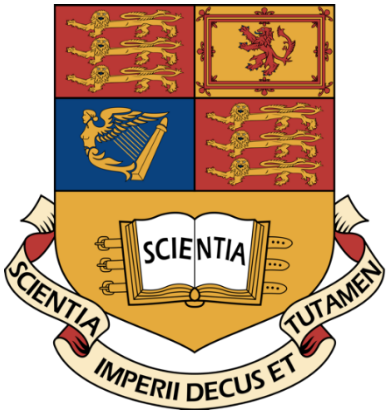

Imperial College London

Developing a Personalised Nutrition toolkit for the nutritional management of individuals at risk of cardiovascular disease

Delyse Tien Shu Yun (CID: 02074052)

August 2022

Department of Metabolism, Digestion and Reproduction

Imperial Clinical Research Facility (CRF) / Hammersmith Campus

Supervisor: Dr Isabel Garcia-Perez

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This research project is submitted in partial fulfilment of the requirements for the degree of MRes in Clinical Research

(Human Nutrition)

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Receipt for Submission of Thesis

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Agreed project contributions of supervisor and student to MRes project

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Contribution to:	By Student (in %)	By Supervisor (in %)
Overall project design*	0	100
Determination of Methodology*	20	80
Collection of specimens/material/patient recruitment	70	30
Conducting experiments/ collation of questionnaires etc	80	20
Data analysis	100	
Write up	100	
Production of submission	100	
Problems encountered if any	Delyse has faced several difficulties in commencing her project: i) it has been very challenging to receive Delyse's Enhanced DBS check with Adults' Barred list, which was essential for her to be able to work with the patients to personalise their diets; ii) The essential consumables for the clinical trial had a three months' delay.	

Any other comments: Delyse is a very bright and hardworking MRes student with phenomenal team working skills. She has become a very valuable member of my team and has developed critical thinking and acquired new skills for metabolomics and data analysis. Specifically, she has been involved in conducted PPI activities and has attended to the Imperial Great Exhibition. Finally, she has acquired experience in conducting clinical trials.

I hereby declare that the attached submission is all my own work, that it has not previously been submitted for assessment, and that I have not knowingly allowed it to be copied by another student. I understand that plagiarism is the presentation of another person's thoughts or words as though they were my own and have clearly identified and reference any such sources. I also understand that suspected plagiarism will be dealt with under the College's Procedure for Dealing with Examinations Offences and may result in a penalty being taken against any student found guilty of plagiarism.

Student Name: ……Delyse Tien …… Candidate Number: …02074052…

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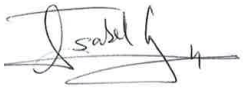
Supervisor Name: …Dr Isabel Garcia-Perez…… Date: 23 August 2022…….

Supervisor Signature……  ……..

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Abstract

Background*:* Dietary modification is a cornerstone for the prevention and treatment of cardiovascular disease (CVD), a condition with leading morbidity and mortality rates in the UK. However, current clinical nutrition strategies are impeded by the inability of dietitians to assess diets accurately due to subjective misreporting, which implicates subsequent delivery of personalised nutrition (PN) advice. Emerging evidence suggest that urinary metabolomics may be a potential solution in guiding PN interventions, given its ability to objectively identify dietary biomarkers and categorise dietary profiles of healthy individuals against nutrition guidelines, according to their metabolite patterns modelled under controlled conditions. Although the model has been validated on healthy free-living populations, there is a need to determine an optimal approach for translating it into clinic, to enhance personalised dietary advice for free-living UK individuals at risk of CVD. This project aims to establish a PN toolkit to facilitate the implementation of metabolically-guided medical nutrition therapy in clinical dietetics practice, comprising of a: 1) urinary metabolic profiling mathematical model specifically designed to accurately assess participants' dietary intake in relation to NICE guidelines; 2) metabolically-personalised nutrition counselling model for the dietary management of participants at risk of CVD, revised based on outcomes from patient and public involvement (PPI).

Methods: To develop the mathematical model, a randomised, controlled, crossover clinical trial was conducted in 18 UK individuals at risk of CVD over two 5-day inpatient periods. Each participant followed two extreme eucaloric dietary interventions (Diet 1 being the most concomitant and Diet 2 the least concomitant with NICE's CVD guidelines) and each intervention lasted 72 hours. 24-hour urine samples were collected daily and analysed using Proton Nuclear Magnetic Resonance spectroscopy. Repeated-measures Monte-Carlo Cross-Validation Partial-Least-Square-Discriminant-Analysis (RM-MCCV-PLSDA) was built to investigate differences in the urinary metabolic profiles between both diets and to predict adherence to NICE dietary guidelines. Thereafter to strengthen the quality of the metabolically-personalised nutrition counselling model, PPI stakeholders (four dietitians and seven patients with CVD risk) were consulted about the counselling approach and tools through a series of activities. This included surveys, focus groups and semi-structured interviews.

