Does Fibre-fix provided to people with irritable bowel syndrome who are consuming a low FODMAP diet improve their gut health, gut microbiome, sleep and mental health? A double-blinded, randomised controlled trial

Ran Yan, Mandy Murphy, Angela Genoni, Evania Marlow, Ian C Dunican, Johnny Lo, Lesley Andrew, Amanda Devine, Claus T Christophersen

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. Diagnosis of IBS is challenging due to the subjective nature of digestive symptoms and is currently based on the Rome IV criteria. This functional disorder typically presents with recurrent abdominal pain with alterations in bowel habits, namely stool consistency and frequency, coexisting bloating, flatulence and abdominal distension. The syndrome is subtyped into four patterns: IBS with predominant constipation or predominant diarrhoea, mixed IBS and un-subtyped. 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. Of those employed, patients with IBS reported 24.3% absenteeism and 86.8% presentism, due to their syndrome.
IBS-related absenteeism and presenteeism cost industry £400–£900 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 LFD.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 al23 observed an increased dysbiosis manifested as altered gut microbial fermentation leading to lower total short-chain fatty acid (SCFA) concentrations24 in patients with IBS. Additionally, a randomised, cross-over trial comparing LFD with a standard Australian diet15 found a marked reduction in butyrate-producing Clostridium cluster XIVa and cluster IV, favourable mucus-associated Akkermansia muciniphila, and an increase in mucus-detrital 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 regulation38 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 al50 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 represents 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 30% 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 al53 concluded that patients with IBS were more likely to develop depression and anxiety than healthy volunteers groups, whereas Sibelli et al54 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, Clostridia, a major class within the phylum Firmicutes, appears at increased abundance in patients with IBS. 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. 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.

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). 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. 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.

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 where a sample size of 50 participants (25 per group) 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% dropout 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-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 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 linacotide) or sleep conditions; follow other restrictive dietary patterns or therapies (eg, low-carbohydrate, keto/Paleo-diet); take any probiotics;
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. 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 is used for removal of all non-biological sequences. DADA2 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) or Genome Taxonomy Database. For fungal classification the UNITE database 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 accelerometry. 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   | 5 5 5 5 10 10 10 20 20 20 20 20 20 20 20 20 20 20 20 |
| Day 2   | 5 5 5 5 10 10 10 20 20 20 20 20 20 20 20 20 20 20 20 |
| Day 3   | 5 5 5 5 10 10 10 20 20 20 20 20 20 20 20 20 20 20 20 |
| Day 4   | 5 5 5 5 10 10 10 20 20 20 20 20 20 20 20 20 20 20 20 |

Yan R, et al. BMJ Open Gastro 2020;7:e000448. doi:10.1136/bmjgast-2020-000448
nor trained personnel. 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. This technology has been widely applied to the sleep-related research. Participants will be required to wear the monitor on the non-dominant 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.

#### Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is a self-rated eight-item questionnaire, designed to measure daytime sleepiness. 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.

#### 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 and produce a total score ranging from 0 to 28, and represents clinical insomnia when it is higher than 14.

#### 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.

#### 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. Each item scales from one to five. The first two items and the last item are reversed scored. The total score ranging

### Table 2

| Sleep measures                  | Acronym | Units       | Abbreviated Measurement Description                                      |
|---------------------------------|---------|-------------|---------------------------------------------------------------------------|
| Time at Sleep Onset Latency     | SOL     | Minutes     | Derived Number of minutes from Time at Lights Out to Time at Sleep Onset.  |
| Time at Sleep Onset             | TASO    | Time of day | Directly measured                                                          |
| Time at Sleep Onset Variance    | TASOV   | Minutes     | Derived Time at Sleep Onset consistency relative to mean Time at Sleep Onset.|
| Wake After Sleep Onset          | WASO    | Minutes     | Directly measured                                                          |
| Fragmentation Index             | FI      | Frequency   | Derived Number of awakenings between Time at Sleep Onset and Time at Wake.  |
| Time at Wake                    | TAW     | Time of day | Directly measured                                                          |
| Time in Bed                     | TIB     | Minutes     | 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.            |

---

**Table 2**: Definitions of sleep measures as extracted from the Readiband (Fatigue Science, Canada) device, based on Dunican et al.\(^{72}\)
from 0 to 100, calculates as: \((\text{RSQ-w average score across completed items−1}) \times 25\). 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. 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 IAB 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.95 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://aboutibbs.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.

