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The rationale and design of a randomized controlled trial testing the effect of metformin administered with adjuvant personalized diet in individuals with prediabetes or type 2 diabetes mellitus

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Abstract

Introduction Metformin and diets aimed at promoting healthy body weight are the first-line in treating type 2 diabetes mellitus (T2DM). Clinical practice, backed by clinical trials, suggests that many individuals do not reach glycaemic targets using this approach alone. The primary aim of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) study is to test the efficacy of personalized diet as adjuvant to metformin in improving glycaemic control in individuals with dysglycemia.

Methods and Analysis PREDICT is a two-arm, parallel group, single-masked randomized controlled trial in adults with prediabetes or early-stage T2DM (with HbA1c up to 8.0%), not treated with glucose-lowering medication. PREDICT is conducted at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney). Enrolment of participants commenced in December 2018 and expected to complete in December 2021. Participants are commenced on metformin (Extended Release, titrated to a target dose of 1500 mg/d) and randomized with equal allocation to either (1) the Personalized Nutrition Project (PNP) algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, designed to provide caloric restriction (75%) in individuals with body mass index >25 kg/m^2. Treatment duration is 6 months and participants visit the Clinical Research Facility 5 times over approximately 7 months. The primary outcome measure is glycated haemoglobin (HbA1c). The secondary outcomes are time of day of interstitial glucose <7.8 mmol/L and glycaemic variability (continuous glucose monitoring), body weight and composition (dual-energy X-ray absorptiometry), markers of cardiovascular disease (blood pressure, lipid profile, pulse wave analysis), liver stiffness and fat (Fibroscan).

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional

Review Board (File 528-3, Rehovot, Israel). The findings will be published in peer-reviewed open access medical journals.

The study has been registered at ClinicalTrials.gov (NCT03558867).

Article Summary

The PREDICT study is set to test a novel diet as an adjuvant to metformin. We expect improved glycaemia with the combined metformin-personalized diet treatment. This study may result in a novel management approach for people with, or predisposed to, type 2 diabetes requiring metformin treatment.

Strengths and limitations of this study

- The randomized controlled design testing a novel diet against standard of care may lead to a novel tool to manage dysglycemia in individuals requiring metformin
- Conducted in an adult Australian population, the study findings may not be applicable to other populations
- The algorithm used to devise the personalized diet relies on accurate recording of the dietary intake by the participants
- The dietary intervention requires use of a smartphone application which may limit its applicability to some populations

Key words: Prediabetes, Type 2 diabetes, Metformin, Algorithm-based diet, Low fat diet, Randomized controlled trial

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Introduction

Type 2 diabetes mellitus (T2DM) and its preceding medical condition, prediabetes are significant risk factors for cardiovascular disease, and most affected individuals demonstrate additional metabolic risk factors, such as hypertension, dyslipidaemia, excess weight and fatty liver[1]. T2DM affects approximately 422 million adults globally, with an additional 352 million individuals at increased risk, having prediabetes[2 3]. Individuals diagnosed with prediabetes or T2DM are encouraged to adopt a healthy lifestyle and, if overweight to lose weight[4]. The majority of individuals with T2DM are treated with metformin, which is the 8th most prescribed medication in the United States[5-7].

Metformin, an oral biguanide, is the first-line treatment for individuals with newly-diagnosed T2DM and, in some cases, for the prevention of diabetes in individuals with prediabetes[6]. Metformin is an ideal medication to initiate for management of T2DM or for prevention of diabetes, because it does not cause hypoglycaemia and has a favourable, albeit modest, effect on body weight[8]. Metformin monotherapy is insufficient to achieve glycaemic control in a large proportion of treated individuals[3 9 10]. Findings from the Diabetes Prevention Program (DPP) in individuals with prediabetes suggested that the glycaemic efficacy of metformin depends on the magnitude of weight loss[11], explaining 64% of the diabetes risk reduction, with additional 17% explained by decreases in fasting insulin and pro-insulin at 3 years of follow up[11 12].

The current treatment guidelines in T2DM recommend prescribing metformin in combination with a healthy lifestyle, enabling weight loss[13]. The most recent nutritional guidelines for individuals with T2DM or prediabetes are no longer supporting a universal ideal dietary macronutrient distribution; instead, the guidelines suggest individualized eating plans[4].

In the pioneering Personalized Nutrition Project (PNP)[14], Segal, Elinav and colleagues developed an algorithm that predicts an individual's postprandial glycaemic response (PPGR) to meals. The algorithm incorporates the individual's personal data (e.g. age, gender, body mass index [BMI]), blood tests (e.g. glycated haemoglobin [HbA1c]), dietary features, continuous glucose monitor (CGM)-derived data, and gut microbiome features, and trained on data previously collected in 800 individuals. Personally-tailored dietary plans based on the algorithm were trialled in a small group of individuals with prediabetes and shown to improve glycaemic variability and PPGR over 7 days[14].

The primary objective of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) Study is to compare glycaemic control, measured by HbA1c, following 6 months of metformin, prescribed with either (1) the PNP algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, based on the Australian Healthy Eating Guide[15] and the American Association of Clinical Endocrinologists guide for medical care of patients with obesity[16], in individuals with prediabetes or early-stage T2DM naive to glucose-lowering pharmacotherapy.

The secondary objectives of the PREDICT study are to compare the effect of metformin when prescribed with the PNP diet versus LFHF diet on: (1) time of interstitial glucose <7.8 mmol/L, (2) glycaemic variability, (3) weight, (4) body composition, (5) resting energy expenditure (REE), (6) markers of cardiovascular disease and (7) liver fat and fibrosis. The exploratory objectives of the study are to test the effect of the treatment on the gut microbiome.

METHODS

Study design, setting and population

The study is a two-arm, parallel group, single-masked randomized controlled trial (RCT). Adults with prediabetes or early-stage T2DM who are not treated with glucose-lowering medications are randomized, with equal allocation, to either the PNP or LFHF arms, both administered with metformin Extended Release (XR) 1500 mg/d for 6 months. All the study visits are performed at the Clinical Research Facility (CRF) at the Garvan Institute of Medical Research (Sydney). Metagenomics and data processing for the personalized dietary interventions are performed at the Weizmann Institute of Science (Rehovot).

Patient and public involvement

Patients or the public were not involved in the design or other aspects of the research.

Eligibility

Adults (20-70 years old) with prediabetes or recently (in the past 6-months) diagnosed T2DM with (HbA1c ≤8.0%), not pregnant or planning to become pregnant during, and for at least 3 months after the study are recruited (Table 1). A wide age range was selected to encompass different populations of individuals managing their prediabetes for short or long durations and to increase the likelihood to recruit the sample size in a timely manner. The HbA1c cap at 8.0% was selected to ensure that individuals with T2DM are relatively well-controlled. Individuals with conditions or treatments that affect glycaemia (e.g. oral steroids), impact weight (e.g. bariatric surgery, weight loss medications), or the gut microbiome (e.g. inflammatory bowel disease, coeliac, frequent antibiotic treatment) will be excluded (Table 1).

Recruitment

The study is advertised in general practitioners (GP), endocrinologists and dieticians' practices in the Sydney metropolitan area, and through targeted social media campaigns. A collaboration with Blacktown Mt Druitt Hospital (Western Sydney) has been established in 2019 for the purpose of recruitment. During a hospital screening program ran between 2016 – 2018, 17.3% and 30.2% of individuals visiting the Emergency Department (ED) at Blacktown Mt Druitt Hospital have had HbA1c values indicative of diabetes and prediabetes, respectively[17]. Since September 2019, individuals visiting the ED who have had a blood test indicative of prediabetes receive a letter prompting them to contact the PREDICT team and encouraging they share the result with a GP.

To-date (February 2020), 38 participants were enrolled. Of the 38 participants enrolled, 20 completed, 13 are ongoing, and 5 withdrew before the end of the treatment (13% drop-out rate). Recruitment of participants is expected to complete in December 2021.

Participants contacting the team receive the participant information sheet via email or post. Experienced clinical research nurse/associate provides details about the study over the phone. Willing participants are referred to a commercial pathology to perform an oral glucose tolerance test (OGTT, 75 g) and HbA1c test. They are asked to sign a consent form after reading the participant information sheet explaining the possible risks of undergoing the OGTT and HbA1c tests prior to performing the blood tests. If the blood tests indicate either T2DM (with HbA1c ≤8.0%), or impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or HbA1c ≥5.7, they are invited to a screening and enrolment visit at the Garvan CRF.

A stool collection kit for metagenomics OMNIgene GUT (OMR-200; DNA Genotek) is mailed to participants prior to the screening/enrolment visit. Participants collect the sample according

to the manufacturer's instructions the day before the visit and keep the sample at room temperature. At the CRF, the sample is vortexed, centrifuged for a few seconds and material aliquoted into cryo vials and kept in -80C freezer. One vial is transferred to facilities at the Weizmann Institute of Science and stored at −20°C until DNA extraction.

The pre-treatment data are collected across the screening/enrolment and the baseline visits.

Screening / enrolment procedures and measurements

During the screening/enrolment visit, participants sign the study informed consent form and undergo medical examination by a physician. Participants have their weight, height, waist and hip circumference and blood pressure measured. Basal metabolic rate (BMR) is estimated using bioelectrical impedance (BIA, used for calculating the energy requirement, see "Energy target"). Blood samples are collected to evaluate liver (liver enzymes) and kidney (creatinine and eGFR) function and full blood count. Glucose monitor (FreeStyle Libre Pro, Abbott, Germany) is attached for a period of 14 days.

A link to download the PNP smartphone application is sent to the participants prior to the screening/enrolment visit. They are asked to log-in to the app with a personal (re-identifiable) code provided by email, and to familiarize with the app in preparation for a training session with the dietician. During the screening/enrolment visit, the dietitian practices with the participants browsing the food database, selecting food and beverage items and indicating the amount consumed. Participants are taught to add frequently consumed foods to a favourites list, which makes future search of food items easier. When the CGM is on, participants are asked to carry on with their usual routine and to record all meals, snacks and drinks using the

app. The period between the screening/enrolment and the baseline visits (4 – 6 weeks) serves as the 'run-in' period.

Randomization

Randomization is performed between the screening/enrolment and baseline visits. Individuals are randomized with 1:1 allocation into the 2 arms in rounds of approximately 4 individuals each, with randomization performed within each round using the minimization program for allocation of subjects to parallel groups, modified from Saghaei et al[18]. They are stratified by gender, age (20-49 or 50-70 years), BMI (<25.0 or >25.1 kg/m^2) and HbA1c (<5.7 or >5.8 %). To avoid bias, the randomization is performed by a study investigator located at the Weizmann Institute who does not interact with the study participants. The study nurses and physicians who have direct contact with the participants are blinded to the randomization order, however due to the nature of the intervention, the study dietician is not blinded to the treatment allocation.

Baseline visit - Measurements

The baseline visit is performed approximately 4-6 weeks after the screening/enrolment visit (Table 2). Participants attend the CRF following an overnight fast. Blood is drawn for serum lipids measurement and anthropometric measures and blood pressure are taken. Arterial stiffness (pulse wave analysis, AtCor Medical, Australia) is measured twice and average recorded, as described[19]. This is followed by measurement of REE, carbohydrate and fat oxidation over 30 minutes by indirect calorimetry (Quark, Cosmed, Italy)[20]. Body composition is assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare). Specifically, total body fat mass and fat-free mass (enCORE software), the android and gynoid region, and visceral fat (CoreScan software, GE Healthcare) are recorded[21]. Liver steatosis

(controlled attenuation parameter, CAP) and liver fibrosis (liver stiffness measurement, LSMs) are assessed using FibroScan (Touch 502 by Echosens) by a trained technician. CAP and LSMs have been reported to correlate closely with steatosis and fibrosis assessed using the gold-standard liver biopsy[22]. A physical activity monitor (ActivePal, Pal Technologies) is applied on the thigh for a period of 14 days. At the end of the baseline visit, the participants practice using the app with the dietician.

Prediction of postprandial glucose response using the algorithm

The prediction of PPGR in PREDICT follows the modelling framework described in Zeevi et al[14] and is performed between the screening/enrolment and baseline visits. Time-stamped food records from the app, CGM and other data collected during the enrolment visit at the Garvan are shared with a mathematician at the Weizmann Institute of Science where data processing occurs, on an Institutional secured server. The data, together with the stool metagenome sequencing data are integrated with the Weizmann Institute's database to develop personalized algorithms for predicting each individual's PPGR. A database of recipes of meals (n=233) and smaller meals ("snacks", n=249) varying in macronutrient composition to generate feedback on the PPGR to pre-consumed meals has been created. Using the participants' features, personalized PPGR are calculated for every meal and snack in the database based on nutrient composition, and energy-adjusted quintile cut-offs of PPGR are used to create personalized meal ratings ranging from 1 to 5 (corresponding with "excellent", "good", "medium", "bad", and "very bad"). The predictive model, originally trained on data collected in an Israeli adult cohort[14], has been shown to be predictive of PPGR in a U.S. cohort of healthy adults (n=327) consuming a Western style diet[23].

Interventions

Both arms use the PNP mobile app to select meals/foods. In 2018, the app, developed at the Weizmann Institute of Science, was adapted to Australian consumers including the Australian food database (AUSNUT 2011–13)[24] of approximately 5,700 food items.

Personalized diet (PNP diet arm)

Participants in the PNP diet arm receive personalized feedback on each of their food item/meal choice and asked to consult with the app in real-time to select the recommended meal for them. The feedback is color-coded with a traffic light system; green ("good" and "excellent"), yellow ("medium") and red ("bad" or "very bad") PPGR (Figure 1 A and B). Participants are advised to aim for as many "good" and "excellent" scores, occasional "medium", and to avoid "bad" and "very bad" scores. When receiving bad scores, they are advised to trial substituting, adding or removing ingredients from the meal to improve the score. In individuals with hypercholesterolemia, a special set of recipes containing reduced saturated fat are uploaded into the smartphone app.

Low-fat high fibre diet (LFHF) arm

The LFHF diet is designed to provide approximately 30% of the total daily energy intake from fat, of which up to 10% of the fat is saturated fat, 50-55% of energy from low glycaemic load carbohydrates, 20-25% from protein and 30 g/d of dietary fibre. A database of recipes (n=110 meals and n=80 snacks) following the LFHF nutrient content has been created based on the AUSNUT 2013 recipes[24]. Food items such as sugary drinks, processed meat, candies, sugar and cream were excluded from the LFHF recipes. Similar to the PNP arm, recommended meals scaled to the individual's energy target are uploaded into the participants' app, taking into account the individual's dietary restrictions and likes. Similar to the PNP arm, participants of the LFHF arm are encouraged to choose from the recipes uploaded for them or browse the food

database (5700 food items) to design their own meals, as long as they follow the general dietary guidelines. Participants of the LFHF arm are instructed to consult with the total daily energy and macronutrient breakdown charts to ensure they follow the recommended diet (Figure 1C).

Energy target and using the app to select meals in real-time (both arms)

The energy requirement calculation is based on BMR estimated by BIA (Tanita, TBF-300 by Wedderburn) and on the Mifflin equation[25]. The two values are multiplied by a physical activity factor of 1.4 (lightly active) then averaged, and the value compared with the average daily energy intake of at least 7 days, extracted from the time-stamped meals recorded using the app. In participants with BMI >25 kg/m^2, energy target of 75% is prescribed. Participants of both arms are encouraged to consume 3 bigger (breakfast, lunch, dinner) and 3 smaller (snack) meals spread throughout the day.

Metformin (both arms)

Metformin (XR) is dispensed by the St Vincent's Hospital Pharmacy (Sydney) at baseline (for a period of 3 months) and at the 3 months visit (to last until the end of the study). The target dose (1500 mg/d) is titrated over 3 weeks to minimize gastrointestinal intolerance. A target dose of 1000 mg/d is set for participants with mild to moderately decreased eGFR (45 – 59 mL/min/1.73m^2), or participants who cannot tolerate the higher dose. A standardized dose of 1500 mg/d, rather than 2000 mg/d, was selected to suit both participants with prediabetes and T2DM, while minimising intolerance. Participants are instructed to take the medication with the evening meal and record it daily using a medication recording screen in the app or in paper log-books.

Monitoring and adherence evaluation (both arms)

The dietician reviews scores calculated programmatically based on the frequency of using the app and on meeting the daily energy target, along with the time-stamped meals consumed by the participants daily. In the PNP arm, the score incorporates the proportion of meals achieving the desired ("excellent" and "good") scores, while in the LFHF arm, the proportion of days in which dietary fat ≤35%, saturated fat ≤10%, carbohydrates ≥45%, and dietary fibre ≥15 g. The dietician contacts individuals who need encouragement to achieve better scores. Participants of the 2 treatment groups receive the same attention according to their adherence. Time devoted to each individual by the dietician is recorded for later analysis purposes. Satisfaction with the diet is assessed using the Diet Satisfaction Questionnaire[26] at 6 months (Table 2).

Adherence to the metformin is based on pill counting at the 6-months visit and on logs of daily dose using the app and/or log-books.

Physical activity and other confounders

Participants are asked to maintain the same level of physical activity throughout the study. Physical activity is monitored at 2 time points during the study using ActivePal (Table 2). The device records time (start and duration) and type (quiet, standing and steps) of activity and 14-days' worth of data, stored in the device, downloaded upon return. Information about background medications and nutritional supplements is collected before the start of the treatment using questionnaires (Table 2). Participants are asked to report any change in medications at each of the CRF visits and using the medication screen in the smartphone app.

Primary and secondary outcomes – Measurements

Participants attend the CRF five times during the study, over approximately 7 months. Primary and secondary endpoint measures are collected before the start of the intervention (across 2 visits: screening/enrolment and baseline) and at 3 and 6 months of treatment. Table 2 outlines the measurements obtained at each of the study visits/events.

Study outcomes

The primary outcome measure is change in HbA1c from baseline to 6 months of treatment. Furthermore, a comprehensive glycaemia assessment is enabled through continuous glucose monitoring. Interstitial glucose concentrations are recorded every 15 min using CGM for 14 days before the start of the intervention (in the run-in period) and after 3- and 6- months of intervention. The sensor stores the data for the duration of the recording, while the participants are blinded to the glucose readings. The data are downloaded upon return of the sensor. Time of the day with glucose readings below 7.8 mmol/L before versus after the treatment will be compared. Glycaemic variability[27], including (1) mean amplitude of glucose excursion (MAGE, a measure of the variation of glucose concentrations from the mean), (2) the standard deviation and the (3) mean postprandial area under the curve will also be assessed. Furthermore, fasting plasma glucose, 1-hr and 2-hr plasma glucose post 75 g glucose assessment are repeated after 6 months and will be compared to the baseline values. HbA1c test is repeated 6 months after treatment cessation (at 12-months, Table 2), along with a short questionnaire, including weight, diet and medication status.

Weight, waist and hip circumferences are recorded at 3-months and 6-months of treatment and compared to baseline. Fat, fat-free mass and android/gynoid fat distribution and visceral fat measurements by DXA are repeated at 6 months treatment[21]. Similarly, REE and fat/carbohydrate oxidation is measured after 6 months of treatment.