Results: 72-hours of adherence to NICE dietary guidelines in a highly controlled environment revealed statistically significant changes in 27 metabolites. MCCV of the

multivariate model for Day 4 demonstrated strong prediction capacity (R^2_Y=1.0, Q^2_Y=0.96) and it was further able to predict the 24-hr urine samples after day 2 and 3 of both diets. For the PPI activities, two emergent themes that shaped the outcomes and impacts were: barriers and facilitators to nutrition care and metabolic profiling, and positive and negative experiences from implementing the metabolic profiling tool in clinical practice. PPI contributions assisted the researchers in identifying challenges and solutions not previously considered, and enabled key learning points to be taken away for future application of PPI.

Conclusion: The inpatient clinical trial and PPI have contributed to the PN toolkit that is ready for application in a subsequent study to deliver PN and objectively assess adherence to diet.

Keywords: cardiovascular disease, metabolomics, urine, personalised nutrition, patient and public involvement

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I would like to express my heartfelt gratitude to Dr Isabel Garcia-Perez for her guidance and assistance in the MRes project, as well as her research team, specifically Lina and Anastasia, for the collaborative effort in making PPI and the conceptualisation of a nutrition counselling model for the clinical trial a success. I would also like to gratefully acknowledge the support of Professor Gary Frost who has kindly provided his expertise in the trial. They have made my MRes journey a truly enjoyable and memorable experience in all respects, despite the few challenges we faced. Finally, I would like to thank my parents for their financial support in making my journey into nutrition research possible and their emotional support throughout the course.

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List of Abbreviations

δ	chemical shift
^{1}H	proton
BIA	Bioelectrical Impedance Analysis
CVD	Cardiovascular disease
CRF	Clinical Research Facility
HDL	High Density Lipoprotein
KDE	Kernel Density Estimate
LDL	Low density Lipoprotein
PLS-DA	Partial least squares Discriminant Analysis
PN	Personalised Nutrition
MP	Metabolic Profiling
MNT	Medical Nutrition Therapy
NCP	Nutrition Care Process
NICE	National Institute of Care and Excellence
NIHR	National Institute of Health and Care Research
NMR	Nuclear Magnetic Resonance
RCT	Randomised controlled trial
TSP	trimethylsilyl-[2,2,3,3,-$^{2}H4$]-propionate

Introduction

Cardiovascular disease and current nutrition strategies

Cardiovascular disease (CVD) is one of the leading cause of morbidity and mortality in the United Kingdom (UK). Although CVD-related deaths have fallen considerably over the past 40 years, there has been a slowdown in improvement since 2011. (Bhatnagar et al., 2016) With one in nine individuals currently living with the disease and CVD-related healthcare costs amounting to approximately £9 billion each year (British Heart Foundation, 2022), it is forecasted that a sustained plateau in mortality decline over the next decade could substantially increase UK's health and social care spending, with the total 10-year cumulative incremental net monetary cost estimated to be £54 billion. (Collins et al., 2021) These patterns implicate a need for optimal strategies to alleviate the socio-economic burden imposed by CVD on the health system and communities across the UK.

Diet has been well-established as a key modifiable risk factor in the prevention and risk reduction of CVD. (Ravera et al., 2016) Current clinical nutrition management within the National Health Service (NHS) involves dietitians utilising the medical nutrition therapy (MNT) for CVD, an individualised nutrition treatment implemented according to evidence-based recommendations such as the National Institute for Health and Care Excellence (NICE) CVD guidelines (Stewart, Manmathan & Wilkinson, 2017), and executed using the Model and Process for Nutrition and Dietetic Practice. (British Dietetic Association, 2020) This standardized framework comprises six steps: assessing nutrition status based on anthropometry, biochemistry, clinical, dietary, environmental/behavioural/social and functional (ABCDEF) parameters, diagnosing CVD-related nutrition problems, collaboratively strategizing and implementing nutrition care plans, monitoring and reviewing dietary intake, and evaluating CVD-related nutritional outcomes. In guiding dietary strategies and implementation, food groups such as wholegrains, fruits and vegetables, nuts, seeds and legumes are often highly encouraged by dietitians, while nutrients such as sodium, saturated and trans fats from fried foods, red and processed meats have been widely discouraged.
In addition, cardioprotective dietary patterns such as the Mediterranean diet and Dietary Approaches to Stop Hypertension (DASH) diet are also commonly advocated due to their efficacy in improving individuals' vascular health. (Butler et al., 2020). A meta-analysis of 20 prospective studies (n=888, 257) highlighted that greater adherence to a Mediterranean diet lowered the incidence (RR=0.73, 95% CI: 0.66-0.80) and mortality (RR: 0.73; 95% CI: 0.68-0.79) from CVD, while 4 randomised

