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## Clinical Nutrition

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## Randomized Control Trials

## The effect of prunes on stool output, gut transit time and gastrointestinal microbiota: A randomised controlled trial

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## SUMMARY

**Background & aim:** Prunes (dried plums) are perceived to maintain healthy bowel function, however their effects on gastrointestinal (GI) function are poorly researched and potential mechanisms of action are not clear. We aimed to investigate the effect of prunes on stool output, whole gut transit time (WGTT), gut microbiota and short-chain fatty acids (SCFA) in healthy adults

**Methods:** We conducted a parallel group, randomised controlled trial with three treatment arms in 120 healthy adults with low fibre intakes and stool frequency of 3–6 stools/wk. Subjects were randomised to 80 g/d prunes (plus 300 ml/d water); 120 g/d prunes (plus 300 ml/d water) or control (300 ml/d water) for 4 weeks. Stool weight was the primary outcome and determined by 7-day stool collection. Secondary outcomes included stool frequency and consistency (stool diary), WGTT (radio-opaque markers), GI symptoms (diary), microbiota (quantitative PCR) and SCFA (gas liquid chromatography). Group assignment was concealed from the outcome assessors.

**Results:** There were significantly greater increases in stool weight in both the 80 g/d (mean + 22.2 g/d, 95% CI –1–45.3) and 120 g/d (+32.8 g/d, 95% CI 13.9–51.7) prune groups compared with control (–0.8 g/d, 95% CI –17.2 to 15.6,  $P = 0.026$ ). Stool frequency was significantly greater following 80 g/d (mean 6.8 bowel movements/wk, SD 3.8) and 120 g/d (5.6, SD 1.9) prune consumption compared with control (5.4, SD 2.1) ( $P = 0.023$ ), but WGTT was unchanged. The incidence of flatulence was significantly higher after prune consumption. There were no significant differences in any of the bacteria measured, except for a greater increase in Bifidobacteria across the groups ( $P = 0.046$ ). Prunes had no effect on SCFA or stool pH.

**Conclusions:** In healthy individuals with infrequent stool habits and low fibre intake, prunes significantly increased stool weight and frequency and were well tolerated. Prunes may have health benefits in populations with low stool weight.

**Clinical trial registry number and website:** ISRCTN42793297 <http://www.isrctn.com/ISRCTN42793297>.

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## 1. Introduction

Diseases and functional disorders of the gastrointestinal (GI) tract, such as colorectal cancer, diverticular disease, haemorrhoids

and constipation, are associated with a significant healthcare burden in developed countries [1]. Low stool weight and delayed gastrointestinal transit time are considered important risk factors [2]. Stool weight is relatively low in developed countries and recommendations have been made to address this through increasing fibre intake [3]. There is substantial epidemiological evidence that high dietary fibre intake is related to lower risk of developing these GI disorders [4]. The effects of fibre on gut function vary according to fibre type and source [4]. Proposed mechanisms for the beneficial effects of fibre include its impact on increasing stool weight, reducing GI transit time and short-chain fatty acid (SCFA) production [5].

**Abbreviations:** ANCOVA, analysis of covariance; ANOVA, analysis of variance; AOAC, Association of Analytical Communities; BMI, body mass index; BMs, bowel movements; BSFS, Bristol Stool Form Scale; CI, confidence interval; GI, gastrointestinal; ITT, intention to treat; NSP, non-starch polysaccharides; qPCR, quantitative polymerase chain reaction; RCT, randomised controlled trial; SCFA, short chain fatty acids; WGTT, whole gut transit time.

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Recommendations for dietary fibre intake vary around the world, including 22.4–28 g/d for females and 28–33.6 g/d for males in the United States [6] and 30 g/d in the United Kingdom [7], with ranges of 25–35 g/d typically recommended [8]. However, actual intakes of fibre fall well below current recommendations in many developed countries [9,10] and increasing the consumption of foods naturally high in dietary fibre represents a major challenge.

Prunes (dried plums) are generally perceived to have a laxative effect [11]. They are high in fibre (~6 g/100 g), including hemicellulose (3.0 g/100 g), pectin (2.1 g/100 g) and cellulose (0.9 g/100 g) [12]. Some fibres resist colonic fermentation (poorly fermented fibres) and increase stool water and volume due to mechanical stimulation of GI peristalsis (e.g. cellulose) [13]. Other fibres are rapidly fermented by colonic microbiota (fermentable fibres), resulting in bacterial proliferation and production of SCFA (e.g. pectin) [8]. Prunes contain other components that may influence colonic health including sorbitol and phenolic chlorogenic acids, both of which are not well absorbed by the small intestine [14,15] and subsequently fermented by the microbiota [15,16]. *In vitro* studies suggest they have prebiotic effects [17,18], whilst sorbitol has a laxative effect [19] by increasing intestinal water volume [20]. Prunes are therefore a promising intervention for increasing fibre intake and impacting GI function.

A systematic review of randomised controlled trials (RCTs) examining the effect of prunes on GI function found only four trials and reported inconsistent results [21]. In one trial, 100 g/d prunes were more effective than psyllium (*Plantago ovata*) for improving stool frequency and consistency in constipation. The remaining trials were conducted in healthy subjects, with GI function measured as a secondary outcome, however, one trial reported that 100 g/d prunes increased stool weight [22], another reported softening of stool consistency [23] and none reported effects on stool frequency or GI symptoms. The included trials were small, of limited quality and used self-reported, subjective measurements of GI function. Therefore, we carried out a RCT examining the effect of prunes, including any dose-dependent effects, on objective markers of GI function. We hypothesised that consumption of 80 g/d or 120 g/d of prunes in addition to normal diet would affect stool weight in healthy adults with infrequent bowel movements and a low fibre intake.