Hepatic steatosis is common in prediabetes and T2DM[1] [28] and prevention of liver steatohepatitis is key target in individuals with prediabetes or T2DM. Metformin primarily targets the liver, inhibiting lipogenesis and increasing fatty acid oxidation; therefore a beneficial effect on liver lipid and fibrosis with metformin has been assumed[29]. However, comprehensive meta-analyses of randomized clinical trials concluded that reduction in both steatosis and fibrosis with metformin were underwhelming[30] [31]. Liver fibroscan measure is repeated after 6 months of treatment.

Blood pressure and pulse wave analysis measurements are repeated at 3 and 6 months of treatment and serum lipids measured after 6 months treatment.

Safety / Adverse events monitoring

Gastrointestinal side effects are the most common adverse effects of metformin and may occur in 20-30% of individuals[32] [33]. Specifically, abdominal discomfort, nausea, diarrhoea and anorexia are common[32]. While the gastrointestinal adverse effects are transient, in approximately 5% of individuals the symptoms may persist and result in cessation of metformin[33]. Vitamin B12 concentrations may be lower with metformin, if metformin is administered for a long duration[34] [35]. The mechanism(s) responsible for the lower plasma B12 concentrations are unclear. A very rare, but potentially fatal complication of metformin use is lactic acidosis, mainly in patients with renal impairment[32] [36]. In PREDICT, individuals with severe renal impairment are excluded. Metformin is titrated over 3 weeks to negate the potential gastrointestinal side effects. Adverse events are recorded and monitored during the study visits.

Statistical analysis

Sample size calculation

Based on the primary outcome measure HbA1c, to detect a clinically meaningful difference of 0.4% in the change of HbA1c from baseline between the study arms at 6 months, assuming SD of 1% for both groups[37], with 80% power at two sided significance level of 0.05, a sample size of 106 for each arm is required. Hence, with an estimated dropout rate of 20%, we aim to enrol 132 individuals to each arm, totalling 264 individuals in the study.

Analysis plan

The intention-to-treat (ITT) approach will be used for efficacy analysis. A likelihood-based mixed model repeated measures (MMRM) approach will be used for the primary efficacy analysis. The primary outcome measure HbA1c at baseline, 3, and 6 months will be the dependent variable and intervention by time interaction will be the fixed effects, and participants will be treated as random effect. The primary time specific comparison will be the difference in least square mean between intervention (PNP) and control (LFHF) diet at 6 months' treatment. The differences between the groups after 3 months of treatment will also be examined. Missing data will be handled directly through maximum-likelihood estimation via mixed modelling. To control for potential confounding effects, demographic and clinical covariates (e.g. age, gender, baseline BMI and background medications) will be adjusted as necessary in the model. Piecewise linear mixed model will be used to compare trend change between arms in different periods (0-3 months, and 3-6 months). Different statistical analysis strategies including t-test, Mann-Whitney U test, Chi-square test, linear/generalized linear regression, and mixed model will be used based on the type and distribution of the outcome measures. Mediation analysis will be carried out to explore if the weight loss mediates the intervention effect on glycaemia, and estimate indirect and direct effects and the proportion

mediated (how much of the total intervention effect works through weight loss). We expect some degree of weight loss in all participants, as has been reported for metformin[3 38 39]. Subgroup analyses can be further performed to explore the intervention effect in specific sub-cohorts, for instance, the group of patients who achieve adherence standard; the group of patients with BMI >25 kg/m^2 at baseline, etc.

Laboratory testing

HbA1c is analysed using high performance liquid chromatography (Bio-Rad D-100, Bio-Rad Laboratories, Inc), plasma glucose using the Cobas 8000 (Roche), and liver and renal function tests using the Atellica platform (Siemens). Serum lipid profile is analysed by a spectrophotometric assay (Advia® 2400 Chemistry System [Siemens Medical Solutions Diagnostics]), with low-density lipoprotein (LDL) calculated using the Friedewald equation. Metagenomic DNA from the stool samples is purified using DNeasy PowerMag Soil DNA extraction kit (Qiagen) optimized for Tecan automated platform. Next-Generation Sequencing (NGS) libraries are prepared using Nextera DNA library prep (Illumina) and sequenced on a NovaSeq sequencing platform (Illumina). Sequencing is performed with 100bp single end reads with the depth of 10 million reads per sample. Host DNA is detected by mapping reads to the Human genome with inclusive parameters, and those reads removed. Bacterial relative abundance estimation is performed by mapping bacterial reads to species-level genome bins (SGB) representative genomes[40]. Mapping is performed using Bowtie[41] and abundance estimated by calculating the mean coverage of unique genomic regions across the 50 percent most densely covered areas, as previously described[42].

Confidentiality and data storage

Each participant is associated with an individual program-generated code used to identify their study documents, data and specimens collected during the study. The re-identifiable code is documented in the participant's record and on all study documents. Study data are collected and managed using REDCap electronic data capture tools[43 44] hosted at the Garvan Institute of Medical Research. Some coded data are shared with essential personnel at the Weizmann Institute of Science on institutional Dropbox. Data collected in the form of paper hard copies are kept in locked cabinets and electronic files on a password protected folder with access granted to the Garvan study team. Re-identifiable blood, stool, plasma and serum samples will be kept at the Garvan Institute's freezer facility. All the study questionnaires are disseminated using REDCap.

Dissemination of results

The results of the study will be disseminated to healthcare professionals via open access publications in medical journals, without any restrictions. Upon completion of data analysis, the participants will be invited to an information session at the Garvan Institute of Medical Research with the study Investigator(s) where the findings of the study will be shared and discussed. Individual letters are disseminated to the study participants after the 6 months treatment visit (approximately 7 – 8 months from study enrolment) summarising individual results (e.g. baseline and post treatment weight, body fat, liver fat, HbA1c, fasting, 1-hr and 2-hr plasma glucose concentrations). The participants are encouraged to share their individual results with the GP.

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional Review Board (File 528-3, Rehovot, Israel). Protocol modifications are communicated to the SVH HREC, the Weizmann IRB, the trial registry (ClinicalTrials.gov), the study investigators and the study participants (if relevant). De-identified participant data that underlie the findings reported in the research article will be available immediately following publication, ending 5 years following the article publication, to researchers who provide a methodologically sound proposal with the aim to achieve the aims reported in the approved project proposal. Data may be obtained from the Principal Investigator Dorit Samocha-Bonet or Associate Investigator Jerry Greenfield upon enquiries directed to d.samochabonet@garvan.org.au or j.greenfield@garvan.org.au.

Authors' contributions

DS-B, JRG, ES and EE contributed to the conception and design of the study, TDH, NG and DS-B drafted the manuscript, NG and ZL contributed to the study design and the statistical plan, ZL contributed to the sample size estimation, NG, DK, RC, KT, EC, MD, JRS and T-MH contributed to the collection of the data. All authors revised and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The study sponsor and funding bodies have no role in the study design, collection, management, analysis, and interpretation of data, writing of the report and the decision to submit the report for publication.

Conflict of interests

Eran Elinav and Eran Segal are paid consultants of the company DayTwo. Mark Danta has received travel support and speaker fees from Gilead, Abbvie and Merck. All other authors declare they have no conflict of interest.

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Figure legends

Figure 1: Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

Table 1: Inclusion and Exclusion criteria

Inclusion criteria
Men and women 20-70 years of age
Prediabetes (IFG* or IGT* and/or HbA1c 5.7 – 6.4%) or Individuals diagnosed with T2DM in the last 6 months with HbA1c ≤ 8.0%
Willingness to provide written informed consent, participate and comply with the study
Exclusion criteria
Women planning pregnancy during the study or 3 months after study completion
Individuals with type 1 diabetes, neoplastic disease (in the last 3 years), cardiovascular event (-6 months), chronic gastrointestinal disorders
Established type 2 diabetes or type 2 diabetes diagnosed at recruitment with HbA1c > 8.0%
Normo-glycaemia
Liver enzymes ALT and/or AST >3 times normal range limit
eGFR<45 mL/min/1.73m^2
Current or recent (-6 months) treatment with an anti-diabetic medication, current treatment with an oral steroid or immunosuppressive medications, antibiotics (-3 months)
Participants who have had bariatric surgery
Participants with conditions that may interfere with the ability to understand the requirements of the study, refuse treatment with metformin or refuse to use the smartphone application

*Impaired fasting glucose (IFG): FPG 5.6-6.9 mmol/L and/or impaired glucose tolerance (IGT): 2-h plasma glucose during 75-g oral glucose tolerance test (OGTT) 7.8-11.0 mmol/L

Table 2: Study timeline of activities and measurements taken at each of the study events and visits

	Location and time	***Measurements***
Pre-screening	Phone -35 +/- 10 days	• Interview (by an experienced staff)
	Local pathology	• 75 g OGTT and HbA1c tests
Screening/ enrolment (Non-fasting)	CRF -30 +/- 15 days	• Informed consent signed • Medical history, medications & medical examination • Blood (full blood count, liver and kidney function tests) • Height and weight (BMI calculated) • BIA (RMR) • Blood pressure • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelope) • Smartphone app training
	Home	• Stool sample • *Study entry questionnaire
Baseline (Fasting)	CRF 0, treatment clock starts	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin 3-months' supply with instructions and log-books • Smartphone app training, according to study arm allocation
3-months (Fasting)	CRF +90 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (HbA1c) • Indirect calorimetry (REE, RQ) • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelop) • Metformin 3-months' supply with log-books • Adverse events recording and monitoring
	Home	• #Stool sample
5.5-months (Non-fasting)	CRF +166 days +/- 10 days	• FreeStyle Libre Pro and ActivePal
	Local pathology	• 75 g OGTT and HbA1c

6-months (Fasting)	CRF +180 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids, HbA1c) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin- unused pills collected and counted • Adverse events recording
	Home	• #Stool sample • ##Diet Satisfaction Questionnaire
12-months	Local pathology	• HbA1c
	Home	• **12-months questionnaire

*Questionnaire includes hunger/fullness, dietary habits and dislikes, physical activity, medications, dietary supplements, personal and family history of disease, food-frequency questionnaire

**Questionnaire includes diet (are you following the diet you were allocated to?), metformin (are you continuing the metformin treatment and dosage) and current body weight

#Gut microbiome features from stool samples collected at 3 and 6 months will be compared to baseline (exploratory outcome)

The Laboratory for the Study of Human Ingestive Behavior, Pennsylvania State University are the copyright holders of the Diet Satisfaction Questionnaire[26]

Abbreviations: BIA, bio impedance analysis; BMR, basal metabolic rate; DXA, dual-energy X-ray absorptiometry; REE, resting energy expenditure; RQ, respiratory quotient

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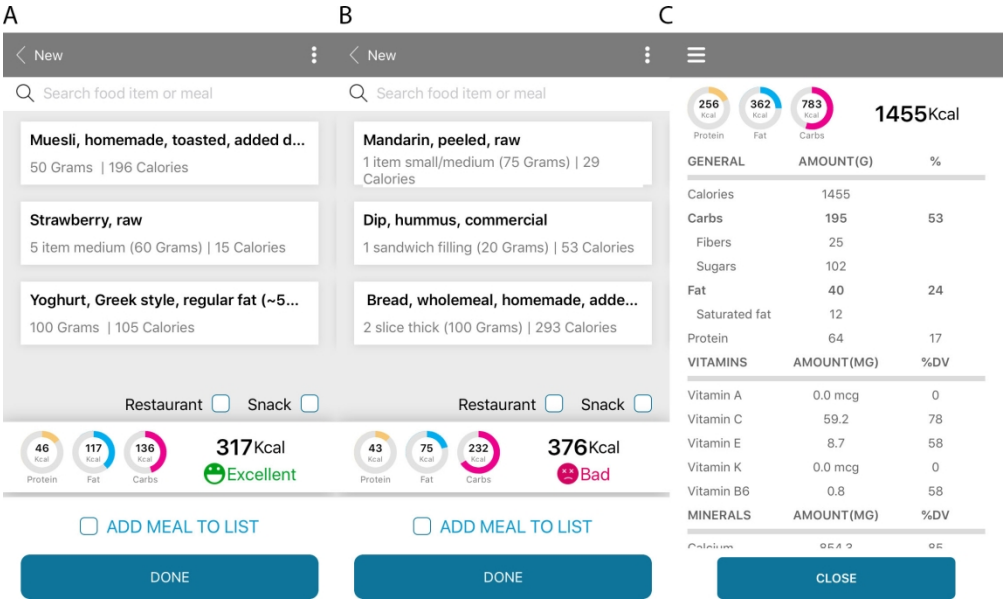

Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

190x112mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 20 (Authors' contribution)
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5 - 6
	6b	Explanation for choice of comparators	5 - 6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6 - 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11 – 13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13 – 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6 – 7 and Table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	15 – 16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7 – 8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9 – 10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9 – 10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9 – 10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9 – 10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14 – 16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14 – 16 and 17 (Statistical plan)

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18 – 19
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16 and Table 2
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approval granted, details page 3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9 and Table 2
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18 – 19
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18 – 19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
	31b	Authorship eligibility guidelines and any intended use of professional writers	20 - 21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not uploaded, but are available upon request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	19

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The rationale and design of a randomized controlled trial testing the effect of personalized diet in individuals with prediabetes or type 2 diabetes mellitus treated with metformin

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Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS, DIABETES & ENDOCRINOLOGY

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The rationale and design of a randomized controlled trial testing the effect of personalized diet in individuals with prediabetes or type 2 diabetes mellitus treated with metformin

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Abstract

Introduction Metformin and diets aimed at promoting healthy body weight are the first-line in treating type 2 diabetes mellitus (T2DM). Clinical practice, backed by clinical trials, suggests that many individuals do not reach glycaemic targets using this approach alone. The primary aim of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) study is to test the efficacy of personalized diet as adjuvant to metformin in improving glycaemic control in individuals with dysglycemia.

Methods and Analysis PREDICT is a two-arm, parallel group, single-masked randomized controlled trial in adults with prediabetes or early-stage T2DM (with HbA1c up to 8.0%), not treated with glucose-lowering medication. PREDICT is conducted at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney). Enrolment of participants commenced in December 2018 and expected to complete in December 2021. Participants are commenced on metformin (Extended Release, titrated to a target dose of 1500 mg/d) and randomized with equal allocation to either (1) the Personalized Nutrition Project (PNP) algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, designed to provide caloric restriction (75%) in individuals with body mass index >25 kg/m^2. Treatment duration is 6 months and participants visit the Clinical Research Facility 5 times over approximately 7 months. The primary outcome measure is glycated haemoglobin (HbA1c). The secondary outcomes are (1) time of interstitial glucose <7.8 mmol/L and (2) glycaemic variability (continuous glucose monitoring), (3) body weight, (4) fat mass and (5) abdominal visceral fat volume (dual-energy X-ray absorptiometry), serum (6) low-density lipoprotein (LDL)-cholesterol (7) high-density lipoprotein (HDL)-cholesterol and (8) triglycerides concentrations, (9) blood pressure, and (10) liver fat (Fibroscan).

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional Review Board (File 528-3, Rehovot, Israel). The findings will be published in peer-reviewed open access medical journals.

The study has been registered at ClinicalTrials.gov (NCT03558867).

Strengths and limitations of this study

- The randomized controlled design testing a novel diet against standard of care may lead to a novel tool to manage dysglycemia in individuals requiring metformin
- Conducted in an adult Australian population, the study findings may not be applicable to other populations
- The algorithm used to devise the personalized diet relies on accurate recording of the dietary intake by the participants
- The dietary intervention requires use of a smartphone application which may limit its applicability to some populations

Key words: Prediabetes, Type 2 diabetes, Metformin, Algorithm-based diet, Low fat diet, Randomized controlled trial

Word count: 3927

Introduction

Type 2 diabetes mellitus (T2DM) and its preceding medical condition, prediabetes are significant risk factors for cardiovascular disease, and most affected individuals demonstrate additional metabolic risk factors, such as hypertension, dyslipidaemia, excess weight and fatty liver[1]. T2DM affects approximately 422 million adults globally, with an additional 352 million individuals at increased risk, having prediabetes[2 3]. Individuals diagnosed with prediabetes or T2DM are encouraged to adopt a healthy lifestyle and, if overweight to lose weight[4]. The majority of individuals with T2DM are treated with metformin, which is the 8th most prescribed medication in the United States[5-7].

Metformin, an oral biguanide, is the first-line treatment for individuals with newly-diagnosed T2DM and, in some cases, for the prevention of diabetes in individuals with prediabetes[6]. Metformin is an ideal medication to initiate for management of T2DM or for prevention of diabetes, because it does not cause hypoglycaemia and has a favourable, albeit modest, effect on body weight[8]. Metformin monotherapy is insufficient to achieve glycaemic control in a large proportion of treated individuals[3 9 10]. Findings from the Diabetes Prevention Program (DPP) in individuals with prediabetes suggested that the glycaemic efficacy of metformin depends on the magnitude of weight loss[11], explaining 64% of the diabetes risk reduction, with additional 17% explained by decreases in fasting insulin and pro-insulin at 3 years of follow up[11 12].

The current treatment guidelines in T2DM recommend prescribing metformin in combination with a healthy lifestyle, enabling weight loss[13]. The most recent nutritional guidelines for individuals with T2DM or prediabetes are no longer supporting a universal ideal dietary macronutrient distribution; instead, the guidelines suggest individualized eating plans[4].

In the pioneering Personalized Nutrition Project (PNP)[14], Segal, Elinav and colleagues developed an algorithm that predicts an individual's postprandial glycaemic response (PPGR) to meals. The algorithm incorporates the individual's personal data (e.g. age, gender, body mass index [BMI]), blood tests (e.g. glycated haemoglobin [HbA1c]), dietary features, continuous glucose monitor (CGM)-derived data, and gut microbiome features, and trained on data previously collected in 800 individuals. Personally-tailored dietary plans based on the algorithm were trialled in a small group of individuals with prediabetes and shown to improve glycaemic variability and PPGR over 7 days[14].

The primary objective of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) Study is to compare glycaemic control, measured by HbA1c, following 6 months of metformin, prescribed with either (1) the PNP algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, based on the Australian Healthy Eating Guide[15] and the American Association of Clinical Endocrinologists guide for medical care of patients with obesity[16], in individuals with prediabetes or early-stage T2DM naive to glucose-lowering pharmacotherapy.

The secondary objectives of the PREDICT study are to compare the effect of the PNP diet versus LFHF diet when prescribed with metformin on: (1) time of interstitial glucose <7.8 mmol/L, (2) glycaemic variability, (3) weight, (4) body fat mass, (5) abdominal visceral fat volume, (6) serum low-density lipoprotein (LDL)-cholesterol concentration (7) serum high-density lipoprotein (HDL)-cholesterol concentration, (8) serum triglycerides concentration, (9) blood pressure (10) liver fat. The exploratory objectives of the study are to test the effect of the treatment on the gut microbiome.