controlled trials (RCT) (n=12,293) demonstrated an average 40% decreased risk of CVD incidence and mortality (RR: 0.59; 95% CI: 0.38-0.93; I^2 = 46%) in the Mediterranean diet group compared with controls. (Grosso et al., 2017) The DASH diet offered similar protective effects, wherein prospective cohorts of 783,732 individuals revealed significant CVD reduction in those who followed a DASH diet (RR=0.80; 95% CI=0.76-0.85). (Chiavaroli et al., 2019)

Unfortunately, the current nutrition strategies are no panacea as it is known that large inter-individual variability exists in response to diets. In the recent PREDICT trial - a tightly controlled clinical experiment involving standardised test-meals consumed by 1002 individuals after 6 hours of fasting, significant heterogeneity (as measured by the population coefficient of variation (s.d./mean, %)) was noted in their postprandial responses of serum triglyceride(TG), glucose and insulin concentrations, compared with fasting values (103% vs 50%, 68% vs 10% and 59% vs 69% respectively). (Berry et al., 2020) These inter-individual variations may be explained by differences in intrinsic factors such as one's genes, sex, age, habitual dietary intake and gut microbiome profile. Indeed, when the researchers examined the genetic and microbiome factors of participants with their postprandial responses, additive genetic factors explained 48% of glycaemic variability$_{iAUC0\text{-}2h}$, while individual's gut microbiome composition explained variation in 7.5% of $TG_{6h\text{-}rise}$, 6.4% of glucose$_{iAUC0\text{-}2h}$ and 5.8% of C-peptide$_{1h\text{-}rise}$. (Berry et al., 2020) Similar observations of inter-individual variability in response to specific dietary components or interventions were outlined in two other review papers. For example when exploring the role of plant food bioactives on cardiometabolic outcomes, Manach et al. (2017) attributed inter-individual response differences to genetic polymorphisms of genes associated with cholesterol trafficking processes, and identified studies that showed better LDL-cholesterol lowering response to plant sterol consumption in subjects carrying the A allele of the ABCG8 gene, while isoflavones from soy consumption induced greater improvements in blood pressure, endothelial function and arterial stiffness among equol producers. Likewise, when examining gut microbiota and host response to dietary interventions, Healey et al. (2017) presented based on several researches that a higher baseline abundance of gut microbiota such as *Prevotella, Akkermansia muciniphilia* and *Lactobacillus/ Leuconostoc/Pediococcus* is respectively associated with better glucose and insulin response, lower LDL-cholesterol and greater weight reduction after dietary interventions. Individuals with higher microbial gene richness may also have a better ability to cope metabolically with changes in dietary intake, leading to a greater potential in influencing health outcomes. (Healey et al., 2017) As the nutritional

complexities underlying major diet-related diseases are increasingly unravelled, researchers are concurrently recognising that the aforementioned one-size-fits-all, population-based dietary approaches may be less effective than intended and a personalised diet that acknowledges individual nutritional differences may be more useful in this respect.

Personalised nutrition (PN): its advantages and limitation

Recent advancements and improved access to health technologies such as genetic testing, microbiome analysis and the use of wearable devices or mobile applications, has enabled the process of dietary personalisation to evolve and become more in-depth. (Adams et al., 2020) While the science is still in its infancy, many PN companies have since emerged, harnessing the power of machine learning, advanced computational methods and data analytics platform to integrate personal data for the provision of dietary advice that claims to work best for the individual. The intention behind PN is to bring about a more sustained improvement in the dietary habits of individuals or groups of individuals with similar traits, and empower them with the overall goal of preventing or treating chronic diseases, as well as advancing human health and well-being. (Ordovas et al., 2018)

To date, few systematic reviews have touted the benefits of PN over conventional nutrition counselling based upon general dietary guidelines, of which include improved diet quality (Jinnette et al., 2021) and cost effectiveness. (Galekop, