Effects of an iso-energetic low glycemic index (GI) diet on liver fat accumulation and gut microbiota composition in patients with non- alcoholic fatty liver disease (NAFLD)

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SYNOPSIS

Title	Effects of an iso-energetic low glycemic index (GI) diet on liver fat accumulation and gut microbiota composition in patients with non- alcoholic fatty liver disease (NAFLD)
Short title	LGI Diet and NAFLD
Chief Investigator	Prof Guru Aithal
Objectives	- To investigate the effects of iso-energetic low GI (LGI) diet versus iso-energetic high GI (HGI) diet on liver fat accumulation in participants with NAFLD. - To investigate the effects of iso-energetic LGI versus iso-energetic HGI diet on gut microbiota composition and its mediated effects on liver outcomes in participants with NAFLD. - To test the efficacy of a dietary intervention by using CAP-Fibroscan™ hence evaluating the latter as a tool to measure intervention outcomes in participants with NAFLD in comparison with MRI/S as a gold standard tool.
Trial Configuration	2x2 Crossover trial
Setting	NIHR Nottingham Clinical Research Facility in Nottingham Digestive Diseases Centre Sir Peter Mansfield Imaging Centre
Sample size estimate	The sample size is calculated using nQuery based on previous completed study by Bawden et al, (2017). Based on their pilot data, baseline liver fat fractions of both diet groups were 2.4 ± 1-1.2%, and after 1 week were HGI (High GI) = 4.7 ± 2 % and LGI = 1.6 ± 0.7%. Using a two-group t-test (Crossover ANOVA) between the post- diet values with a 0.05 two-sided significance level, this will produce a sample size of n=8 in each diet sequence (a total sample size of 16). This sample size will have 80% power to detect a difference in means of 3.1, with the assumption that the Crossover ANOVA sqrt (MSE) is 2.828 (the Standard deviation of differences, σ, is 4). Assuming that 40% of people would drop out from cross-over study design, it is expected that at least 24 participants would need to be recruited.
Number of participants	We will continue recruitment until a total of 16 have completed the study.

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Eligibility criteria	**Inclusion Criteria**: • Adult males and females aged from 18 to 65 years (balanced number). • Detected NAFLD by Controlled Attenuation Parameter (CAP)-FibroScan™ >288dB/m or by Magnetic resonance imaging proton density fat fraction (MRI-PDFF) > 5% fat of liver weight. • Body mass index ≥ 25 kg/m^2 • Have current moderate to high GI diet intake of ≥ 60 (This can only be assessed on completion of food diary). • Abdominal obesity (Waist circumference > 102 cm for males and > 88 cm for females) • Able to give informed consent. • Able to undergo MRI/S and CAP-FibroScan™. **Exclusion Criteria**: • Current smokers and excessive alcohol drinkers (> 14 units week). • Perimenopausal (irregular periods) women. • Participants with other liver abnormalities. • Participants with history of gastrointestinal surgeries, depression, eating disorders or difficulties. • Participants using pharmacologic agents for obesity or NAFLD. • Participants with type 1 diabetes. • Participants with type 2 diabetes on second line medications (eg GLP-1 analogues, sulfonylureas). • Participation in any other trial in the last 3 months. • Participants on any special diets (eg vegetarians). • Intolerance or a dislike of foods included in the diet plan.
Description of interventions	• Three-day cycle menu of LGI diet and HGI diet will be designed and supplied to each participant in an amount designed to meet their estimated energy requirements. The percentage energy from protein, fat and carbohydrate will be the same in the two diets. Each diet period of the trial will last for two weeks. • Medical history and sociodemographic characteristics • Anthropometric measures. • Assessment of level of activity using International Physical Activity Questionnaire (IPAQ) to improve estimate of energy requirement. • Transient elastography using CAP-FibroScan™ . • MRI/S to assess liver fat fraction • Stool sampling for 16S rRNA to analyse gut microbiota composition. • Blood sampling (for metabolic biomarkers). • A 7-day food diary records and EPIC-FFQ (European Prospective Investigation of Cancer food frequency questionnaire)

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	• Continuous interstitial glucose monitoring.
Duration of study	4 years
Randomisation and blinding	The diet group sequence for each participant will be randomised by one of the trial co-ordinators who has no contact with the participants. Allocation will be via on-site computerized system. The participants and trial investigator will be aware of the diet prescribed after randomisation, but the trial statistician and MRI team will remain blinded when analysing data.
Outcome measures	Primary outcome: - To assess the difference in liver fat content between LGI and HGI diet groups in participants with NAFLD. Secondary outcomes: - To assess the difference in blood biomarkers between LGI and HGI diet groups in participants with NAFLD. - To assess the difference in gut microbiota composition between LGI and HGI diet groups in participants with NAFLD. - To test the efficacy of the CAP-Fibroscan™ as a new tool to evaluate efficacy of intervention compared with MRI/S in participants with NAFLD.
Statistical methods	The differences in liver fat levels, blood biomarkers and gut microbiome diversity between the two dietary interventions will be assessed by matched-pair Student's t-test. Two-way repeated measures ANOVA test will be used to evaluate the effects of diets and time sequences on liver fat content, as well as the effects of dietary interventions on gut microbial composition and blood biomarkers and/or interactions with liver fat content, gut microbiota composition and blood biomarkers. Data will be presented as mean± standard deviation and the significance of difference will be set at $p < 0.05$.

