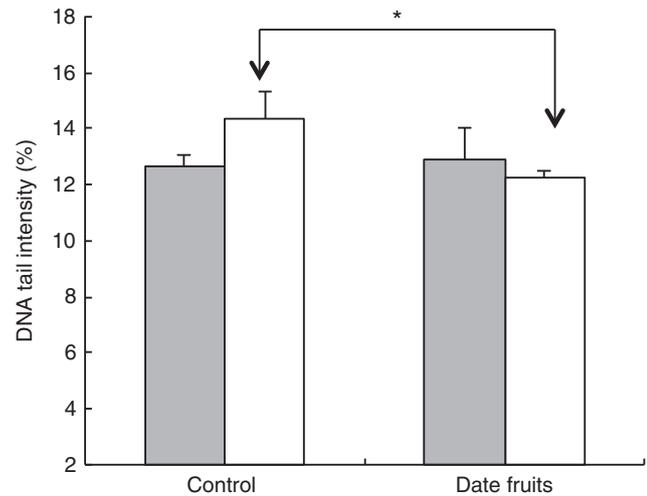
† Bacterial metabolites in stool samples measured by HPLC are expressed as mmol.



**Fig. 2.** Ammonia concentrations in faecal water of twenty-one volunteers in five visits over the human study period. Bacterial metabolites in stool samples measured by the plate reader spectrophotometer expressed as mean values micromoles. Mean values at treatment time points were significantly different from mean values at pretreatment time points (<sup>a</sup>  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; two-way ANOVA and the least significant difference test). ■, Pretreatment; □, treatment.

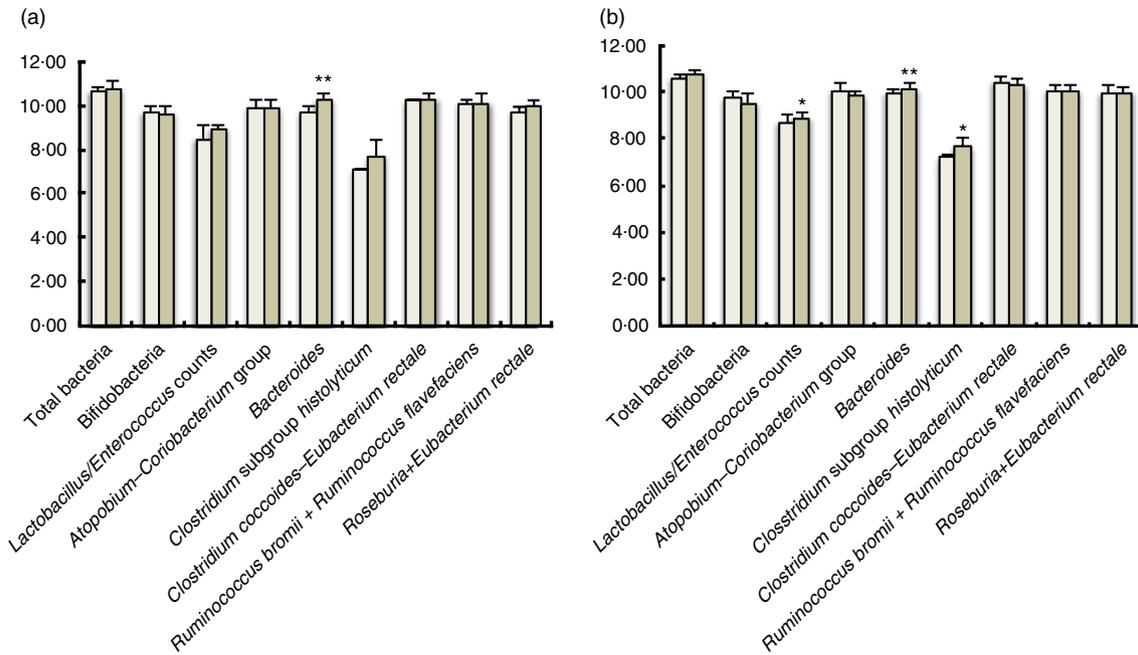


**Fig. 4.** HT29 growth inhibition tested in faecal water of twenty-one volunteers before and after treatment (control and date fruit consumption) over the human study period. Percentage was measured with a spectrophotometer at 570 nm. Changes in mean values at treatment time points are significantly different from mean values at pretreatment time points (<sup>a</sup>  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; two-way repeated measures ANOVA and the least significant difference test). ■, Pretreatment; □, treatment.



