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Effects of inulin supplementation on inflammatory biomarkers and clinical symptoms of women with obesity and depression on a calorie-restricted diet: a randomised controlled clinical trial

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Abstract

Major depressive disorder (MDD) is regarded as an inflammatory disorder. Gut microbiota dysbiosis, observed in both MDD and obesity, leads to endotoxemia and inflammatory status, eventually exacerbating depressive symptoms. Manipulation of gut microbiota by prebiotics might help alleviate depression. The present study aimed to investigate the effects of inulin supplementation on psychological outcomes and biomarkers of gut permeability, endotoxemia, inflammation, and brain-derived neurotrophic factor (BDNF) in women with obesity and depression on a calorie-restricted diet. In a double-blind randomised clinical trial, forty-five women with obesity and MDD were allocated to receive 10 g/d of either inulin or maltodextrin for 8 weeks; all the patients followed a healthy calorie restricted diet as well. Anthropometric measures, dietary intakes, depression, and serum levels of zonulin, lipopolysaccharide (LPS), inflammatory biomarkers (TNF-α, IL-10, monocyte chemoattractant protein-1, toll-like receptor-4 and high-sensitivity C-reactive protein), and BDNF were assessed at baseline and end of the study. Weight and Hamilton Depression Rating Scale (HDRS) scores decreased in both groups; between-group differences were non-significant by the end of study ($P = 0{\cdot}333$ for body weight and $P = 0{\cdot}500$ for HDRS). No between-group differences were observed for the other psychological outcomes and serum biomarkers ($P > 0{\cdot}05$). In this short-term study, prebiotic supplementation had no significant beneficial effects on depressive symptoms, gut permeability, or inflammatory biomarkers in women with obesity and depression.

Keywords: **Calorie restriction: Inflammation: Inulin: Major depressive disorder: Obesity**

Affecting about 300 million people, major depressive disorder (MDD) is the most prevalent psychiatric disorder and the leading cause of disability worldwide; women are generally more affected than men[1]. There is a bidirectional association between depression and obesity. Patients with depression are 58 % more likely to become obese, and those with obesity are not only at 55 % greater risk of developing depression but also respond poorly to antidepressants[2].

MDD is regarded as an inflammatory disease, as some of its well-known risk factors (including psychological stress, sedentary lifestyle and obesity) are associated with chronic low-grade inflammation[3]. Gut microbiota dysbiosis and increased gut permeability, recently implicated in the pathophysiology of depression, might also affect mood partly through dysregulating immune responses of the host[4]. High gut permeability, specified by augmented serum levels of zonulin (a modulator of the intercellular tight junctions), allows for easier translocation of lipopolysaccharide (LPS; a gram-negative bacterial component) from the gut lumen into the circulation and eventually leads to increased expression of pro-inflammatory

Abbreviations: BDNF, brain-derived neurotrophic factor; HDRS, Hamilton Depression Rating Scale; LPS, lipopolysaccharide; MDD, major depressive disorder.

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cytokines[5,6]. One proposed mechanism through which inflammation affects depressive symptoms is down-regulation of brain-derived neurotrophic factor (BDNF, a neurotrophin essential for neuron plasticity)[7]. Higher levels of circulating zonulin, LPS (endotoxin) and pro-inflammatory cytokines have been reported in both obesity and depression, compared with control samples[8–11].

Novel treatment strategies are needed for those with concurrent depression and obesity. Because, the current medications for the management of depression have shown insufficient effectiveness[12] and have many side effects. Moreover, prevention of cardiovascular complications very much depends on management of both obesity and depression in patients suffering from both disorders[13]. Calorie restriction, as the first-line treatment for obesity, has shown favourable effects on gut microbiota composition, makers of gut permeability and inflammation, as well as depressive symptoms[14,15]. Prebiotics, defined as 'substrates that are selectively utilised by host micro-organisms conferring a health benefit', also help restore healthy gut microbiota ecosystem[16]. Studies have revealed that these substances might facilitate weight loss and improve gut barrier function, endotoxemia and inflammation[17,18]. Some beneficiary effects of prebiotics on depressive symptoms have been reported as well[19].

Depression is generally believed to be worsened by strict long-term calorie restriction, and individuals with depression are assumed to have lower compliance to dietary interventions[20,21]; therefore, clinical trials investigating the effects of weight loss diets or dietary supplements usually exclude patients with depression. This is despite the fact that these subjects are at a great risk for metabolic disease and CVD and would benefit from moderate dietary interventions[22,23]. Studies on antidepressant and anti-inflammatory effects of calorie restriction and prebiotic supplementation among patients with concurrent obesity and depression are scarce. Thus, we aimed in the present clinical trial, to assess the effects of a calorie-restricted diet in combination with inulin – a dietary fibre with established prebiotic properties[24] – on depression, serum BDNF, gut permeability, endotoxemia and inflammatory biomarkers of women suffering from obesity and MDD.