METHODS

Study design, setting and population

The study is a two-arm, parallel group, single-masked randomized controlled trial (RCT). Adults with prediabetes or early-stage T2DM who are not treated with glucose-lowering medications are randomized, with equal allocation, to either the PNP or LFHF diet arms, in both arms participants are commenced on metformin Extended Release (XR) 1500 mg/d treatment for 6 months. All the study visits are performed at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney). Metagenomics and data processing for the personalized dietary interventions are performed at the Weizmann Institute of Science (Rehovot).

Patient and public involvement

Patients or the public were not involved in the design or other aspects of the research.

Eligibility

Adults (20-70 years old) with prediabetes or recently (in the last 6-months) diagnosed T2DM with (HbA1c ≤8.0%), not pregnant or planning to become pregnant during, and for at least 3 months after the study are recruited (Table 1). A wide age range was selected to encompass different populations of individuals managing their prediabetes for short or long durations and to increase the likelihood to recruit the sample size in a timely manner. The HbA1c cap at 8.0% was selected to ensure that individuals with T2DM are relatively well-controlled. Blood tests indicative of normo-glycaemia, HbA1c above 8.0%, liver enzymes (ALT and/or AST) over 3 times the normal range limit and eGFR lower than 45 mL/min/1.73m^2 are grounds for exclusion. Individuals treated with glucose-lowering medication other than metformin in the

last 24 months, or metformin in the last 3 months, will be excluded. Individuals with conditions or treatments that affect glycaemia (e.g. oral steroids), impact weight (e.g. bariatric surgery, weight loss medications), or the gut microbiome (e.g. inflammatory bowel disease, coeliac, frequent antibiotic treatment) will be excluded. Participants who have had a cardiovascular event in the previous 6 months, or received an investigational new drug within the last 6 months will be excluded. Participants with conditions that may interfere with the ability to understand the requirements of the study and those who refuse treatment with metformin or refuse to use the smartphone application will be excluded (Table 1).

Recruitment

The study is advertised in general practitioners (GP), endocrinologists and dieticians' practices in the Sydney metropolitan area, and through targeted social media campaigns. A collaboration with Blacktown Mt Druitt Hospital (Western Sydney) has been established in 2019 for the purpose of recruitment. During a hospital screening program ran between 2016 – 2018, 17.3% and 30.2% of individuals visiting the Emergency Department (ED) at Blacktown Mt Druitt Hospital have had HbA1c values indicative of diabetes and prediabetes, respectively[17]. Since September 2019, individuals visiting the ED who have had a blood test indicative of prediabetes receive a letter prompting them to contact the PREDICT team and encouraging they share the result with a GP.

To-date (February 2020), 38 participants were enrolled. Of the 38 participants enrolled, 20 completed, 13 are ongoing, and 5 withdrew before the end of the treatment (13% drop-out rate). Recruitment of participants is expected to complete in December 2021.

Participants contacting the team receive the participant information sheet (Supplementary material) via email or post. Experienced clinical research nurse/associate provides details about the study over the phone. Willing participants are referred to a commercial pathology to perform an oral glucose tolerance test (OGTT, 75 g) and HbA1c test. They are asked to sign a consent form after reading the participant information sheet explaining the possible risks of undergoing the OGTT and HbA1c tests prior to performing the blood tests. If the blood tests indicate either T2DM (with HbA1c ≤8.0%), or impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or HbA1c ≥5.7, they are invited to a screening and enrolment visit at the Garvan Clinical Research Facility.

A stool collection kit for metagenomics OMNIgene GUT (OMR-200; DNA Genotek) is mailed to participants prior to the screening/enrolment visit. Participants collect the sample according to the manufacturer's instructions the day before the visit and keep the sample at room temperature. At the Clinical Research Facility, the sample is vortexed, centrifuged for a few seconds and material aliquoted into cryo vials and kept in -80C freezer. One vial is transferred to facilities at the Weizmann Institute of Science and stored at −20°C until DNA extraction.

The pre-treatment data are collected across the screening/enrolment and the baseline visits.

Screening / enrolment procedures and measurements

During the screening/enrolment visit, participants sign the study informed consent form (Supplementary material) and undergo medical examination by a physician. Participants have their weight, height, waist and hip circumference and blood pressure measured. Basal metabolic rate (BMR) is estimated using bioelectrical impedance (BIA, used for calculating the energy requirement, see "Energy target"). Blood samples are collected to evaluate liver

(liver enzymes) and kidney (creatinine and eGFR) function and full blood count. Glucose monitor (FreeStyle Libre Pro, Abbott, Germany) is attached for a period of 14 days.

A link to download the PNP smartphone application is sent to the participants prior to the screening/enrolment visit. They are asked to log-in to the app with a personal (re-identifiable) code provided by email, and to familiarize with the app in preparation for a training session with the dietician. During the screening/enrolment visit, the dietitian practices with the participants browsing the food database, selecting food and beverage items and indicating the amount consumed. Participants are taught to add frequently consumed foods to a favourites list, which makes future search of food items easier. When the CGM is on, participants are asked to carry on with their usual routine and to record all meals, snacks and drinks using the app. The period between the screening/enrolment and the baseline visits (4 – 6 weeks) serves as the 'run-in' period.

Randomization

Randomization is performed between the screening/enrolment and baseline visits. Individuals are randomized with 1:1 allocation into the 2 arms in rounds of approximately 4 individuals each, with randomization performed within each round using the minimization program for allocation of subjects to parallel groups, modified from Saghaei et al[18]. They are stratified by gender, age (20-49 or 50-70 years), BMI (<25.0 or >25.1 kg/m^2) and HbA1c (<5.7 or >5.8 %). To avoid bias, the randomization is performed by a study investigator located at the Weizmann Institute who does not interact with the study participants. The study nurses and physicians who have direct contact with the participants are blinded to the randomization order, however due to the nature of the intervention, the study dietician is not blinded to the treatment allocation.

Baseline visit - Measurements

The baseline visit is performed approximately 4-6 weeks after the screening/enrolment visit (Table 2). Participants attend the Clinical Research Facility following an overnight fast. Blood is drawn for serum lipids measurement and anthropometric measures and blood pressure are taken. Arterial stiffness (pulse wave analysis, AtCor Medical, Australia) is measured twice and average recorded, as described[19]. This is followed by measurement of resting energy expenditure, carbohydrate and fat oxidation over 30 minutes by indirect calorimetry (Quark, Cosmed, Italy)[20]. Body composition is assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare). Specifically, total body fat mass and fat-free mass (enCORE software), the android and gynoid region, and visceral fat (CoreScan software, GE Healthcare) are recorded[21]. Liver steatosis (controlled attenuation parameter, CAP) and liver fibrosis (liver stiffness measurement, LSMs) are assessed using FibroScan (Touch 502 by Echosens) by a trained technician. CAP and LSMs have been reported to correlate closely with steatosis and fibrosis assessed using the gold-standard liver biopsy[22]. A physical activity monitor (ActivePal, Pal Technologies) is applied on the thigh for a period of 14 days. At the end of the baseline visit, the participants practice using the app with the dietician.

Prediction of postprandial glucose response using the algorithm

The prediction of PPGR in PREDICT follows the modelling framework described in Zeevi et al[14] and is performed between the screening/enrolment and baseline visits. Time-stamped food records from the app, CGM and other data collected during the enrolment visit at the Garvan are shared with a mathematician at the Weizmann Institute of Science where data processing occurs, on an Institutional secured server. The data, together with the stool metagenome sequencing data are integrated with the Weizmann Institute's database to develop personalized

algorithms for predicting each individual's PPGR. A database of recipes of meals (n=233) and smaller meals ("snacks", n=249) varying in macronutrient composition to generate feedback on the PPGR to pre-consumed meals has been created. Using the participants' features, personalized PPGR are calculated for every meal and snack in the database based on nutrient composition, and energy-adjusted quintile cut-offs of PPGR are used to create personalized meal ratings ranging from 1 to 5 (corresponding with "excellent", "good", "medium", "bad", and "very bad"). The predictive model, originally trained on data collected in an Israeli adult cohort[14], has been shown to be predictive of PPGR in a U.S. cohort of healthy adults (n=327) consuming a Western style diet[23].

Interventions

Both arms use the PNP mobile app to select meals/foods. In 2018, the app, developed at the Weizmann Institute of Science, was adapted to Australian consumers including the Australian food database (AUSNUT 2011–13)[24] of approximately 5,700 food items.

Personalized diet (PNP diet arm)

Participants in the PNP diet arm receive personalized feedback on each of their food item/meal choice and asked to consult with the app in real-time to select the recommended meal for them. The feedback is color-coded with a traffic light system; green ("good" and "excellent"), yellow ("medium") and red ("bad" or "very bad") PPGR (Figure 1 A and B). Participants are advised to aim for as many "good" and "excellent" scores, occasional "medium", and to avoid "bad" and "very bad" scores. When receiving bad scores, they are advised to trial substituting, adding or removing ingredients from the meal to improve the score. In individuals with hypercholesterolemia, a special set of recipes containing reduced saturated fat are uploaded into the smartphone app.

Low-fat high fibre diet (LFHF) arm

The diet comparator chosen in the present study (the standard of care, LFHF) follows the Australian Healthy Eating Guide[15] principles. The LFHF diet is designed to provide approximately 30% of the total daily energy intake from fat, of which up to 10% of the fat is saturated fat, 50-55% of energy from low glycaemic load carbohydrates, 20-25% from protein and 30 g/d of dietary fibre. The diet is rich in legumes, poor in white grains and added sugar. Overall, the comparator diet chosen is considerably different from the diet of the average adult Australian[25]. A database of recipes (n=110 meals and n=80 snacks) following the LFHF nutrient content has been created based on the AUSNUT 2013 recipes[24]. Food items such as sugary drinks, processed meat, candies, sugar and cream were excluded from the LFHF recipes. Similar to the PNP arm, recommended meals scaled to the individual's energy target are uploaded into the participants' app, taking into account the individual's dietary restrictions and likes. Similar to the PNP arm, participants of the LFHF arm are encouraged to choose from the recipes uploaded for them or browse the food database (5700 food items) to design their own meals, as long as they follow the general dietary guidelines. Participants of the LFHF arm are instructed to consult with the total daily energy and macronutrient breakdown charts to ensure they follow the recommended diet (Figure 1C).

Energy target and using the app to select meals in real-time (both arms)

The energy requirement calculation is based on BMR estimated by BIA (Tanita, TBF-300 by Wedderburn) and on the Mifflin equation[26]. The two values are multiplied by a physical activity factor of 1.4 (lightly active) then averaged, and the value compared with the average daily energy intake of at least 7 days, extracted from the time-stamped meals recorded using the app. In participants with BMI >25 kg/m^2, energy target of 75% is prescribed. Participants

of both arms are encouraged to consume 3 bigger (breakfast, lunch, dinner) and 3 smaller (snack) meals spread throughout the day.

Metformin (relevant to both study arms)

Metformin (XR) is dispensed by the St Vincent's Hospital Pharmacy (Sydney) at baseline (for a period of 3 months) and at the 3 months visit (to last until the end of the study). The target dose (1500 mg/d) is titrated over 3 weeks to minimize gastrointestinal intolerance. A target dose of 1000 mg/d is set for participants with mild to moderately decreased eGFR (45 – 59 mL/min/1.73m^2), or participants who cannot tolerate the higher dose. A standardized dose of 1500 mg/d, rather than 2000 mg/d, was selected to suit both participants with prediabetes and T2DM, while minimising intolerance. Participants are instructed to take the medication with the evening meal and record it daily using a medication recording screen in the app or in paper log-books.

Monitoring and adherence evaluation (relevant to both study arms)

The dietician reviews scores calculated programmatically based on the frequency of using the app and on meeting the daily energy target, along with the time-stamped meals consumed by the participants daily. In the PNP arm, the score incorporates the proportion of meals achieving the desired ("excellent" and "good") scores, while in the LFHF arm, the proportion of days in which dietary fat ≤35%, saturated fat ≤10%, carbohydrates ≥45%, and dietary fibre ≥15 g. The dietician contacts individuals who need encouragement to achieve better scores. Participants of the 2 treatment groups receive the same attention according to their adherence. Time devoted to each individual by the dietician is recorded for later analysis purposes. Satisfaction with the diet is assessed using the Diet Satisfaction Questionnaire[27] at 6 months (Table 2).

Adherence to the metformin is based on pill counting at the 6-months visit and on logs of daily dose using the app and/or log-books.

Physical activity and other confounders

Participants are asked to maintain the same level of physical activity throughout the study. Physical activity is monitored at 2 time points during the study using ActivePal (Table 2). The device records time (start and duration) and type (quiet, standing and steps) of activity and 14-days' worth of data, stored in the device, downloaded upon return. Information about background medications and nutritional supplements is collected before the start of the treatment using questionnaires (Table 2). Participants are asked to report any change in medications at each of the study visits and using the medication screen in the smartphone app.

Primary and secondary outcomes – Measurements

Participants attend the Clinical Research Facility five times during the study, over approximately 7 months. Primary and secondary endpoint measures are collected before the start of the intervention (across 2 visits: screening/enrolment and baseline) and at 3 and 6 months of treatment. Table 2 outlines the measurements obtained at each of the study visits/events.

Study outcomes

The primary outcome measure is change in HbA1c from baseline to 6 months of treatment. Furthermore, a comprehensive glycaemia assessment is enabled through continuous glucose monitoring. Interstitial glucose concentrations are recorded every 15 min using CGM for 14 days before the start of the intervention (in the run-in period) and after 3- and 6- months of

intervention. The sensor stores the data for the duration of the recording, while the participants are blinded to the glucose readings. The data are downloaded upon return of the sensor. Time of the day with glucose readings below 7.8 mmol/L before versus after the treatment will be compared. Glycaemic variability[28], including (1) mean amplitude of glucose excursion (MAGE, a measure of the variation of glucose concentrations from the mean), (2) the standard deviation and the (3) mean postprandial area under the curve will also be assessed. Furthermore, fasting plasma glucose, 1-hr and 2-hr plasma glucose post 75 g glucose assessment are repeated after 6 months and will be compared to the baseline values. HbA1c test is repeated 6 months after treatment cessation (at 12-months, Table 2), along with a short questionnaire, including weight, diet and medication status.

Weight, waist and hip circumferences are recorded at 3-months and 6-months of treatment and compared to baseline. Fat, fat-free mass and android/gynoid fat distribution and visceral fat measurements by DXA are repeated at 6 months treatment[21]. Similarly, resting energy expenditure and fat/carbohydrate oxidation is measured after 6 months of treatment.

Hepatic steatosis is common in prediabetes and T2DM[1 29] and prevention of liver steatohepatitis is key target in individuals with prediabetes or T2DM. Metformin primarily targets the liver, inhibiting lipogenesis and increasing fatty acid oxidation; therefore a beneficial effect on liver lipid and fibrosis with metformin has been assumed[30]. However, comprehensive meta-analyses of randomized clinical trials concluded that reduction in both steatosis and fibrosis with metformin were underwhelming[31 32]. Liver fibroscan measure is repeated after 6 months of treatment.

Blood pressure and pulse wave analysis measurements are repeated at 3 and 6 months of treatment and serum lipids measured after 6 months treatment.

Safety / Adverse events monitoring

Gastrointestinal side effects are the most common adverse effects of metformin and may occur in 20-30% of individuals[33 34]. Specifically, abdominal discomfort, nausea, diarrhoea and anorexia are common[33]. While the gastrointestinal adverse effects are transient, in approximately 5% of individuals the symptoms may persist and result in cessation of metformin[34]. Vitamin B12 concentrations may be lower with metformin, if metformin is administered for a long duration[35 36]. The mechanism(s) responsible for the lower plasma B12 concentrations are unclear. A very rare, but potentially fatal complication of metformin use is lactic acidosis, mainly in patients with renal impairment[33 37]. In PREDICT, individuals with severe renal impairment are excluded. Metformin is titrated over 3 weeks to negate the potential gastrointestinal side effects. Adverse events are recorded and monitored during the study visits.

Statistical analysis

Sample size calculation

Based on the primary outcome measure HbA1c, to detect a clinically meaningful difference of 0.4% in the change of HbA1c from baseline between the study arms at 6 months, assuming SD of 1% for both groups[38], with 80% power at two sided significance level of 0.05, a sample size of 106 for each arm is required. Hence, with an estimated dropout rate of 20%, we aim to enrol 132 individuals to each arm, totalling 264 individuals in the study.

Analysis plan

The intention-to-treat (ITT) approach will be used for efficacy analysis. A likelihood-based mixed model repeated measures (MMRM) approach will be used for the primary efficacy analysis. The primary outcome measure HbA1c at baseline, 3, and 6 months will be the dependent variable and intervention by time interaction will be the fixed effects, and participants will be treated as random effect. The primary time specific comparison will be the difference in least square mean between intervention (PNP) and control (LFHF) diet at 6 months' treatment. The differences between the groups after 3 months of treatment will also be examined. Missing data will be handled directly through maximum-likelihood estimation via mixed modelling. To control for potential confounding effects, demographic and clinical covariates (e.g. age, gender, baseline BMI and background medications) will be adjusted as necessary in the model. To account for reduced metformin dose due to intolerance or eGFR 45 – 60 mL/min/1.73m^2, metformin dose status (1500 mg/d [normal] dose / reduced dose) will be also adjusted in the model. Piecewise linear mixed model will be used to compare trend change between arms in different periods (0-3 months, and 3-6 months). Different statistical analysis strategies including t-test, Mann-Whitney U test, Chi-square test, linear/generalized linear regression, and mixed model will be used based on the type and distribution of the outcome measures. Mediation analysis will be carried out to explore if the weight loss mediates the intervention effect on glycaemia, and estimate indirect and direct effects and the proportion mediated (how much of the total intervention effect works through weight loss). We expect some degree of weight loss in all participants, as has been reported for metformin[3 39 40]. The effect of the diet intervention mediated by metformin adherence on the study outcomes will also be tested. Subgroup analyses can be further performed to explore the intervention effect in specific sub-cohorts, for instance, the group of participants who have diabetes at baseline, the group of participants who achieve adherence standard and maintain the desired metformin dose; the group of participants with BMI >25 kg/m^2 at baseline, etc. The potential impact of

the COVID-19 pandemic on the study outcomes may be explored, including comparisons of adherence and outcomes across groups of participants enrolled and followed-up pre-, during, and post- pandemic period. Sensitivity analysis related to the impact of COVID-19 may be conducted.

Laboratory testing

HbA1c is analysed using high performance liquid chromatography (Bio-Rad D-100, Bio-Rad Laboratories, Inc), plasma glucose using the Cobas 8000 (Roche), and liver and renal function tests using the Atellica platform (Siemens). Serum lipid profile is analysed by a spectrophotometric assay (Advia® 2400 Chemistry System [Siemens Medical Solutions Diagnostics]), with low-density lipoprotein (LDL) calculated using the Friedewald equation. Metagenomic DNA from the stool samples is purified using DNeasy PowerMag Soil DNA extraction kit (Qiagen) optimized for Tecan automated platform. Next-Generation Sequencing (NGS) libraries are prepared using Nextera DNA library prep (Illumina) and sequenced on a NovaSeq sequencing platform (Illumina). Sequencing is performed with 100bp single end reads with the depth of 10 million reads per sample. Host DNA is detected by mapping reads to the Human genome with inclusive parameters, and those reads removed. Bacterial relative abundance estimation is performed by mapping bacterial reads to species-level genome bins (SGB) representative genomes[41]. Mapping is performed using Bowtie[42] and abundance estimated by calculating the mean coverage of unique genomic regions across the 50 percent most densely covered areas, as previously described[43].