**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

### Table 3 Study assessment schedule

| Study item                                      | Baseline period | 3-week dietary intervention | Post-intervention |
|------------------------------------------------|-----------------|-----------------------------|-------------------|
| Demographic information                        | ✓               | W1                           | W2 W3             |
| 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. Significance level is set at p≤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. 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 fourweeks (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. 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: 201900619-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.

Twitter Amanda Devine @AdMandydevine and Claus T Christophersen @ctguthealth

Acknowledgements The authors would like to express their gratitude to Dr Michael Stein for grammar and language editing; to Kim Luu, Cathy Latino, Rhys Woollard, Finna Rohadhia, Joanna Rees, Sheridan Barnett, Sheerey Syson and Karina Vaswani for kindly offering their time to help with preparation of the intervention and placebo sachets.

Contributors RY, MM, AD, CC, AG conceived and designed the work. ICD, EM, AG, JL and LA supported with expertise in the study design. RY and MM drafted the manuscript. All authors contributed to refinement of the protocol and the review and editing the manuscript. All authors have read and approved the final manuscript.

Funding RY was supported by the China Scholarship Council (CSC 201808230427). Currently, this research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
REFERENCES

1. Rodríguez-Janeiro BK, Vicario M, Alonso-Coteron C, et al. A review of microbiota and irritable bowel syndrome: future in therapies. Adv Ther 2016;39:389–409.

2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol 2012;10:712–21.

3. Drossman DA, Hasler WL. Rome iv-functional GI disorders: disorders of gut-brain interaction. Gastroenterology 2016;150:1257–61.

4. Lacy B, Ayyagari R, Guerin A, et al. Factors associated with more frequent diagnostic tests and procedures in patients with irritable bowel syndrome. Therap Adv Gastroenterol 2019;12:175628411881326.

5. Bugnon JL, Carson RT, Flores NM. Health-related quality of life, work productivity, and indirect costs among patients with irritable bowel syndrome with diarrhea. Health Qual Life Outcomes 2017;15:35.

6. Lacy BE, Patel H, Guérin A, et al. Variation in care for patients with irritable bowel syndrome in the United States. PLoS One 2016;11:e0154258.

7. Frändemark Åsa, Törnblom H, Jakobsson S, et al. Work productivity and activity impairment in irritable bowel syndrome (IBS): a multifaceted problem. Am J Gastroenterol 2018;113:1540–9.

8. Canavan C, West J, Card T. Review article: the economic impact of the irritable bowel syndrome. Aliment Pharmacol Ther 2014;40:1023–44.

9. Tack J, Stanghellini V, Mearin F, et al. Economic burden of moderate to severe irritable bowel syndrome with constipation in six European countries. BMC Gastroenterol 2019;19:69.

10. Barrett JS, Gibson GR. Development and validation of a comprehensive semi-quantitative food frequency questionnaire that includes FODMAP intake and glycemic index. J Am Diet Assoc 2010;110:1469–76.

11. Halmos EP, Power VA, Shepherd SJ, et al. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology 2014;146:67–75.

12. Mitchell H, Porter J, Gibson PR, et al. Review article: implementation of a diet low in FODMAPs for patients with irritable bowel syndrome—directions for future research. Aliment Pharmacol Ther 2019;49:1245–59.

13. Whelan K, Martin LD, Staudacher HM, et al. The low FODMAP diet in the management of irritable bowel syndrome: an evidence-based review of FODMAP restriction, reintroduction and personalisation in clinical practice. J Hum Nutr Diet 2018;31:239–55.

14. Werlang ME, Palmer WC, Lacy BE. Irritable bowel syndrome and dietary interventions. Gastroenterol Hepatol 2019;15:16–26.

15. Halmos EP, Christophersen CT, Bird AR, et al. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. Gut 2015;64:93–100.

16. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell 2016;167:1339–53.

17. Davis CD, Mitiner JA. Gastrointestinal microflora, food components and colon cancer prevention. J Nutr Biochem 2009;20:743–52.

18. Sonnenburg ED, Smits SA, Tilková M, et al. Diet-induced extinctions in the gut microbiota compound over generations. Nature 2016;529:212–5.

19. Ou J, Carbonero F, Zoetendal EG, et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr 2013;98:111–20.

20. Staудacher HM, Lomer MCE, Farquharson FM, et al. A diet low in FODMAPs reduces symptoms in patients with irritable bowel syndrome and a probiotic restores Bifidobacterium species: a randomized controlled trial. Gastroenterology 2017;153:936–47.

21. Ooi SL, Corrêa D, Park SC. Prebiotics and low FODMAP diet for irritable bowel syndrome - what is the current evidence? Complement Ther Med 2019;43:73–80.

22. Sloan TJ, Jalanja J, Major GAD, et al. A low FODMAP diet is associated with changes in the microbiota and reduction in breath hydrogen but not colonic volume in healthy subjects. PLoS One 2018;13:e0201140.

23. Bennet SMP, Böhn L, Storsrud S, et al. Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs. Gut 2018;67:872–81.

24. Valeur J, Rosseth AG, Knudsen T, et al. Fecal fermentation in irritable bowel syndrome: influence of dietary restriction of fermentable oligosaccharides, disaccharides, monosaccharides and polyols. Digestion 2016;94:50–6.

25. Hustoft TN, Hausken T, Ystad SO, et al. Effects of varying dietary content of fermentable short-chain carbohydrates on symptoms,ecal microbiome, and cytokine profiles in patients with irritable bowel syndrome. Neurogastroenterol Motil 2017;29:e12969.

26. Zhou Z, Zhang Y, Zhang P, et al. Starch structure modulates metabolic activity and gut microbiota profile. Anaerobe 2013;24:71–8.

27. Warren FJ, Fukuma NM, Mikkelsen D, et al. Food starch structure impacts gut microbiome composition. mSphere 2018;3:e00086–18.

28. Rogovski A, Briggs JA, Mortimer JC, et al. Glycan complexity dictates microbial resource allocation in the large intestine. Nat Commun 2015;6:7481.

29. Williams BA, Grant LJ, Gidley MJ, et al. Gut fermentation of dietary fibres: Physico-chemistry of plant cell walls and implications for health. Int J Mol Sci 2017;18:2203.

30. Shinozaka H, Ohashi Y, Kawasumi K, et al. Effect of apple intake on fecal microbiota and metabolites in humans. Anaerobe 2010;16:510–5.

31. Salyers AA, Vercellotti JR, West SE, et al. Fermentation of mucin and plant polysaccharides by strains of Bacteroides from the human colon. Appl Environ Microbiol 1977;33:319–22.

32. Williams LA, Eastman AC, Singh A, et al. Glycemic index of dietary fibers: Physico-chemistry of plant cell walls and implications for health. Int J Mol Sci 2017;18:2203.

33. Hooda S, Bolier BM, Serao MCR, et al. 454 pyrosequencing reveals a shift in fecal microbiota of healthy adult men consuming polydextrose or soluble corn fiber. J Nutr 2012;142:1259–65.

34. De Filippo C, Cavaliere D, Di Paola M, et al. Impact of diet on shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691–6.

35. Williams BA, Mikkelsen D, Flanagan BM, et al. “Dietary fibre”: moving beyond the “soluble/insoluble” classification for monogastric nutrition, with an emphasis on humans and pigs. J Anim Sci Biotechnol 2019:10:212.

36. Bugaut M, Bentéjac M. Biological effects of short-chain fatty acids in nonruminant mammals. Annu Rev Nutr 1993;13:217–41.

37. Pryde SE, Duncan SH, Hold GL, et al. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett 2002;171:133–9.

38. Sonnenburg ED, Sonnenburg JL, Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metab 2014;20:779–86.

39. Leone V, Gibbons SM, Martinez K, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host Microbe 2015;17:681–93.

40. Pant K, Yadav AK, Gupta P, et al. Butyrate induces ROS-mediated apoptosis by modulating miR-22-SIRT-1 pathway in hepatic cancer cells. Redox Biol 2017;12:340–9.

41. Szentirmai EV, Miliccan NS, Massie AR, et al. Butyrate, a metabolite of intestinal bacteria, enhances sleep. Sci Rep 2019;9:7035.

42. Vege SS, Locke GR, Weaver AL, et al. Functional gastrointestinal disorders among people with sleep disturbances: a population-based study. Mayo Clin Proc 2004;79:1501–6.

43. Rotem AV, Sperber AD, Kruglik P, et al. Polysomnographic and actigraphic evidence of sleep fragmentation in patients with irritable bowel syndrome. Sleep 2003;26:742–7.

44. Wang B, Duan R, Duan L. Prevalence of sleep disorder in irritable bowel syndrome. Saudi J Gastroenterol 2018;24:141–50.

45. Patel A, Hasak S, Gaskell J, et al. Effects of disturbed sleep on gastrointestinal and somatic pain symptoms in irritable bowel syndrome. Aliment Pharmacol Ther 2016;44:246–58.
Hyun MK, Baek Y, Lee S. Association between digestive symptoms and sleep disturbance: a cross-sectional community-based study. *BMC Gastroenterol* 2019;19:34.

Baloul A, Alt EI, Hon E, et al. Sleep disturbances are commonly reported among patients presenting to a gastroenterology clinic. *Dig Dis Sci* 2018;63:2983–91.

Tu Q, Heitkemper MM, Jarrett ME, et al. Sleep disturbances in irritable bowel syndrome: a systematic review. *Neurogastroenterol Motil* 2017;29. doi:10.1111/nmo.12948

Coddier-Franch P, Gombert M. Circadian rhythms in the pathogenesis of gastrointestinal diseases. *World J Gastroenterol* 2018;24:4297–303.

Parkar SG, Kalsbeek A, Cheeseman JF. Potential role for the gut microbiota in modulating host circadian rhythms and metabolic health. *Microorganisms* 2019;7. doi:10.3390/microorganisms7020041

Smith RP, Easson C, Lyle SM, et al. Gut microbiome diversity is associated with sleep physiology in humans. *PLoS One* 2014;14:e022939.

Turnbaugh PJ, R ida va KY, Faith JJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009;1:6ra14.

Verdam FJ, Fuentes S, de Jonge C, et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity* 2013;21:E607–15.

Fond G, Loundou A, Hamdani N, et al. Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. *Eur Arch Psychiatry Clin Neurosci* 2014;264:651–60.

Sibelli A, Ch al der T, E r eit H, et al. A systematic review with meta-analysis of the role of anxiety and depression in irritable bowel syndrome onset. *Psychol Med* 2016;46:3065–80.

Guthrie E, Creed F, Fernandes L, et al. Cluster analysis of symptoms and health seeking behaviour differentiate subgroups of patients with severe irritable bowel syndrome. *Gut* 2003;52:1616–22.

Clarke G, Fitzgerald P, Cryan JF, et al. Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort. *BMC Gastroenterol* 2009;9:6.

Bhattarai Y, Muniz Pedroga D, K ash yac PC. Irritable bowel syndrome: a gut microbiota-related disorder? *Am J Physiol Gastrointest Liver Physiol* 2017;32;G52–62.

Duan R, Zhu S, Wang B, et al. Alterations of gut microbiota in patients with irritable bowel syndrome based on 16S rRNA-targeted sequencing: a systematic review. *Clin Transl Gastroenterol* 2019;10:e00012.

Labus JS, Hollister EB, Jacobs J, et al. Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. *Microbiome* 2017;5:49.

Pearson-Leary J, Zhao C, Bittinger K, et al. The gut microbiome regulates the increases in depressive-type behaviors and inflammatory processes in the ventral hippocampus of stress-vulnerable rats. *NPJ Psychiatry 2020;5:1057.

Akkash Geh, Kashani-Poor Z, Tajabadi-Ebrahimi M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *Nutrition* 2016;32:315–20.

Sampson TR, Mmanaman SK. Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe* 2015;17:565–76.

Bransite V, Al-Ashamkh M, Kowal C, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014;6:263ra158.

McCrust AL, Miller RB, Bird AR, et al. Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J Nutr* 2011;141:883–9.

Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features, and Rome IV. *Gastroenterology* 2016;150:1262–79.

Stinson LF, Boyce MC, Payne MS, et al. The not-so-stereol eb: evidence that the human fetus is exposed to bacteria prior to birth. *Front Microbiol* 2019;10:1124.

Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13:581–3.

Cole JR, Wang Q, Fish JA, et al. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014;42:D633–42.

Parks DH, Chuvshina M, Waite DW, et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.

Nilsson RH, Larsson K-H, Taylor AF, et al. The unite database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res* 2019;47:D259–64.

Dunican IC, Murray K, Slater JA, et al. Laboratory and home comparison of wrist-activity monitors and polysomnography in mid-life women before and after the menopause. *Chronobiol Int* 2016;33:967–76.

O’Donnell S, Bird S, Jacobson G, et al. Sleep and stress hormone responses to training and competition in elite female athletes. *Eur J Sport Sci* 2018;18:611–8.

Dunican IC, Martin DT, Haison SL, et al. The effects of the removal of electronic devices for 48 hours on sleep in elite judo athletes. *J Strength Cond Res* 2017;31:2832–9.

Dennis J, Dawson B, Heasman J, et al. Sleep patterns and injury occurrence in elite Australian footballers. *J Sci Med Sport* 2016;19:113–6.

Dunican IC, Higgins CC, Jones MJ, et al. Caffeine use in a super rugby game and its relationship to post-game sleep. *Eur J Sport Sci* 2018;18:513–23.

Buysse DJ, Reynolds CF, Monk TH, et al. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatr Res* 1989;28:193–213.

Johns MW. A new method for measuring daytime sleepiness: the Epworth Sleepiness scale. *Sleep* 1991;14:540–5.

Chervin RD, Aldrich MS, Pickett R, et al. Comparison of the results of the Epworth Sleepiness scale and the multiple sleep latency test. *J Psychosom Res* 1997;42:145–55.

Bastien CH, Valli è res A, Morin CM. Validation of the insomnia severity index as an outcome measure for insomnia research. *Sleep Med* 2001;2:297–307.

Mastin DF, Bryson J, Corwyn R. Assessment of sleep hygiene using the sleep hygiene index. *J Behav Med* 2006;29:223–7.

Drake CL, Hays RD, Morlock R, et al. Development and evaluation of a measure to assess restorative sleep. *J Clin Sleep Med* 2014;10:733–41.

Henry JD, Crawford JR. The short-form version of the depression anxiety stress scales (DASS-21): construct validity and normative data in a large non-clinical sample. *Br J Psychol* 2005;44:227–38.

Lee J, Lee E-H, Moon SH. Systematic review of the measurement properties of the depression anxiety stress Scales-21 by applying updated COSMIN methodology. *Qual Life Res* 2019;28:2355–39.

Crawford J, Cayley C, Lovibond PF, et al. Percentile norms and accompanying interval estimates from an Australian general adult population sample for self-report mood scales (BAI, bdi, crsd, CES-D, dass, dass-21, stai-x, stai-y, srds, and sras). *Aust Psychol* 2011;46:3–14.

Labus JS, Mayer EA, Chang L, et al. The central role of gastrointestinal-specific anxiety in irritable bowel syndrome: further validation of the visceral sensitivity index. *Psychosom Med* 2007;69:89–98.

Labus JS, Bolus R, Chang L, et al. The visceral sensitivity index: development and validation of a gastrointestinal-symptom-specific anxiety scale. *Aliment Pharmacol Ther* 2004;20:89–97.

Patrick DL, Drossman DA, Frederick IO, et al. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. *Dig Dis Sci* 1998;43:400–11.

Topp CW, Østergaard SD, Søndergaard S, et al. The WHO-5 well-being index: a systematic review of the literature. *Psychother Psychosom* 2015;84:167–76.

Stewart A, Marfell-Jones M, Olds T. International standards for anthropometric assessment. *International Society for the Advancement of Kinanthropometry*, 2011.

Bhattacharya A, Pal B, Mukherjee S, et al. Assessment of nutritional status using anthropometric variables by multivariate analysis. *BMC Public Health* 2019;19:1045.

Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr* 2002;75:453–67.

Wilkend JK, Fullerton S, Hawick CJ, et al. An irritable bowel syndrome-specific symptom questionnaire: development and validation. *Scand J Gastroenterol* 2003;38:947–54.

Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 1997;11:385–92.

IBM. IBM spss statistics for windows. 25.0 ed. Armonk, NY: IBM Corp. 2017.
Kumar D, Thompson PD, Wingate DL, et al. Abnormal REM sleep in the irritable bowel syndrome. Gastroenterology 1992;103:12–17.