**Fig. 3.** Faecal water genotoxicity of twenty-one volunteers before and after treatment (control and date fruit consumption) over the human study period. DNA tail intensity was measured by single electrophoresis expressed as a percentage of damage. Mean values at treatment time points were significantly different from mean values at pretreatment time points (<sup>a</sup>  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; two-way repeated measures ANOVA and the least significant difference test). ■, Pretreatment; □, treatment.

behind high bacterial counts in certain faecal samples before starting the intervention (Fig. 5(a) and (b)) by analysing the 4-d diet diaries of food intake. Indeed, high habitual intake of dietary fibre (18.5 g/d) in many of our volunteers may explain why faecal samples at baseline encounter higher numbers of the tested bacterial strains (Table 7), in comparison with the seven volunteers consuming low amounts of fibre, an average of 6 g/d (Table 8). In previous human trials of this nature, even though bacterial numbers were significantly changed, SCFA were rarely altered by bacterial fermentation of plant-based foods, as seen previously with whole-grain consumption<sup>(36)</sup>. The main reason for this seems to be the fact that SCFA are absorbed very quickly<sup>(19)</sup>, and thus are difficult to detect. With respect to SCFA concentrations, our data are in agreement with those of previous human trials carried out with inulin extracted from artichoke and maize-based cereals, despite changes in the microbiota<sup>(37,38)</sup>. Little information is found on the role of whole-fruit consumption in changing the gut microbiota, which was mostly seen in apples *in vitro*<sup>(39)</sup>, and in humans<sup>(14)</sup>,



**Fig. 5.** Comparison between the faecal bacterial numbers of two selected groups with high fibre intake (*n* 14) and low fibre intake (*n* 7) before intervention (pretreatment) with control (a) and date fruit (b). Bacterial counts in stool samples as determined by fluorescence *in situ* hybridisation are expressed as mean log<sub>10</sub> cells/g faeces. Changes in mean values on treatment were measured using the paired (one-tailed) *t* test. \* *P* < 0.05, \*\* *P* < 0.01 (paired (two-tailed) *t* test). □, Low fibre intake; ■, high fibre intake.

**Table 7.** Faecal bacterial numbers in faecal samples of volunteers with high fibre intake (*n* 14) over the human study period\*† (Mean values and standard deviations)

	High fibre intake							
	Control				Date fruit			
	Pretreatment		Treatment		Pretreatment		Treatment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bacteria	10.76	0.19	10.81	0.35	10.72	0.37	10.72	0.25
Bifidobacteria	9.48	0.45	9.61	0.42	9.62	0.39	9.52	0.42
Lactobacillus/Enterococcus counts	8.88	0.28	8.84	0.28	8.93	0.20	8.68	0.48
Atopobium-Coriobacterium group	9.82	0.18	9.96	0.24	9.82	0.40	9.88	0.21
Bacteroides	10.07	0.30	10.08	0.38	10.20	0.34	9.98	0.49
Clostridium subgroup histolyticum	7.65	0.43	7.22	0.25	7.70	0.70	7.46	0.48
Clostridium coccooides-Eubacterium rectale	10.25	0.35	10.30	0.16	10.28	0.27	10.23	0.10
Ruminococcus bromii + Ruminococcus flavefaciens	10.00	0.28	9.51	0.92	10.09	0.45	9.86	0.39
Roseburia + Eubacterium rectale	9.95	0.28	9.95	0.39	9.96	0.31	9.95	0.28

\* Mean values for the date treatment were compared with those of the control treatment and/or baseline using two-way repeated-measures ANOVA and the least significant difference test.

† Bacterial counts in stool samples determined by fluorescence *in situ* hybridisation expressed as mean log<sub>10</sub> cells/g faeces.

showing a significant alteration in bifidobacteria in a small number of volunteers. Thus, there is still a huge shortage of data with regard to whole fruits and vegetables and their actions on modulating the gut microbiota.