Confidentiality and data storage

Each participant is associated with an individual program-generated code used to identify their study documents, data and specimens collected during the study. The re-identifiable code is documented in the participant's record and on all study documents. Study data are collected and managed using REDCap electronic data capture tools[44 45] hosted at the Garvan Institute of Medical Research. Some coded data are shared with essential personnel at the Weizmann Institute of Science on institutional Dropbox. Data collected in the form of paper hard copies are kept in locked cabinets and electronic files on a password protected folder with access granted to the Garvan study team. Re-identifiable blood, stool, plasma and serum samples will be kept at the Garvan Institute's freezer facility. All the study questionnaires are disseminated using REDCap.

Dissemination of results

The results of the study will be disseminated to healthcare professionals via open access publications in medical journals, without any restrictions. Upon completion of data analysis, the participants will be invited to an information session at the Garvan Institute of Medical Research with the study Investigator(s) where the findings of the study will be shared and discussed. Individual letters are disseminated to the study participants after the 6 months treatment visit (approximately 7 – 8 months from study enrolment) summarising individual results (e.g. baseline and post treatment weight, body fat, liver fat, HbA1c, fasting, 1-hr and 2-hr plasma glucose concentrations). The participants are encouraged to share their individual results with the GP.

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional Review Board (File 528-3, Rehovot, Israel). Protocol modifications are communicated to the

SVH HREC, the Weizmann IRB, the trial registry (ClinicalTrials.gov), the study investigators and the study participants (if relevant). De-identified participant data that underlie the findings reported in the research article will be available immediately following publication, ending 5 years following the article publication, to researchers who provide a methodologically sound proposal with the aim to achieve the aims reported in the approved project proposal. Data may be obtained from the Principal Investigator Dorit Samocha-Bonet or Associate Investigator Jerry Greenfield upon enquiries directed to d.samochabonet@garvan.org.au or j.greenfield@garvan.org.au.

Authors' contributions

DS-B, JRG, ES and EE contributed to the conception and design of the study, TDH, NG and DS-B drafted the manuscript, NG and ZL contributed to the study design and the statistical plan, ZL contributed to the sample size estimation, NG, DK, RC, KT, EC, MD, JRS and T-MH contributed to the collection of the data. All authors revised and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The study sponsor and funding bodies have no role in the study design, collection, management, analysis, and interpretation of data, writing of the report and the decision to submit the report for publication.

Conflict of interests

Eran Elinav and Eran Segal are paid consultants of the company DayTwo. Mark Danta has received travel support and speaker fees from Gilead, Abbvie and Merck. All other authors declare they have no conflict of interest.

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Figure legends

Figure 1: Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

Table 1: Inclusion and Exclusion criteria

Inclusion criteria
Men and women 20-70 years of age
Prediabetes (IFG* or IGT* and/or HbA1c 5.7 – 6.4%) or Individuals diagnosed with T2DM in the last 6 months with HbA1c ≤ 8.0%
Willingness to provide written informed consent, participate and comply with the study
Exclusion criteria
Women planning pregnancy during the study or 3 months after study completion
Individuals with type 1 diabetes, neoplastic disease (in the last 3 years), cardiovascular event (-6 months), chronic gastrointestinal disorders
Liver enzymes ALT and/or AST >3 times normal range limit
eGFR<45 mL/min/1.73m^2
Normo-glycaemia
HbA1c > 8.0%
Current or recent (within 24 months) treatment with a glucose lowering medication other than metformin, current or recent (within 3 months) treatment with metformin, current treatment with an oral steroid, immunosuppressive medications, antibiotics (within 3 months)
Alcohol or substance abuse
Participants who had received an investigational new drug within the last 6 months
Participants involved in another clinical study
Participants who have had bariatric surgery
Participants who actively lose weight
Participants with conditions that may interfere with the ability to understand the requirements of the study, refuse treatment with metformin or refuse to use the smartphone application

*Impaired fasting glucose (IFG): FPG 5.6-6.9 mmol/L and/or impaired glucose tolerance (IGT): 2-h plasma glucose during 75-g oral glucose tolerance test (OGTT) 7.8-11.0 mmol/L

Table 2: Study timeline of activities and measurements taken at each of the study events and visits

	Location and time	***Measurements***
Pre-screening	Phone -35 +/- 10 days	• Interview (by an experienced staff)
	Local pathology	• 75 g OGTT and HbA1c tests
Screening/ enrolment (Non-fasting)	Clinical Research Facility -30 +/- 15 days	• Informed consent signed • Medical history, medications & medical examination • Blood (full blood count, liver and kidney function tests) • Height and weight (BMI calculated) • BIA (RMR) • Blood pressure • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelope) • Smartphone app training
	Home	• Stool sample • *Study entry questionnaire
Baseline (Fasting)	Clinical Research Facility 0, treatment clock starts	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin 3-months' supply with instructions and log-books • Smartphone app training, according to study arm allocation
3-months (Fasting)	Clinical Research Facility +90 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (HbA1c) • Indirect calorimetry (REE, RQ) • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelop) • Metformin 3-months' supply with log-books • Adverse events recording and monitoring
	Home	• #Stool sample
5.5-months (Non-fasting)	Clinical Research Facility +166 days +/- 10 days	• FreeStyle Libre Pro and ActivePal
	Local pathology	• 75 g OGTT and HbA1c

6-months (Fasting)	Clinical Research Facility +180 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids, HbA1c) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin- unused pills collected and counted • Adverse events recording
	Home	• #Stool sample • ##Diet Satisfaction Questionnaire
12-months	Local pathology	• HbA1c
	Home	• **12-months questionnaire

*Questionnaire includes hunger/fullness, dietary habits and dislikes, physical activity, medications, dietary supplements, personal and family history of disease, food-frequency questionnaire

**Questionnaire includes diet (are you following the diet you were allocated to?), metformin (are you continuing the metformin treatment and dosage) and current body weight

#Gut microbiome features from stool samples collected at 3 and 6 months will be compared to baseline (exploratory outcome)

The Laboratory for the Study of Human Ingestive Behavior, Pennsylvania State University are the copyright holders of the Diet Satisfaction Questionnaire[27]

Abbreviations: BIA, bio impedance analysis; BMR, basal metabolic rate; DXA, dual-energy X-ray absorptiometry; REE, resting energy expenditure; RQ, respiratory quotient

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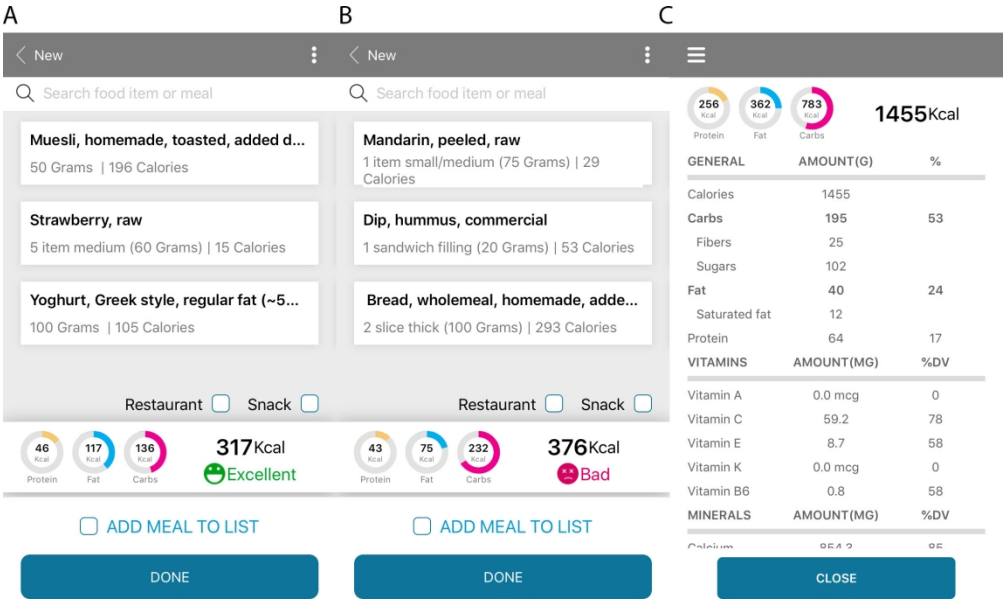

Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

190x112mm (300 x 300 DPI)

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

Garvan Institute of Medical Research

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s) *(if required by institution)*	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location *(where CPI/PI will recruit)*	*Garvan Institute of Medical Research, Sydney*

Part 1 What does my participation involve?

1 Introduction

Type 2 diabetes affects 8.5% of the world's adult population. Prediabetes is a condition developing many years before type 2 diabetes, and is diagnosed by raised glucose (sugar) in the blood stream, either when measured first thing in the morning (fasting) or when measured after a meal. Approximately 30 - 40 % of the world's adult population is affected by prediabetes. Individuals with prediabetes have a higher risk of diabetes and heart disease. Treatment with the most commonly used sugar-lowering medication metformin does not always improve blood sugar levels in individuals with prediabetes or type 2 diabetes. This means that after the treatment, blood sugar concentrations are still higher than normal and the risk of diabetes remains increased.

You are invited to take part in this research project because you either have prediabetes or you have recently been diagnosed with type 2 diabetes. The research project is testing the effect of a personalised diet combined with the medication metformin on sugar control, body weight, and other risk factors.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your General Practitioner.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

• Understand what you have read

• Consent to take part in the research project

• Consent to have the tests and treatments that are described

• Consent to the use of your personal and health information as described

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

The overall aim of the study is to improve the treatment of individuals with prediabetes or type 2 diabetes with a special nutritional intervention.

In particular, we would like to find out whether a personalised diet administered with a medication commonly used to treat diabetes named metformin will achieve better results when compared with a low fat low sugar diet (the type of diet commonly recommended to individuals with prediabetes or type 2 diabetes) administered with metformin.

We will compare the effects of the different treatments on sugar control, body weight, body fat, blood lipids, blood pressure and the amount of fat in the liver.

The medication used in this research project and listed below is approved in Australia to treat type 2 diabetes. While it is not routinely prescribed to treat prediabetes, metformin has been used to treat individuals with prediabetes in a large American trial and has been proven to be safe in individuals with prediabetes (the Diabetes Prevention Program, United States).

<u>The following medication/treatments will be used in the study:</u>

1. Metformin Extended Release (XR) + Low fat low sugar diet (“healthy” diet).
2. Metformin XR + “Personalised diet” (see further details below).

In both study arms, if your weight falls in the overweight or obese category (i.e. body mass index [BMI] > 25 kg/m^2), we will recommend a reduction of approximately 500 Kcal/d from the dietary energy you are currently on. This will result in moderate weight loss. If your weight is within the recommended BMI range (equals to or under 25 kg/m^2), we will recommend the same level of energy intake you are currently consuming. This will result in weight maintenance.

<u>Method of administration of medication:</u>

Metformin XR – Daily tablet (oral ingestion 1500 mg/d) with evening meal

<u>Method of administration of diet:</u>

The diet will be delivered using a smartphone application. You will be guided and supported by an Accredited Practising Dietitian

You will be participating in a randomised research project. Sometimes we do not know which treatment is best for treating a condition. To find out, we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better than the other. To try to make sure the groups are the same, each participant is put into a group by chance (random).

We will administer 1 medication in combination with 2 different nutritional interventions. There is a 1 in 2 chance that you will be allocated to each study arm.

This research has been initiated by the study doctors, Dr Dorit Samocha-Bonet and Professor Jerry R Greenfield.

This research has been funded by a Foundation requesting to remain anonymous, funds from the Garvan Research Foundation and a grant from the St Vincent's Clinic Foundation.

3 What does participation in this research involve?

If you agree to participate in this study, you will be asked to sign the Participant Consent Form. We will ask you to sign a separate Participant Consent Form before having blood tests at a commercial pathology.

Participation in this project will involve the following:

Initially we will ask you questions regarding your health and medical history over the telephone. If it sounds like you may be eligible for the study we will ask you to attend a commercial pathology service in your area for an oral sugar test (oral glucose tolerance test), testing your blood sugar after drinking a sugary drink, and a blood test measuring glycated haemoglobin (HbA1c, a marker of average blood sugar levels over the past 3 months). This involves having a blood sample taken prior to drinking a sugary drink (containing 75 grams of sugar), followed by blood samples taken one and two hours later.

You will be asked to remain in the blood collection centre for the duration of the test (2 hours).

Overall the commercial Pathology will collect 20 mL (about 1.5 tablespoons) of blood.

These blood tests will tell us if you have diabetes or prediabetes and will help us determine whether or not you are eligible to participate in our study. We will call you and discuss the test results and your eligibility to participate in the study within 1 week. We will provide you with a letter summarising the results which you will be able to share with your General Practitioner.

If you are eligible to participate in the study, we will invite you to a Screening / Enrolment visit. We will send you a special kit for collection of stool at home, with detailed instructions on how to collect the sample. We will ask you to bring the sample with you to your next visit.

Prior to the study we will ask you to reply to questionnaires asking about your usual eating and physical activity habits and your health. You will be asked to download a smartphone application which you will use during the study.

Screening / Enrolment visit (approximately 1.5 hours)

- We will ask that you arrive to the Clinical Research Facility (clinic) at the Garvan Institute of Medical Research, Darlinghurst. During this visit you will have time to ask questions and if you agree to participate, you will sign the consent form. You will change into a hospital gown. The study doctor will ask you about your health and perform a physical examination. We will measure body weight and height, blood pressure, waist and hip circumferences, and body fat composition (estimated using Tanita scales).
- Blood will be drawn to assess your liver and kidney health and your blood cells count. The volume of blood that will be taken during this visit is 20 mL (about 1.5 tablespoons).
- We will attach a small device (sensor) that measures sugar levels for a period of 14 days to your arm. The size of the device is of a fifty cents coin. We will apply the sensor on to the back of the upper arm with a disposable applicator. When the sensor is applied, a thin, flexible sterile fibre is inserted just under the skin. It is held in place with a small adhesive pad. Most people feel no pain when applying the sensor. You will not have to do anything while the sensor measures sugar level. All the information will be stored in the sensor. We will also attach a physical activity sensor. Both sensors are designed to wear for up to 14 days. We will ask that you remove and send the sensors to us after 14 days in a prepaid envelop. You will have your usual diet and record what you eat in a smartphone application.

During this visit, or soon after, you will meet the dietitian (face-to-face or using a video meeting tool like Zoom or Skype). The dietitian will teach you to use the smartphone application. You will use this application to log the food that you eat, and optionally, to log your physical activity (type and duration), and the medications that you take. We will provide you with a light meal at the end of this visit.

After this visit we will randomise you to one of 2 treatment arms.

Baseline visit (2 hours, we will ask that you fast from 10 pm the night before this visit)

The baseline visit will be performed approximately 6 – 8 weeks after the Screening / Enrolment visit. You will arrive at the clinic in the morning. The following measurements and procedures will take place,

- You will undergo dual-energy X-ray absorptiometry (DXA) at St Vincent's Hospital (next door to the Garvan). DXA will be used to evaluate the amount of fat in your body.
- Liver fibroscan, performed at St Vincent's Hospital. This measurement will provide information about the amount of fat and fibrosis (scarring) in the liver.
- A team member will measure your weight, height, waist and hip circumference and blood pressure, while wearing a hospital gown.
- We will measure how many calories you burn while resting using a plastic hood placed over your shoulders while you are resting in bed (takes 30 minutes).
- We will measure the stiffness of an artery in the wrist (radial artery) using a non-invasive device which looks like a pen. This measurement takes approximately 5 minutes and will provide information about the stiffness of your arteries.
- We will collect blood samples. Overall we will collect 20 mL (2 tablespoons) of blood during this visit.
- We will collect a sample of bacteria from the mouth by swabbing a special collection stick over the gums. We will store this sample for testing the effect of the treatment on the mouth bacteria.

At the end of the visit we will provide you with 3-months' worth of medication and medication logbooks. We will ask you to take the metformin every evening **with dinner.** You will start with taking a small dose and depending on how you feel (some people experience diarrhoea, nausea, and flatulence, mostly at the beginning of the treatment), we will gradually ask you to increase the dose until you reach the target dose. You will be guided by the study investigator / dietitian regarding using the smartphone application during the treatment period.

We will call you at a time of your convenience once a month or fortnightly to follow up on your progress with the treatment. We will ask you how you feel and whether you have any general health changes that you would like to report (whether you think they are related or unrelated to the treatment), as well as any changes to your usual medications, other than the study treatment.. Follow-up may be performed using Zoom, Skype, text, or over the phone.

4-months visit (2 hours, similar to the baseline visit, we will ask that you fast from 10 pm the night before this visit)

This visit will be similar to the baseline visit (described above) and include the same procedures, except that you will not have a DXA and fibroscan. We will attach another sugar sensor to the arm, and ask you to send it back to us in a prepaid envelope after 14 days. During this visit we will also take a blood sample that will be used to measure the level of metformin in the blood. We will ask you to bring the medication log books.

6.5-months visits

We will invite you to the Garvan to attach the sugar sensor and activity monitor. We will refer you again to perform a sugar challenge test, as you have done before the treatment in a commercial pathology in your area. We will provide you with a special kit for collection of stool and ask you to bring the sample with you to your next visit at the Garvan.

7-months (end-of treatment, performed 6 months from the start of the treatment) visit

Identical to the baseline visit, with the exception that we will collect a blood sample to measure the concentration of the medication in the blood and will ask you to bring back the medication and the logbooks.

12-months survey and blood test (includes a questionnaire and a blood test at a local commercial pathology)

6-months after the completion of the treatment, we will send you a survey and ask you to tell us what your current body weight is, whether you remained on the diet, and whether you are treated with a sugar-lowering medication (and if relevant to specify the medication and dose). We will also ask you to perform a blood test in a commercial pathology in your area. The blood test will measure sugar control and does not require you to be fasting. You will not have to visit the Garvan for this event.

In summary, you will be required to visit and maintain contact with the study team over a period of approximately 6 months, while you're treated. The overall duration of the study is approximately 13 – 14 months.

There are no costs associated with participating in this research project, nor will you be paid. All medications, dietary advice, tests, including all blood tests, some meals and medical care required as part of the research project will be provided to you free of charge.

You will be provided a $100 AUD retail voucher. Parking will be provided at the Garvan carpark on days of visits.

If you have a General Practitioner, we strongly recommend that you inform them of your participation in this research project.

4 What do I have to do?

During the study period, the study team will instruct you regarding what you should be doing before each of the visits/study events. For example, we may ask you to avoid drinking alcohol or to avoid vigorous physical activity before some of the visits.

During the study you will be able to continue taking your regular medications, but if you will require a sugar-lowering medication other than metformin (prescribed in the study), you will have to withdraw from the study.