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AE	Adverse Event
BIA	Bio-electrical Impedance Analysis
BMI	Body Mass Index
BRC	Biomedical Research Centre
CI	Chief Investigator overall
CRF	Case Report Form
CAP	Controlled Attenuation Parameter
DMC	Data Monitoring Committee
EPIC	European Prospective Investigation of Cancer
FFQ	Food Frequency Questionnaire
GCP	Good Clinical Practice
GI	Glycemic Index
GP	General Practitioner
HGI	High Glycemic Index
ICF	Informed Consent Form
IPAQ	International Physical Activity Questionnaire
LGI	Low Glycemic Index
MRI-PDFF	Magnetic Resonance Imaging Proton Density Fat Fraction
NAFLD	Non-alcoholic Fatty Liver Disease
NASH	Non-alcoholic Steatohepatitis
NDDC	Nottingham Digestive Diseases Centre

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NHS	National Health Service
NIHR	National Institute for Health Research
NUH	Nottingham University Hospital
PI	Principal Investigator at a local centre
PIS	Participant Information Sheet
QMC	Queens Medical Centre
REC	Research Ethics Committee
RCT	Randomised Control Trial
R&D	Research and Development department
SAE	Serious Adverse Event
SCFA	Short Chain Fatty Acids
SPIMC	Sir Peter Mansfield Imaging Centre
UoN	University of Nottingham

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Non-alcoholic fatty liver disease (NAFLD) is one of the growing global health problems and considered the leading cause of chronic liver disease worldwide. It is associated with rising incidences of obesity and diabetes [1, 2]. Alarming figures for NAFLD prevalence have been shown among obese adults with type 2 diabetes mellitus [3 – 5] and obese children [6, 7]. Recent reviews indicated that one billion worldwide are affected by NAFLD with the highest prevalence rate, ranging from 20% to 30% prevalence, reported in Western countries [8, 9]. NAFLD is characterized by fat accumulation in hepatocytes exceeding 5% of liver weight. It encompasses a spectrum of fatty liver diseases including steatosis, steatohepatitis, cirrhosis and hepatocellular carcinoma. Clinical risk factors that have been highly associated with NAFLD are obesity, insulin resistance, type 2 diabetes, and metabolic syndrome [8, 10]. Western dietary habits along with sedentary lifestyle and genetic factors can lead to insulin resistance, adipocyte proliferation and gut microbiota alteration (dysbiosis or dysbiota) indicating that NAFLD is a complex multifactorial disease [11].

Dysbiosis induced by diet has been correlated with a number of diseases including obesity [12, 13] metabolic syndrome [14 -16], type 2 diabetes [17], immunological dysfunctions [18], coronary vascular disease and inflammatory bowel diseases [19]. Currently, the study of the gut microbiota roles in NAFLD management has gained attention. Dysbiota in diet-induced NAFLD has been shown to stimulate hepatic fat disposition and promote NAFLD progression to non-alcoholic steatohepatitis (NASH). Due to gut microbiota involvement in gut permeability modulation, low-grade inflammation and immune balance, dietary choline metabolism modulation, bile acid metabolism regulation and endogenous ethanol production [20, 21]. Through the gut-liver axis, modulated gut microbiota by diet increases the energy extraction from the breakdown of non-absorbable polysaccharides with the production of monosaccharides and short chain fatty acids (SCFAs). Both monosaccharides and SCFAs are substrates for hepatic de novo lipogeneses and carbohydrates biosynthesis [22]. This demonstrates the mechanism by which diet modulates gut microbiota as a means of NAFLD prevention or alleviation [7, 23].

To date, there are no approved drugs for treating NAFLD, and most of the current literature suggests that lifestyle modifications including modification of dietary patterns together with increased physical activity is the initial step to treat this disease [24]. Accordingly, several dietary interventions have been conducted on patients with NAFLD. In a prospective clinical study, a high protein hypo-energetic diet was positively associated with improvement in lipid profile, glucose homeostasis and liver enzymes in 48 stable NAFLD patients but their concurrent decrease in carbohydrate intake confounded with these outcomes [25]. Evidence from systematic review and meta-analysis of randomized control trials (RCT) on patients with NAFLD have shown that lifestyle-induced weight loss of ≥ 7% improved histological disease activity as well as cardiometabolic risk profile in only less than 50% of study population (n=373 patients) [26]. A recent systematic review has concluded that a reduction in saturated and trans fatty acids, carbohydrates, and animal-based protein intake and increased intake of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), plant- based

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proteins, and antioxidants are the most beneficial dietary approaches to manage NAFLD [9]. Despite the abundance of the interventional studies on dietary management in NAFLD, still there is a lack of evidence-based recommendations and few of them have measured liver-related outcomes [8]. Moreover, the dietary effects on liver-related outcomes that are mediated by gut microbiome have not been studied in these patients as well [27].