Materials and methods

Participants

The participants of the present study were women with MDD (based on DSM-5 criteria; Diagnostic and Statistical Manual of Mental Disorders, 5th Edition), who met the following inclusion criteria: pre-menopausal; age of 20–50 years; BMI: 30–40 kg/m^2; a score of 8–23 (mild to moderate depression) on the seventeen-item Hamilton Depression Rating Scale (HDRS)[25]; being on a stable antidepressants regimen for ≥ 6 months prior to the study; and willingness to participate in the study. All the patients were interviewed by a psychiatrist for confirmation of MDD diagnosis. Pregnant or lactating women, drug addicts or smokers, and those with a history of following particular diets during the last year or using synthetic/herbal drugs for weight loss were excluded from the study. Co-morbidity with thyroid dysfunctions or other major psychiatric/neurological diseases including psychosis, bipolar disorder, multiple sclerosis or epilepsy were also among exclusion criteria. Patients who had any changes in type/dosage of any medications or nutritional supplements during the study, those using fibre supplements or taking more than 25 g/d of dietary fibre, and patients who took antibiotic or prebiotic/probiotic products/supplements daily 2 months prior to or during the study were deemed ineligible in our study as well. Patients who experienced significant events during the study which could affect their psychological health were also excluded.

Study subjects were outpatients recruited from Bozorghmehr and Sharif Psychiatry Centers as well as the polyclinics of twenty-nine Bahman Hospital, affiliated to Tabriz University of Medical Sciences, Tabriz, Iran. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human patients were approved by the Ethics Committee of Research Vice-Chancellor of Tabriz University of Medical Sciences, Tabriz, Iran (Ethics code: IR.TBZMED.REC.1397·189). The patients were given a comprehensive explanation of the study objectives and procedures, and written informed consent was obtained from them. This clinical trial was registered in the Iranian Registry of Clinical Trials available at www.irct.ir (IRCT20100209003320N15).

Study procedures

The present study was a double-blind placebo-controlled randomised parallel-group clinical trial. At the time this study was designed, no previous papers on the effect of prebiotics on zonulin (gut permeability) or BDNF in obesity and depression had been published. Therefore, the sample size was calculated based on mean and standard deviation of TNF-α (a main inflammatory biomarker in our study) reported by Dehghan *et al.*[26] We considered type I (α) and type II (β) errors of 0·05 and 0·20 (power = 80 %) in two-sided tests, respectively. The estimated sample size was 22 per each group. HDRS was the primary outcome in our study. There were no previous studies on the effect of prebiotics on HDRS in patients with depression, at the time we designed this study. Therefore, we used an estimate of variability in HDRS (SD = 2·19) from a previous meta-analysis[27] to calculate a sample size that provided at least 90 % power with two-sided type I error of 0·05 to detect a mean difference of 3 (minimal clinically important change in HDRS) in the intervention group[28]:

$$\text{Effectsize (ES)} = \frac{3}{2.19} = 1.37$$

$$\text{N} = 2\left(\frac{Z1 - \frac{\alpha}{2} + Z1 - \beta}{ES}\right)2 = 2\left(\frac{1.96 + 1.282}{1.37}\right)2 = 11.2\sim12$$

This sample size was lower than the sample size calculated based on TNF-α. Therefore, we chose the sample size estimated based on TNF-α (n 22). Considering a probable 40 % dropout rate, this number was increased to 31.

The participants were randomly assigned into one of the two study groups (1:1) by a research assistant not otherwise involved in the study. The randomised block procedure of size 4 was used, and the sequence was generated using the Random Allocation Software (RAS)[29]. Randomisation was stratified by

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depression severity (mild *v.* moderate) and BMI (< 35 kg/m^2 *v.* ≥ 35 kg/m^2). Three-digit codes were given to each of the two intervention sachets (prebiotic and placebo) by the person (entirely unrelated to the trial) who prepared them. The sachets were completely identical in all aspects (colour and weight) other than the assigned codes. All the patients and the study team were blind to the randomisation and allocation until the end of the study and completion of statistical analyses.