## 2. Materials and methods

### 2.1. Subjects and recruitment

Subjects were recruited from London, United Kingdom, via emails to students and staff within London universities, advertising on websites, local newspaper and posters, and online clinical trial databases. Inclusion criteria were subjects aged between 18 and 65 years with a stool frequency of 3–6 bowel movements per week. Exclusion criteria were: functional constipation or irritable bowel syndrome determined by Rome III criteria; habitual high fibre intake (>1 × 80 g/d whole grain cereal and >3 × 80 g/d fruit and vegetables; or >5 × 80 g/d fruit and vegetables; or >3 × 80 g/d whole grain cereal; or >2 × 30 g/month dried fruit); prune dislike/allergy/intolerance; use of prebiotic, probiotic, antibiotic or medication known to affect gut motility for four weeks before or during the study; use of laxatives or fibre supplements (>6 × in previous 3 months); GI or other major disease or surgery that may affect gut motility (e.g. cardiovascular, endocrine, renal); eating disorders; pregnant or lactating women; and abdominal exposure to ionising radiation in last 12 months. Potential volunteers were screened via telephone and completed a 7-day stool frequency diary in order to assess eligibility.

### 2.2. Study design and protocol

This was a 9 week, single center, 3-arm, parallel-group, randomised controlled trial. Eligible subjects were provided with verbal and written study information and gave their informed consent. After a one week baseline period, subjects were randomly allocated to one of three groups: control (no prunes plus 300 ml/d water); 80 g/d prunes (plus 300 ml/d water); 120 g/d prunes (plus 300 ml/d water). Previous studies have reported an impact on stool weight and/or frequency at a dose of 100 g of prunes per day [21], and portions either side of this were selected in order to determine a dose–response relationship, should one exist.

Prunes were of Californian origin and provided by the California Dried Plum Board (US) in 80 g and 120 g sealed bags (pitted, edible portion). Water was not provided but subjects were given plastic beakers with instructions on measuring the correct volume of water. Subjects were instructed to consume the intervention in addition to their normal diet every day for 4 weeks and to cease consumption after the intervention period. During the first week of the intervention, subjects were instructed to consume only half of their allocated prunes and/or water in order to slowly increase tolerance to fibre and to consume the prunes and water together as a snack, in divided doses, half in the morning (before 12:00) and half in the afternoon/evening (after 15:00). Subjects were requested to maintain their regular lifestyle, diet, and physical activity throughout the study but to refrain from using excluded dietary supplements.

The primary outcome was stool weight. Secondary outcomes were stool frequency, stool consistency, GI symptoms, GI transit time, faecal microbiota, faecal SCFA concentrations and faecal pH. Data on diet (7-d food diary), compliance and acceptability of the intervention, and confounding variables including physical activity (Recent Physical Activity Questionnaire, RPAQ) were also collected. All outcomes were measured at baseline and week 4 of the intervention. Stool frequency, consistency and GI symptoms were also measured at week 2 of the intervention. Compliance was measured during the final 3 weeks of the intervention when subjects were advised to consume the full dose of prunes.

Double blinding was not achievable because it was not possible to develop a placebo identical to prunes but without any active components. However, blinding of subjects was attempted by using water in all groups (including the control group) and advertising the study as an investigation of ‘fibre or fluids’ on GI health, therefore subjects in the water group were unaware they were in the control group. Blinding of data and laboratory samples was achieved by the recoding of subject identification and group allocation by an independent researcher.

Randomisation was by permuted block design, stratified by gender. Sequence generation was undertaken by a researcher uninvolved with the current study using an online random number generator with a 1:1:1 allocation using fixed block sizes of 18. The allocation sequence was concealed from the researchers in sequentially numbered, opaque, sealed envelopes.

### 2.3. Outcome measures

#### 2.3.1. Stool output and GI symptoms

The primary outcome of stool weight was calculated from total stool collections made on 7 consecutive days. Subjects were provided with verbal and written instructions on how to collect stools and provided with a stool collection kit and plastic storage boxes. Briefly, subjects passed a stool onto the stool collection device and then stored it in a sealed plastic bag inside an air tight pouch. Stools were labelled and returned to King's College London at a

convenient time or at the next scheduled study visit and weighed on a laboratory balance accurate to 0.1 g.

Stool frequency, consistency and GI symptoms were recorded in a 7-day diary. Subjects contemporaneously recorded each bowel movement and its consistency using the Bristol Stool Form Scale (BSFS) [24]. The number of bowel movements (BMs) was recorded over 7 days. The dates and times of the BMs recorded in the diary were checked against 7-day stool collection data and any discrepancies resolved with the subject during study visits. Stool consistency was reported as the mean BSFS score of all stools over the 7 days and as the percentage of type 3 or type 4 over the 7 days.

At the end of each diary day, subjects completed the Gastrointestinal Symptom Rating Scale [25], recording the presence and severity of 14 common GI symptoms (heartburn, acid reflux, nausea, belching, borborygmus, bloating, pain/discomfort, flatulence, hard stools, constipation, diarrhoea, loose stools, urgency, incomplete evacuation) on a Likert scale as absent (score 0), mild (score 1), moderate (score 2) or severe (score 3). The incidence of each symptom (mild, moderate or severe) and the incidence of moderate and severe symptoms over the 7 days were reported.

### 2.3.2. Whole gut transit time

Radio-opaque markers followed by abdominal fluoroscopy were used to measure whole gut transit time (WGTT), using the Metcalf method [26] at Queen Mary University of London. Subjects were provided with 6 gelatin capsules, each containing 10 polyurethane ring markers consisting of 40% barium sulphate (P & A Mauch, Switzerland). Subjects were provided with verbal and written instructions to ingest 2 capsules at a predetermined time each day for the first three days of the 7-d stool collection/diary period. On the fourth day an abdominal fluoroscopy was taken at the same predetermined time. If subjects retained  $\geq 30$  radio-opaque markers, a second X-ray was taken on the seventh day. WGTT was calculated using the formula:  $TT = n_i \times (t/N)$ , where  $n_i$  = number of markers observed at fluoroscopy (first and second fluoroscopy combined);  $t$  = time between each marker ingestion in hours; and  $N$  = number of markers ingested each day.

### 2.3.3. Compliance and acceptability

Compliance was measured using both self-report and return of unused prunes. Firstly, a diary was used in which subjects prospectively recorded the time and the quantity of prunes and/or water consumed each day. Secondly, subjects were provided with a known number of bags of prunes, each of a known weight and were asked to return all uneaten bags, which were checked against the diary entries. Compliance was defined as the number of subjects who consumed at least 80% of their prunes or water during the final 3-weeks of the intervention period.

Subjects in the intervention groups were asked to rate “How much do you like the prunes” and “How difficult was it to eat the prunes” on a 100 mm visual analogue scale on the first (d1) and final (d28) day of the intervention. The controls were asked equivalent questions about water.