More recently, the type of carbohydrates and the glycemic index (GI) of food has been considered as an important predictor of NAFLD progression [9,28]. Animal studies have shown that low GI (LGI) food consumption reduced the glycemic excursions and food intake [29] and had a strong relationship with the parameters of metabolic syndrome and type 2 diabetes mellitus [30]. Whilst prolonged consumption of a high GI (HGI) diet was associated with a marked increase in body fat mass as well as liver fat, and impaired fat oxidation [31]. Diets of the majority of NAFLD patients were characterized as containing high levels of HGI foods and were correlated with insulin resistance, hepatic fat accumulation [9] increased inflammation and liver dysfunction [32]. Review articles have revealed that limiting sugar intake and HGI foods had preventive and therapeutic effects on patients with NAFLD [33, 34]. In addition, HGI dietary habits have been significantly associated with high-grade liver steatosis in patients with insulin resistance [35]. Further, previous pilot data showed that only one-week consumption of HGI diet caused significant increase in hepatic stores of fat when compared with LGI diet consumption [8]. However, there are no available clinical trials examining the effects of LGI diet specifically on hepatic fat accumulation in patients with NAFLD. Also, the association of LGI diet consumption on gut microbiota composition have not been studied in this population.

As described earlier, both insulin resistance and dysbiosis induced by dietary factors leads to hepatic fat accumulation, and diet composition affects the host gut microbiota. Therefore, the study of the association between LGI food consumption, hepatic fat stores and gut microbiota composition is highly relevant and of importance. Through this project we will assess the effects of LGI diet consumption on hepatic fat content and its effects on gut microbiota composition in participants with NAFLD compared with a high GI (HGI) diet as a control diet. The diets will be designed to meet the energy requirements of the study participants, and the macronutrient composition of the diets will be carefully controlled hence ensuring difference in glycaemic load are due to differences in glycaemic index.

The hepatic fat content will be assessed using Magnetic Resonance Imaging Spectroscopy (MRI/S) and the gut microbiome composition will be assessed using 16S sequencing of rRNA extracted from faecal samples [36, 37]. All biochemical parameters concentrations will be assessed by a central laboratory that use standard methods. The homeostasis model assessment-insulin resistance index (HOMA-IR) will be calculated as well, according to the equation [HOMA-IR = (FPI× FPG)/22.5] [38]. We will assess if the effect of LGI diet is modified by the gut microbiota present.

This research will extend our understanding in Four important areas. First, it will provide a population-based evidence on the effects of a LGI diet on liver fat accumulation in NAFLD and may serve as a complementary tool for reducing hepatic fat accumulation in these patients. Second, it will provide information about the nutritional and protective functions of gut microbiota composition in this medical condition. Thirdly, it will contribute to open the way

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to a novel strategy for NAFLD monitoring and therapy through understanding the dietary-mediated effects of specific gut microbiota. And, finally, it will validate; for the first time, the use of CAP-Fibroscan™ as a new tool for assessing liver fat content in dietary interventions.

TRIAL OBJECTIVES AND PURPOSE

PURPOSE

To assess the effects of the short-term consumption of an iso-energetic with LGI food on liver fat accumulation and gut microbiome composition in participants with NAFLD.

PRIMARY OBJECTIVE

1. To investigate the effects of LGI versus HGI diet on liver fat accumulation in participants with NAFLD whilst controlling for the macronutrient composition of the diet.

SECONDARY OBJECTIVES

1. To investigate the differences in blood metabolic biomarkers between LGI and HGI diet groups in participants with NAFLD.
2. To investigate the effects of LGI versus HGI diet on gut microbiota composition and its mediating effects on liver outcomes in participants with NAFLD.
3. To test the efficacy of a dietary intervention by using CAP-Fibroscan™ hence evaluating the latter as a tool to measure intervention outcomes in participants with NAFLD in comparison with MRI/S as a gold standard tool.

DETAILS OF PRODUCT

Not applicable. This is a dietary intervention trial and only normal food from local supermarkets will be provided to the participants.

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TRIAL DESIGN

TRIAL CONFIGURATION

This is a randomized 2 x 2 cross-over mechanistic trial that will be conducted through the NIHR Nottingham Clinical Research Facility (Nottingham Digestive Diseases Centre) in collaboration with Sir Peter Mansfield Imaging Centre in University of Nottingham.

Primary endpoint

The liver fat content after iso-energetic LGI and HGI diet consumption using MRI/S.

Secondary endpoints

- Blood metabolic biomarkers.
- Gut microbiota composition.
- CAP-Fibroscan™ score.

Safety endpoints

Adverse events (AEs), discontinuations due to AEs, and special investigations such as clinical laboratory data and results of MRI/S scans will be the safety endpoints of this trial.

Stopping rules and discontinuation

This dietary intervention is widely used in practice and known to be safe and well-tolerated. There are thus no expected causes for entire trial discontinuation. At an individual level, participants experiencing any AEs related to the trial intervention may be withdrawn. They will be made aware that they can withdraw their consent at any time during the trial. If a participant chooses to leave the trial early, the primary reason for discontinuation will be determined and recorded in as much detail as possible. Should they withdraw, they will have been made aware via the participant information sheet (PIS) and consent form, that data collected to date cannot be erased and may still be used in the final analysis.

RANDOMIZATION AND BLINDING

Enrolled participants who meets the eligibility criteria will be randomized to the group receiving LGI or HGI diet. The random allocation sequence (AB or BA) will be generated by one of the trial co-ordinators; who will have no contact with the participants, using block randomization by impartial biostatistics. The online randomisation package (http://randomization.com) will be used to randomly allocate the sequences of the diet groups to the participants in equal numbers. Once the random number is generated, both trial investigators and participants will be aware of the diet sequence (i.e. whether the participant is going to start with LGI diet or HGI diet). The diets will then be delivered to the participant according to the sequence. The trial outcome measures will be determined by blinded researcher using codes on samples that will be sent for 16S rRNA analysis, blood markers analyses and MRI/CAP-FibroScan™ analysers.

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Maintenance of randomisation codes and procedures for breaking code

As trial investigators and participants, will be aware of the diet group randomisation, no emergency un-blinding procedure will be required. The list of participants random intervention allocations will be concealed in sequentially numbered opaque sealed envelopes and kept securely beforehand. After participants' enrolment and consenting the seal will be broken to reveal which intervention the participant has been allocated. The blinded allocation will be provided to the study statistician at the end of the study and once the analysis has been completed, the study statistician will be provided with the un-blinded allocation.

TRIAL MANAGEMENT

The study will form part of PhD project undertaken by researcher Amina Alawadi whose role will include responsibility for the day to day running of the study, recruiting and consenting patients and performing MRI data analysis. Amina is part of Nottingham BRC and will have a research passport and appropriate training to attend clinics via NUH and will have access to NUH IT system thus ensuring that patients data will be secure. She will be complying with BRC clinical research facility policy and regulations. Access to the scarred liver project database will supervised by Dr. Jane Grove and Dr. Neil Guha.
The Chief Investigator (Prof Guru Aithal) will have the overall responsibility for the study and shall oversee all study management. The data custodian will be the Chief Investigator (Prof Guru Aithal).

DURATION OF THE TRIAL AND PARTICIPANT INVOLVEMENT

Trial Duration: Participants will be recruited over an estimated time of 3.5 years until the target sample size has been reached.

Participant Duration: Eligible participants will be in the trial for up to 3 months, to include 3 weeks for screening visit 1, 7-day diary completion and 2 weeks for analysis of diary for eligibility and attending screening visit 2.
Eligible participants involvement will end after each participant has completed both periods of the dietary intervention for 8-weeks including washout period (4 weeks).

End of the Trial

The end of the trial will be the last trial visit of the last participant.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

We aim to recruit 16 participants to complete both phases of 2x2 cross-over trials. We will select people with a CAP score of >288dB/m for inclusion in the study (this value correlates with MRI-PDFF > 5% fat of liver weight which indicates that they have a fatty liver) [38].

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NAFLD patients will be recruited as follows:

- Patients who have attended appointments at liver clinics at the Queens Medical Centre (QMC) day-case unit, Nottingham University Hospital NHS Trust and had a CAP-Fibroscan will be identified by the research nurse who is also part of the patients' clinical care team. Any patients with NAFLD will be provided by post or at their clinic appointment with a copy of the participant information sheet (PIS).

- Suitable participants from a list of those included in the existing "The Scarred Liver Project database" in the UK who have given consent to be contacted to participate in future research (Community study approved by East Midlands-Leicester Research ethics committee ref/B/EM/0123, sponsored by UoN) or participants from public (advert in local and social media). The research team will send a letter by post inviting them to the study and contact them by phone. Detailed information about the study will be sent to each participant before their screening visit.

Our contact information will be provided in PIS as well as study advert sheet. If interested they will be invited to attend at NIHR Nottingham Biomedical Research Centre (BRC) in QMC. At this screening visit, participants will have chance to discuss the study and ask any questions, if willing to take part they will sign the consent form and basic demography details will be recorded. Participants will also complete a Food Frequency Questionnaire (FFQ) and be provide with a 7-day diary for completion. They will be asked to send back the 7-day diary records by post after one week.

The investigator will inform the participant of all aspects pertaining to participation in the study.

We will first characterise the baseline GI diet intake of all recruited patients using a 7-day food records. Participants will also be asked to complete a FFQ which will be validated against the 7-day food records. After completion of diary records and FFQ, participant's eligibility will be reassessed based on analysis of diet information. Those with low GI diet intake (GI < 60) will be excluded from the study. We will enrol patients who have only moderate to high GI diet intake (GI ≥ 60) and assign them randomly to either HGI diet (HGI group) or LGI group and then crossover to either HGI/LGI diets.

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

Eligibility criteria

Inclusion criteria

- Adult males and females aged from 18 to 65 years (balanced number).
- Detected NAFLD by CAP-FibroScan™ >288dB/m or by MRI-PDFF) > 5% fat of liver weight.
- Body mass index ≥ 25 kg/m^2
- Have current moderate to high GI diet intake of ≥ 60 (Assessed from a completed 7-day food diary).

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- Abdominal obesity (Waist circumference > 102 cm for males and > 88 cm for females)
- Able to give informed consent.
- Able to undergo MRI/S and CAP-FibroScan™.

Exclusion criteria

- Current smokers and excessive alcohol drinkers (> 14 units/week).
- Perimenopausal (irregular periods) women.
- Participants with other liver abnormalities.
- Participants with history of gastrointestinal surgeries, depression, eating disorders or difficulties.
- Participants using pharmacologic agents for obesity or NAFLD.
- Participants with type 1 diabetes.
- Participants with type 2 diabetes on second line medications (eg GLP-1 analogues, sulfonylureas).
- Participation in any other trial in the last 3 months.
- Participants on any special diets (e.g. vegetarians).
- Intolerance to foods included in the diet plan.