The study duration of the present clinical trial was 8 weeks. All the patients were seeking weight loss; thus, weight loss diet (as the primary treatment for obesity) was planned for them. Total energy expenditure of the patients was estimated by adding up their RMR (assessed by indirect calorimetry), physical activity level (approximated based on data obtained from the short form of International Physical Activity Questionnaire; IPAQ) and thermic effect of food (10 % of total energy expenditure)[30]. To reach weight loss, dietary plans were prepared based on 75 % of the total energy expenditure. This extent of calorie restriction has been found to show favourable antidepressive effects, especially in short term[15]. Macronutrient distribution was 55 %, 30 % and 15 % of energy from carbohydrates, fat and protein, respectively. The patients were allowed to consume non-starchy vegetables *ad libitum*. Subjects were provided with weight loss meal plans and instructed on how to use food exchange lists in case they did not have access to the foods within their dietary plans. The food-based dietary guidelines for Iranians (available at: http://www.fao.org/nutrition/education/food-based-dietary-guidelines/regions/countries/iran/fr/) were also fully described for the participants. Subjects in the prebiotic group received 10 g/d of Frutafit® IQ (Sensus Co., Batch No: 2510802557). Frutafit® IQ is a native inulin/oligofructose. It is a food ingredient that is extracted from chicory roots. It is an agglomerated powder with excellent dispersibility and wettability. Inulin from chicory is a polydisperse mixture of linear fructose polymers with mostly a terminal glucose unit coupled by β (2–1) bonds. The number of units (degree of polymerisation) can vary between 2 and 60. Patients in the placebo groups received 10 g/d of maltodextrin (FIC Co., China). The participants were asked to dissolve the contents of a sachet in a glass of water and drink it after lunch. Inulin was administered at the dose of 10 g/d, as it is well tolerated by the gastrointestinal tract[31] and has been found to augment faecal *Bifidobacteria* in only 2 weeks[32,33]. Every 2 weeks, the participants returned to the clinic to receive their supplements and new food plans (for more diversity and flexibility of the diet). At these sessions, the participants discussed with a nutritionist, any inconvenience they felt with the supplements or diet, worked out solutions for these issues, and were put back on track. Adherence to supplements was evaluated by counting the unused sachets, at every visit; it was considered as non-compliance if more than 10 % of the administered supplements were returned to the study staff during the whole 8 weeks. If the patients had non-compliance to the supplements, they would be excluded from the analyses. We used a short questionnaire designed for this study to ask the study participants about any adverse effects related to the study interventions at every visit.

A demographic questionnaire was completed for the patients at baseline. To assess the patients' physical activity level, IPAQ-short form was completed for them; metabolic equivalents (MET; minutes/week) were calculated according to the manual[34]. Patients were asked to maintain their usual physical activities throughout the study. For assessment of dietary intakes, the subjects completed three food records (two non-consecutive weekdays and a weekend) before starting the calorie-restricted diet and the supplements at baseline, and another three food records during the last week at the end point of the study. The dietary records were based on estimated values in household measurement. Data on food intake were analysed by Nutritionist IV software (First Databank) modified for Iranian foods.

Body weight (to the nearest 100 g) and height (to the nearest 0·5 cm) were measured using a Seca scale, with minimal clothing and no shoes on. BMI was calculated as weight (kg) divided by height squared (m^2). RMR was evaluated by indirect calorimetry using Fitmate Pro; the basic guidelines were followed before performing the RMR measurement[35]. A single trained nutritionist performed all these measurements at both ends of the study to eliminate inter-individual errors.

Anxiety was assessed by Spielberger's State-Trait Anxiety Inventory Form Y (STAI-Y). This questionnaire consists of two sections, each containing twenty questions. The first part assesses state anxiety (i.e. how the subject feels at the moment of completing the questionnaire), and the second part measures trait (habitual) anxiety. The answers to all questions are scored based on Likert scale from 1 to 4; weighted scores, in which anxiety-absent items are reversely scored (i.e. 4 to 1), are added up for a final score on each of the two sections[36]. The Persian translation of this inventory has shown acceptable validity and reliability among Iranian populations[37–40]. For clinical assessment of depression, HDRS and Beck Depression Inventory-II (BDI-II) were used. HDRS is a seventeen-item clinician-administered questionnaire[41] and was completed for the patients during an interview by a psychiatrist. Based on the severity of the symptoms assessed during the interview, the psychiatrist marks one (from among 3 or 5) of the statements that best describes the patient; the statements have a score range of 0–2 or 0–4 points, and the maximum score for HDRS-17 is 54[25]. BDI-II, a twenty-one-item self-administered scale of depression[42], was completed by the patients. Each item consists of a response set of four sentences describing the extent of depressive symptoms during the preceding 2 weeks; scoring is based on Likert scale from 0 (absent or mild) to 3 (severe). A total score is calculated by adding up the scores for all the items (range: 0–63)[43]. Validity and reliability of both BDI-II and HDRS have been confirmed among Iranian subjects[43–45]. WHO-5, a widely used self-rated instrument developed by the WHO for the assessment of overall psychological well-being based on only five questions, was also completed by the patients. This questionnaire includes five positively worded sentences, and the patients are instructed to score on a six-point Likert scale (0–5) how often they had these positive feeling during the last 2 weeks; the scores are summed and reported as percent[46]. This questionnaire has sufficient validity and reliability for use in psychiatric studies among Iranians as well[46].

After a 12-h overnight fasting, 10 ml of venous blood was drawn at baseline and end of the study and immediately

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centrifuged at 3500 rpm for 10 min. Serum was removed, aliquoted in microtubes and stored at −80°C until analysis.

Laboratory analysis

Serum LPS, zonulin, BDNF, TNF-α and IL-10 were measured by relevant ELISA kits (Crystal Day Bio-Tec) according to manufacturer's instructions. Monocyte chemoattractant protein-1 (R&D Systems) and toll-like receptor-4 (Elabscience®) were measured using ELISA method as well. Turbidometric method and commercial kit (Pars Azmun) was used to measure serum high-sensitivity C-reactive protein.

