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ABSTRACT

Introduction A diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) is an effective way to reduce gut symptoms in people with irritable bowel syndrome (IBS). This diet reduces the intake of fermentable fibres, leading to changes of the gut microbiota and insufficient fermentation in the large bowel, resulting in reduced production of short-chain fatty acids (SCFAs), such as butyrate, which has unfavourable implications for gut health, sleep and mental health. This study will examine the effect of Fibre-fix, a supplement containing a mix of dietary fibres, on the human gut microbiome composition, fermentative capacity, sleep, quality of life (QOL) and mental health of people with IBS who consume a low FODMAP diet (LFD).

Methods and analysis A randomised, double-blind, placebo-controlled, study design is proposed to examine whether Fibre-fix added to an existing LFD may help modulate gastrointestinal function, improve markers of sleep, mental health and promote QOL in patients with IBS. Participants will provide stool and blood samples, daily bowel symptoms diaries and 3-day diet records. Additionally, they will complete validated questionnaires relating to FODMAP intake, sleep, mental health and QOL before and after a 3-week intervention. Gut health will be assessed via faecal microbiome composition, faecal pH and SCFA levels. Alteration of sleep will be recorded using an actigraphy device worn by all participants over the whole study. Multivariate analysis will be used to examine the gut microbiome and repeated measures Analysis of variance (ANOVA) will be used for dependent variables from questionnaires related to bowel symptoms, stool type, sleep, mental health and QOL to assess the differences between intervention and control groups after adjustment for confounding variables.

Ethics and dissemination Ethics approval was obtained from the Human Research Ethics Committee of Edith Cowan University (2019-00619-YAN). Results will be disseminated in peer-review journal publications, and conference presentations. Participants will be provided with a summary of findings once the study is completed. If Fibre-fix is shown to result in favourable changes in gut microbial composition, SCFA production, sleep and mental well-being without exacerbating symptoms, this will provide additional dietary management options for those with IBS following an LFD.

Trial registration number ACTRN12620000032954.

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most frequently diagnosed functional gastrointestinal disorders, affecting approximately 11.2% of the global adult population.[1 2] Diagnosis of IBS is challenging due to the subjective nature of digestive symptoms and is currently based on the Rome IV criteria.[3] This functional disorder typically presents with recurrent abdominal pain with alterations in bowel habits, namely stool consistency and frequency, coexisting bloating, flatulence and abdominal distention. The syndrome is subtyped into four patterns: IBS with predominant constipation or predominant diarrhoea, mixed IBS and un-subtyped.[4] Due to the dominance of chronic symptoms and frequently present comorbidities, somatic and psychological, IBS imposes a heavy burden on individuals and communities both economically and socially.[5 6] Of those employed, patients with IBS reported 24.3% absenteeism and 86.8% presentism, due to their syndrome.[7] In two independent studies it were estimated that

IBS-related absenteeism and presenteeism cost industry £400–£900[8] or to €937–€2108 annually.[9]

A diet that is low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP)[10] alias a low FODMAP diet (LFD), is an effective dietary intervention for IBS. A blinded and placebo-controlled trial found that approximately three-quarters of patients with IBS benefit from an LF.[11] The LFD reduces food (fibre) compounds that are poorly absorbed in the small intestine, rapidly fermentable in the proximal colon, and thereby contribute to the gastroenterological symptoms. The LFD includes a selective elimination diet for 2–6 weeks followed by a reintroduction phase of FODMAP containing foods followed by personalisation of a diet that minimises symptoms.[12 13] Despite the positive effects of the LFD in reducing gut symptoms and improving quality of life (QOL) in those with IBS, it only treats the symptoms of IBS. Studies suggest potentially negative impacts of long term adherence to an LFD, including nutritional inadequacy, potential increased risk of gastrointestinal complications and imbalance of the gastrointestinal microbiome.[12 14 15] Evidence from both animal and human studies has demonstrated that a low intake of dietary fibres can reduce microbiota diversity leading to increased cancer risk.[16–19] Reintroduction or restoration of dietary fibres to an LFD diet can be difficult with whole food due to the coexistence of a range of fibres in individual foods. This study therefore reintroduces dietary fibre using a supplement however this process should be done gradually and continuous, otherwise unwanted symptoms such as gas, flatulence and cramps may impact adherence.