Expected duration of participant participation

Study participants will be participating in the study for three months including screening visits (2 weeks), one week before the start of the intervention periods (standardization week) and a washout period of 4 weeks in between the two diet intervention periods.

Removal of participants from therapy or assessments/Participant Withdrawal

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator where necessary. The participants will be made aware that this will not affect their future care. Participants will have been made aware (via the PIS and consent form) that should they withdraw, data collected to date cannot be erased and may still be used in the final analysis.

Informed consent

All participants will provide written informed consent. The Informed Consent Form will be signed and dated by the participant before they enter the trial. The Investigator will explain the details of the trial and provide a Participant Information Sheet (PIS), ensuring that the participant has sufficient time to consider whether to participate or not. The Investigator will answer any questions that the participant has concerning study participation.

As a University we can not give any clinical diagnoses. If we did find anything abnormal on a participant's scan, the investigator would arrange for an appropriately qualified doctor, from a healthcare provider e.g. a NHS Trust or a private doctor, to look at them. That specialist doctor would contact their GP to explain the situation, so that the GP could then advise you.

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Informed consent will be collected from each participant before they undergo any interventions (including history taking) related to the study. One copy of this will be kept by the participant and one will be kept by the Investigator.

Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the study, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

TRIAL TREATMENT AND REGIMEN

A schematic diagram of the trial design is shown below (Figure 1).

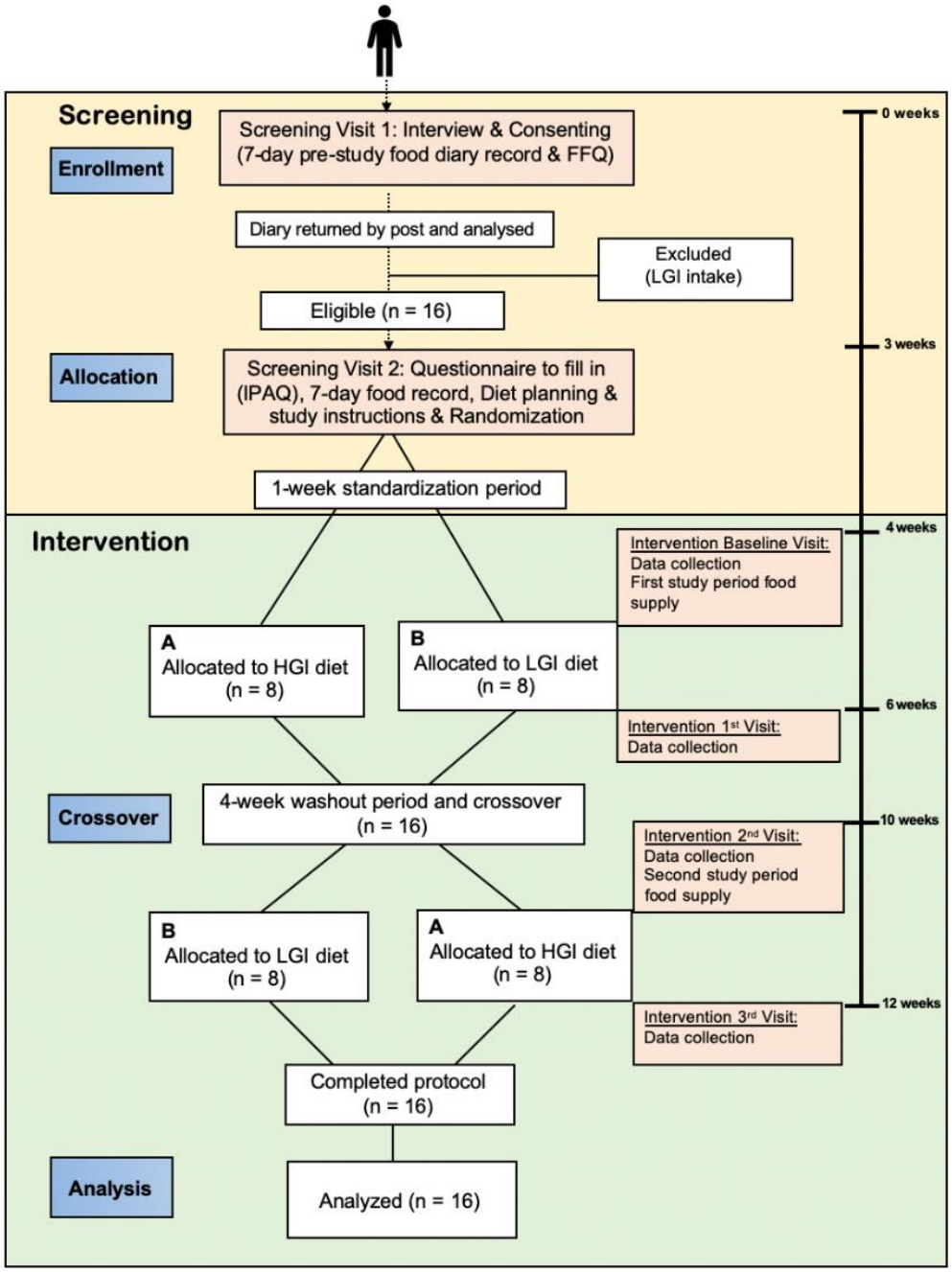

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Figure 1: Schematic diagram for crossover trial of LGI diet versus HGI diet effects on liver fat accumulation in participants with NAFLD.