Statistical analysis

IBM SPSS Statistics for Windows, version 26 (IBM Corp.) and Per protocol (PP) principles were used for the statistical analyses. Normality of data distribution was tested by Kolmogorov–Smirnov test. Logarithmic transformation was used in attempt to achieve normality for the data not normally distributed; non-parametric tests were used if normality was not achieved. Data were expressed as mean (SD) and median (25th and 75th percentiles) for variables with normal distribution and otherwise, respectively. Independent-samples *t* test and Mann–Whitney *U* test were applied for comparing the groups at baseline. Qualitative data were presented as frequency (percentage); trend Chi-square test was used for assessment of between-group differences. We were interested in testing the between-group differences at the end of the study after adjusting for baseline values and covariates. Therefore, we used ANCOVA for this aim when the data were normally distributed. When the data were not normally distributed, we used quantile regression to look at median differences (rather than mean differences). This approach is useful in understanding outcomes that are not normally distributed[47,48] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054530/. Mean difference (MD) and 95 % CI were reported for normally distributed data, and coefficient, and 95 % CI was reported for the data not normally distributed. For evaluating clinical importance of prebiotic supplementation, number needed to treat was calculated using the standard method (inverse of the risk difference) and based on ≥ 3 points reduction in HDRS score[49]:

$$\text{Risk difference} = \frac{a}{b} - \frac{c}{d}$$

where *a*: patients (N) in the prebiotic group, with ≥ 3 points reduction in HDRS score; *b*: patients (N) in the prebiotic group; *c*: patients (N) in the placebo group, with ≥ 3 points reduction in HDRS score; and *d*: patients (N) in the prebiotic group:

$$NNT = \frac{1}{\text{Risk of difference}}$$

Statistical significance was set at $P < 0{\cdot}05$.

Results

The study was conducted between 2018 June and 2018 September. Figure 1 presents the study flow chart. From among those who had been randomised to treatment groups at baseline (*n* 62), seven patients could not be contacted after the first visit and ten patients claimed that they could not comply with the dietary plans they were given. A total of forty-five patients completed the study. Among those who completed the study, compliance to prebiotic and placebo supplements were 91·67 (2·86) and 91·09 (2·39) per cent, respectively; no significant difference was observed between the two groups ($P = 0{\cdot}464$). We had no patients fulfilling the non-compliance criterion for the supplements in our study. Five patients in the prebiotic group reported gastrointestinal complaints (flatulence and soft stool), which was resolved after 2 weeks; no further study-related adverse events were described by the study subjects.

There was no significant difference between the study groups in terms of demographic characteristics, BMI and depression status at baseline (Table 1). Data on dietary intakes and physical activity of the patients are summarised in Table 2. There were no significant differences between the two groups at baseline for the dietary components and physical activity. After adjusting for baseline values, calories and protein intake was lower following the intervention in the treatment group compared with the placebo group ($P < 0{\cdot}05$). However, we observed no significant difference between the two groups for the calorie percentage from proteins and dietary fibre per 1000 cal of energy intake at week 8 after adjusting for baseline values. Calorie and protein intake can impact inflammatory biomarkers[50,51]. There is also evidence that calorie and protein intake can affect depression and mood[52,53]. Since there was significant difference between the two groups by the end of the study for these two dietary variables, we included them as covariates when looking at the effect of the study treatments on mental health outcomes and serum biomarkers, to make sure that we obtain a net effect of the treatment when doing either ANCOVA or quantile regression.

Physical activity of the participants did not change significantly throughout the study. There was no significant effect of prebiotic supplementation on weight (MD = –0·62, 95 % CI − 1·91, 0·66, $P = 0{\cdot}333$).

Table 3 presents the results for psychological outcomes; we observed no significant effect of the intervention on any of these outcomes. Twelve patients in each group experienced ≥ 3 points improvement in HDRS; thus, the calculated number needed to treat for 8-week inulin (10 g/d) supplementation to reach a minimum decrease of three points on HDRS score was 42 (95 % CI: −3·00, 4·00). We found no significant effect of the intervention on LPS, zonulin, BDNF or inflammatory biomarkers (Table 4).

Discussion

According to the results of the present clinical trial in women with depression and obesity who were on a calorie-restricted diet, no significant effects of 8-week prebiotic supplementation were found on body weight, depression (HDRS score), BDNF and biomarkers of endotoxemia, gut permeability, and inflammation compared with placebo.