We will ask you to inform us if and when you started taking a new medication, or if and when there has been a change in dose of any medication you are taking regularly.

During the study we will ask you not to donate blood or participate in another study.

We will expect you to be compliant with the study instructions and if any problem arises, we expect that you will contact us.

5 Other relevant information about the research project

We plan to enrol approximately 260 men and women to this study. All participants will be enrolled at a single site, the Garvan Institute of Medical Research, Darlinghurst, Sydney.

Some tests will be performed at the adjacent St Vincent's Hospital.

The project involves researchers from a number of academic institutes working in collaboration. The research team includes researchers and endocrinologists from the Diabetes Division at the Garvan Institute of Medical Research and St Vincent's Hospital (Sydney), researchers from the Endocrinology Department at Blacktown & Mount Druitt Hospital (Sydney), and researchers from the Immunology Department and the Computer Science Department at the Weizmann Institute of Science (Israel).

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Garvan Institute of Medical Research or St Vincent's Hospital.

7 What are the alternatives to participation?

Other treatment options for your condition are available; these include dietary counselling and physical activity advice, and / or a sugar-lowering medication. Your study doctor will discuss

these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your General Practitioner.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research. You will have access to a medication regulating blood sugar level that is primarily offered to type 2 diabetes patients and you may lose weight (1 – 5 kg).

We are hoping that the information generated in this study will help improve treatment of your condition.

9 What are the possible risks and disadvantages of taking part?

Taking a medication may involve some risks, as detailed below. We will monitor any adverse events on a regular basis and you will have access to a clinical staff member at all time during the study.

There are potential risks relating to the procedures we will utilise in the course of the study. We have listed them below.

Risks related to the medication

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects regularly.

In case you need to have any diagnostic procedure requiring contrast, most radiological services recommend routine withholding of metformin for 48 hours prior to and after the procedure. Please let your doctor know that you are taking metformin.

There may be side effects that the researchers do not expect and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly in the first few weeks of the treatment or after the treatment ends. However, sometimes side effects can be serious. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

The medication we will administer in the present study, metformin, has been administered to people with type 2 diabetes over many years and has proven to be generally safe. We have listed below the potential side effects of the medication administered.

- Mild gastrointestinal symptoms such as diarrhoea, nausea, vomiting, abdominal pain and loss of appetite are the most frequent reactions to metformin, occurring in approximately 10% of individuals (10 in 100), especially during the initial treatment period. These symptoms are generally transient and resolve spontaneously during continued treatment.
- Taste disturbance may occur in 3% (3 in 100).

- Rare adverse reactions occur in less than 0.01% (1 in 10000) of individuals include,
  - Lactic acidosis, often with mild nonspecific symptoms such as feeling of sickness, sleepiness, shortness of breath, and nonspecific abdominal distress.
  - Abnormalities of liver tests have been reported. Liver function tests will be monitored during the treatment.

Risks related to the personalised nutrition

The Personalised Nutrition Project diet (http://newsite.personalnutrition.org/WebSite/Home.aspx) has been trialled in various studies for over 5 years by the study co-Investigators Professors Elinav and Segal from the Weizmann Institute in Israel. The nutritional recommendation is different from person to person. This is because it is based on the individual's features and measurements, including sugar rise in response to food. Co-Investigators Elinav and Segal have reported that the individual response depends on blood markers, body measurements and different features of microorganisms in the gut. The individual food recommendation is based on "real" foods.

The effects of the personalised diet on the level of blood lipids in individuals with prediabetes and type 2 diabetes is being investigated. If we find that the baseline level of low-density lipoprotein (LDL)-cholesterol in the blood is elevated, a modified personalised diet containing less saturated fat (the type of dietary fat known to increase the level of LDL) will be prescribed.

Risks relating to procedures

Blood sampling

You may have pain, light-headedness, minor infection, bleeding or bruising at the sites of the blood sampling; however, the staff will use proper technique while drawing blood samples in order to reduce the risk for these unwanted effects. These effects are usually temporary, are easily treated, and are expected to resolve completely.

Dual-energy X-ray absorptiometry (DXA) scan

This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 0.04 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal.

Fibroscan test

The fibroscan is a non-invasive method of assessing liver scarring, termed fibrosis. The test is performed at the bedside in a clinic. A mechanical pulse is generated at the skin surface, which is propagated through the liver. The velocity of the wave is measured by ultrasound. The speed of this wave correlates with the stiffness of the liver, which in turn reflects the degree of fibrosis – the stiffer the liver is, the greater the degree of fibrosis. This test also estimates the amount of fat in the liver. This test utilises an ultrasound device and carries no risk.

Body sugar monitoring

The device we use in the study is used to monitor blood glucose levels, mostly in diabetes patients. Most people feel no pain when applying the sensor. The sensor is designed for a safe wear of up to 14 days. You can shower and exercise while wearing the sensor. Some individuals

develop a skin reaction around the device on the arm. This typically resolves spontaneously over time; an over the counter cream may be recommended if the skin reaction persists. If you experience a skin reaction, we will likely decide to omit this measurement from the follow-up events.

Indirect calorimetry

A clear plastic hood is placed loosely over your head. Room air flows through the hood and the machine measures how much oxygen you are using and how much carbon-dioxide you produce. This test carries no significant risk. However, some participants may experience claustrophobia. If this occurs, the hood will be removed immediately.

Arterial stiffness

The stiffness of an artery in your wrist will be measured using a small device, looking like a pen, by applying light pressure to your wrist. There are no risks related to this method.

Tanita scales

The scales estimate how much energy your body requires based on your body fat and muscle composition. When you stand on a Tanita monitor, a very low, safe electrical signal is sent from four metal electrodes through your feet to your legs and abdomen. The electrical signal passes quickly through water that is present in muscle tissue but meets resistance when it hits fat. This resistance, known as impedance, is measured and used in validated equations to calculate body composition. This measurement will take around 20 seconds. Information from the Tanita measurement will be used to estimate your recommended daily energy intake.

People with an electronic medical implant, such as a pacemaker, should not use a body composition monitor because the electrical signal travelling through the body may interfere with its operation.

Pregnancy

The effects of the medication metformin on the unborn child and on the newborn baby are not known. Because of this, it is important that study participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in this study if you are pregnant or trying to become pregnant, or breast-feeding. If you are female and child-bearing is a possibility, you will be required to undergo a pregnancy test prior to commencing the research project, and before each of the DXA scans.

Female participants must avoid pregnancy during the course of the research and for a period of 3 months after completion of the research project. You should discuss methods of effective contraception with your study doctor.

If you do become pregnant whilst participating in the research project, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

If you are a male, it is safe to father a child or donate sperm whilst taking the study medication.

10 What will happen to my test samples?

During the study we will store blood and stool samples in -80°C freezer. In this research project, collecting blood and stool samples are mandatory.

The blood, oral and stool samples collected are for research purposes, as specified in this document.

All the samples we will collect from you will be coded so they are not identifiable as your sample. Your identification linked to the sample code will be stored on a password protected computer and physical hardcopies in a locked filing cabinet at the Garvan Institute of Medical Research. Only staff involved in this study at the site will have access to this data to maintain privacy/confidentiality.

Some analyses in blood will be performed in commercial Laboratories or at St Vincent's Hospital. These specimens will be de-identified (coded). Your clinical information will not be sent with the specimens. The results of these analyses will be returned to the Investigators at the Garvan Institute of Medical Research. The external sites may retain your re-identifiable, analysed data in their records. After analysis of your specimen is complete, these external sites will destroy any remaining tissue or return remaining sample to us for storage.

We will store the samples we collect for 15 years.

Some samples of your blood, mouth and stool obtained for the purpose of this research project will be transferred to the Weizmann Institute of Science in Israel. These samples will be de-identified by coding. Information such as your gender, age, ethnicity, medical history, medications, food sensitivities and eating habits will be shared with researchers at the Weizmann Institute for planning your diet. This information will be de-identified.

Your samples will not be sold and the Garvan Institute of Medical Research will not knowingly transfer your samples to anyone who has expressed intent to sell the samples.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he / she will explain the reasons and arrange for your regular health care to continue.

When we assess your suitability to participate in the study we may uncover a medical condition of which you may be unaware. While we may decide that you are not able to participate in the study because of this new condition, we will refer you to your General Practitioner for further investigation and management.

If during the screening visit we diagnose a previously unknown condition, for example type 2 diabetes, this may affect insurance in the future.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies. You should also tell your study doctor about any changes to these during your participation in the research project.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

Sometimes research projects trialling medications need to stop prematurely because of unexpected side effects. However, this research project is unlikely to be stopped unexpectedly, because the medication administered has been studied and is routinely administered in type 2 diabetes and to a lesser degree in people with prediabetes. The medication administered here has been proven safe in type 2 diabetes patients.

15 What happens when the research project ends?

The medication administered in the present study will not be available after the research finishes through the study (but may be prescribed by your General Practitioner if required and decided). At study completion, you will be able to continue using the app and follow your study diet. We will offer you nutritional counselling on healthy lifestyle that will help you maintain a healthy body weight and decrease your risk of developing diabetes.

After you complete the study treatment (approximately 7 months from when you start), we will send you a detailed results letter, with measurements collected at baseline and after the 6 months' treatment, that will include your weight, BMI, blood pressure, fasting blood sugar, glycated haemoglobin (HbA1c), and the amount of fat in your body and liver.

After we finalise the study of all the participants and analyse the data (approximately in 2022), we will invite you to an information evening at the Garvan Institute of Medical Research (Darlinghurst, Sydney) to share with you the study findings. You will have opportunity to discuss the findings with the study Investigators.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All the clinical information and the data that will be collected will be coded. It can be re-identified; however, your identification matching your code will be secured, accessible only by research staff. Data allowing re-identification of your data will be kept on a password protected computer and a locked filing cabinet at the Garvan Institute of Medical Research. Only staff involved in this study will have access to this data to maintain privacy/confidentiality.

All data will be stored for 15 years, after which it will be destroyed.

Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities and authorised representatives of the Sponsor, the Garvan Institute of Medical Research, the institution relevant to this Participant Information Sheet, St Vincent's Hospital, Sydney, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

It is anticipated that the results of this research project will be published and presented in a variety of forums. In any publication and presentation, information will be provided in such a way that you cannot be identified. No individual data will be reported in any publication or presentation.

Data collected in the study may be made available to other researchers. The data will be coded and will not reveal your personal details. The data will be made available subject to a review of applications submitted by interested researchers performed by the study Investigators Dorit Samocha-Bonet and/or Jerry R Greenfield. Only researchers aiming to achieve the aims of the project will be considered. Upon approval, access to the de-identified data will be available for a period of 5 years after publishing the study findings.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant Australian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

In the event of loss or injury, the parties involved in this study have agreed to provide compensation if the participants' injury or complication is caused by the study procedures or by the negligence of any of the parties involved in the study. If you suffer any distress or psychological injury as a result of this study, you should contact the study team as soon as possible, who will assist you in arranging appropriate treatment and support.

18 Who is organising and funding the research?

This research project is being conducted by Dr Samocha-Bonet and Professor Greenfield.

Dr Samocha-Bonet, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu and Ms Godneva have no conflict of interest to disclose.

The study co-Investigators, Prof Eran Elinav and Prof Eran Segal of the Weizmann Institute of Science are paid consultants of the company DayTwo. DayTwo, established in Israel, provides personalised nutrition to individuals. The product is based on the technology arising from research conducted by Prof Elinav and Prof Segal at the Weizmann Institute of Science. The research findings were published in the journal *Cell* in 2015.

The Garvan Institute of Medical Research may benefit financially from this research project if, for example, the project assists Garvan Institute of Medical Research to obtain approval for a new treatment tool.

By taking part in this research project you agree that samples of your blood or stool (or data generated from analysis of these materials) may be provided to the Weizmann Institute of Science (Israel).

The Weizmann Institute of Science may directly or indirectly benefit financially from your samples or from knowledge acquired through analysis of your samples.

You will not benefit financially from your involvement in this research project even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to the Garvan Institute of Medical Research.

In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to the Garvan Institute of Medical Research or the Weizmann Institute of Science, the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of St. Vincent's Hospital Sydney (HREC reference number 17/080).

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor Dr Dorit Samocha-Bonet on 02-9295 8309 or 0416-316 111.

Clinical contact person

Name	Professor Jerry R Greenfield
Position	Associate Investigator
Telephone	SVH Switchboard 8382 1111, ask to page Prof Jerry Greenfield **24-hour medical contact**
Email	predict@garvan.org.au

Research Nurse

Name	Ms Renee Richens
Position	Research Nurse
Telephone	02-92958215 (Working days: Tuesday, Wednesday, Thursday)
Email	predict@garvan.org.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Research Office Manager
Position	Research Office Manager
Telephone	02 8382 4960
Email	SVHS.Research@svha.org.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Research Officer details

Reviewing HREC name	St Vincent's Hospital HREC
HREC Research Officer	Research Officer
Telephone	02 8382 4960
Email	SVHS.Research@svha.org.au

Research Governance Officer

Name	Ms Therese Yim
Position	Research Governance Officer
Telephone	02-9295 8173
Email	t.yim@garvan.org.au

Consent Form - *Adult providing own consent*

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s)	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location	*Garvan Institute of Medical Research*

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Garvan Institute of Medical Research concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

I consent to the storage and use of blood and tissue samples taken from me for use, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project
- Other research that is closely related to this research project

I consent to sharing de-identified (coded) data collected during the study with other researchers aiming to achieve the study aims as listed, subject to approval by the study Principal Investigator Dorit Samocha-Bonet and/or Associate Investigator Jerry R Greenfield.

Name of Participant (please print)_____

Signature _____ Date _____

Name of Witness* to Participant's Signature (please print)

_____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print)

_____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s)	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location	*Garvan Institute of Medical Research*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with the Garvan Institute of Medical Research.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print)	
Signature	Date

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 20 (Authors' contribution)
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5 - 6
	6b	Explanation for choice of comparators	5 - 6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6 - 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11 – 13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13 – 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6 – 7 and Table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	15 – 16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7 – 8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9 – 10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9 – 10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9 – 10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9 – 10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14 – 16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14 – 16 and 17 (Statistical plan)

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18 – 19
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16 and Table 2
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approval granted, details page 3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9 and Table 2
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18 – 19
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18 – 19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
	31b	Authorship eligibility guidelines and any intended use of professional writers	20 - 21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	19

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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The rationale and design of a randomized controlled trial testing the effect of personalized diet in individuals with prediabetes or type 2 diabetes mellitus treated with metformin

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Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS, DIABETES & ENDOCRINOLOGY

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The rationale and design of a randomized controlled trial testing the effect of personalized diet in individuals with prediabetes or type 2 diabetes mellitus treated with metformin

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Abstract

Introduction Metformin and diets aimed at promoting healthy body weight are the first line in treating type 2 diabetes mellitus (T2DM). Clinical practice, backed by clinical trials, suggests that many individuals do not reach glycaemic targets using this approach alone. The primary aim of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) study is to test the efficacy of personalized diet as adjuvant to metformin in improving glycaemic control in individuals with dysglycemia.

Methods and Analysis PREDICT is a two-arm, parallel group, single-masked randomized controlled trial in adults with prediabetes or early-stage T2DM (with glycated haemoglobin [HbA1c] up to 8.0% [64 mmol/mol]), not treated with glucose-lowering medication. PREDICT is conducted at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney). Enrolment of participants commenced in December 2018 and expected to complete in December 2021. Participants are commenced on metformin (Extended Release, titrated to a target dose of 1500 mg/d) and randomized with equal allocation to either (1) the Personalized Nutrition Project (PNP) algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, designed to provide caloric restriction (75%) in individuals with body mass index >25 kg/m^2. Treatment duration is 6 months and participants visit the Clinical Research Facility 5 times over approximately 7 months. The primary outcome measure is HbA1c. The secondary outcomes are (1) time of interstitial glucose <7.8 mmol/L and (2) glycaemic variability (continuous glucose monitoring), (3) body weight, (4) fat mass and (5) abdominal visceral fat volume (dual-energy X-ray absorptiometry), serum (6) low-density lipoprotein (LDL)-cholesterol (7) high-density lipoprotein (HDL)-cholesterol and (8) triglycerides concentrations, (9) blood pressure, and (10) liver fat (Fibroscan).

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional Review Board (File 528-3, Rehovot, Israel). The findings will be published in peer-reviewed open access medical journals.

The study has been registered at ClinicalTrials.gov (NCT03558867).

Strengths and limitations of this study

- The randomized controlled design testing a novel diet against standard of care may lead to a novel tool to manage dysglycemia in individuals requiring metformin
- Conducted in an adult Australian population, the study findings may not be applicable to other populations
- The algorithm used to devise the personalized diet relies on accurate recording of the dietary intake by the participants
- The dietary intervention requires use of a smartphone application which may limit its applicability to some populations

Key words: Prediabetes, Type 2 diabetes, Metformin, Algorithm-based diet, Low fat diet, Randomized controlled trial

Word count: 3927

Introduction

Type 2 diabetes mellitus (T2DM) and its preceding medical condition, prediabetes are significant risk factors for cardiovascular disease, and most affected individuals demonstrate additional metabolic risk factors, such as hypertension, dyslipidaemia, excess weight and fatty liver[1]. T2DM affects approximately 422 million adults globally, with an additional 352 million individuals at increased risk, having prediabetes[2 3]. Individuals diagnosed with prediabetes or T2DM are encouraged to adopt a healthy lifestyle and, if overweight to lose weight[4]. The majority of individuals with T2DM are treated with metformin, which is the 8th most prescribed medication in the United States[5-7].

Metformin, an oral biguanide, is the first-line treatment for individuals with newly-diagnosed T2DM and, in some cases, for the prevention of diabetes in individuals with prediabetes[6]. Metformin is an ideal medication to initiate for management of T2DM or for prevention of diabetes, because it does not cause hypoglycaemia and has a favourable, albeit modest, effect on body weight[8]. Metformin monotherapy is insufficient to achieve glycaemic control in a large proportion of treated individuals[3 9 10]. Findings from the Diabetes Prevention Program (DPP) in individuals with prediabetes suggested that the glycaemic efficacy of metformin depends on the magnitude of weight loss[11], explaining 64% of the diabetes risk reduction, with additional 17% explained by decreases in fasting insulin and pro-insulin at 3 years of follow up[11 12].

The current treatment guidelines in T2DM recommend prescribing metformin in combination with a healthy lifestyle, enabling weight loss[13]. The most recent nutritional guidelines for individuals with T2DM or prediabetes are no longer supporting a universal ideal dietary macronutrient distribution; instead, the guidelines suggest individualized eating plans[4].

In the pioneering Personalized Nutrition Project (PNP)[14], Segal, Elinav and colleagues developed an algorithm that predicts an individual's postprandial glycaemic response (PPGR) to meals. The algorithm incorporates the individual's personal data (e.g. age, gender, body mass index [BMI]), blood tests (e.g. glycated haemoglobin [HbA1c]), dietary features, continuous glucose monitor (CGM)-derived data, and gut microbiome features, and trained on data previously collected in 800 individuals. Personally-tailored dietary plans based on the algorithm were trialled in a small group of individuals with prediabetes and shown to improve glycaemic variability and PPGR over 7 days[14].

The primary objective of the Personalised Medicine in Prediabetes – Towards Preventing Diabetes in Individuals at Risk (PREDICT) Study is to compare glycaemic control, measured by HbA1c, following 6 months of metformin, prescribed with either (1) the PNP algorithm-based diet or (2) low-fat high dietary fibre (LFHF) diet, based on the Australian Healthy Eating Guide[15] and the American Association of Clinical Endocrinologists guide for medical care of patients with obesity[16], in individuals with prediabetes or early-stage T2DM naive to glucose-lowering pharmacotherapy.

The secondary objectives of the PREDICT study are to compare the effect of the PNP diet versus LFHF diet when prescribed with metformin on: (1) time of interstitial glucose <7.8 mmol/L, (2) glycaemic variability, (3) weight, (4) body fat mass, (5) abdominal visceral fat volume, (6) serum low-density lipoprotein (LDL)-cholesterol concentration (7) serum high-density lipoprotein (HDL)-cholesterol concentration, (8) serum triglycerides concentration, (9) blood pressure, and (10) liver fat. The exploratory objectives of the study are to test the effect of the treatment on the gut microbiome.

METHODS

Study design, setting and population

The study is a two-arm, parallel group, single-masked randomized controlled trial (RCT). Adults with prediabetes or early-stage T2DM who are not treated with glucose-lowering medications are randomized, with equal allocation, to either the PNP or LFHF diet arms, in both arms participants are commenced on metformin Extended Release (XR) 1500 mg/d treatment for 6 months. All the study visits are performed at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney). Metagenomics and data processing for the personalized dietary interventions are performed at the Weizmann Institute of Science (Rehovot).

Patient and public involvement

Patients or the public were not involved in the design or other aspects of the research.

Eligibility

Adults (20-70 years old) with prediabetes or recently (in the last 6-months) diagnosed T2DM with (HbA1c ≤8.0% [64 mmol/mol]), not pregnant or planning to become pregnant during, and for at least 3 months after the study are recruited (Table 1). A wide age range was selected to encompass different populations of individuals managing their prediabetes for short or long durations and to increase the likelihood to recruit the sample size in a timely manner. The HbA1c cap at 8.0% [64 mmol/mol] was selected to ensure that individuals with T2DM are relatively well-controlled. Blood tests indicative of normo-glycaemia, HbA1c above 8.0% [64 mmol/mol], liver enzymes (ALT and/or AST) over 3 times the normal range limit and eGFR[17] lower than 45 mL/min/1.73m^2 are grounds for exclusion. Individuals treated with glucose-

lowering medication other than metformin in the last 24 months, or metformin in the last 3 months, will be excluded. Individuals with conditions or treatments that affect glycaemia (e.g. oral steroids), impact weight (e.g. bariatric surgery, weight loss medications), or the gut microbiome (e.g. inflammatory bowel disease, coeliac, frequent antibiotic treatment) will be excluded. Participants who have had a cardiovascular event in the previous 6 months or received an investigational new drug within the last 6 months will be excluded. Participants with conditions that may interfere with the ability to understand the requirements of the study and those who refuse treatment with metformin or refuse to use the smartphone application will be excluded (Table 1).

Recruitment

The study is advertised in general practitioners (GP), endocrinologists and dieticians' practices in the Sydney metropolitan area, and through targeted social media campaigns. A collaboration with Blacktown Mt Druitt Hospital (Western Sydney) has been established in 2019 for the purpose of recruitment. During a hospital screening program ran between 2016 – 2018, 17.3% and 30.2% of individuals visiting the Emergency Department (ED) at Blacktown Mt Druitt Hospital have had HbA1c values indicative of diabetes and prediabetes, respectively[18]. Since September 2019, individuals visiting the ED who have had a blood test indicative of prediabetes receive a letter prompting them to contact the PREDICT team and encouraging they share the result with a GP.

To-date (February 2020), 38 participants were enrolled. Of the 38 participants enrolled, 20 completed, 13 are ongoing, and 5 withdrew before the end of the treatment (13% drop-out rate). Recruitment of participants is expected to complete in December 2021.

Participants contacting the team receive the participant information sheet (Supplementary material) via email or post. Experienced clinical research nurse/associate provides details about the study over the phone. Willing participants are referred to a commercial pathology to perform an oral glucose tolerance test (OGTT, 75 g) and HbA1c test. They are asked to sign a consent form after reading the participant information sheet explaining the possible risks of undergoing the OGTT and HbA1c tests prior to performing the blood tests. If the blood tests indicate either T2DM (with HbA1c ≤8.0% [64 mmol/mol]), or impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or HbA1c ≥5.7 [39 mmol/mol], they are invited to a screening and enrolment visit at the Garvan Clinical Research Facility.

A stool collection kit for metagenomics OMNIgene GUT (OMR-200; DNA Genotek) is mailed to participants prior to the screening/enrolment visit. Participants collect the sample according to the manufacturer's instructions the day before the visit and keep the sample at room temperature. At the Clinical Research Facility, the sample is vortexed, centrifuged for a few seconds and material aliquoted into cryo vials and kept in -70C freezer. One vial is transferred to facilities at the Weizmann Institute of Science and stored at −20°C until DNA extraction.

The pre-treatment data are collected across the screening/enrolment and the baseline visits.

Screening / enrolment procedures and measurements

During the screening/enrolment visit, participants sign the study informed consent form (Supplementary material) and undergo medical examination by a physician. Participants have their weight, height, waist and hip circumference and blood pressure measured. Basal metabolic rate (BMR) is estimated using bioelectrical impedance (BIA, used for calculating the energy requirement, see "Energy target"). Blood samples are collected to evaluate liver

(liver enzymes) and kidney (creatinine and eGFR) function and full blood count. Glucose monitor (FreeStyle Libre Pro, Abbott, Germany) is attached for a period of 14 days.

A link to download the PNP smartphone application is sent to the participants prior to the screening/enrolment visit along with a short video demonstrating the app use. They are asked to log-in to the app with a personal (re-identifiable) code provided by email, and to familiarize with the app in preparation for a training session with the dietician. During the screening/enrolment visit, the dietitian practices with the participants browsing the food database, selecting food and beverage items and indicating the amount consumed. Participants are taught to add frequently consumed foods to a favourites list, which makes future search of food items easier. When the CGM is on, participants are asked to carry on with their usual routine and to record all meals, snacks and drinks using the app. The period between the screening/enrolment and the baseline visits (4 – 6 weeks) serves as the 'run-in' period.

Randomization

Randomization is performed between the screening/enrolment and baseline visits. Individuals are randomized with 1:1 allocation into the 2 arms in rounds of 4 – 6 individuals each, with randomization performed within each round using the minimization program for allocation of subjects to parallel groups, modified from Saghaei et al[19]. They are stratified by gender, age (20-49 or 50-70 years), BMI (<25.0 or >25.1 kg/m^2) and HbA1c (<5.7 [39 mmol/mol] or >5.8 % [40 mmol/mol]). To avoid bias, the randomization is performed by a study investigator located at the Weizmann Institute who does not interact with the study participants. The study nurses and physicians who have direct contact with the participants are blinded to the randomization order, however due to the nature of the intervention, the study dietician is not blinded to the treatment allocation.

Baseline visit - Measurements

The baseline visit is performed approximately 4-6 weeks after the screening/enrolment visit (Table 2). Participants attend the Clinical Research Facility following an overnight fast. Blood is drawn for serum lipids measurement and anthropometric measures and blood pressure are taken. Arterial stiffness (pulse wave analysis, AtCor Medical, Australia) is measured twice and average recorded, as described[20]. This is followed by measurement of resting energy expenditure, carbohydrate and fat oxidation over 30 minutes by indirect calorimetry (Quark, Cosmed, Italy)[21]. Body composition is assessed using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare). Specifically, total body fat mass and fat-free mass (enCORE software), the android and gynoid region, and visceral fat (CoreScan software, GE Healthcare) are recorded[22]. Liver steatosis (controlled attenuation parameter, CAP) and liver fibrosis (liver stiffness measurement, LSMs) are assessed using FibroScan (Touch 502 by Echosens) by a trained technician. CAP and LSMs have been reported to correlate closely with steatosis and fibrosis assessed using the gold-standard liver biopsy[23]. A physical activity monitor (ActivePal, Pal Technologies) is applied on the thigh for a period of 14 days. At the end of the baseline visit, the participants practice using the app with the dietician.

Prediction of postprandial glucose response using the algorithm

The prediction of PPGR in PREDICT follows the modelling framework described in Zeevi et al[14] and is performed between the screening/enrolment and baseline visits. Time-stamped food records from the app, CGM and other data collected during the enrolment visit at the Garvan are shared with a mathematician at the Weizmann Institute of Science where data processing occurs, on an Institutional secured server. The data, together with the stool metagenome sequencing data are integrated with the Weizmann Institute's database to develop personalized

algorithms for predicting each individual's PPGR. A database of recipes of meals (n=233) and smaller meals ("snacks", n=249) varying in macronutrient composition to generate feedback on the PPGR to pre-consumed meals has been created. Using the participants' features, personalized PPGR are calculated for every meal and snack in the database based on nutrient composition, and energy-adjusted quintile cut-offs of PPGR are used to create personalized meal ratings ranging from 1 to 5 (corresponding with "excellent", "good", "medium", "bad", and "very bad"). The predictive model, originally trained on data collected in an Israeli adult cohort[14], has been shown to be predictive of PPGR in a U.S. cohort of healthy adults (n=327) consuming a Western style diet[24].

Interventions

Both arms use the PNP mobile app to select meals/foods. In 2018, the app, developed at the Weizmann Institute of Science, was adapted to Australian consumers including the Australian food database (AUSNUT 2011–13)[25] of approximately 5,700 food items.

Personalized diet (PNP diet arm)

Participants in the PNP diet arm receive personalized feedback on each of their food item/meal choice and asked to consult with the app in real-time to select the recommended meal for them. The feedback is color-coded with a traffic light system; green ("good" and "excellent"), yellow ("medium") and red ("bad" or "very bad") PPGR (Figure 1 A and B). Participants are advised to aim for as many "good" and "excellent" scores, occasional "medium", and to avoid "bad" and "very bad" scores. When receiving bad scores, they are advised to trial substituting, adding or removing ingredients from the meal to improve the score. In individuals with hypercholesterolemia, a special set of recipes containing reduced saturated fat are uploaded

into the smartphone app. Special recipes are available for individuals practicing vegetarianism or avoiding dairy, eggs and fish/seafood.

Low-fat high fibre diet (LFHF) arm

The diet comparator chosen in the present study (the standard of care, LFHF) follows the Australian Healthy Eating Guide[15] principles. The LFHF diet is designed to provide approximately 30% of the total daily energy intake from fat, of which up to 10% of the fat is saturated fat, 50-55% of energy from low glycaemic load carbohydrates, 20-25% from protein and 30 g/d of dietary fibre. The diet is rich in legumes, poor in white grains and added sugar. Overall, the comparator diet chosen is considerably different from the diet of the average adult Australian[26]. A database of recipes (n=110 meals and n=80 snacks) following the LFHF nutrient content has been created based on the AUSNUT 2013 recipes[25]. Food items such as sugary drinks, processed meat, candies, sugar and cream were excluded from the LFHF recipes. Similar to the PNP arm, recommended meals scaled to the individual's energy target are uploaded into the participants' app, taking into account the individual's dietary restrictions and likes. Similar to the PNP arm, participants of the LFHF arm are encouraged to choose from the recipes uploaded for them or browse the food database (5700 food items) to design their own meals, as long as they follow the general dietary guidelines. Participants of the LFHF arm are instructed to consult with the total daily energy and macronutrient breakdown charts to ensure they follow the recommended diet (Figure 1C).

Energy target and using the app to select meals in real-time (both arms)

The energy requirement calculation is based on BMR estimated by BIA (Tanita, TBF-300 by Wedderburn) and on the Mifflin equation[27]. The two values are multiplied by a physical activity factor of 1.4 (lightly active) then averaged, and the value compared with the average

daily energy intake of at least 7 days, extracted from the time-stamped meals recorded using the app. In participants with BMI >25 kg/m^2, energy target of 75% is prescribed. Participants of both arms are encouraged to consume 3 bigger (breakfast, lunch, dinner) and 3 smaller (snack) meals spread throughout the day. An email, along with a short video summarising the principles of the diet and app use is sent to the participants (different sets of email and video to each arm) in the first week of the treatment period.

Metformin (relevant to both study arms)

Metformin (XR) is dispensed by the St Vincent's Hospital Pharmacy (Sydney) at baseline (for a period of 3 months) and at the 3 months visit (to last until the end of the study). The target dose (1500 mg/d) is titrated over 3 weeks to minimize gastrointestinal intolerance. A target dose of 1000 mg/d is set for participants with mild to moderately decreased eGFR (45 – 59 mL/min/1.73m^2), or participants who cannot tolerate the higher dose. A standardized dose of 1500 mg/d, rather than 2000 mg/d, was selected to suit both participants with prediabetes and T2DM, while minimising intolerance. Participants are instructed to take the medication with the evening meal and record it daily using a medication recording screen in the app or in paper logbooks.

Monitoring and adherence evaluation (relevant to both study arms)

The dietician reviews scores calculated programmatically based on the frequency of using the app and on meeting the daily energy target, along with the time-stamped meals consumed by the participants daily. In the PNP arm, the score incorporates the proportion of meals achieving the desired ("excellent" and "good") scores, while in the LFHF arm, the proportion of days in which dietary fat ≤35%, saturated fat ≤10%, carbohydrates ≥45%, and dietary fibre ≥15 g. The dietician contacts individuals who need encouragement to achieve better scores. Participants

of the 2 treatment groups receive the same attention according to their adherence. Time devoted to each individual by the dietician is recorded for later analysis purposes. Satisfaction with the diet is assessed using the Diet Satisfaction Questionnaire[28] at 6 months (Table 2).

Adherence to the metformin is based on pill counting at the 6-months visit and on logs of daily dose using the app or logbooks.

Physical activity and other confounders

Participants are asked to maintain the same level of physical activity throughout the study. Physical activity is monitored at 2 time points during the study using ActivePal (Table 2). The device records time (start and duration) and type (quiet, standing and steps) of activity and 14-days' worth of data, stored in the device, downloaded upon return. Information about background medications and nutritional supplements is collected before the start of the treatment using questionnaires (Table 2). Participants are asked to report any change in medications at each of the study visits and using the medication screen in the smartphone app.

Primary and secondary outcomes – Measurements

Participants attend the Clinical Research Facility five times during the study, over approximately 7 months. Primary and secondary endpoint measures are collected before the start of the intervention (across 2 visits: screening/enrolment and baseline) and at 3 and 6 months of treatment. Table 2 outlines the measurements obtained at each of the study visits/events.

Study outcomes

The primary outcome measure is change in HbA1c from baseline to 6 months of treatment. Furthermore, a comprehensive glycaemia assessment is enabled through continuous glucose monitoring. Interstitial glucose concentrations are recorded every 15 min using CGM for 14 days before the start of the intervention (in the run-in period) and after 3- and 6- months of intervention. The sensor stores the data for the duration of the recording, while the participants are blinded to the glucose readings. The data are downloaded upon return of the sensor. Time of the day with glucose readings below 7.8 mmol/L before versus after the treatment will be compared. Glycaemic variability[29], including (1) mean amplitude of glucose excursion (MAGE, a measure of the variation of glucose concentrations from the mean), (2) the standard deviation and the (3) mean postprandial area under the curve will also be assessed. Furthermore, fasting plasma glucose, 1-hr and 2-hr plasma glucose post 75 g glucose assessment are repeated after 6 months and will be compared to the baseline values. HbA1c test is repeated 6 months after treatment cessation (at 12-months, Table 2), along with a short questionnaire, including weight, diet and medication status.

Weight, waist and hip circumferences are recorded at 3-months and 6-months of treatment and compared to baseline. Fat, fat-free mass and android/gynoid fat distribution and visceral fat measurements by DXA are repeated at 6 months treatment[22]. Similarly, resting energy expenditure and fat/carbohydrate oxidation is measured after 6 months of treatment.

Hepatic steatosis is common in prediabetes and T2DM[1] [30] and prevention of liver steatohepatitis is key target in individuals with prediabetes or T2DM. Metformin primarily targets the liver, inhibiting lipogenesis and increasing fatty acid oxidation; therefore a beneficial effect on liver lipid and fibrosis with metformin has been assumed[31]. However, comprehensive meta-analyses of randomized clinical trials concluded that reduction in both

steatosis and fibrosis with metformin were underwhelming[32 33]. Liver fibroscan measure is repeated after 6 months of treatment.

Blood pressure and pulse wave analysis measurements are repeated at 3 and 6 months of treatment and serum lipids measured after 6 months treatment.

Safety / Adverse events monitoring

Gastrointestinal side effects are the most common adverse effects of metformin and may occur in 20-30% of individuals[34 35]. Specifically, abdominal discomfort, nausea, diarrhoea and anorexia are common[34]. While the gastrointestinal adverse effects are transient, in approximately 5% of individuals the symptoms may persist and result in cessation of metformin[35]. Vitamin B12 concentrations may be lower with metformin, if metformin is administered for a long duration[36 37]. The mechanism(s) responsible for the lower plasma B12 concentrations are unclear. A very rare, but potentially fatal complication of metformin use is lactic acidosis, mainly in patients with renal impairment[34 38]. In PREDICT, individuals with severe renal impairment are excluded. Metformin is titrated over 3 weeks to negate the potential gastrointestinal side effects. Adverse events are recorded and monitored over the phone and during the study visits.

Statistical analysis

Sample size calculation

Based on the primary outcome measure HbA1c, to detect a clinically meaningful difference of 0.4% in the change of HbA1c from baseline between the study arms at 6 months, assuming SD of 1% for both groups[39], with 80% power at two sided significance level of 0.05, a sample size

of 106 for each arm is required. Hence, with an estimated dropout rate of 20%, we aim to enrol 132 individuals to each arm, totalling 264 individuals in the study.