Research suggests a low FODMAP intake rapidly and negatively changes the gut microbial community, abundance and diversity.[15 20 21] In healthy people, a 1-week LFD resulted in an alteration of the gut microbiota, reduced beneficial bacteria such as Actinobacteria, predominantly *Bifidobacterium*, and a lower overall total bacterial count.[22] After a 4-week LFD, Bennet *et al*[23] observed an increased dysbiosis manifested as altered gut microbial fermentation leading to lower total short-chain fatty acid (SCFA) concentrations[24] in patients with IBS. Additionally, a randomised, cross-over trial comparing LFD with a standard Australian diet[15] found a marked reduction in butyrate-producing Clostridium cluster XIVa and cluster IV, favourable mucus-associated *Akkerkmansia muciniphila*, and an increase in mucus-detrimental *Ruminococcus torques*. In another study, *Bifidobacterium and Faecalibacterium prausnitzii* associated with butyrate production were significantly decreased following a 3-week LFD.[25] Taken together these results suggest the lack of fibre associated with LFD may explain the microbial changes. Human gut microbiota is able to recognise and degrade different forms of complex carbohydrate.[26–28] A diet rich in dietary fibres with different extents of fermentability and solubility is recommended, which means more varied and complex dietary fibres in the diet leads to a more dynamic, diverse and stable gut microbiota.[29] Various purified dietary fibres are capable of nourishing specific gut bacteria, such as *Bifidobacteria, F. prausnitzii* and *Eubacterium hallii*.[26 30–32] Dietary fibre improves the human gut microbiota by providing a substrate for fermentation, and subsequently increases production of SCFA. It is well established in the literature that higher levels of SCFA can be obtained from a higher intake of fermentable dietary fibres.[19 33 34] Butyrate, one of the major SCFA throughout the colon, provides the primary fuel for colonic cells to maintain growth and integrity and thereby improve gut health.[35–37] Furthermore, research suggests that butyrate can positively affect circadian rhythm regulation[38 39] and enhance sleep via interplay between gut and brain.[40] Therefore, this study will increase the amount of fibre in the diet of patients with IBS to restore the gut microbiome and its metabolite profile to potentially prevent increasing the risk of patient's developing other more severe gastrointestinal diseases.

Thirty-three per cent of patients with IBS reported they had sleep problems, such as sleep fragmentation, poor sleep quality, reduced sleep time and frequent awakening.[41 42] Disordered sleep or sleep disturbances are also recorded with a greater prevalence in IBS sufferers compared with healthy individuals.[43 44] Despite unknown causal relationship between impacted sleep and IBS, the close association between gastrointestinal symptoms and sleep disturbances has been identified by others.[45–47] The gut microbiota is suggested to play a pivotal role and affect multiple mechanisms in this complex relationship between human sleep disturbances and gastrointestinal disease.[48 49] Smith *et al*[50] found that gut microbial diversity was positively associated with total sleep time, as well as sleep efficiency which also were positively correlated with phyla richness of Bacteroidetes and Firmicutes. Their findings indicate that an diet intervention represent a promising way to improve sleep by manipulating the gut microbiota to promote sleep-related phyla and taxa in the human gut microbiome.[50] In addition, it has been suggested that gut microbial composition could be altered by sleep fragmentation, resulting in a 50% reduction in Actinobacteria and 20% in Bacteroidetes, but a 20% increase in Firmicutes, which is similar to the microbial profile of obese individuals.[51 52]

Many research studies have demonstrated the significant association between IBS and mental health, even though the causation relationship is still unclear. A meta-analysis of Guillaume Fond *et al*[53] concluded that patients with IBS were more likely to develop depression and anxiety than healthy volunteers groups, whereas Sibelli *et al*[54] found that depression and anxiety doubled the risk of IBS onset. It is estimated that 44% of patients with IBS have associated mental health conditions, such as depression and anxiety.[55] Patients with IBS have been observed with gut microbial alterations related to depression, including greater rates of kynurenine (a deleterious metabolite of tryptophan), an elevated kynurenine/tryptophan ratio,[56] and declined *Lactobacillus* and *Bifidobacterium* which are also less abundant in patients with major

depressive disorder.[57] Clostridia, a major class within the phylum Firmicutes, appears at increased abundance in patients with IBS.[58 59] This link to an animal study showing abundance of Clostridia were significantly higher after stress-related stimuli in stress-vulnerable rats compared with stress-resilient rats.[60] Demonstrating that gut microbial communities respond to stress differently among animals with distinct stress vulnerability. Furthermore, the researchers suggest a Bacilli to Clostridia ratio can reflect stress effects, with a higher value indicating less stress-derived inflammatory reactions.[60]

Some inflammatory markers in human blood are associated with both human gut health and mental health and provide a potential mechanism for the role of dietary fibre in mitigating mental health. A randomised controlled trial in patients with serious depression demonstrated serum concentration of high-sensitivity C reactive protein (hs-CRP) and scores of Beck Depression Inventory questionnaire significantly decreased after taking a probiotic supplement (*Lactobacillus* spp and *Bifidobacterium bifidum*).[61] Additionally, proinflammatory cytokines, like tumor necrosis factor alpha (TNF-α), interleukin (IL-6) and IL-1β, are able to cross the blood-brain barrier (BBB), and their entry and following influences can have a negative effect on mental health.[62] The entry of the cytokines, however, can be reduced by improving the integrity of blood brain barrier. The permeability of BBB can be decreased by the SCFA butyrate which is produced in the gut via gut bacterial fermentation of fermentable carbohydrate residue escaping from small intestinal digestion.[63]

In summary, a healthier gut microbiota altered by a dietary fibre intervention or supplement in patients with IBS may not only improve gut health but also sleep, mental health and QOL. The objective of this research study is to determine, in patients with diagnosed IBS and on an LFD, whether Fibre-fix, compared with a placebo control, improves gut microbial composition, faecal SCFA levels, sleep quality, QOL, markers inflammation and of mental well-being, without exacerbation of IBS symptoms. This study will be the first to explore the bidirectional relationship between dietary fibres supplement and sleep modulated by the gut microbiota in patients with IBS following the LFD.

METHODS AND ANALYSIS
Study design
The study is designed as a randomised, double-blinded, placebo-controlled trial, with a 3-week intervention period (figure 1). The total time required for participant involvement is 4 weeks, including a 1-week baseline and a 3-week intervention.

Sample size
The a priori sample size for the proposed study was determined based on the results obtained by McOrist *et al*,[64] where a sample size of 50 participants (25 per group)
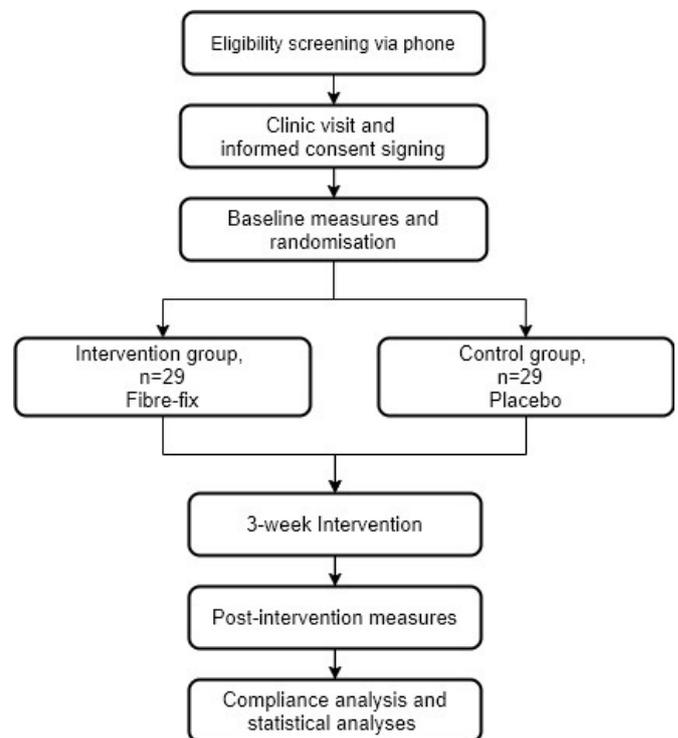

Figure 1 Flow chart showing study design overview.

was required to detect a change in log SCFA concentration of 0.4 at 80% power and 5% level of significance. Allowing for a 15% drop-out rate, the total sample size of 58 subjects, 29 per group is required. This sample size is sufficient to detect at least a medium between-within group interaction effect (Cohen's f=0.25) in sleep improvement at 80% power and 5% significance level, whereby the corresponding sample size requirement is 34.

Participants
Fifty-eight people (n=58) with IBS on an LFD will be recruited. Participants must be between 18 and 65 years old and have been clinically diagnosed with IBS, using the Rome IV edition diagnostic criteria[65] by a gastroenterologist or other medical professional. Participants will be on an LFD for 1 month prior to the intervention. Additionally, participants will need to be available to attend the local clinic visits and be willing to consume the Fibre-fix supplement or matched placebo.

Participants will be excluded if they are current smokers; pregnant or planning to become pregnant; have a known diagnosis of other gastrointestinal illness (eg, inflammatory bowel disease, malabsorption of any macronutrients, bowel resection, coeliac disease); have had previous abdominal or gastrointestinal surgeries, severe mental health and sleep-related conditions (eg, insomnia), renal or hepatic diseases, and major medical illness; currently use pharmaceutical agents that could modify or treat IBS (eg, probiotics, antibiotics, eluxadoline, lubiprostone and linaclotide) or sleep conditions; follow other restrictive dietary patterns or therapies (eg, low-carbohydrate, keto/Paleo-diet); take any prebiotics;

have any other disease, condition or habit that may interfere with completion of study.

Recruitment

Participants will be recruited through networks of registered Western Australian based dietitians and gastroenterologists who will be provided with information flyers to promote the study to potential participants. Information flyers will be posted on websites including social media groups relating to IBS or FODMAP. A university webpage will advertise study information.

Allocation, blinding and compliance

A computer-generated list of random numbers provided by a statistician will be used to randomly assign participants to either the intervention or control group. The participants will receive a resealable snap-lock bag, labelled A or B, containing 40 sachets of either Fibre-fix or placebo. Both participant and researcher will be blinded from the group allocation. Bag labelling will be completed by an independent person. The participants will be required to return unused sachets to calculate compliance. An online daily checklist, together with a weekly text/email reminder, will be provided to participants to record time of consuming the intervention; a daily tick-list/calendar will be created for participants to follow. Consumption of >80% of the sachets (32 sachets) during the 3-week period will be considered compliant.

Intervention

Fibre-fix consists of one soluble dietary fibre and one insoluble fermentable fibre, which will be provided to participants in 40 separate sachets with gradually increasing amount (table 1). After baseline data collection, participants will be required to consume Fibre-fix as per the labels on the sachets, one sachet per day for the first 2 days and two for the remaining 19 days according to the schedule (table 1). For participants' convenience, all sachets have been labelled with day and time (AM or PM) on the package, for example DAY 3 AM, and will be provided orderly in resealable plastic bags. The placebo sachet contains a combination of the same soluble dietary fibre and highly digestible fibre and will be delivered in the same way as the intervention.

Primary outcomes
Faecal SCFA and gut microbiota

Faecal SCFA levels and gut microbiota will be assessed through 24 hours stool samples which will be obtained at baseline and at the end of the intervention. Participants will be provided with the stool collection kit, including a portable cooler bag, frozen icepacks and an instruction

sheet. All stool samples collected with the 24 hours period will be pooled and homogenised if the number of individual samples is more than one. On receipt, stool samples will be immediately weighed and stored at −80°C. Individual's samples will be thawed at 4°C and kept at this temperature during homogenised and aliquoting for all planned analysis and re-frozen at −80°C until analyses.

The concentrations of bacterial metabolites in faeces, such as SCFA (acetate, propionate and butyrate) will be determined by gas chromatography.[66] In brief, an acidified aqueous methanol solution will be used to extract SCFA from faecal samples, followed by separating SCFA by gas chromatography with a fatty acid column using a Thermo Scientific TG-Wax column (30 m x 0.25 mm x 0.25 μm). The SCFA level will be qualified via internal standards.

Microbial analyses will be performed at the WA Human Microbiome Collaboration Centre, Curtin University, Western Australia. DNA will be extracted using the QIAamp PowerFecal DNA kit (Qiagen) using Qiacube extraction platform. Microbiome signatures are generated using the Illumina MiSeq platform using uniquely barcoded 16S rRNA gene primers (515-806(V4)) for bacterial and ITS2 primers for fungal profiling (pending on funding), following PCR inhibition assessment of each DNA extract. PCR-free ligation protocol is thereafter deployed for the process of library building. Samples will be sequenced to a depth of minimum 50 000 reads, which is sufficient to identify microbes to a genus/species level. Quality control and mock community samples are included in the analysis from sample collection to sequence analysis. Sequence read quality is initially assessed with FastQC before demultiplexing and preprocessing by GHAPv2, an in-house tool. Cutadapt[67] is used for removal of all non-biological sequences. DADA2[68] is then used for quality filtering, error correction, amplicon sequence variants (ASVs) picking. A trained naïve bayes classifier then assigns ASVs to genus/species against a curated database of microbial reference sequences such as the Ribosomal Database Project (RDP)[69] or Genome Taxonomy Database.[70] For fungal classification the UNITE database[71] will be used.

Secondary outcomes
Objective measures of sleep

Participants will be provided with the Readiband V.5 (Readiband, Fatigue Science, Canada), a wrist-activity monitor that has been validated and objectively assesses sleep using accelerometery.[72] Compared against polysomnography—the gold standard of sleep measurement, the wrist-activity monitor does not require laboratory setup

Table 1 Grams of dietary fibre supplement in each sachet provided to participants during 3-week interventional period

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
AM	5	5	5	5	10	10	10	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PM	\	\	5	5	10	10	10	10	10	10	20	20	20	20	20	20	20	20	20	20	20

Table 2 Definitions of sleep measures as extracted from the Readiband (Fatigue Science, Canada) device, based on Dunican et al[72]

Sleep measures	Acronym	Units	Abbreviated	Measurement	Description
Time at Lights Out	TALO	Time of day	hh:mm	Directly measured	Time at Sleep Onset minus Sleep Onset Latency.
Sleep Onset Latency	SOL	Minutes	min	Derived	Number of minutes from Time at Lights Out to Time at Sleep Onset.
Time at Sleep Onset	TASO	Time of day	hh:mm	Directly measured	Time of day when the first epoch of sleep occurs between Time at Lights Out and Time at Wake.
Time at Sleep Onset Variance	TASOV	Minutes	min	Derived	Time at Sleep Onset consistency relative to mean Time at Sleep Onset.
Sleep Duration	SD	Minutes	min	Derived	Number of minutes from Time at Sleep Onset to Time at Wake, minus number of minutes awake (WASO).
Wake After Sleep Onset	WASO	Minutes	min	Directly measured	Number of minutes awake after Time at Sleep Onset.
Fragmentation Index	FI	Frequency	number	Directly measured	Number of awakenings between Time at Sleep Onset and Time at Wake.
Time at Wake	TAW	Time of day	hh:mm	Directly measured	Time of day when awake with no further Sleep Duration.
Time in Bed	TIB	Minutes	min	Derived	The total time spent in bed, from Time at Lights Out to Time at Wake.
Sleep Efficiency	SE	Percentage	%	Derived	Sleep Duration divided by Time in Bed multiplied by 100.

nor trained personnel.[72] Moreover, the Readiband can automatically identify time at lights out using a proprietary algorithm, which not only eases the burden of sleep diary but also avoids the potential bias from self-reported data of recalling time for bed.[72] This technology has been widely applied to the sleep-related research.[73–76] Participants will be required to wear the monitor on the nondominant wrist for 24 hours per day during the study. The monitor-derived sleep measure data will be downloaded via the automated Readiband Sync software (table 2).

Subjective measures of sleep
Participants will be required to complete five validated questionnaires related to sleep at baseline, and at the end of the intervention.

Pittsburgh Sleep Quality Index
The Pittsburgh Sleep Quality Index (PSQI) retrospectively assesses sleep quality and relevant disturbances over the previous month. This self-administered questionnaire has been validated in a population-based setting to measure sleep quality. The summary score is calculated as the summation of 19 items grouped into seven components, ranging from 0 (better) to 21 (worse); a score >5 indicates poor sleep quality.[77]

Epworth Sleepiness Scale
The Epworth Sleepiness Scale (ESS) is a self-rated eight-item questionnaire, designed to measure daytime sleepiness.[78] Participants score each question from 0 (high

chance of dozing) to 3 (would never doze), which yield a global score of ESS ranging from 0 to 24. Scores higher than nine reflect excessive daytime sleepiness and severe problems with daytime somnolence.[79]