Patients with depression are usually assumed to poorly comply with weight loss diets[20]. Interestingly, despite having lower protein intake (a satiating nutrient)[54] and no differences in

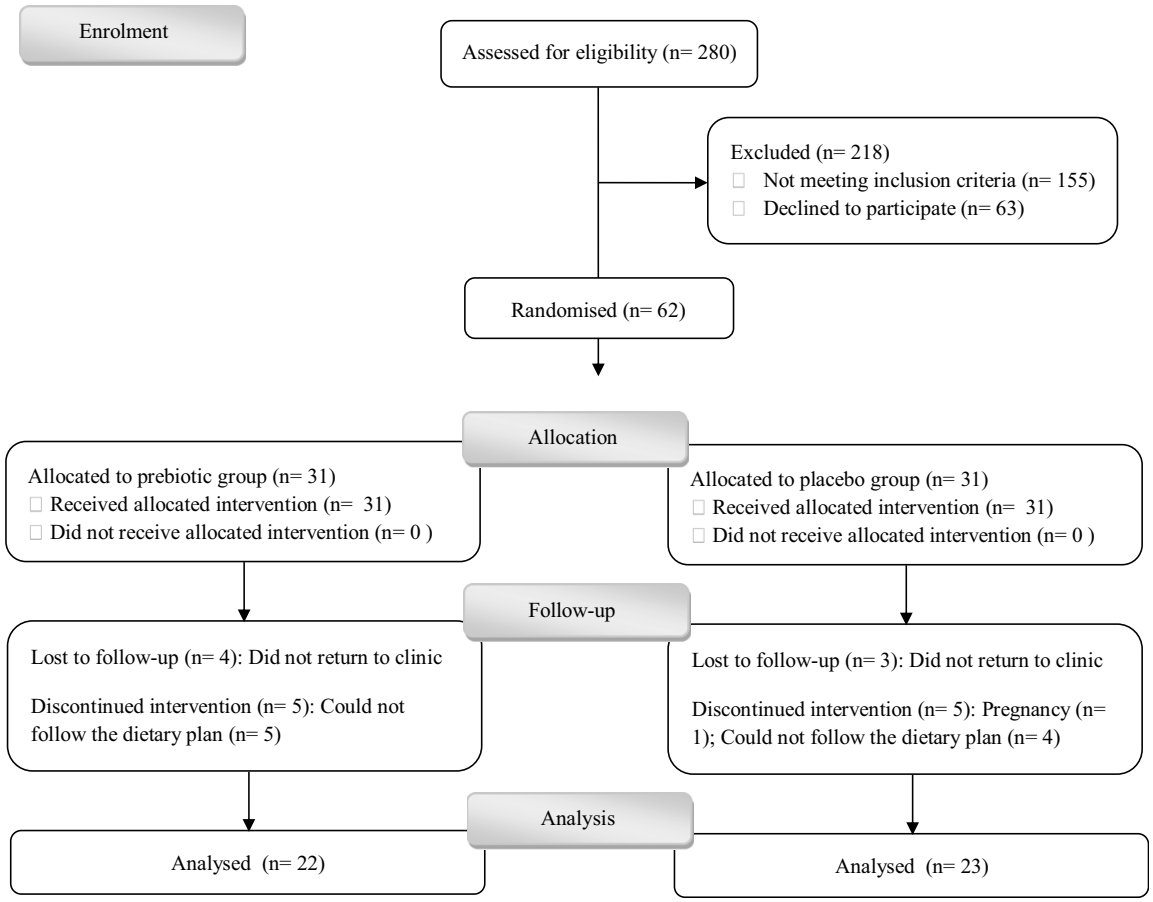

Fig. 1. Flow chart of the study.

Table 1. Baseline characteristics
(Mean values and standard deviations)

	Inulin (*n* 22)		Maltodextrin (*n* 23)		
Variables	Mean	SD	Mean	SD	*P*
Age (years)	38·55	6·32	40·96	8·01	0·270*
BMI	34·49	4·01	33·57	3·91	0·436*
	Median	25th and 75th percentiles	Median	25th and 75th percentiles	
Depression years	4·00	2·75, 6·00	6·00	3·00, 8·00	0·196†
	n	%	*n*	%	
Marital status					0·489‡
Single	0	0·0	0	0·0	
Married	21	95·5	23	100·0	
Divorced or Widow	1	4·5	0	0·0	
Education					0·753‡
Illiterate	0	0·0	1	4·3	
Diploma and lower	17	77·3	17	73·9	
Bachelors and higher	5	22·7	5	21·7	
Occupation					0·287‡
Homemaker	19	86·4	22	95·7	
Employee	2	9·1	1	4·3	
Self-employed	1	4·5	0	0·0	
Depression severity‖					0·459§
Mild	19	86·4	17	73·9	
Moderate	3	13·6	6	26·1	

* *P* based on independent-samples *t* test.
† *P* based on Mann–Whitney *U* test.
‡ *P* based on trend χ^2 test.
§ *P* based on Fisher's exact test.
‖ Based on HDRS (Hamilton Depression Rating Scale) scores: mild (7–17) and moderate (18–24).