## 2.4. Laboratory methods

During each 7-day stool collection period, one fresh stool sample was collected from subjects within 1 h of evacuation. The stool was immediately homogenised in a stomacher (Steward laboratory blender stomacher 400) for 4 min and aliquots taken for the analysis of microbiota, SCFA, pH and water content.

### 2.4.1. Gut microbiota

Gut microbiota were analysed using quantitative polymerase chain reaction (qPCR). Fresh stool was diluted 1:3 with sterile

phosphate buffered saline/30% glycerol buffer, homogenised and frozen at  $-80^\circ\text{C}$  until analysis. DNA was extracted from defrosted stool using the FastDNA Spin for soil kit and the FastPrep-24 bead homogeniser (MP Biomedicals, Solon, Ohio, USA) following manufacturer's instructions (Qbiogene, MP Biomedicals, Illkirch, France).

DNA was diluted to  $2.5\text{ ng}/\mu\text{l}$  in  $5\text{ mg/ml}$  herring sperm DNA and amplified with primers (Online supplemental material, Table S1). Primers were selected to measure total bacteria, dominant phyla and genera and those previously shown to be responsive to dietary change. Amplified bacterial 16S rRNA genes served as standard templates and standard curves were prepared with five standard concentrations of  $10^6$  to  $10^2$  gene copies/ $\mu\text{l}$  in  $5\text{ g/ml}$  herring sperm DNA. A qPCR analysis of faecal DNA was performed in triplicate using iTaq Universal SYBR Green Supermix (Biorad) in a total volume of  $10\ \mu\text{l}$  that comprised  $5\ \mu\text{l}$  SYBR green,  $2\ \mu\text{l}$  H<sub>2</sub>O,  $0.2\ \mu\text{l}$  forward primer,  $0.2\ \mu\text{l}$  reverse primer and  $2\ \mu\text{l}$  DNA standard template or control ( $5\ \mu\text{g/ml}$  herring sperm DNA and H<sub>2</sub>O) in 384-well PCR plates sealed with an optical adhesive cover. Amplification was performed with the 900HT fast real time PCR system (Life Technologies) with the following protocol: one cycle of  $95^\circ\text{C}$  for 3 min, 40 cycles of  $95^\circ\text{C}$  and the annealing temperature for each primer (online supplemental material, Table S1) for 30 s each, one cycle of  $95^\circ\text{C}$  for 10 s, and a stepwise increase from 65 to  $95^\circ\text{C}$  (at 5 s per  $0.5^\circ\text{C}$ ) to obtain melt curve data. Data were analysed using SDS version 2.4.1 ABI 7900HT Applied Biosystems sequence detection system (Life Technologies). Absolute quantification was determined by comparison of the Cq values of the target template against the standard curve.

Bacterial concentrations were corrected for stool water content and were expressed as  $\log_{10}/\text{dry}$  faeces using data for stool water content measured using lyophilisation. Briefly, weighed and frozen ( $-20^\circ\text{C}$ ) stool samples were lyophilised at  $-45^\circ\text{C}$  at a vacuum of approximately 4 mBar for 4 days (LyolabA; LSL Secroid SA, Aclens-Lausanne, Switzerland), then weighed daily until constant weight (within 0.01 g). Bacterial concentrations were expressed as  $\log_{10}/\text{dry}$  faeces using lyophilisation data.

### 2.4.2. Short-chain fatty acids and pH

Homogenised fresh stools were frozen at  $-80^\circ\text{C}$  until analysis. SCFA were extracted from defrosted stool as previously described using extraction buffer containing an internal standard 2, 2-dimethylbutyric acid [27]. Extracted SCFA ( $0.2\ \mu\text{l}$ ) were separated on a 7890A Aligent Technology gas-liquid chromatography system equipped with a  $220\ \mu\text{m}$  internal diameter, 25 m fused silica capillary column with a film thickness of  $0.25\ \mu\text{m}$  (ID-BP21, SGE, Australia). The initial oven temperature was  $80^\circ\text{C}$ , increasing to  $145^\circ\text{C}$  at  $10^\circ\text{C}/\text{min}$  and then increasing to  $200^\circ\text{C}$  at  $100^\circ\text{C}/\text{min}$ . All chromatograms were automatically integrated using the Agilent Chromatogram database (Agilent Technologies, US).

For stool pH analysis, fresh stool was diluted 1:4 (vol:vol) in pH buffer ( $1 \times 10^{-5}\text{ mol/L}$   $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $0.1\ \text{g HgCl}_2$ ), homogenised for 4 min and incubated at room temperature for 1 h. pH was measured using a calibrated pH meter (Hanna Instruments, Portable pH meter), equipped with a glass electrode specifically designed for slurries (Beckman Coulter pHRESH Electrode).

## 2.5. Ethics

Ethical approval was granted from the West London NHS Research Ethics Committee and the research was conducted according to the Declaration of Helsinki. All subjects gave their written informed consent. There were no important protocol changes once the trial commenced. The trial was registered in advance of trial commencement (ISRCTN42793297).

## 2.6. Statistical analysis

To detect a difference in the primary outcome of stool weight between the three groups with a power of 0.95 and a 0.01 level of significance, a total sample size of 108 (36 subjects in each group) was required. This was based on variance estimates of change in stool weight of mean 6.0 g/d (range 1.4–19.6) per 1 g of fruit and vegetable fibre [28]. We estimated the standard deviation (3.0) from the range using the range formula (range/6) for large samples. We aimed to recruit 120 subjects (40 in each group) to allow for an estimated 10% attrition.

The primary analysis was based on the intention to treat (ITT) data set, including all randomised subjects in the group to which they were allocated regardless of protocol deviations or attrition and last observation carried forward was used to account for missing data. A per protocol analysis was also conducted on the primary outcome only, which included only subjects who completed the intervention and who were compliant with the study protocol.

Data were checked for normality and outliers using histograms and box plots and logarithmic transformation was applied to non-normally distributed data. Differences between the groups were assessed using analysis of covariance (ANCOVA) with baseline value

as a covariate or the Kruskal–Wallis test if transformation did not result in sufficiently normal distribution. Changes from baseline were tested using one way analysis of variance (ANOVA) or the Kruskal–Wallis test if transformation did not result in sufficiently normal distribution. Categorical variables were assessed using the chi-squared test. Planned orthogonal comparisons (both prune interventions combined vs control and 120 g/d prunes vs 80 g/d prunes) were used instead of *post hoc* tests since they provide direct tests of the hypotheses of interest, control the type 1 error and are more statistically powerful than *post hoc* testing [29].