Insomnia Severity Index
Insomnia will be assessed through the self-reported Insomnia Severity Index (ISI) comprising seven items. Participants are required to rate each question on a 5-point Likert scale as per their own experience over the past 2 weeks. The total score ranges from 0 to 28, and represents clinical insomnia when it is higher than 14.[80]

Sleep Hygiene Index
The Sleep Hygiene Index (SHI) is a self-reported instrument for assessing individual behaviours in sleep hygiene. The 13 items that comprise SHI are rated on a 5-point Likert scale and produce a total score ranging from 0 to 52 with higher scores representing poorer status of sleep-behavioural hygiene.[81]

Restorative Sleep Questionnaire weekly version
The Restorative Sleep Questionnaire weekly version (RSQ-w) is composed of nine questions completed on restorative aspects of the sleep during the past week, and whose reliability and validity has been published.[82] Each item scales from one to five. The first two items and the last item are reversed scored. The total score ranging

from 0 to 100, calculates as: (RSQ-w average score across completed items−1)×25. The higher total scores indicate better restorative sleep.

Mental health and QOL assessment

The condition of mental health and QOL in both groups will be assessed using in total four validated questionnaires at baseline and at the end of the intervention.

Depression Anxiety Stress Scale

The Depression Anxiety Stress Scale (DASS-21) is a validated self-reported questionnaire designed to measure three subscales which are depression, anxiety and stress with seven items for each dimension.[83] Higher scores are indicative of poorer mental condition and severity of symptoms, but the DASS-21 is not a clinically diagnostic instrument. Nonetheless, DASS-21 has broad applicability and free availability and has been validated among the general population and for patients with chronic disease.[84 85]

Scores above 14, 10 and 17 in the three dimensions indicate severe depression, anxiety and stress, respectively. In such instances, participants will be referred to their medical practitioner for clinical care.

Visceral Sensitivity Index

The Visceral Sensitivity Index (VSI) is a validated self-reported questionnaire and will be employed to measure gastrointestinal specific anxiety. The total VSI score is generated from all 15 items, each defined on a 6-point Likert scale. Patients with a higher score will be regarded as experiencing severe gastrointestinal symptom-specific anxiety.[86 87]

IBS Quality of Life

For assessment of participants' QOL, IBS-QOL questionnaire will be used at the stages of baseline clinic, prior to and after intervention. The IBS-specific questionnaire is a validated measurement tool, generating one total and eight subscale scores with 34 items covering dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual activity and relationships.[88]

WHO (Five) Well-Being Index

WHO (Five) Well-Being Index (WHO-5) is a validated self-reported questionnaire consisting of five items that measures mental well-being in relation to the past 2 weeks. Responders rate each item on a 6-point Likert scale. The result will be calculated by multiplying the raw total score, ranging from 0 to 25, by four. The higher scores represent those with a better *imaginable* well-being condition.[89]

All questionnaires will be collated in the software Qualtrics and administered online to reduce participant burden.

Demographic information

Participants will complete a demographic questionnaire which requires personal information: gender, age, nationality, marital status, area of residence, mobile number, email address, smoking history, alcohol consumption, birth delivery mode, dietary pattern and physical activity.

Anthropometric measurements

Participants' height (cm) and weight (kg) will be measured to the nearest 0.1 cm and 0.1 kg, respectively, by an SECA 763 digital column scale (SECA, USA), where circumferences of waist and hips will be measured in accordance with international operating procedures for anthropometric assessment.[90] Body mass index (BMI) and waist/hip ratio will be calculated.[91] Percentage of lean and fat mass will be obtained using the BOD POD (Cosmed, Rome, Italy), an Air Displacement Plethysmograph using whole body densitometry, to determine body composition (fat vs lean % fat mass),[92] and conducted following manufacturer protocols for measurement. This will require subjects to fast for 8 hours prior to testing and wear tightly fitted gym clothes for measurement. Blood pressure (mm Hg) will be measured using an Omron IA1B Automated Blood Pressure Device (Omron Healthcare, Japan). All measurements will be carried out at baseline and end of intervention.