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Table 2. Dietary intake and physical activity of the patients throughout study (Mean values and standard deviations)

	Inulin (*n* 22)		Maltodextrin (*n* 23)				
Variables	Mean	SD	Mean	SD	MD	95 % CI	*P**
Energy (cal)							
Baseline	1822·7	254·02	1846·6	306·53			
End	1292·6	269·03	1509·4	301·21	–201·45	–335·83, –67·07	0·004
Protein (g)							
Baseline	61·89	20·01	64·55	16·34			
End	41·20	10·59	49·27	13·34	–7·72	–14·95, –0·49	0·037
Protein (% of cal)							
Baseline	13·29	3·16	13·98	2·76			
End	13·08	3·68	13·11	2·76	–0·13	–2·08, 1·83	0·896
CHO (g)							
Baseline	247·93	53·96	260·96	53·40			
End	185·01	60·91	221·93	74·00	–27·99	–63·03, 7·05	0·114
CHO (% of cal)							
Baseline	54·29	9·21	56·55	7·51			
End	56·56	12·22	57·98	11·34	0·68	–7·73, 6·36	0·845
Fat (g)							
Baseline	67·87	18·20	62·94	19·96			
End	46·17	16·89	51·12	18·23	–6·18	–16·58, 4·22	0·237
Fat (% of cal)							
Baseline	33·94	9·87	30·66	7·72			
End	32·40	10·62	31·24	10·85	0·43	–6·11, 6·98	0·895
MUFA (g)							
Baseline	15·95	4·42	14·94	6·10			
End	10·50	5·05	11·33	4·93	–1·14	–4·02, 1·74	0·430
	Median	25th and 75th percentiles	Median	25th and 75th percentiles	Coefficient	95 % CI	*P*†
Cholesterol (mg)							
Baseline	146·65	85·21, 422·42	116·50	77·88, 315·10			
End	66·21	5·37, 143·92	71·27	36·00, 301·10	–69·32	–176·34, 37·70	0·198
SFA (g)							
Baseline	11·92	8·47, 16·18	12·70	8·65, 15·47			
End	7·81	4·67, 10·28	9·05	6·69, 11·97	–1·30	–4·88, 2·27	0·466
PUFA (g)							
Baseline	25·02	18·87, 37·55	19·47	11·16, 25·33			
End	19·19	10·42, 28·31	19·93	9·66, 22·59	–0·33	–7·54, 6·87	0·926
Dietary fibre (g)							
Baseline	12·53	9·41, 14·74	11·94	9·23, 14·75			
End	9·11	6·09, 15·30	10·27	8·15, 11·75	–1·02	–4·62, 2·58	0·571
Dietary fibre (g/1000 cal)							
Baseline	7·01	4·90, 8·26	6·58	5·26, 7·39			
End	7·59	5·25, 9·67	6·58	5·69, 7·64	0·18	–0·34, 0·70	0·487
Physical activity (MET)							
Baseline	766·50	334·50, 1853·20	840·00	0·00, 1680·00			
End	792·00	229·50, 1719·00	720·00	0·00, 1680·00	–79·47	–254·62, 95·68	0·365

MD, mean/median of difference; Cal, calories; CHO, carbohydrate; MET: metabolic equivalents (MET-minutes/week).
* *P* based on ANCOVA adjusted for baseline values.
† *P* based on quantile regression adjusted for baseline values.

Table 3. Psychological outcomes of the patients throughout study
(Mean values and standard deviations)

	Inulin (*n* 22)		Maltodextrin (*n* 23)				
Variables	Mean	SD	Mean	SD	MD	95 % CI	*P*
STAI-state							
Baseline	43·54	10·36	47·00	14·71	−3·45	−11·14, 4·23	0·370*
End	43·04	10·71	46·52	13·31	−0·81	−7·42, 5·79	0·804†
STAI-trait							
Baseline	47·14	7·41	52·13	10·60	−4·99	−10·52, 0·53	0·075*
End	45·10	9·52	47·96	9·43	0·87	−3·90, 5·65	0·872†
HDRS							
Baseline	12·86	3·96	13·87	4·87	−1·01	−3·68, 1·67	0·452*
End	9·36	3·24	11·04	4·08	−0·59	−2·35, 1·17	0·500†
BDI-II							
Baseline	18·14	9·28	22·78	10·43	−4·65	−10·59, 1·66	0·149*
End	15·32	8·66	19·78	11·45	−0·48	−5·62, 4·70	0·853†
WHO-5							
Baseline	49·27	22·40	42·78	23·46	6·49	−7·31, 20·29	0·348*
End	56·18	21·47	50·61	23·15	1·44	−12·63, 15·51	0·837†

MD, mean difference; STAI, State-Trait Anxiety Inventory; HDRS, Hamilton Depression Rating Scale, BDI, Beck Depression Inventory.
* *P* based on independent-samples *t* test for comparison of the baseline values between groups.
† *P* based on ANCOVA adjusted for baseline values and changes in calorie and protein intake.