As illustrated above, after consenting at screening visit 1, a FFQ will be completed. The participants will be given a 7-day food diary to complete and return by post. The food diary will be analysed using Nutritics software (Nutritics Ltd, Dublin, Ireland, 2018) and participants included if they have a GI of ≥ 60. Those who have low GI intake (< 60) will be excluded from the study. Then at screening visit 2, each eligible participant will be assigned randomly to undergo both study diet groups. The intervention period will have four visits in order to collect necessary data from participants. Habitual food intake at baseline visit will be assessed following the second screening visit by completion of a 7-day diary records. Energy expenditure will be estimated for each participant using the individual's basal metabolic rate (BMR) estimated using Henry-modified Schofield formula [39] multiplied by an activity factor based on the International Physical Activity Questionnaire (IPAQ). An individual personalised menu of meals for 3 days will then be devised (these meals will then be repeated over 2 weeks) by adjusting a template according to their estimated energy requirement in order to minimise weight change during the intervention phase.

The diet will consist of normal food resembling that described by Bawden et al, (2017) study. A diet template of 2000 kcal/day with energy proportions distributed as 55% from carbohydrates, 15% from protein and 30% from fat [40] will be designed and food quantities will be modified to supply the energy requirements for each participant. Three-day cycle menu plans will be designed for each diet (LGI/ HGI). An example template menu is included in appendix 1 which will be adjusted to meet participant energy requirements. The dietary GI of the menu will be evaluated. The GL of the diet will be estimated based on the dietary GI and the amount of carbohydrate provided (the same in both diets). Food composition analysis and evaluation will be performed using Nutritics software (Nutritics Ltd, Dublin, Ireland, 2018).

Participants will be provided with the food at the start of each diet period (i.e at/after intervention baseline visit and intervention visit 2 as shown on figure 1). This will be either on-site collection or home delivered each week and at subsequent points through the study. They will be clearly instructed on safe food storage. They will be required to complete a dietary compliance sheet. Dietary counselling on compliance will be given between the two study periods and the dietary compliance records will be reviewed regularly during the intervention to assess the compliance of participants. For one week before the first period of the intervention (standardization period) and during washout period, participants will be asked to avoid eating probiotics, yogurt, cultured milk drink and oligosaccharides to standardize their gut microbiota composition. Meal-timing will be standardised among all participants as much as possible.

Data Point Collection

The following measurements will be taken at all intervention visits (participants will come fasted):

1. MRI/S in Sir Peter Mansfield Imaging Centre to detect their liver fat fraction.
2. CAP-Fibroscan™ for liver fat quantification (a quantitative and non-invasive specialized ultrasound machine for liver fat quantification and stiffness with a new algorithm that calculates the Controlled Attenuation Parameter from the ultrasound signals).
3. Stool samples for gut microbial composition analysis using 16S rRNA.

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4. Blood samples for analyses of metabolic analytes such as: glycaemic, insulinemic, liver, lipid, hematologic profiles, gut hormones, and plasma SCFAs.
5. Dietary compliance records (to be completed during the two intervention periods).
6. Anthropometric measurements (height, weight, waist circumference and BMI and body composition by Bio-electrical Impedance analysis (BIA).
7. Continuous interstitial glucose monitoring using iPRO™ 2 (a small discreet device that measures and records glucose levels every 5 minutes, up to 6 days, revealing hidden glucose excursions and trends which finger sticks miss).
8. Visual analogue scales (VAS) will be used to assess the participant's subjective appetite (hunger and fullness) during the intervention. Participants will be provided with a booklet to record their subjective appetite at each intervention visit.

Enrolment visit 2 is expected to take place three weeks after recruitment. Intervention baseline visit will be after 4 weeks, 1st intervention visit after 6 weeks, 2nd intervention visit after 10 weeks and 3rd intervention visit (end of study) after 12 weeks. Screening visits are expected to last 1 hour each, and intervention visits are expected to last 2 hours each. After each intervention visit, we will provide the participants a snack.

Compliance

Participants will complete dietary compliance sheets during the intervention. These will be checked at each visit and reference will be made to them during twice weekly phone calls. It will be emphasised to participants that they must consume the prescribed foods. Participants who report either failing to consume all the foods or eating non prescribed foods on a regular basis will be withdrawn from the study.

Criteria for terminating trial

In the event of a participant withdrawing from trial participation or being unable to continue the intervention periods after randomisation, a replacement will be recruited who will be assigned to the same diet group to ensure a 1:1 ratio for the 2 diet groups. In order to complete this study, we will continue recruiting until 16 participants have fully completed the study. We anticipate that the drop-out rate is 40% for dietary intervention so we expect to recruit at least 24 participants.

Transport and Storage Of The Tissues

Samples will be stored in a linked anonymised format and labelled using a combination of study reference, unique study identifier and cross referenced with location code numbers to permit accurate linkage to study data and the consent form. The master database will be held by the study investigators in a password encrypted file.

Blood and stool samples will be processed in the Nottingham Digestive Diseases Centre (NDDC) laboratories, University of Nottingham (located in the QMC) or SPMIC laboratories (on University Park Campus). Samples will be transported from SPMIC following local standard operating procedures and stored securely in the NDDC laboratories at -80°C prior to analysis in an HTA licensed freezer. The analysis of samples will take place either in the

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NDDC laboratories, University of Nottingham or else samples will be shipped to commercial providers for specialist analyses with a material transfer agreement arranged.

Once the study is completed and analysis has taken place, any residual samples will be stored within the Nottingham Digestive Diseases Centre Biomedical Research Unit Research Tissue Bank for future research (DI William Dunn- Licence Number 12265) if participants are agreeable and sign the optional clause on the consent form. Where participants do not agree to the future use of the samples, they will be destroyed in accordance with the Human Tissue Act, 2004.

Laboratory Analyses

Blood samples (not more than 80 mL at each visit) will be taken by a qualified practitioner. Each participant will be asked to collect stool sample in a container and bring it to study visit.

Standard operating procedures (SOPs) will be followed for all laboratory assays to ensure the high quality and reliability of our generated data. Routine blood tests will be analysed by the accredited laboratories. Samples will be stored in -80˚C freezers, under yearly service contract and monitoring system. Analysis will be done according to assay manufacturer advice and will be completed by technicians and research staff who are formally trained or outsourced to collaborators or external suppliers where necessary. Investigators will be blinded to the patient identity and treatment group. DNA will be extracted from stool samples for microbiome analysis.

STATISTICS

Methods

The researcher will conduct the statistical analyses, with the supervision and support of Professor Guru Aithal and trial statistician Dr Lu Ban. Data input, cleaning and analysis will be conducted using Stata software package. All analyses will be conducted on UoN computers/laptops that are regularly backed up to UoN servers.

Baseline characteristics of participants, outcome measures at baseline (liver fat content, blood biomarkers and gut microbial composition) at each period time-point will be summarized by diet group using descriptive statistics. The differences between the two interventions (LGI and HGI diet) will be tested by matched-pair Student's t-test. Two-way repeated measures ANOVA test will be used to evaluate the effects of diets and time sequences on liver fat content, as well as on gut microbial composition and blood biomarkers and/or interactions with liver fat content, gut microbial composition and blood biomarkers. Data will be presented as mean± standard deviation and the significance of difference will be set at $p < 0.05$. Any changes in the planned statistical methods will be documented in the trial report.

Sample size and justification

The sample size is calculated using nQuery based on previous completed study by Bawden et al, (2017). Based on their pilot data, baseline liver fat fractions of both diet groups were 2.4 ± 1-1.2%, and after 1 week were HGI (High GI) = 4.7 ± 2 % and LGI = 1.6 ± 0.7%. Using a two-group t-test (Crossover ANOVA) between the post- diet values with a

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0.05 two-sided significance level, this will produce a sample size of n=8 in each diet sequence (a total sample size of 16). This sample size will have 80% power to detect a difference in means of 3.1 (the difference between a Treatment 1, μ_1, of 4.7 and a Treatment 2, μ_2, of 1.6) with the assumption that the Crossover ANOVA sqrt (MSE) is 2.828 (the Standard deviation of differences, σ, is 4). Assuming that 40% of people would drop out from cross-over study design, it is expected that at least 24 participants would need to be recruited.

The effect from Bawden, et al, (2017) pilot study(n=7) was an average 1.6% ± 0.7% decrease in liver fat fraction after following a one-week low glycaemic index diet ($p < 0.05$); Given that baseline liver fat fraction was 2.4%, in order to see such change with 80% power ($p< 0.05$) we have doubled the participant numbers to n=16 using nQuery software to detect statistically significant differences [35]. Our group has recently completed a study (ClinicalTrials.gov Identifier: NCT03844165) in which 84 participants with NAFLD followed either a low GI food enriched diet or their normal diet. Patients who changed their diet were found to have a significant decrease in liver fat after 16 weeks (from 20.4% to 18.8%) and had a statistically significant decrease in fasting glucose levels after the diet compared to the control group patients following their normal diet. This provides strong support to our hypothesis and demonstrates that a simple dietary change can have measurable impact on NAFLD symptoms in a short time period. In another previous randomized crossover trial, metabolic changes were seen within two-weeks in a dietary intervention with 10 participants (Farshchi, Tylor & Macdonald, 2005).

Based on these different approaches, we conclude that a sample size of 16 at the end of the study is sufficient. This calculation was confirmed by our study statistician Dr Lu Ban, the biomedical research centre statistical advisor who is a senior research fellow in epidemiology. We should also clarify that we do expect dropouts and it is likely to be necessary to enrol n=24 NAFLD participants to achieve n=16 with two diet-interventions completed assuming a 33% drop-out rate generally reported in interventional studies.
For the first one week prior to the intervention(one-week standardization), and during the four-week washout period, participants will be asked to avoid eating probiotics, yogurt and cultured milk drink; if any, to standardize their gut microbiota and avoid any carry-over effects as shown in previous studies (Mano et al., 2018).

Assessment of efficacy

Endpoint	Variable	Timings	Parameter
Primary	Liver Fat Content	At baseline, 1st , 2nd 3rd visits	Difference in means between two diet groups
Secondary	Blood biomarkers (glycemic, insulinemic, liver, lipid, hematologic profiles, gut hormones, and plasma SCFAs)	At baseline, 1st , 2nd 3rd visits	Difference in means between two diet groups
Secondary	Gut microbial composition	At baseline, 1st , 2nd 3rd visits	Difference in means between two diet groups

Assessment of safety

As this is a dietary intervention trial the primary and secondary efficacy variables are identified as safety endpoints and will be analysed as specified above.