Analysis plan

The intention-to-treat (ITT) approach will be used for efficacy analysis. A likelihood-based mixed model repeated measures (MMRM) approach will be used for the primary efficacy analysis. The primary outcome measure HbA1c at baseline, 3, and 6 months will be the dependent variable and intervention by time interaction will be the fixed effects, and participants will be treated as random effect. The primary time specific comparison will be the difference in least square mean between intervention (PNP) and control (LFHF) diet at 6 months' treatment. The differences between the groups after 3 months of treatment will also be examined. Missing data will be handled directly through maximum-likelihood estimation via mixed modelling. To control for potential confounding effects, demographic and clinical covariates (e.g. age, gender, baseline BMI and background medications) will be adjusted as necessary in the model. To account for reduced metformin dose due to intolerance or eGFR 45 – 60 mL/min/1.73m^2, metformin dose status (1500 mg/d [normal] dose / reduced dose) will be also adjusted in the model. Piecewise linear mixed model will be used to compare trend change between arms in different periods (0-3 months, and 3-6 months). Different statistical analysis strategies including t-test, Mann-Whitney U test, Chi-square test, linear/generalized linear regression, and mixed model will be used based on the type and distribution of the outcome measures. Mediation analysis will be carried out to explore if the weight loss mediates the intervention effect on glycaemia and estimate indirect and direct effects and the proportion mediated (how much of the total intervention effect works through weight loss). We expect some degree of weight loss in all participants, as has been reported for metformin[3 40 41]. The effect of the diet intervention mediated by metformin adherence on the study outcomes will

also be tested. Subgroup analyses can be further performed to explore the intervention effect in specific sub-cohorts, for instance, the group of participants who have diabetes at baseline, the group of participants who achieve adherence standard and maintain the desired metformin dose; the group of participants with BMI >25 kg/m^2 at baseline, etc. The potential impact of the COVID-19 pandemic on the study outcomes may be explored, including comparisons of adherence and outcomes across groups of participants enrolled and followed-up pre-, during, and post- pandemic period. Sensitivity analysis related to the impact of COVID-19 may be conducted.

Laboratory testing

HbA1c is analysed using high performance liquid chromatography (Bio-Rad D-100, Bio-Rad Laboratories, Inc), plasma glucose using the Cobas 8000 (Roche), and liver and renal function tests using the Atellica platform (Siemens). Serum lipid profile is analysed by a spectrophotometric assay (Advia® 2400 Chemistry System [Siemens Medical Solutions Diagnostics]), with low-density lipoprotein (LDL) calculated using the Friedewald equation. Metagenomic DNA from the stool samples is purified using DNeasy PowerMag Soil DNA extraction kit (Qiagen) optimized for Tecan automated platform. Next-Generation Sequencing (NGS) libraries are prepared using Nextera DNA library prep (Illumina) and sequenced on a NovaSeq sequencing platform (Illumina). Sequencing is performed with 100bp single end reads with the depth of 10 million reads per sample. Host DNA is detected by mapping reads to the Human genome with inclusive parameters, and those reads removed. Bacterial relative abundance estimation is performed by mapping bacterial reads to species-level genome bins (SGB) representative genomes[42]. Mapping is performed using Bowtie[43] and abundance estimated by calculating the mean coverage of unique genomic regions across the 50 percent most densely covered areas, as previously described[44].

Confidentiality and data storage

Each participant is associated with an individual program-generated code used to identify their study documents, data and specimens collected during the study. The re-identifiable code is documented in the participant's record and on all study documents. Study data are collected and managed using REDCap electronic data capture tools[45 46] hosted at the Garvan Institute of Medical Research. Some coded data are shared with essential personnel at the Weizmann Institute of Science on institutional Dropbox. Data collected in the form of paper hard copies are kept in locked cabinets and electronic files on a password protected folder with access granted to the Garvan study team. Re-identifiable blood, stool, plasma and serum samples will be kept at the Garvan Institute's freezer facility. All the study questionnaires are disseminated using REDCap.

Dissemination of results

The results of the study will be disseminated to healthcare professionals via open access publications in medical journals, without any restrictions. Upon completion of data analysis, the participants will be invited to an information session at the Garvan Institute of Medical Research with the study Investigator(s) where the findings of the study will be shared and discussed. Individual letters are disseminated to the study participants after the 6 months treatment visit (approximately 7 – 8 months from study enrolment) summarising individual results (e.g. baseline and post treatment weight, body fat, liver fat, HbA1c, fasting, 1-hr and 2-hr plasma glucose concentrations). The participants are encouraged to share their individual results with the GP.

Ethics and dissemination The study has been approved by the St Vincent's Hospital Human Research Ethics Committee (File 17/080, Sydney, Australia) and the Weizmann Institutional Review Board (File 528-3, Rehovot, Israel). Protocol modifications are communicated to the SVH HREC, the Weizmann IRB, the trial registry (ClinicalTrials.gov), the study investigators and the study participants (if relevant). De-identified participant data that underlie the findings reported in the research article will be available immediately following publication, ending 5 years following the article publication, to researchers who provide a methodologically sound proposal with the aim to achieve the aims reported in the approved project proposal. Data may be obtained from the Principal Investigator Dorit Samocha-Bonet or Associate Investigator Jerry Greenfield upon enquiries directed to d.samochabonet@garvan.org.au or j.greenfield@garvan.org.au.

Authors' contributions

DS-B, JRG, ES and EE contributed to the conception and design of the study, TDH, AG and DS-B drafted the manuscript, AG and ZL contributed to the study design and the statistical plan, ZL contributed to the sample size estimation, AG, DK, RR, KT, EC, MD, JRS and T-MH contributed to the collection of the data. All authors revised and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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The study sponsor and funding bodies have no role in the study design, collection, management, analysis, and interpretation of data, writing of the report and the decision to submit the report for publication.

Conflict of interests

Eran Elinav and Eran Segal are paid consultants of the company DayTwo. Mark Danta has received travel support and speaker fees from Gilead, Abbvie and Merck. All other authors declare they have no conflict of interest.

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Figure legends

Figure 1: Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

Table 1: Inclusion and Exclusion criteria

Inclusion criteria
Men and women 20-70 years of age
Prediabetes (IFG* or IGT* and/or HbA1c 5.7 – 6.4% [39 – 46 mmol/mol]) or Individuals diagnosed with T2DM in the last 6 months with HbA1c ≤ 8.0% [64 mmol/mol]
Willingness to provide written informed consent, participate and comply with the study
Exclusion criteria
Women planning pregnancy during the study or 3 months after study completion
Individuals with type 1 diabetes, neoplastic disease (in the last 3 years), cardiovascular event (-6 months), chronic gastrointestinal disorders
Liver enzymes ALT and/or AST >3 times normal range limit
eGFR**<45 mL/min/1.73m^2
Normo-glycaemia
HbA1c > 8.0% [64 mmol/mol]
Current or recent (within 24 months) treatment with a glucose lowering medication other than metformin, current or recent (within 3 months) treatment with metformin, current treatment with an oral steroid, immunosuppressive medications, antibiotics (within 3 months)
Alcohol or substance abuse
Participants who had received an investigational new drug within the last 6 months
Participants involved in another clinical study
Participants who have had bariatric surgery
Participants who actively lose weight
Participants with conditions that may interfere with the ability to understand the requirements of the study, refuse treatment with metformin or refuse to use the smartphone application

*Impaired fasting glucose (IFG): FPG 5.6-6.9 mmol/L and/or impaired glucose tolerance (IGT): 2-h plasma glucose during 75-g oral glucose tolerance test (OGTT) 7.8-11.0 mmol/L

**eGFR calculated as reported[17]

Table 2: Study timeline of activities and measurements taken at each of the study events and visits

	Location and time	***Measurements***
Pre-screening	Phone -35 +/- 10 days	• Interview (by an experienced staff)
	Local pathology	• 75 g OGTT and HbA1c tests
Screening/ enrolment (Non-fasting)	Clinical Research Facility -30 +/- 15 days	• Informed consent signed • Medical history, medications & medical examination • Blood (full blood count, liver and kidney function tests) • Height and weight (BMI calculated) • BIA (RMR) • Blood pressure • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelope) • Smartphone app training
	Home	• Stool sample • *Study entry questionnaire
Baseline (Fasting)	Clinical Research Facility 0, treatment clock starts	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin 3-months' supply with instructions and logbooks • Smartphone app training, according to study arm allocation
3-months (Fasting)	Clinical Research Facility +90 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (HbA1c) • Indirect calorimetry (REE, RQ) • FreeStyle Libre Pro (to remove and send back after 14 days in a pre-paid envelop) • Metformin 3-months' supply with logbooks • Adverse events recording and monitoring
	Home	• #Stool sample
5.5-months (Non-fasting)	Clinical Research Facility +166 days +/- 10 days	• FreeStyle Libre Pro and ActivePal
	Local pathology	• 75 g OGTT and HbA1c

6-months (Fasting)	Clinical Research Facility +180 +/- 10 days	• Anthropometry • Blood pressure, pulse wave analysis • Blood (lipids, HbA1c) • Indirect calorimetry (REE, RQ) • DXA, FibroScan • Metformin- unused pills collected and counted • Adverse events recording
	Home	• #Stool sample • ##Diet Satisfaction Questionnaire
12-months	Local pathology	• HbA1c
	Home	• **12-months questionnaire

*Questionnaire includes hunger/fullness, dietary habits and dislikes, physical activity, medications, dietary supplements, personal and family history of disease, food-frequency questionnaire

**Questionnaire includes diet (are you following the diet you were allocated to?), metformin (are you continuing the metformin treatment and dosage) and current body weight

#Gut microbiome features from stool samples collected at 3 and 6 months will be compared to baseline (exploratory outcome)

The Laboratory for the Study of Human Ingestive Behavior, Pennsylvania State University are the copyright holders of the Diet Satisfaction Questionnaire[28]

Abbreviations: BIA, bio impedance analysis; BMR, basal metabolic rate; DXA, dual-energy X-ray absorptiometry; REE, resting energy expenditure; RQ, respiratory quotient

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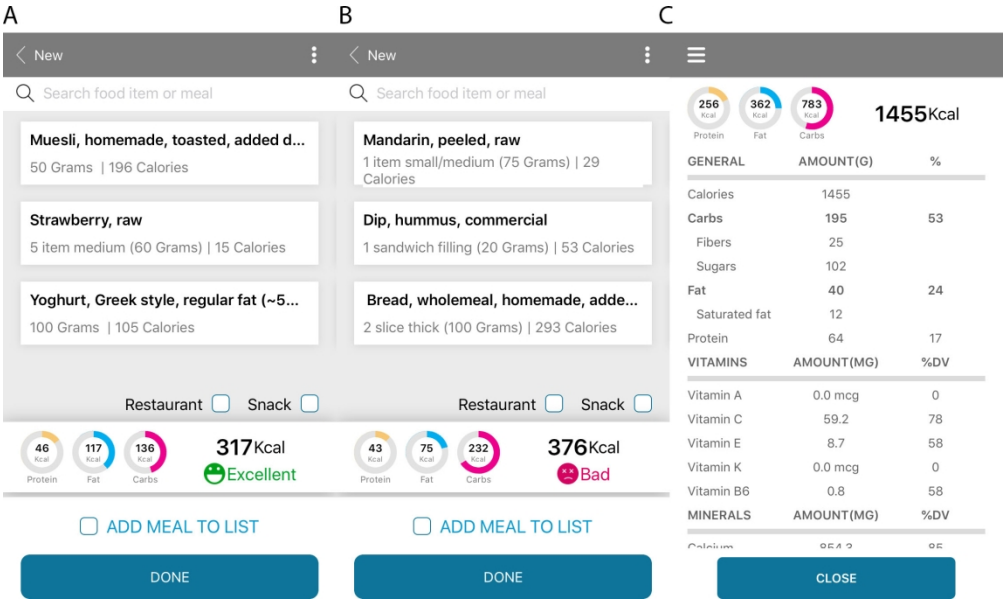

Screenshots of the smartphone application used daily by participants in the PREDICT study. Participants randomized to the personalized diet arm receive scores for each meal. Panels A and B depict two meal options selected by an individual in the study where 2 iso-caloric breakfasts are predicted to result in modest (A) or exaggerated (B) postprandial glycaemic responses. The daily energy intake and macronutrient breakdown are provided to each of the study participants (C).

190x112mm (300 x 300 DPI)

Participant Information Sheet/Consent Form

Interventional Study - *Adult providing own consent*

Garvan Institute of Medical Research

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s) *(if required by institution)*	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location *(where CPI/PI will recruit)*	*Garvan Institute of Medical Research, Sydney*

Part 1 What does my participation involve?

1 Introduction

Type 2 diabetes affects 8.5% of the world's adult population. Prediabetes is a condition developing many years before type 2 diabetes, and is diagnosed by raised glucose (sugar) in the blood stream, either when measured first thing in the morning (fasting) or when measured after a meal. Approximately 30 - 40 % of the world's adult population is affected by prediabetes. Individuals with prediabetes have a higher risk of diabetes and heart disease. Treatment with the most commonly used sugar-lowering medication metformin does not always improve blood sugar levels in individuals with prediabetes or type 2 diabetes. This means that after the treatment, blood sugar concentrations are still higher than normal and the risk of diabetes remains increased.

You are invited to take part in this research project because you either have prediabetes or you have recently been diagnosed with type 2 diabetes. The research project is testing the effect of a personalised diet combined with the medication metformin on sugar control, body weight, and other risk factors.

This Participant Information Sheet/Consent Form tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your General Practitioner.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

• Understand what you have read

• Consent to take part in the research project

• Consent to have the tests and treatments that are described

• Consent to the use of your personal and health information as described

You will be given a copy of this Participant Information and Consent Form to keep.

2 What is the purpose of this research?

The overall aim of the study is to improve the treatment of individuals with prediabetes or type 2 diabetes with a special nutritional intervention.

In particular, we would like to find out whether a personalised diet administered with a medication commonly used to treat diabetes named metformin will achieve better results when compared with a low fat low sugar diet (the type of diet commonly recommended to individuals with prediabetes or type 2 diabetes) administered with metformin.

We will compare the effects of the different treatments on sugar control, body weight, body fat, blood lipids, blood pressure and the amount of fat in the liver.

The medication used in this research project and listed below is approved in Australia to treat type 2 diabetes. While it is not routinely prescribed to treat prediabetes, metformin has been used to treat individuals with prediabetes in a large American trial and has been proven to be safe in individuals with prediabetes (the Diabetes Prevention Program, United States).

<u>The following medication/treatments will be used in the study:</u>

1. Metformin Extended Release (XR) + Low fat low sugar diet (“healthy” diet).
2. Metformin XR + “Personalised diet” (see further details below).

In both study arms, if your weight falls in the overweight or obese category (i.e. body mass index [BMI] > 25 kg/m^2), we will recommend a reduction of approximately 500 Kcal/d from the dietary energy you are currently on. This will result in moderate weight loss. If your weight is within the recommended BMI range (equals to or under 25 kg/m^2), we will recommend the same level of energy intake you are currently consuming. This will result in weight maintenance.

<u>Method of administration of medication:</u>

Metformin XR – Daily tablet (oral ingestion 1500 mg/d) with evening meal

<u>Method of administration of diet:</u>

The diet will be delivered using a smartphone application. You will be guided and supported by an Accredited Practising Dietitian

You will be participating in a randomised research project. Sometimes we do not know which treatment is best for treating a condition. To find out, we need to compare different treatments. We put people into groups and give each group a different treatment. The results are compared to see if one is better than the other. To try to make sure the groups are the same, each participant is put into a group by chance (random).

We will administer 1 medication in combination with 2 different nutritional interventions. There is a 1 in 2 chance that you will be allocated to each study arm.

This research has been initiated by the study doctors, Dr Dorit Samocha-Bonet and Professor Jerry R Greenfield.

This research has been funded by a Foundation requesting to remain anonymous, funds from the Garvan Research Foundation and a grant from the St Vincent's Clinic Foundation.

3 What does participation in this research involve?

If you agree to participate in this study, you will be asked to sign the Participant Consent Form. We will ask you to sign a separate Participant Consent Form before having blood tests at a commercial pathology.

Participation in this project will involve the following:

Initially we will ask you questions regarding your health and medical history over the telephone. If it sounds like you may be eligible for the study we will ask you to attend a commercial pathology service in your area for an oral sugar test (oral glucose tolerance test), testing your blood sugar after drinking a sugary drink, and a blood test measuring glycated haemoglobin (HbA1c, a marker of average blood sugar levels over the past 3 months). This involves having a blood sample taken prior to drinking a sugary drink (containing 75 grams of sugar), followed by blood samples taken one and two hours later.

You will be asked to remain in the blood collection centre for the duration of the test (2 hours).

Overall the commercial Pathology will collect 20 mL (about 1.5 tablespoons) of blood.

These blood tests will tell us if you have diabetes or prediabetes and will help us determine whether or not you are eligible to participate in our study. We will call you and discuss the test results and your eligibility to participate in the study within 1 week. We will provide you with a letter summarising the results which you will be able to share with your General Practitioner.

If you are eligible to participate in the study, we will invite you to a Screening / Enrolment visit. We will send you a special kit for collection of stool at home, with detailed instructions on how to collect the sample. We will ask you to bring the sample with you to your next visit.

Prior to the study we will ask you to reply to questionnaires asking about your usual eating and physical activity habits and your health. You will be asked to download a smartphone application which you will use during the study.

Screening / Enrolment visit (approximately 1.5 hours)

- We will ask that you arrive to the Clinical Research Facility (clinic) at the Garvan Institute of Medical Research, Darlinghurst. During this visit you will have time to ask questions and if you agree to participate, you will sign the consent form. You will change into a hospital gown. The study doctor will ask you about your health and perform a physical examination. We will measure body weight and height, blood pressure, waist and hip circumferences, and body fat composition (estimated using Tanita scales).
- Blood will be drawn to assess your liver and kidney health and your blood cells count. The volume of blood that will be taken during this visit is 20 mL (about 1.5 tablespoons).
- We will attach a small device (sensor) that measures sugar levels for a period of 14 days to your arm. The size of the device is of a fifty cents coin. We will apply the sensor on to the back of the upper arm with a disposable applicator. When the sensor is applied, a thin, flexible sterile fibre is inserted just under the skin. It is held in place with a small adhesive pad. Most people feel no pain when applying the sensor. You will not have to do anything while the sensor measures sugar level. All the information will be stored in the sensor. We will also attach a physical activity sensor. Both sensors are designed to wear for up to 14 days. We will ask that you remove and send the sensors to us after 14 days in a prepaid envelop. You will have your usual diet and record what you eat in a smartphone application.

During this visit, or soon after, you will meet the dietitian (face-to-face or using a video meeting tool like Zoom or Skype). The dietitian will teach you to use the smartphone application. You will use this application to log the food that you eat, and optionally, to log your physical activity (type and duration), and the medications that you take. We will provide you with a light meal at the end of this visit.

After this visit we will randomise you to one of 2 treatment arms.

Baseline visit (2 hours, we will ask that you fast from 10 pm the night before this visit)

The baseline visit will be performed approximately 6 – 8 weeks after the Screening / Enrolment visit. You will arrive at the clinic in the morning. The following measurements and procedures will take place,

- You will undergo dual-energy X-ray absorptiometry (DXA) at St Vincent's Hospital (next door to the Garvan). DXA will be used to evaluate the amount of fat in your body.
- Liver fibroscan, performed at St Vincent's Hospital. This measurement will provide information about the amount of fat and fibrosis (scarring) in the liver.
- A team member will measure your weight, height, waist and hip circumference and blood pressure, while wearing a hospital gown.
- We will measure how many calories you burn while resting using a plastic hood placed over your shoulders while you are resting in bed (takes 30 minutes).
- We will measure the stiffness of an artery in the wrist (radial artery) using a non-invasive device which looks like a pen. This measurement takes approximately 5 minutes and will provide information about the stiffness of your arteries.
- We will collect blood samples. Overall we will collect 20 mL (2 tablespoons) of blood during this visit.
- We will collect a sample of bacteria from the mouth by swabbing a special collection stick over the gums. We will store this sample for testing the effect of the treatment on the mouth bacteria.