Even though modulation of gut ecology is involved in CRC aetiology, in human intervention studies the focus is mostly on the ability of dietary ingredients to induce changes in both genotoxicity and toxic metabolites<sup>(6)</sup>. Studying the impact of food components on cancer risk is extremely difficult as it is clearly impractical to use cancer as an end point in human intervention trials because of the length of time needed for

cancer development. There are few, if any, well-validated biomarkers of cancer available for use as markers of cancer progression<sup>(40)</sup>. On the other hand, there is a range of experimental biomarkers with potential mechanistic links to cancer, which include cell models (Caco-2 and HT29 cell lines), molecular techniques<sup>(41)</sup> and Western blotting, as well as the identification and quantification of the activity of certain proteins (ERK, JNK, P-38, Akt)<sup>(42)</sup>, inflammatory enzymes (COX-1 and COX-2)<sup>(43)</sup> and DNA damage, which is strongly associated with protein metabolic products<sup>(44)</sup>. Our previous *in vitro* work on Ajwa dates have shown that date polyphenols

**Table 8.** Faecal bacterial numbers in faecal samples of volunteers with low fibre intake (*n* 7) over the human study period‡ (Mean values and standard deviations)

	Low fibre intake							
	Control				Date fruit			
	Pretreatment		Treatment		Pretreatment		Treatment	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bacteria	10.57	0.18	10.68	0.41	10.61	0.19	10.92**	0.16
Bifidobacteria	9.72	0.27	9.55	0.32	9.68	0.31	9.80†	0.29
<i>Lactobacillus/Enterococcus</i> counts	8.68	0.32	8.90	0.37	8.47	0.59	8.87*	0.43
<i>Atopobium–Coriobacterium</i> group	10.04	0.36	10.19	0.22	9.89	0.35	10.14	0.23
<i>Bacteroides</i>	9.96	0.16	10.05	0.37	9.67	0.31	10.11*	0.26
<i>Clostridium</i> subgroup <i>histolyticum</i>	7.20	0.16	7.40*	0.33	7.11	0.02	7.26	0.19
<i>Clostridium</i> <i>coccoides–Eubacterium rectale</i>	10.41	0.20	10.38	0.27	10.20	0.07	10.49**	0.20
<i>Ruminococcus bromii</i> + <i>Ruminococcus flavefaciens</i>	10.01	0.26	10.12	0.19	10.01	0.28	10.30*	0.29
<i>Roseburia</i> + <i>Eubacterium rectale</i>	9.93	0.35	10.04	0.32	9.70	0.31	9.97**	0.43

Mean values for the date treatment were significantly different from those for the control treatment and/or baseline: \* *P* < 0.05, \*\* *P* < 0.01, † *P* > 0.05 (borderline significance) (two-way repeated-measures ANOVA and least significant difference test).

‡ Bacterial counts in stool samples determined by fluorescence *in situ* hybridisation expressed as mean log<sub>10</sub> cells/g faeces.

and whole date extracts were effective in inhibiting the growth of colon adenocarcinoma cell growth, something that was reduced when they were initially exposed to the faecal bacteria prior to cell exposure<sup>(22)</sup>, which is accompanied with current work. Furthermore, with regard to fruits rich in fibre, human trials have identified anti-cancer actions of cruciferous vegetables, such as Brussels sprouts, watercress and mixed types, resulting in significant reductions in DNA damage and oxidation<sup>(45)</sup>. With regard to fruits rich in polyphenols, a number of human interventions on berries showed a reducing action on cell proliferation and polyp percentages, in addition to an increase in cell apoptosis in CRC patients<sup>(46)</sup>.

In the current study, colon health was first assessed to show the significant effect of date fruit consumption on increased frequency of bowel movements. This parameter has been seen to be strongly associated with CRC patients<sup>(47)</sup>. Previous data strongly link prebiotic intake to significant reductions in constipation, which may be associated with reducing colon cancer risks through an increase in faecal genotoxicity and carcinogen levels<sup>(48)</sup>. Indeed, we show that significant reductions in the genotoxicity of faecal water were apparent following date consumption compared with control. DNA damage in colon epithelial cells has been postulated to contribute to the progression of CRC<sup>(49)</sup>, and has been shown to be reduced following the intake of prebiotics and other dietary compounds<sup>(26)</sup>, and also in polyp patients following synbiotic treatments<sup>(44)</sup>. Following our previous data on inhibiting colon cancer cell proliferation<sup>(22)</sup>, utilising similar assays, an increase in cancer growth inhibition was also detected after the consumption of date fruits, but the results were not statistically significant. Most of the cancer-related studies were carried out *in vitro*, which demonstrated that polyphenols have the potential to inhibit DNA mutations in colon cancer cell lines and to inactivate enzymes such as protein kinases and other pro-oxidant enzymes<sup>(17)</sup>, which was previously observed with olive oil<sup>(50)</sup> and in clinical human trials discussed earlier.