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Procedures for missing, unused and spurious data

All efforts will be made to ensure the capture of all necessary outcome data. Any missing data will not be imputed on an individual level. The reasons for missed data, if any, will be sought. If outcome data are missing for a diet period, the participant will be labelled as non-responder for that period.

Definition of populations analyzed

Full Analysis set: All randomised participants, who participated in at least one treatment and for whom at least one post-baseline assessment of the primary endpoint is available.

ADVERSE EVENTS

As this is a dietary intervention trial and non-invasive procedures will be used, no serious adverse events or any side-effects are expected. Allergies may be an adverse event in this intervention and participants will be asked if they have any food allergies prior the intervention. All safety procedures will be taken into consideration before blood taking and MRI scanning. As part of the screening process we will select only participants who are able to undergo MRI. Adverse events (serious and non-serious) will not be collected in this study.

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Research Ethics Committee (REC), the respective National Health Service (NHS) or other healthcare provider's Research & Development (R&D) department, and the Health Research Authority (HRA) if required. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol

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amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the UK Department of Health Policy Framework for Health and Social Care, 2017.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms before screening.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Case Report Forms

Each participant will be assigned a study identity code number, allocated at randomisation if appropriate, for use on CRFs other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy).

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in accordance with regulatory requirements and for follow-up as required

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CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Sample Labelling

Each participant will be assigned a trial identity code number for use on the samples, consent forms and other study documents and the electronic database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy).

Samples for NHS pathology analysis will be labelled in accordance with local NHS procedures.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall made be available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g. DH, Human Tissue Authority).

DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 2018. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above). Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).
Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

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QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham as research Sponsor indemnifies its staff, research participants and research protocols with both public liability insurance and clinical trials insurance. These policies include provision for indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct may be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; accountability of trial materials and equipment calibration logs.

The Trial Coordinator/Academic Supervisor, or where required shall carry out a site system audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator/Academic Supervisor, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition, the subsequent capture of the data on the trial database will be checked. Where corrections are required these will carry a full audit trail and justification.

Trial data and evidence of monitoring and systems audits will be made available for inspection by REC as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study.

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These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

If information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

Data from this trial are intended to be presented at local and international conferences. Within five years' timeframe the data will be displayed in the trial websites for trial participants. Data will also be used as part of a Philosophy Doctorate thesis. Publications will be in peer reviewed journals. No participant will be identified in any of these publications

USER AND PUBLIC INVOLVEMENT

Ahead of this application the BRC liver and gastrointestinal disorders Patient Advisory Group has been consulted at meetings including NAFLD patients in 2017 and 2018. They expressed strong support for the research objectives and the recent meeting the group considered the intervention feasible and acceptable to participants.

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STUDY FINANCES

Funding source

This study is funded by University of Nottingham studentship to Amina Al-Awadi and support from Nottingham BRC.

Participant stipends and payments

Participants will not be paid to participate in the trial and all food will be provided for them during the intervention periods. An inconvenience allowance to participate in the study (£120) at the end of the trial. All food will be provided for the participants during the intervention periods. Travel expenses will not be offered for any visits incurred as a result of participation.

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SIGNATURE PAGE

Signatories to Protocol:

Chief Investigator: (name)_____Guru Aithal_____

Signature: 

Date: _____27.5.2019_____

Co- investigator: (name) _____Jane Grove_____

Signature: 

Date: ____27.5.2019_____

Trial Statistician: (name)_____Lu Ban_____

Signature:_____  _____

Date: _____May 24th 2019_____

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APPENDIX 1

Illustration of template diet menu for use in the study.
This will be adjusted according to participant's preferences and local food supplies

Meal	High GI Diet – Sample Menu 1						
	Weight (g)	Energy (Kcal)	Fat (g)	Protein (g)	Carbohydrate (g)	Fibre (g)	GI
Breakfast							
Sultana Bran	90	293	2	8	60	12	90
Skimmed milk	180	56	1	5	7	0	48
Glucose	10	39	0	0	10	0	100
Water	170						
Boiled egg, 1	50	71	5	7	0	0	0
Sub-Total		459	8	20	77	12	87.5*
Lunch							
Strawberry, low fat Yogurt	125	104	1	3	20	1	85
Fruit Loaf	144	400	6	10	77	5	90
Margarine	25	191	19	0	0	0	0
Skimmed Milk	120	37	1	3	5	0	48
Roast Turkey Breast Slices (2 slices)	44	50	1	10	0	0	0
Sub-Total		782	28	26	102	6	87*
Dinner							
Cottage Pie ready meal	300	240	8	15	26	4	65
Medium Cheddar	35	144	12	9	0	0	0
Strawberry yogurt	125	100	1	3	20	1	85
Glucose	10	39	0	0	10	0	100
Water	170						
Mars Bar	51	228	8.5	2.2	35.3	0	68
Sub-Total		751	29.5	29.2	91.3	5	74.5*
Day Total		1992	65.5	75.2	270.3	23	84.3*
Energy Proportions			30%	15%	55%		
*Calculated from GI proportional contribution to total carbohydrates using the following equation: $GI = \sum(C_{food}/C_{total}) \times GI_{food}$, [35].							

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