Blood biomarkers

The venous blood samples will be collected after an overnight fast at baseline and at the end of the intervention. Blood samples will be centrifuged and processed within half an hour after collection for separating plasma and serum, and frozen at −80°C after being aliquoted into 2 mL vials. Analysis of fasting lipids, glucose and glycated haemoglobin will be performed by a pathology laboratory in accordance with the protocols from the National Association of Testing Laboratories. The outcome measures of hs-CRP, TNF-α, IL-6 and IL-1β will be analysed if funding is made available.

Gut symptoms

Participants will complete a bowel symptom questionnaire at baseline and postintervention to assess changes in symptom severity. The questionnaire consists of Gastrointestinal Symptom Rating Scale for IBS (GSRS-IBS)[93] and the IBS Severity Scoring System (IBS-SSS),[94] which have been validated in clinical trials. The 13-item GSRS-IBS uses a 7-point Likert scale for severity of symptoms characteristic of IBS including abdominal pain, diarrhoea, constipation and bloating satiety. This instrument is short and simple, and will assist researchers to determine the specific symptoms encountered by patients.[93] The IBS-SSS point, with a maximum of 500 (100 for each item), is also used for classification of patients as remission (<75), mild (75–175), moderate (176–300) or severe (>300). Participants will use a visual analogue scale to score each of the five questions regarding symptom severity which include pain severity and duration, abdominal distention,

bowel dysfunction and QOL. For this study, a reduction of more than 50 points of IBS-SSS is defined as symptoms improvement.[13]

Daily Symptoms checklist

Each participant will be provided with an online daily bowel symptom checklist to report the sachet-consuming time and daily symptoms throughout the entire study period (Adapted from the not for profit, International Foundation for Functional Gastrointestinal Disorders (IFFGD) Personal Daily Diary: https://aboutibs.org/symptom-diary.html). The checkbox items include Bristol stool chart type, time and amount of fibre added to meals, bowel movement number of motions, stress level and menstrual cycle. Adverse symptoms monitoring, scoring symptoms (0–10), will be reported by participants daily and include abdominal pain, constipation, diarrhoea, bloating, flatus, eructation, headache, nausea and vomiting.

Food records

Dietary intake will be assessed using a 3-day weighed food record preintervention and postintervention. This will be completed by participants via a free downloaded smart-phone application, Research Food Diary (Xyris, Queensland, Australia). Participants will be provided with a set of scales (Propert, Supertex Industries) and instructed on the correct recording methods for weighed diet records. The Monash University Comprehensive Nutrition Assessment Questionnaire will be used to specifically quantify individuals' FODMAP intake.

Table 3 schedule of primary and secondary endpoints that will be measured over the study.

Statistical analyses

Baseline participant demographics and primary and secondary outcome variables will be described and compared for differences by group. Descriptive statistics in the form of mean±SD will be used to describe continuous variables, and frequencies and proportions for nominal and ordinal variables. All continuous outcome and demographic variables will be examined normality using the Shapiro-Wilk test. Median±IQR range will be presented instead for non-normal continuous variables.

Change in individual and total SCFA levels and faecal pH, will be examined using mixed-model Analysis of variance (ANOVA) between-groups and within-groups. Covariates, including gender and age, will be entered into the model as confounders. To analyse the gut microbiota profiles, multivariate analysis, Primer7 and PERMANOVA+ (PRIMER-E, Plymouth) and various R packages, will be used. Principal coordinates analysis will be deployed to visualise data. Distance-based linear

Study item	Baseline period	3-week dietary intervention			Post-intervention
		W1	W2	W3	
Demographic information	✓				
BMI, body fat, waist/hip ratio	✓				✓
Gut symptoms questionnaire (IBS-SSS+GSRS IBS)	✓				✓
Pittsburgh Sleep Quality Index	✓				✓
Epworth Sleepiness Scale	✓				✓
Sleep Hygiene Index	✓				✓
Insomnia Severity Index	✓				✓
Restorative Sleep Questionnaire weekly version	✓				✓
Depression Anxiety Stress Scale	✓				✓
Visceral Sensitivity Index	✓				✓
IBS-Quality of Life	✓				✓
WHO (Five) Well-Being Index	✓				✓
Monash University: Comprehensive Nutrition Assessment Questionnaire	✓				✓
Blood sample	✓				✓
Stool sample	✓				✓
Three-day diet record (via research food diary)	✓			✓	
Sleep monitor—Readiband wrist wearable device	✓	✓	✓	✓	
Daily symptoms checklist	✓	✓	✓	✓	