Increased risk for CRC has also been linked to the consumption of red or processed meats<sup>(40)</sup>, something thought to be linked to the impact of bacterial metabolic products of protein metabolism (secondary bile acids, ammonia, phenol and cresol) on colon epithelial cells<sup>(19)</sup>. Previous trials carried in polypectomised patients indicated the presence of such types of carcinogens<sup>(51)</sup> and their impact on genotoxicity<sup>(44)</sup>. Date intake in our study leads to a significant reduction in faecal ammonia concentrations, indicating that a daily consumption of seven pieces of date fruit was utilised by the gut microbiota as its energy supply and to reduce toxic metabolites, because of preferred carbohydrate degradation and reduced utilisation of protein products<sup>(52)</sup>. There are other biomarkers related to cancer risk, such as secondary bile acids, which were significantly reduced with prebiotics or probiotics<sup>(53)</sup>, and ammonia, both of which are known risk factors for colon cancer incidence, in association with epidemiology<sup>(48)</sup>. Date consumption may have also modulated bacterial enzymatic activity ( $\beta$ -glucuronidase,  $\beta$ -glycosidase and nitroreductase) and caused the suppression of ammonia and other genotoxic compounds, which is worth investigating in future work<sup>(54)</sup>.

The current study is considered a preliminary one demonstrating a possible specific property of date fruits in enhancing colon movements and metabolism and in reducing toxicity. Data successfully confirmed significant reduction in the DNA tail percentage of colonocytes and ammonia, and an increase in faecal frequency among twenty-one volunteers after the consumption of date fruit on a daily basis. Because dates contain high amounts of fibre and polyphenol compared with other types of fruit, it can be easily consumed as a snack and has a long shelf life. The current study follows the same concept as discussed by Tuohy *et al.*<sup>(4)</sup>, and is similar to the FLAVURS clinical trial of 2013<sup>(55)</sup>, in that it refers to the consumption of specific types of fruit and vegetables, rather than a generic recommendation, to induce significant changes on the gut microbiota and health. Future approach must consider the fact that bacterial ecology in the gut is altered in populations at

higher risk<sup>(56)</sup>, and trials are showing promising impacts on immunity and in reducing inflammatory markers<sup>(57)</sup>. Thus, targeting populations with altered gut ecology and assessing the impact of whole-food consumption at the microbiological, cellular, metabolic and clinical levels must be undertaken to obtain a novel health claim, wherein the gut microbiome can be used as a marker/remedy in clinical practice, which has been seen mostly with obesity and the metabolic syndrome<sup>(58)</sup>.

More advanced techniques must be applied in future work, such as bacterial enumeration using flow cytometry<sup>(59)</sup>, or rRNA gene sequencing, which is excellent in measuring the abundance of rRNA copies<sup>(60)</sup>, when the FISH technique may have limited the detection of different bacterial groups. With regard to SCFA characterisation, GC is a better approach for volatiles, even though the HPLC method used was validated using a column that overcomes volatile compounds<sup>(37,38)</sup>. Indeed, in those trials, no changes in SCFA were seen after the consumption of inulin or maize-based cereals, despite changes in microbial numbers. On the other hand, such a method has shown significant alterations in SCFA in our *in vitro* work<sup>(22)</sup>. One reason could be the type of fermented faecal material analysed in human trials, which affects SCFA amounts and availability and/or the detectors used in HPLC, wherein electrochemical detectors are more precise and accurate<sup>(61)</sup>. Epidemiological studies on diet-related health issues for populations living in the Middle East and North Africa do not only rely on the diet, and consuming staple foods, such as date fruits, but genetics may play a role as well. This was seen in the Collaborative Gene–Environment Study, where different populations were stratified according to their diet, genes and other factors<sup>(62)</sup>. Future work on the prebiotic potential of whole foods can be optimised with new recommendations by dietitians for reducing DNA damage<sup>(63)</sup>, CRC risk<sup>(64)</sup> and other chronic illnesses<sup>(65)</sup>.

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