Results are presented as means and SD or median and inter-quartile range (IQR), as appropriate. All tests were two-tailed and P values < 0.05 were considered statistically significant. Data were analysed using IBM SPSS Statistics for Windows, version 22.

## 3. Results

### 3.1. Subject characteristics

In total, 343 subjects were screened for eligibility between January 2012 and September 2013, of whom 120 were randomised (40 in each group) and included in the ITT analysis (Fig. 1). Of these,

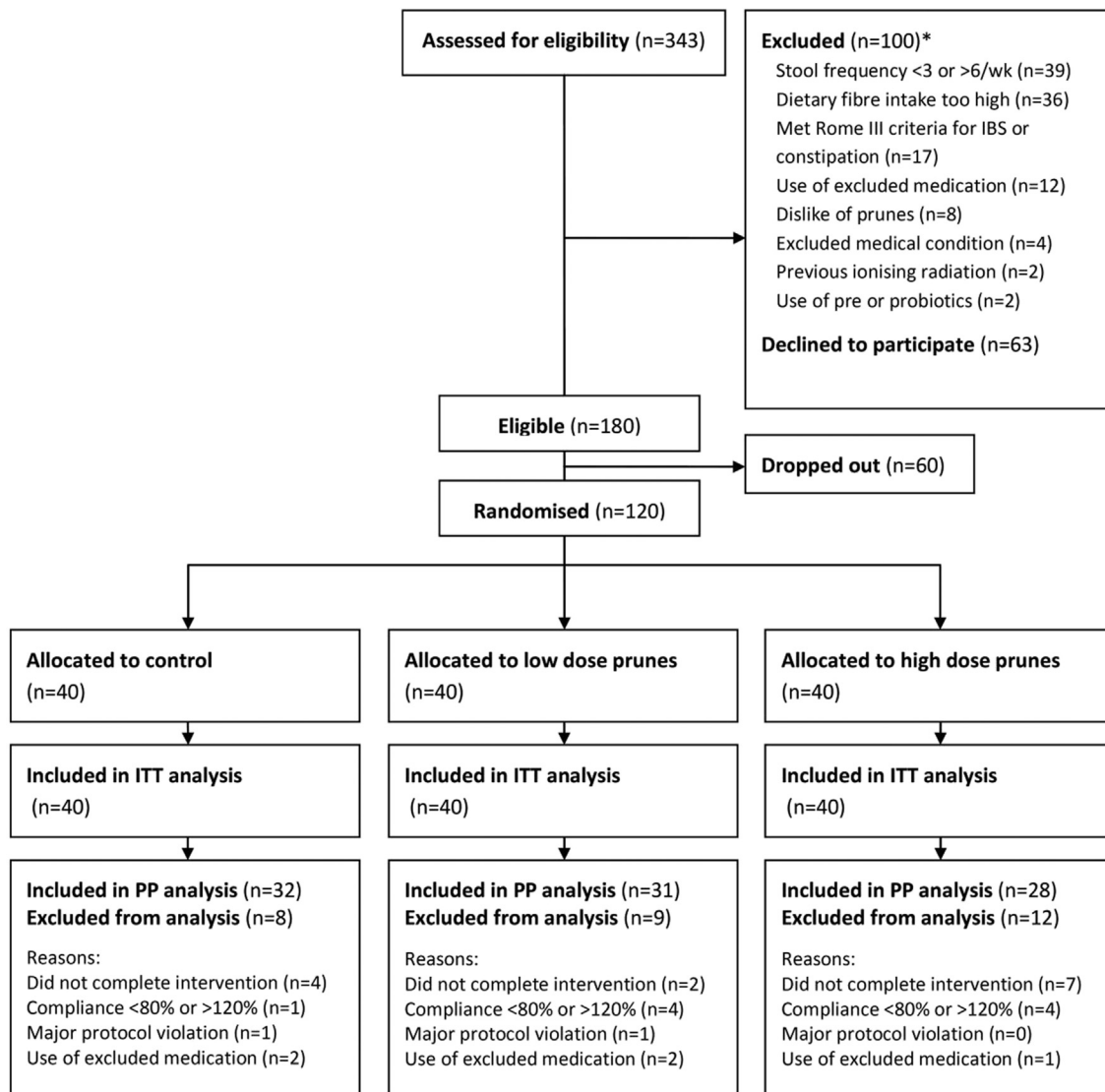


Fig. 1. Consort diagram. ITT = intention to treat; PP = per protocol.\*subjects may appear in more than one exclusion category.



29 were not compliant with the protocol and therefore 91 were included in the per protocol analysis of the primary outcome. Baseline characteristics are shown in Table 1.

### 3.2. Compliance, acceptability and nutrient intake

During the final 3 weeks of intervention, 90% of subjects in the 80 g/d prune group, 88% in the 120 g/d prune group and 97% in the control group reported compliance with the prune or water regimens respectively. In both prune groups, there was a significant decrease in liking of the prunes and subjects found it significantly more difficult to eat the prunes on day 28 than day 1 of the intervention (data not shown). However, there were no significant changes in self-reported compliance between weeks 2 and 4 ( $P = 0.427$ ).

**Table 1**  
Baseline characteristics of subjects by intervention group.

	Control (n = 40)	80 g/d prunes (n = 40)	120 g/d prunes (n = 40)
Age (y)	36.5 (13.5)	33.5 (13.1)	34.4 (11.8)
Females, n (%)	28 (70)	29 (73)	29 (73)
Post-menopausal, n (%)	6 (15)	3 (8)	1 (3)
BMI (kg/m <sup>2</sup> )	25.4 (4.9)	23.9 (4.5)	25.2 (4.5)
Physical activity energy expenditure (kJ/kg/d) (RPAQ)	35.2 (18.7)	40.7 (25.7)	34.0 (21.2)
Stool weight (g/d)	110.9 (46.1)	87.9 (44.2)	89.9 (51.6)
Stool frequency (per week)	5.4 (1.4)	5.1 (2.1)	4.9 (2.0)
Stool consistency (Bristol stool form scale)	4.0 (1.1)	3.5 (1.0)	3.2 (1.2)
Whole gut transit time (h)	40.7 (27.7)	41.8 (28.4)	46.8 (28.2)

Values are mean (SD) unless otherwise stated.  
BMI body mass index.