BMI, body mass index; GSRS-IBS, Gastrointestinal Symptom Rating Scale for irritable bowel syndrome; IBS-SSS, irritable bowel syndrome Severity Scoring System.

models and distance-based redundancy analysis will be used to integrate microbiome findings with clinical, diet intake, immune function and other relevant data that might help explain the relationship between the microbiome findings and other outcomes. If a significant relationship or difference is established at the multivariate level, multiple linear regression (MLR) will be conducted at the univariate level to determine the association between the gut microbiota composition and SCFA levels, faecal pH, diet intake, sleep, mental health. The covariates including gender, age, intervention compliance and IBS subtype will be considered as confounding variables and are adjusted in the MLR modelling. False discovery rate correction will be applied to account for testing of multiple outcomes.

For the effect of Fibre-fix on IBS symptomology, dependent variables (DV) from the Bristol Stool Chart type and symptom severity scores collected from daily symptom checklist will be examined by group. Differences between-groups and within-groups in these outcomes will be assessed using mixed-model repeated measures ANOVA, adjusting for sex and age. The DV from the questionnaires for sleep, mental health and QOL include PSQI, ESS, ISI, SHI, RSQ-w, DASS-21, VSI, IBS-QOF and WHO-5 will be analysed in the same way as mentioned above.

All data analyses, other than the microbiome multivariate data, will be conducted using SPSS V.25.0.[95] Significance level is set at $p \leq 0.05$. Cohen's effect size will be presented, where appropriate, to provide a measure practical/clinical significance.

DISCUSSION

The double-blinded, randomised controlled trial aims to examine whether Fibre-fix, in patients with IBS following an LFD, can improve gut microbiome composition, faecal SCFA concentrations, sleep quality, QOL and mental well-being, without exacerbation of IBS symptoms. Negative impacts on the gut microbiota of the LFD have been emerging, despite positive effects on IBS symptoms control.[12] Therefore, how to maintain a low level of IBS symptoms and concurrently improve the gut microbiome requires further research. Fibre-fix, a combination of fibres, may improve gut health of IBS subjects through greater distal fermentation in the colon. This combination may avoid triggering IBS symptoms, promote gut fermentation and SCFA (particularly butyrate) production which increases the biosynthesis and metabolism of neurotransmitters that could improve sleep and mental conditions. If Fibre-fix can improve the gut microbiota as expected, this will propose a long-term dietary solution for those with IBS. The proposed mental health and sleep benefits may have a flow-on effect in terms of lowering the occurrence of other comorbidities, such as depression, anxiety and work absenteeism, which have economic benefits. In addition, if the cost-effective dietary fibre administration is well-tolerated, it may also significantly lower the economic and mental burden originating from IBS comorbidities on patients, the healthcare system as well as the wider community.

Limitations

This is the first study to assess the effects of Fibre-fix on patients with IBS and as a result there are limitations which broadly include: time, funding and participants' burden. Specifically, this study will not explore hormonal changes relating to melatonin and serotonin caused by the dietary fibre supplement which may be helpful to further understand the mechanism and association of gut-brain axis. Moreover, the long-term effects of dietary fibre supplement will not be determined in this study because the study period is four weeks (1-week baseline and 3-week intervention). The gut microbiome and its metabolite profile (SCFA) are primary outcomes in this study as they will be valuable predictors of an improved gut health because of the reactive nature of the gut microbiome to diet changes. Therefore, such changes can be used to predict the value of Fibre-fix in lowering the risk of more severe gastrointestinal diseases in the future, but such findings would need to be validated in a long-term study. For sleep assessment, this study does not apply polysomnography. Therefore, changes in specific sleep stages will not be available, despite abnormality in rapid-eye movement among patients with IBS has been reported.[96] Future studies would therefore be required to explore the long-term and mechanistic relationship between human gut health, diet, sleep and mental health.

DISSEMINATION
Declarations ethics approval and consent to participate

This study has been approved by the Human Research Ethics Committee of Edith Cowan University (ID: 2019-00619-YAN) with additional approval provided by the Radiation, Biosafety and Hazardous Substances Committee. All participants will be provided with an information letter and a written consent form in digital or hard copy format for signing before commencement of the study.

The results will be disseminated in peer-review journal publications, and conference presentations. Participants will be given information about the findings once the study is completed.

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