At the end of the visit we will provide you with 3-months' worth of medication and medication logbooks. We will ask you to take the metformin every evening **with dinner.** You will start with taking a small dose and depending on how you feel (some people experience diarrhoea, nausea, and flatulence, mostly at the beginning of the treatment), we will gradually ask you to increase the dose until you reach the target dose. You will be guided by the study investigator / dietitian regarding using the smartphone application during the treatment period.

We will call you at a time of your convenience once a month or fortnightly to follow up on your progress with the treatment. We will ask you how you feel and whether you have any general health changes that you would like to report (whether you think they are related or unrelated to the treatment), as well as any changes to your usual medications, other than the study treatment.. Follow-up may be performed using Zoom, Skype, text, or over the phone.

4-months visit (2 hours, similar to the baseline visit, we will ask that you fast from 10 pm the night before this visit)

This visit will be similar to the baseline visit (described above) and include the same procedures, except that you will not have a DXA and fibroscan. We will attach another sugar sensor to the arm, and ask you to send it back to us in a prepaid envelope after 14 days. During this visit we will also take a blood sample that will be used to measure the level of metformin in the blood. We will ask you to bring the medication log books.

6.5-months visits

We will invite you to the Garvan to attach the sugar sensor and activity monitor. We will refer you again to perform a sugar challenge test, as you have done before the treatment in a commercial pathology in your area. We will provide you with a special kit for collection of stool and ask you to bring the sample with you to your next visit at the Garvan.

7-months (end-of treatment, performed 6 months from the start of the treatment) visit

Identical to the baseline visit, with the exception that we will collect a blood sample to measure the concentration of the medication in the blood and will ask you to bring back the medication and the logbooks.

12-months survey and blood test (includes a questionnaire and a blood test at a local commercial pathology)

6-months after the completion of the treatment, we will send you a survey and ask you to tell us what your current body weight is, whether you remained on the diet, and whether you are treated with a sugar-lowering medication (and if relevant to specify the medication and dose). We will also ask you to perform a blood test in a commercial pathology in your area. The blood test will measure sugar control and does not require you to be fasting. You will not have to visit the Garvan for this event.

In summary, you will be required to visit and maintain contact with the study team over a period of approximately 6 months, while you're treated. The overall duration of the study is approximately 13 – 14 months.

There are no costs associated with participating in this research project, nor will you be paid. All medications, dietary advice, tests, including all blood tests, some meals and medical care required as part of the research project will be provided to you free of charge.

You will be provided a $100 AUD retail voucher. Parking will be provided at the Garvan carpark on days of visits.

If you have a General Practitioner, we strongly recommend that you inform them of your participation in this research project.

4 What do I have to do?

During the study period, the study team will instruct you regarding what you should be doing before each of the visits/study events. For example, we may ask you to avoid drinking alcohol or to avoid vigorous physical activity before some of the visits.

During the study you will be able to continue taking your regular medications, but if you will require a sugar-lowering medication other than metformin (prescribed in the study), you will have to withdraw from the study.

We will ask you to inform us if and when you started taking a new medication, or if and when there has been a change in dose of any medication you are taking regularly.

During the study we will ask you not to donate blood or participate in another study.

We will expect you to be compliant with the study instructions and if any problem arises, we expect that you will contact us.

5 Other relevant information about the research project

We plan to enrol approximately 260 men and women to this study. All participants will be enrolled at a single site, the Garvan Institute of Medical Research, Darlinghurst, Sydney.

Some tests will be performed at the adjacent St Vincent's Hospital.

The project involves researchers from a number of academic institutes working in collaboration. The research team includes researchers and endocrinologists from the Diabetes Division at the Garvan Institute of Medical Research and St Vincent's Hospital (Sydney), researchers from the Endocrinology Department at Blacktown & Mount Druitt Hospital (Sydney), and researchers from the Immunology Department and the Computer Science Department at the Weizmann Institute of Science (Israel).

6 Do I have to take part in this research project?

Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

If you do decide to take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the Garvan Institute of Medical Research or St Vincent's Hospital.

7 What are the alternatives to participation?

Other treatment options for your condition are available; these include dietary counselling and physical activity advice, and / or a sugar-lowering medication. Your study doctor will discuss

these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your General Practitioner.

8 What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research. You will have access to a medication regulating blood sugar level that is primarily offered to type 2 diabetes patients and you may lose weight (1 – 5 kg).

We are hoping that the information generated in this study will help improve treatment of your condition.

9 What are the possible risks and disadvantages of taking part?

Taking a medication may involve some risks, as detailed below. We will monitor any adverse events on a regular basis and you will have access to a clinical staff member at all time during the study.

There are potential risks relating to the procedures we will utilise in the course of the study. We have listed them below.

Risks related to the medication

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects regularly.

In case you need to have any diagnostic procedure requiring contrast, most radiological services recommend routine withholding of metformin for 48 hours prior to and after the procedure. Please let your doctor know that you are taking metformin.

There may be side effects that the researchers do not expect and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly in the first few weeks of the treatment or after the treatment ends. However, sometimes side effects can be serious. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you.

The medication we will administer in the present study, metformin, has been administered to people with type 2 diabetes over many years and has proven to be generally safe. We have listed below the potential side effects of the medication administered.

- Mild gastrointestinal symptoms such as diarrhoea, nausea, vomiting, abdominal pain and loss of appetite are the most frequent reactions to metformin, occurring in approximately 10% of individuals (10 in 100), especially during the initial treatment period. These symptoms are generally transient and resolve spontaneously during continued treatment.
- Taste disturbance may occur in 3% (3 in 100).

- Rare adverse reactions occur in less than 0.01% (1 in 10000) of individuals include,
  - Lactic acidosis, often with mild nonspecific symptoms such as feeling of sickness, sleepiness, shortness of breath, and nonspecific abdominal distress.
  - Abnormalities of liver tests have been reported. Liver function tests will be monitored during the treatment.

Risks related to the personalised nutrition

The Personalised Nutrition Project diet (http://newsite.personalnutrition.org/WebSite/Home.aspx) has been trialled in various studies for over 5 years by the study co-Investigators Professors Elinav and Segal from the Weizmann Institute in Israel. The nutritional recommendation is different from person to person. This is because it is based on the individual's features and measurements, including sugar rise in response to food. Co-Investigators Elinav and Segal have reported that the individual response depends on blood markers, body measurements and different features of microorganisms in the gut. The individual food recommendation is based on "real" foods.

The effects of the personalised diet on the level of blood lipids in individuals with prediabetes and type 2 diabetes is being investigated. If we find that the baseline level of low-density lipoprotein (LDL)-cholesterol in the blood is elevated, a modified personalised diet containing less saturated fat (the type of dietary fat known to increase the level of LDL) will be prescribed.

Risks relating to procedures

Blood sampling

You may have pain, light-headedness, minor infection, bleeding or bruising at the sites of the blood sampling; however, the staff will use proper technique while drawing blood samples in order to reduce the risk for these unwanted effects. These effects are usually temporary, are easily treated, and are expected to resolve completely.

Dual-energy X-ray absorptiometry (DXA) scan

This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 0.04 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal.

Fibroscan test

The fibroscan is a non-invasive method of assessing liver scarring, termed fibrosis. The test is performed at the bedside in a clinic. A mechanical pulse is generated at the skin surface, which is propagated through the liver. The velocity of the wave is measured by ultrasound. The speed of this wave correlates with the stiffness of the liver, which in turn reflects the degree of fibrosis – the stiffer the liver is, the greater the degree of fibrosis. This test also estimates the amount of fat in the liver. This test utilises an ultrasound device and carries no risk.

Body sugar monitoring

The device we use in the study is used to monitor blood glucose levels, mostly in diabetes patients. Most people feel no pain when applying the sensor. The sensor is designed for a safe wear of up to 14 days. You can shower and exercise while wearing the sensor. Some individuals

develop a skin reaction around the device on the arm. This typically resolves spontaneously over time; an over the counter cream may be recommended if the skin reaction persists. If you experience a skin reaction, we will likely decide to omit this measurement from the follow-up events.

Indirect calorimetry

A clear plastic hood is placed loosely over your head. Room air flows through the hood and the machine measures how much oxygen you are using and how much carbon-dioxide you produce. This test carries no significant risk. However, some participants may experience claustrophobia. If this occurs, the hood will be removed immediately.

Arterial stiffness

The stiffness of an artery in your wrist will be measured using a small device, looking like a pen, by applying light pressure to your wrist. There are no risks related to this method.

Tanita scales

The scales estimate how much energy your body requires based on your body fat and muscle composition. When you stand on a Tanita monitor, a very low, safe electrical signal is sent from four metal electrodes through your feet to your legs and abdomen. The electrical signal passes quickly through water that is present in muscle tissue but meets resistance when it hits fat. This resistance, known as impedance, is measured and used in validated equations to calculate body composition. This measurement will take around 20 seconds. Information from the Tanita measurement will be used to estimate your recommended daily energy intake.

People with an electronic medical implant, such as a pacemaker, should not use a body composition monitor because the electrical signal travelling through the body may interfere with its operation.

Pregnancy

The effects of the medication metformin on the unborn child and on the newborn baby are not known. Because of this, it is important that study participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in this study if you are pregnant or trying to become pregnant, or breast-feeding. If you are female and child-bearing is a possibility, you will be required to undergo a pregnancy test prior to commencing the research project, and before each of the DXA scans.

Female participants must avoid pregnancy during the course of the research and for a period of 3 months after completion of the research project. You should discuss methods of effective contraception with your study doctor.

If you do become pregnant whilst participating in the research project, you should advise your study doctor immediately. Your study doctor will withdraw you from the research project and advise on further medical attention should this be necessary. You must not continue in the research if you become pregnant.

If you are a male, it is safe to father a child or donate sperm whilst taking the study medication.

10 What will happen to my test samples?

During the study we will store blood and stool samples in -80°C freezer. In this research project, collecting blood and stool samples are mandatory.

The blood, oral and stool samples collected are for research purposes, as specified in this document.

All the samples we will collect from you will be coded so they are not identifiable as your sample. Your identification linked to the sample code will be stored on a password protected computer and physical hardcopies in a locked filing cabinet at the Garvan Institute of Medical Research. Only staff involved in this study at the site will have access to this data to maintain privacy/confidentiality.

Some analyses in blood will be performed in commercial Laboratories or at St Vincent's Hospital. These specimens will be de-identified (coded). Your clinical information will not be sent with the specimens. The results of these analyses will be returned to the Investigators at the Garvan Institute of Medical Research. The external sites may retain your re-identifiable, analysed data in their records. After analysis of your specimen is complete, these external sites will destroy any remaining tissue or return remaining sample to us for storage.

We will store the samples we collect for 15 years.

Some samples of your blood, mouth and stool obtained for the purpose of this research project will be transferred to the Weizmann Institute of Science in Israel. These samples will be de-identified by coding. Information such as your gender, age, ethnicity, medical history, medications, food sensitivities and eating habits will be shared with researchers at the Weizmann Institute for planning your diet. This information will be de-identified.

Your samples will not be sold and the Garvan Institute of Medical Research will not knowingly transfer your samples to anyone who has expressed intent to sell the samples.

11 What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he / she will explain the reasons and arrange for your regular health care to continue.

When we assess your suitability to participate in the study we may uncover a medical condition of which you may be unaware. While we may decide that you are not able to participate in the study because of this new condition, we will refer you to your General Practitioner for further investigation and management.

If during the screening visit we diagnose a previously unknown condition, for example type 2 diabetes, this may affect insurance in the future.

12 Can I have other treatments during this research project?

Whilst you are participating in this research project, you may not be able to take some of the medications or treatments you have been taking for your condition or for other reasons. It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies. You should also tell your study doctor about any changes to these during your participation in the research project.

13 What if I withdraw from this research project?

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected by the sponsor up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them before you join the research project.

14 Could this research project be stopped unexpectedly?

Sometimes research projects trialling medications need to stop prematurely because of unexpected side effects. However, this research project is unlikely to be stopped unexpectedly, because the medication administered has been studied and is routinely administered in type 2 diabetes and to a lesser degree in people with prediabetes. The medication administered here has been proven safe in type 2 diabetes patients.

15 What happens when the research project ends?

The medication administered in the present study will not be available after the research finishes through the study (but may be prescribed by your General Practitioner if required and decided). At study completion, you will be able to continue using the app and follow your study diet. We will offer you nutritional counselling on healthy lifestyle that will help you maintain a healthy body weight and decrease your risk of developing diabetes.

After you complete the study treatment (approximately 7 months from when you start), we will send you a detailed results letter, with measurements collected at baseline and after the 6 months' treatment, that will include your weight, BMI, blood pressure, fasting blood sugar, glycated haemoglobin (HbA1c), and the amount of fat in your body and liver.

After we finalise the study of all the participants and analyse the data (approximately in 2022), we will invite you to an information evening at the Garvan Institute of Medical Research (Darlinghurst, Sydney) to share with you the study findings. You will have opportunity to discuss the findings with the study Investigators.

Part 2 How is the research project being conducted?

16 What will happen to information about me?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All the clinical information and the data that will be collected will be coded. It can be re-identified; however, your identification matching your code will be secured, accessible only by research staff. Data allowing re-identification of your data will be kept on a password protected computer and a locked filing cabinet at the Garvan Institute of Medical Research. Only staff involved in this study will have access to this data to maintain privacy/confidentiality.

All data will be stored for 15 years, after which it will be destroyed.

Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities and authorised representatives of the Sponsor, the Garvan Institute of Medical Research, the institution relevant to this Participant Information Sheet, St Vincent's Hospital, Sydney, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and regulatory authorities as noted above.

It is anticipated that the results of this research project will be published and presented in a variety of forums. In any publication and presentation, information will be provided in such a way that you cannot be identified. No individual data will be reported in any publication or presentation.

Data collected in the study may be made available to other researchers. The data will be coded and will not reveal your personal details. The data will be made available subject to a review of applications submitted by interested researchers performed by the study Investigators Dorit Samocha-Bonet and/or Jerry R Greenfield. Only researchers aiming to achieve the aims of the project will be considered. Upon approval, access to the de-identified data will be available for a period of 5 years after publishing the study findings.

Information about your participation in this research project may be recorded in your health records.

In accordance with relevant Australian privacy and other relevant laws, you have the right to request access to your information collected and stored by the research team. You also have the right to request that any information with which you disagree be corrected. Please contact the study team member named at the end of this document if you would like to access your information.

Any information obtained for the purpose of this research project that can identify you will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

In the event of loss or injury, the parties involved in this study have agreed to provide compensation if the participants' injury or complication is caused by the study procedures or by the negligence of any of the parties involved in the study. If you suffer any distress or psychological injury as a result of this study, you should contact the study team as soon as possible, who will assist you in arranging appropriate treatment and support.

18 Who is organising and funding the research?

This research project is being conducted by Dr Samocha-Bonet and Professor Greenfield.

Dr Samocha-Bonet, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu and Ms Godneva have no conflict of interest to disclose.

The study co-Investigators, Prof Eran Elinav and Prof Eran Segal of the Weizmann Institute of Science are paid consultants of the company DayTwo. DayTwo, established in Israel, provides personalised nutrition to individuals. The product is based on the technology arising from research conducted by Prof Elinav and Prof Segal at the Weizmann Institute of Science. The research findings were published in the journal *Cell* in 2015.

The Garvan Institute of Medical Research may benefit financially from this research project if, for example, the project assists Garvan Institute of Medical Research to obtain approval for a new treatment tool.

By taking part in this research project you agree that samples of your blood or stool (or data generated from analysis of these materials) may be provided to the Weizmann Institute of Science (Israel).

The Weizmann Institute of Science may directly or indirectly benefit financially from your samples or from knowledge acquired through analysis of your samples.

You will not benefit financially from your involvement in this research project even if, for example, your samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to the Garvan Institute of Medical Research.

In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to the Garvan Institute of Medical Research or the Weizmann Institute of Science, the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19 Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of St. Vincent's Hospital Sydney (HREC reference number 17/080).

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor Dr Dorit Samocha-Bonet on 02-9295 8309 or 0416-316 111.

Clinical contact person

Name	Professor Jerry R Greenfield
Position	Associate Investigator
Telephone	SVH Switchboard 8382 1111, ask to page Prof Jerry Greenfield **24-hour medical contact**
Email	predict@garvan.org.au

Research Nurse

Name	Ms Renee Richens
Position	Research Nurse
Telephone	02-92958215 (Working days: Tuesday, Wednesday, Thursday)
Email	predict@garvan.org.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Research Office Manager
Position	Research Office Manager
Telephone	02 8382 4960
Email	SVHS.Research@svha.org.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Research Officer details

Reviewing HREC name	St Vincent's Hospital HREC
HREC Research Officer	Research Officer
Telephone	02 8382 4960
Email	SVHS.Research@svha.org.au

Research Governance Officer

Name	Ms Therese Yim
Position	Research Governance Officer
Telephone	02-9295 8173
Email	t.yim@garvan.org.au

Consent Form - *Adult providing own consent*

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s)	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location	*Garvan Institute of Medical Research*

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to Garvan Institute of Medical Research concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I understand that I will be given a signed copy of this document to keep.

I consent to the storage and use of blood and tissue samples taken from me for use, as described in the relevant section of the Participant Information Sheet, for:

- This specific research project
- Other research that is closely related to this research project

I consent to sharing de-identified (coded) data collected during the study with other researchers aiming to achieve the study aims as listed, subject to approval by the study Principal Investigator Dorit Samocha-Bonet and/or Associate Investigator Jerry R Greenfield.

Name of Participant (please print)_____

Signature _____ Date _____

Name of Witness* to Participant's Signature (please print)

_____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print)

_____

Signature _____ Date _____

† A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title	*Personalised medicine in prediabetes – Towards preventing diabetes in individuals at risk*
Short Title	*Personalised medicine in prediabetes (PREDICT)*
Protocol Number	*17/080*
Project Sponsor	*Garvan Institute of Medical Research*
Coordinating Principal Investigator/ Principal Investigator	*Dr Dorit Samocha-Bonet*
Associate Investigator(s)	*Prof Segal, Prof Elinav, Prof Greenfield, Dr Hng, Dr Snaith, Dr Liu, Ms Godneva*
Location	*Garvan Institute of Medical Research*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with the Garvan Institute of Medical Research.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/ Senior Researcher† (please print)	
Signature	Date

† A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 20 (Authors' contribution)
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5 - 6
	6b	Explanation for choice of comparators	5 - 6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6 - 7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11 – 13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13 – 14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6 – 7 and Table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	15 – 16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7 – 8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9 – 10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9 – 10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9 – 10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9 – 10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14 – 16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14 – 16 and 17 (Statistical plan)

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18 – 19
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	16 and Table 2
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Approval granted, details page 3
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	3

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9 and Table 2
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18 – 19
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18 – 19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
	31b	Authorship eligibility guidelines and any intended use of professional writers	20 - 21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Uploaded
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	19

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.