During week 4, intakes of energy, carbohydrate, protein, fat and fibre were significantly higher in the prune intervention groups (80 g/d and 120 g/d groups combined) than control (Table 2). Intake of carbohydrate was higher in the 120 g/d prune group than the 80 g/d prune group. Despite the higher energy intake in the prune groups, there were no significant differences in body weight between the groups at week 4 (Table 2). There were no significant differences in physical activity energy expenditure between the controls (mean 35.0 kJ/kg/d, SD 24.5), 80 g/d (42.8 kJ/kg/d, 30.4) or 120 g/d (35.8 kJ/kg/d, 22.5) groups at week 4.

### 3.3. Stool output and whole gut transit time

In the ITT analysis, stool weight was not significantly different between the groups at week 4 ( $P = 0.072$ ) (Table 3). However, in the per protocol analysis (only subjects who completed, and were compliant with, the intervention), stool weight was significantly different between the groups ( $P = 0.039$ ), and in the planned contrasts was significantly higher following prune consumption compared with control, but not between 80 g/d and 120 g/d prunes. In both the ITT and per protocol analyses, there was a significantly greater change in stool weight from baseline to week 4 between the groups ( $P = 0.026$ ), and in the planned contrasts this was significantly higher for subjects consuming prunes compared with controls, but not different between 80 g/d and 120 g/d prunes (Fig. 2).

Stool frequency was significantly different between the groups ( $P = 0.023$ ), and in the planned contrasts, the prune groups reported significantly more frequent bowel movements than controls, but there were no significant differences between 80 g/d and 120 g/d prunes (Table 3). In terms of stool consistency, there were no significant differences between groups in BSFS score at week 4 of the intervention, and no significant differences in the percentage

**Table 2**  
Total energy, macronutrient, fibre intakes and body weight at baseline and week 4 (end of intervention).

Values <sup>a</sup>	Control (n = 40)		80 g/d prunes (n = 40)		120 g/d prunes (n = 40)		P values		
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention	Intervention <sup>b</sup>	Prunes vs. Control <sup>c</sup>	80 g/d vs. 120 g/d <sup>c</sup>
Energy (kcal/d)	1868 (469)	1570 (345)	1869 (597)	1880 (623)	1873 (532)	1950 (628)	<0.001	<0.001	0.390
Protein (g/d)	68.9 (18.2)	62.0 (17.0)	68.3 (21.8)	70.4 (23.7)	70.4 (23.4)	66.3 (22.6)	0.041	0.046	0.116
Fat (g/d)	78.0 (23.2)	63.2 (18.5)	78.2 (26.0)	73.5 (28.6)	79.0 (28.9)	74.2 (33.6)	0.034	0.009	0.959
Carbohydrate (g/d)	224 (61)	188 (50)	223 (91)	232 (81)	214 (59)	251 (71)	<0.001	<0.001	0.021
AOAC fibre (g/d)	15.3 (4.8)	13.5 (4.5)	15.3 (6.9)	19.8 (7.7)	14.5 (4.6)	20.9 (6.8)	<0.001	<0.001	0.109
Non-starch polysaccharide (g/d)	10.8 (3.6)	9.6 (3.2)	10.4 (4.6)	14.0 (28.9)	10.1 (3.4)	15.2 (5.0)	<0.001	<0.001	0.079
Body weight (kg)	71.1 (13.7)	71.1 (13.9)	68.1 (12.7)	68.5 (12.8)	71.0 (13.7)	71.1 (13.5)	0.229	–	–

<sup>a</sup> Values are mean (SD).

<sup>b</sup> P values are the result of an Analysis of Covariance (ANCOVA) for the intervention data using baseline data as a covariate.

<sup>c</sup> P values are the result of planned contrasts.

**Table 3**  
Stool output and consistency and transit time at baseline (week 0) and end of the intervention (week 4).

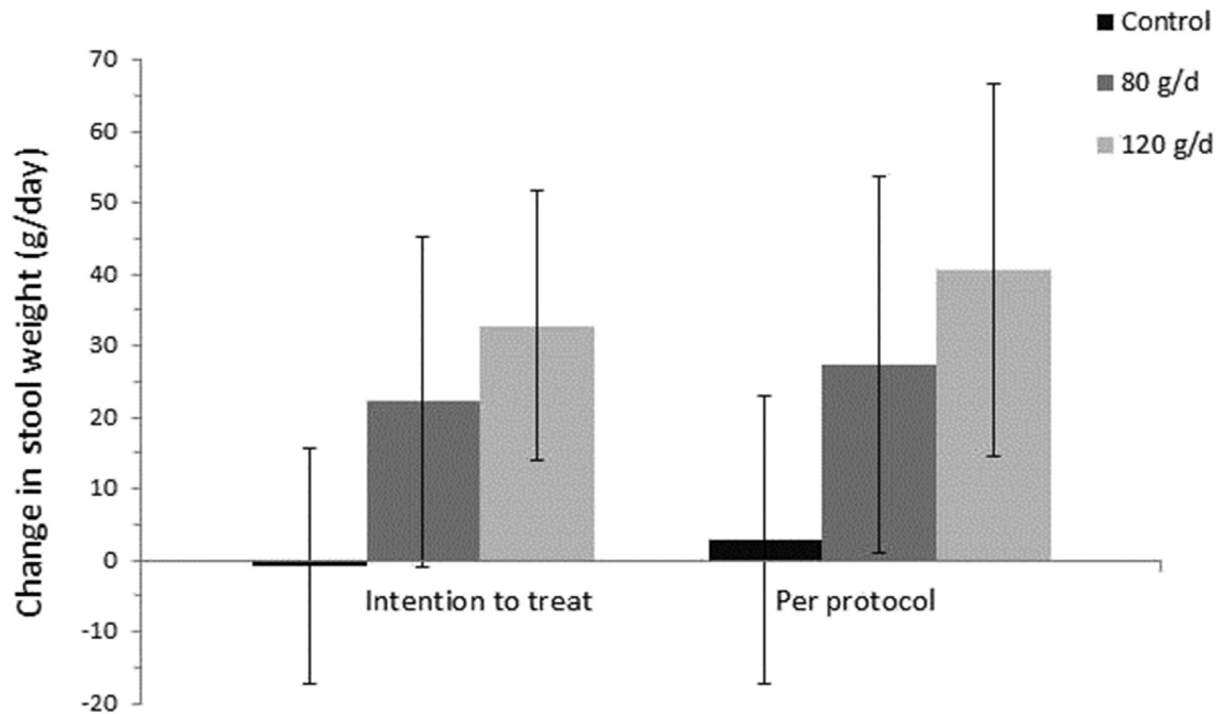
Values <sup>a</sup>	Control (n = 40)		80 g/d prunes (n = 40)		120 g/d prunes (n = 40)		P values <sup>b</sup>		
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention	Intervention <sup>b</sup>	Prunes vs. control <sup>c</sup>	80 g/d vs. 120 g/d <sup>c</sup>
Stool weight (g/d)	111 (46)	110 (62)	87.9 (44.2)	111 (69)	89.9 (50.7)	123 (71)	0.072	–	–
Stool weight (g/d) (per protocol, n = 91)	110 (44)	112 (63)	87.4 (37.8)	115 (72)	99.2 (56.1)	140 (74)	0.039	0.035	0.132
Stool frequency (BM/wk)	5.4 (1.4)	5.4 (2.1)	5.1 (2.1)	6.8 (3.8)	4.9 (2.0)	5.6 (1.9)	0.023	0.031	0.089
Stool consistency (BSFS)	4.0 (1.4)	4.1 (1.7)	3.5 (1.3)	3.8 (1.9)	3.2 (1.6)	3.0 (3.0)	0.259	–	–
Percentage type 3/4 stools	46.5 (65.1)	50.0 (45.9)	59.2 (49.1)	45.0 (77.7)	50.0 (71.9)	61.3 (65.1)	0.372	–	–
Whole gut transit time (h)	40.7 (27.3)	35.3 (28.5)	41.8 (28.4)	33.9 (27.1)	46.6 (27.5)	41.0 (26.7)	0.507	–	–

ITT: intention to treat; PP: per protocol.

<sup>a</sup> Values are mean (SD), except for stool consistency score where values are median (IQR). All analyses are on the intention to treat population (n = 120), except for stool weight which is given as both ITT (n = 120) and per protocol (n = 91).

<sup>b</sup> P values are the result of an Analysis of Covariance (ANCOVA) for the intervention data using baseline data as a covariate, except for stool consistency score which is the result of a Kruskal–Wallis test.

<sup>c</sup> P values are the result of planned contrasts.



**Fig. 2.** Change from baseline in stool weight (g/d) during consumption of prunes under the intention to treat and per protocol analyses. Data are presented as mean and distribution bars are 95% confidence intervals. ITT Analysis of variance, ITT Analysis of variance:  $F_{(2,117)} [3.75]$ ,  $P = 0.026$ ; planned contrasts, prunes vs control ( $P = 0.014$ ) and 80 g/d vs 120 g/d ( $P = 0.259$ ). PP Analysis of variance:  $F_{(2,88)} [3.35]$ ,  $P = 0.040$ ; planned contrasts, prunes vs control ( $P = 0.016$ ) and 80 g/d vs 120 g/d ( $P = 0.367$ ).

of type 3 or 4 stools at week 4 (Table 3). There were no significant differences in WGIT between the groups (Table 3).

### 3.4. Gastrointestinal symptoms

There were no significant differences between the groups at week 4 for any of the symptoms recorded, except for acid reflux (overall incidence) and flatulence (overall incidence and incidence of moderate or severe symptoms) (Table 4). Following the planned contrasts for these symptoms, there were significantly higher values in subjects consuming prunes than those in the control

group, but no significant differences between 80 g/d and 120 g/d prunes.

### 3.4. Faecal microbiota, SCFA and pH

There were no significant differences between groups at the end of the intervention for any of the bacterial groups measured (Table 5). However there was a significant difference in the change in Bifidobacteria from baseline between the control (median change 0.0, IQR 0.8), 80 g/d (0.0, 1.1) and 120 g/d (+0.3, 0.6) ( $P = 0.046$ , Kruskal–Wallis test), although neither of the planned

**Table 4**  
Incidence and severity of gastrointestinal symptoms at week 4.

	Incidence (d/wk) <sup>a</sup>				Incidence of moderate and severe symptoms (d/wk) <sup>a</sup>			
	Control (n = 40)	80 g/d prunes (n = 40)	120 g/d prunes (n = 40)	P value <sup>b</sup>	Control (n = 40)	80 g/d prunes (n = 40)	120 g/d prunes (n = 40)	P value <sup>b</sup>
Heartburn	0.2 (0.7)	0.2 (0.5)	0.5 (1.5)	0.731	0.1 (0.2)	0.1 (0.2)	0.0 (0.2)	0.813
Acid reflux	0.1 (0.2)	0.3 (0.8)	0.6 (1.3)	0.038*	0.0 (0.0)	0.0 (0.2)	0.1 (0.3)	0.068
Nausea	0.4 (0.8)	0.5 (1.5)	0.7 (1.1)	0.088	0.1 (0.3)	0.1 (0.5)	0.1 (0.3)	0.907
Belching	1.8 (2.8)	1.8 (2.5)	1.8 (2.2)	0.714	0.2 (0.5)	0.3 (1.2)	0.2 (0.7)	0.937
Borborygmi	1.7 (2.5)	2.0 (2.3)	2.4 (2.5)	0.267	0.2 (0.4)	0.4 (1.4)	0.5 (1.4)	0.459
Abdominal bloating	1.8 (2.5)	2.2 (2.6)	2.3 (2.6)	0.575	0.3 (0.7)	0.6 (1.6)	0.6 (1.4)	0.899
Abdominal pain/discomfort	0.7 (1.3)	1.5 (2.2)	1.6 (2.2)	0.303	0.2 (0.4)	0.4 (1.1)	0.6 (1.2)	0.203
Flatulence	3.0 (2.8)	4.7 (2.7)	4.4 (2.6)	0.015*	0.4 (1.2)	1.4 (1.9)	1.7 (2.3)	0.005*
Hard stools	0.5 (1.2)	0.4 (0.8)	0.4 (0.8)	0.958	0.0 (0.2)	0.1 (0.8)	0.1 (0.3)	0.220
Constipation	0.5 (1.2)	0.6 (1.5)	0.8 (1.5)	0.603	0.2 (0.8)	0.3 (1.0)	0.2 (0.6)	0.303
Diarrhoea	0.2 (0.5)	0.5 (1.4)	0.2 (0.7)	0.775	0.1 (0.3)	0.3 (1.1)	0.1 (0.3)	0.898
Loose stools	0.7 (1.3)	1.0 (1.9)	0.5 (1.2)	0.213	0.2 (0.5)	0.3 (1.1)	0.2 (0.5)	0.826
Urgency	1.0 (1.3)	1.0 (1.6)	1.1 (1.5)	0.952	0.3 (0.6)	0.3 (0.6)	0.6 (0.8)	0.625
Incomplete evacuation	0.6 (1.4)	1.1 (1.9)	1.0 (1.9)	0.515	0.2 (0.8)	0.3 (1.0)	0.2 (0.5)	0.804

\*Planned contrasts: Incidence acid reflux Prunes vs control ( $P = 0.026$ ) and 80 g/d vs 120 g/d ( $P = 0.273$ ); Incidence of flatulence Prunes vs control ( $P = 0.001$ ) and 80 g/d vs 120 g/d ( $P = 0.469$ ); Incidence of moderate and severe flatulence Prunes vs control ( $P = 0.001$ ) and 80 g/d vs 120 g/d ( $P = 0.721$ ).

<sup>a</sup> Values are mean (SD) days/week of GI symptoms measured during the final week of the intervention (week 4). Mean values are shown in spite of the data not being normally distributed, because most median values were zero and hence uninformative.

<sup>b</sup> P values are the result of a Kruskal–Wallis test.

**Table 5**Concentrations of bacteria (log<sub>10</sub>/g dry faeces) at baseline (week 0) and end of the intervention (week 4).

Bacterial groups <sup>a</sup>	Control (n = 39)		80 g/d prunes (n = 40)		120 g/d prunes (n = 38)		P value <sup>b</sup> (intervention)	P value <sup>b</sup> (change from baseline)
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention		
All bacteria	10.7 (0.4)	10.8 (0.6)	10.5 (0.6)	10.8 (0.7)	10.7 (0.3)	10.8 (0.5)	0.854	0.770
Bifidobacteria	9.1 (0.5)	9.1 (0.6)	8.8 (0.9)	9.3 (0.9)	8.9 (0.8)	9.0 (0.6)	0.554	0.046*
<i>B. longum</i>	8.9 (0.6)	8.8 (1.2)	8.7 (1.2)	8.9 (1.3)	8.4 (0.9)	9.1 (0.8)	0.264	0.096
<i>B. adolescentis</i>	8.3 (3.9)	8.4 (3.9)	8.1 (2.1)	8.1 (92.3)	7.9 (3.7)	8.4 (2.9)	0.907	0.619
Bacteroidetes	9.9 (0.4)	10.0 (0.8)	9.8 (0.6)	10.1 (0.8)	9.9 (0.4)	10.0 (0.6)	0.881	0.972
<i>Bacteroides</i> spp.	9.7 (0.5)	9.7 (0.8)	9.5 (0.6)	9.7 (0.5)	9.8 (0.5)	9.6 (0.5)	0.784	0.741
<i>Prevotella</i> spp.	7.9 (3.9)	7.9 (3.6)	7.5 (3.5)	7.3 (3.8)	7.7 (3.2)	8.1 (2.9)	0.882	0.725
Clostridia Cluster XIVa	9.8 (1.0)	9.6 (1.1)	9.6 (1.4)	9.9 (1.5)	10.1 (0.7)	10.1 (0.8)	0.784	0.661
<i>Roseburia</i> spp.	9.4 (0.7)	9.5 (0.7)	9.2 (0.7)	9.5 (0.5)	9.4 (0.5)	9.4 (0.6)	0.996	0.111
Cluster IV Ruminococcus	8.6 (1.0)	8.4 (1.6)	8.4 (1.3)	8.6 (1.6)	8.6 (0.9)	9.0 (1.2)	0.179	0.661
<i>Lactobacillus</i> spp.	6.7 (2.5)	6.7 (2.8)	6.2 (2.2)	6.2 (2.4)	6.5 (2.3)	6.7 (1.0)	0.256	0.711
<i>Faecalibacterium prausnitzii</i>	9.5 (0.7)	9.5 (1.1)	9.4 (0.7)	9.5 (0.8)	9.6 (0.6)	9.6 (0.7)	0.524	0.367

\*Planned contrasts for change in bifidobacteria from baseline: Prunes vs control (P 0.057) and 80 g/d vs 120 g/d (P 0.121).

<sup>a</sup> Values are median (IQR) Bacteria in log<sub>10</sub>/g dry faeces measured at baseline and at week 4. Due to a technical issue with the qPCR assays for three subjects, data for 117 subjects were analysed for gut microbiota concentration.<sup>b</sup> P values are the result of a Kruskal–Wallis test.

contrasts showed a significant difference (Table 5). There were no significant differences between groups in change from baseline for any other bacteria measured, nor for absolute values of SCFA, stool pH or stool water at the end of the intervention (data not shown).

#### 4. Discussion

This study has demonstrated that prunes resulted in a greater increase in stool weight. However, there were no significant differences between groups in absolute stool weight under the ITT analysis, despite significant differences in the per protocol analysis, which might be explained by the ITT analysis producing conservative effect size estimates because of the use of 'last observation carried forward' for missing data. The change in stool weight equated to an increase of approximately 27.5 g/d per 100 g of prunes (Fig. 2), an effect size that is consistent with a previous study that demonstrated a 26 g/d increase in stool weight with 100 g/d prunes [22]. Based on these results, the hypothesis that consumption of prunes at doses of 80 g/d or 120 g/d in addition to the normal diet affects stool weight can be accepted.