Table 4. Endotoxemia, gut permeability, BDNF and inflammatory biomarkers of the patients throughout study
(Median values and percentiles)

	Inulin (*n* 22)		Maltodextrin (*n* 22)				
Variables	Median	25th and 75th percentiles	Median	25th and 75th percentiles	Coefficient	95 % CI	*P**
LPS (EU/L)							
Baseline	207·25	156·10, 451·40	203·30	175·60, 315·20			
End	186·55	157·10, 415·92	199·40	167·70, 271·20	17·72	−32·42, 67·85	0·479
Zonulin (ng/ml)							
Baseline	4·00	2·80, 8·72	3·80	2·70, 6·20			
End	3·85	2·97, 7·60	3·60	3·00, 6·10	0·20	−0·77, 1·18	0·676
BDNF (ng/ml)							
Baseline	1·50	1·10, 2·32	1·40	1·20, 2·00			
End	1·60	1·20, 2·07	1·60	1·40, 1·80	−0·02	−0·32, 0·28	0·892
IL-10 (ng/ml)							
Baseline	125·45	84·37, 179·08	133·70	109·30, 152·30			
End	132·65	99·25, 192·48	137·20	104·50, 162·30	13·13	−11·62, 37·89	0·290
TNF-α (ng/l)							
Baseline	54·25	41·55, 161·05	46·10	42·20, 106·10			
End	52·45	38·92, 134·60	48·10	43·30, 109·40	2·38	−6·59, 11·35	0·595
MCP-1 (pg/ml)							
Baseline	207·88	146·72, 394·74	395·75	219·99, 542·15			
End	207·52	108·73, 313·07	299·97	183·41, 429·26	−1·74	−46·99, 43·51	0·938
TLR-4 (pg/ml)							
Baseline	232·48	69·82, 464·15	529·63	341·58, 911·11			
End	224·82	46·02, 403·70	346·63	240·80, 509·49	9·25	−116·42, 134·92	0·882
hs-CRP (mg/l)							
Baseline	6·10	4·17, 9·05	4·30	3·20, 15·40			
End	4·70	3·75, 9·70	4·30	2·10, 10·40	−0·30	−2·41, 1·81	0·775

BDNF, brain-derived neurotrophic factor; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; TLR-4, toll-like receptor-4; hs-CRP, high-sensitivity C-reactive protein.
* *P* based on quantile regression adjusted for baseline values and changes in calorie and protein intake.

dietary fibre intake (also a satiating nutrient)[55], after adjusting for baseline values energy intake was lower following the intervention in the inulin group compared with the placebo at the end of our study. This finding might add to the evidence that inulin may promote satiety and lower energy intakes, which in turn could potentially improve weight loss maintenance and hence psychological outcomes over a longer time period. Prebiotics might decrease calorie intake by suppressing ghrelin and enhancing peptide YY (PYY), two hormones playing major roles in appetite regulation[56]. A role for SCFA has also been suggested in mediating the appetite regulatory effects of fermentable carbohydrates including prebiotics, but most of the evidence comes from animal studies and there are conflicting results from human studies[57].

Gut microbiota dysbiosis, increased gut permeability (leaky gut), endotoxemia and inflammation contribute to depression.

Inflammation disrupts blood–brain barrier function, induces hyperactivity of hypothalamic–pituitary–adrenal axis, activates enzymes responsible for metabolising tryptophan (the precursor of serotonin) and reduces BDNF expression; these are all implicated in depression pathophysiology[7,58]. Prebiotics can modulate gut microbiota composition, and some clinical trials have shown that serum levels of zonulin, LPS and inflammatory cytokines can be reduced following supplementation with various types of prebiotics (e.g. inulin, oligofructose-enriched inulin, resistant dextrin, etc.)[19,59,60]. Supplementing women with overweigh/obesity suffering from type 2 diabetes with 10 g/d of Nutriose (a resistant dextrin) resulted in a significant reduction in LPS, IFN-γ and IFN-γ/IL-10 ratio and a significant increase in CD8 and IL-10 compared with the control group after 8 weeks[19]. In a well-designed cross-over study with a 2-week run-in period, two 5-week study periods and a washout period of 8 weeks, Russo *et al.* showed that inulin-rich pasta intake could significantly decrease serum zonulin and increase glucagon-like peptide 2 (an essential molecule in the regulation of intestinal barrier function) levels in healthy young subjects compared with the control pasta; the urinary lactulose/mannitol excretion ratio was also significantly reduced in the inulin-enriched pasta group compared with the control group, indicating decreased gut permeability[59]. Oligofructose-enriched inulin supplementation for 8 weeks could significantly decrease IL-6, TNF-α and plasma LPS compared with placebo in women who were diabetic and overweight/obese[60]. Our study was not in agreement with these studies as we found no significant effect of prebiotic supplementation on LPS, gut permeability or inflammatory biomarkers.

It is speculated that through restoring gut microbiota balance and reversing the consequences of gut dysbiosis, prebiotics might help alleviate depressive symptoms. Unlike the promising results coming from animal models, clinical trials investigating antidepressant effects of prebiotics supplementation have not come up with consistent results. Abbasalizad-Farhangi *et al.* reported that receiving 10 g/d of resistant maltodextrin for 8 weeks significantly improved depression score in patients with diabetes[19]. However, the other clinical trials which assessed the effects of prebiotics on depressive symptoms of healthy or clinical samples found no significant effect of the treatments on depression scores; no data on LPS or inflammation were presented in these studies[61–63]. The only clinical trial in MDD that evaluated the effects of prebiotics (10 g/d galactooligosaccharide for 8 weeks) on BDI scores of non-obese patients reported no significant decreases of the score in the prebiotic arm[64]. In agreement with these studies, we found no significant effect of prebiotic supplementation on psychological outcomes. At the end of the study, our analyses revealed that the SD for HDRS was higher in our study from both study arms, compared with the SD = 2·19 that we had used in sample size calculation. This might have led to reduced power of our study to detect significant changes in the intervention group for the HDRS by the end of study. It is also probable that prebiotics can improve depression only if they can decrease gut permeability, LPS and inflammation in the upstream of the suggested pathway, described above. Moreover, the baseline values for these biomarkers were not far outside of normal range in our study sample. Higher baseline values of these biomarkers in the samples of Abbasalizad-Farhangi study might have offered more room for improvement. The non-significant difference between the two groups for the study outcomes at the end of the study could also be attributed to the Hawthorne effect; all the patients were regularly visited, and they all received the same dietary advice[65]. Moreover, the patients talked to the psychiatrist freely about their personal life and problems; this could affect their mood, as WHO 'Let's Talk' quote for depression has emphasised the importance of talking to a confident other in battling depression.

To the best of our knowledge, this was the first clinical trial that investigated the effects of a combination of weight loss diet and prebiotic supplementation, compared with diet alone, among women with obesity and MDD. Providing individualised dietary plans to the participants, frequent contact with the patients through visit sessions and telephone, and assessment of psychological response by three questionnaires (including both self-administered and clinician-rated inventories) were some of the strength points of our study. Moreover, we assessed some of the major biomarkers (gut permeability, endotoxemia, inflammation and BDNF) that are postulated to act in sequence to affect mood. As for any study, our work was not devoid of weak points. Our study duration was short. Moreover, funding constraints did not allow for gut microbiota analysis. This limitation is of great importance, because a recent systematic review of the clinical trials that looked at the effects of inulin on gut microbiome concluded that although there are some concordant changes in gut microbes following inulin supplementation, these taxonomic alterations are not associated with increase in SCFA[66]. SCFA are believed to contribute to the anti-inflammatory effects of prebiotics[67], but there is also some emerging evidence that prebiotics might lead to gut microbial dysbiosis and exacerbation of inflammatory bowel diseases, specially through increasing the SCFA butyrate production[68]. Therefore, it is crucial to assess gut microbiota and metabolites (including SCFA) when doing an inulin intervention study in a given population, whose baseline gut microbiome might be unique in a disease-specific manner. All our patients wished to lose weight, and calorie-restricted diets are the first treatment of choice for obesity. Since ethics would not approve of depriving subjects with obesity from an established treatment, we could not include a study group that received no weight loss diet. Moreover, there is some recent evidence that the commercial zonulin ELISA might not be an adequate measure of intestinal permeability and the biomarker of zonulin[69]. It is also noteworthy that by using ELISA technique, we measured LPS concentrations and not activity. There is evidence that LPS extracted from different bacteria can have different levels of activity and therefore induce TNF-α to varying extents[70], and some LPS might even have anti-inflammatory activities[71]. Since we were mainly interested in whether prebiotics can diminish LPS-driven inflammation, having measured LPS concentrations might be another limitation of our study.

Conclusion

Ten grmas per d inulin supplementation had no significant effects on gut permeability, endotoxemia, inflammatory status

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or depression of patients with obesity and MDD. We suggest that future studies focus on finding optimal type and dosage of prebiotics as well as supplementation duration for patients suffering from both depression and obesity. Furthermore, considering the probable benefits of calorie restriction for mental health outcomes especially in those with overweight/obesity, we encourage well-designed clinical trials including a proper control group, to look at the effects of these dietary intervention on anthropometric and psychological measures of these patients. Adopting more accurate indicators of gut permeability and LPS activity are also highly recommended. Well-designed *in vitro* and animal studies would also shed more light on the pathways and mechanisms through which dietary interventions and prebiotic substances might affect mood.

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The authors' contributions are as follows: E. V. M was the principal investigator and contributed to the study conceptualisation, design, data collection, data analysis, and interpretation of the findings and wrote the manuscript. M. E. M and F. R supervised the study and contributed to the study design and administration and results interpretation. R. H. and M. B contributed to the study administration, data collection, data analysis, and results interpretation. M. A. J contributed to the study design, data analysis and results interpretation. All authors read and approved the final version of the manuscript.

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