We also found that prunes significantly increased stool frequency. This was expected because stool frequency correlates with stool weight [30] and confirms a previous study in constipation in which 100 g/d prunes increased stool frequency by one BM per week [31]. However, three previous studies in people without constipation reported prunes had no effect on stool frequency [22,23,32], although subjects in two of these studies had a baseline frequency greater than one BM per day and stool frequency has been reported to respond to dietary fibre only when less than once daily [33]. The mechanisms through which fibre impacts on stool weight and frequency are thought to include an increase in non-digestible, non-fermentable bulk in the colon which has a greater water-holding capacity, together with supporting bacterial metabolism and bacterial numbers in the colon.

Fibre is thought to mediate its effect on stool output by reducing WGTT, however, we did not find any differences in WGTT between the groups. This was unexpected because stool weight has an inverse relationship with transit time [34] and similar increases in fibre intake to those in the current study (e.g. 9 g/d wheat fibre) have decreased WGTT [35]. However, the effect of fibre on transit time may be influenced by fibre type. Pectin, one of the fibre components of prunes, has little effect on WGTT [36,37] but delays gastric emptying approximately twofold, possibly due to its viscous, gel-forming properties [38]. Fibre has

been found to normalise transit time [30,39] with the greatest reductions in those with slowest transit [4]. In the current study, the mean baseline WGTT was within the normal range [40], which may account for the lack of effect of prunes on this outcome.

Although prunes were generally well tolerated, they caused a small increased incidence of flatulence. This is likely the result of increased colonic fermentation of components in prunes such as pectin and sorbitol, although there was no measurable change in faecal SCFA or pH. Previously, 10 g of pure sorbitol induced gas and bloating in healthy subjects [41]. Although statistically significant the effect size was relatively small, with the difference in incidence of moderate/severe flatulence being 1.3 days/week for the 120 g/d group compared with control.

A difference in change in bifidobacteria was detected across the groups, with +0.3 log<sub>10</sub> increase occurring in the 120 g/d prune group. This is of interest because Bifidobacteria exert a number of effects that may contribute towards host health [42]. Although the increase was small in logarithmic terms, this can correspond to a substantial increase in absolute bacterial numbers which may alter biological activity [43]. Moreover, the increase in bifidobacteria observed is in the same order of magnitude as that observed in some studies investigating the prebiotic effects of inulin [44] and fructo-oligosaccharides [45]. The effect of prunes on the microbiota may be mediated by dietary fibre (e.g. pectin), sorbitol or phenolic compounds [17,18,46] but more research is required to confirm this finding and to establish which prune components are responsible for the increase in bifidobacteria.

Dietary analysis revealed that subjects in both prune groups consumed more energy than controls, despite no differences in body weight during the study. This was due to a reduction in reported energy intake from diet in all groups between baseline and week 4, with prunes intakes (reported in separate compliance diaries) resulting in additional energy intake. It is possible that subjects under-reported dietary intake, a problem widely acknowledged in dietary assessment [47].

An increase in dietary fibre consumption is the first line treatment for constipation [48], however, the effectiveness of food-based dietary fibre for treating constipation has been evaluated by few trials [49] and a recent systematic review identified that the quality of studies for fibre supplementation were either low or very low [50]. In the present study, both prune groups experienced an increase in stool weight and frequency, in line with a previous study reporting that prunes were superior to psyllium in



constipation [31]. Another study reported that prunes/flixweed significantly prevented constipation in pilgrims [51]. These results suggest that prunes could be useful in the prevention or management of constipation.

Epidemiological data suggest that stool weights >150 g/d and fibre intake of >24 g/d are associated with a reduced risk of colorectal cancer [3]. At the end of the 120 g/d prune intervention, mean stool weight was 123 g/d and fibre intake was 21 g/d, therefore at the group level not achieving these thresholds. The effect of fibre on stool weight varies according to fibre type. Raw wheat bran produces the greatest change in stool weight per gram of non-starch polysaccharides (NSP) consumed (7.2 g), whereas pectin appears to have the least effect (1.3 g per g NSP consumed) [28]. In the present study, stool weight increased by 4.5 g/d per g of prune NSP consumed, equivalent to 3.3 g/d per g of AOAC fibre. Therefore increasing fibre intake with prunes will increase stool weight, but doing so in conjunction with other fibre-rich foods could have a greater impact.

The strengths of the study include the randomised design, large sample size and blinded data analysis. The findings of the per protocol analyses were generally consistent with those from the primary ITT analysis, although as expected, the ITT analysis produced conservative effect size estimates for stool weight.

Limitations included the difficulty in double blinding, because prunes are easily identifiable and it was impossible to develop a control identical in appearance but without any active components. Therefore, blinding of subjects was attempted by using water as the control and advertising the study as an investigation of the effect of 'fibre or fluids' on GI health, making the intervention and control ambiguous. The success of subject blinding is unclear, and the possibility of a placebo response cannot be ruled out. However, subjective outcomes (e.g. GI symptoms) are particularly susceptible to placebo responses, and for this reason numerous objective measures were included (e.g. stool weight, WGT). At baseline the population had an average of 5 BMs per week and fibre intake of approximately 15 g/d (AOAC), both of which are below the average for the general population, but which were specifically chosen to reflect the intended consumer who may wish to increase fibre intakes.

In conclusion, this study has demonstrated that a four-week intervention of 80 g/d or 120 g/d prunes resulted in greater increases in stool weight. Prunes also increased stool frequency, were relatively well tolerated in terms of GI symptoms and did not lead to weight gain. Therefore, they have potential health benefits in populations where stool weights are low, including potentially contributing to the prevention of constipation and other disorders associated with low fibre intake. Whether the effects are mediated by dietary fibre, sorbitol or phenolics, or the combination, remains to be established.

#### Author's contributions

EL, PWE and KW designed the research; EL conducted the research; EL analysed data; PWE and KW supervised the research and interpreted all data. SMS supervised and interpreted the transit time studies. PL supervised and interpreted the microbiota analysis. EL, PWE and KW wrote the paper; KW had primary responsibility for final content. All authors read and approved the final manuscript.

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#### Conflict of interest

This trial formed part of a California Dried Plum Board PhD studentship. A preliminary version of these data were presented at the Nutrition Society Summer Meeting 2014 as: Lever E, Scott SM, Emery PW, Whelan K. The effect of prunes on stool output, whole gut transit time and gastrointestinal symptoms: a randomised controlled trial. *Proc Nutr Soc.* 2015; 74 (OCE1): E2.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.01.003>.

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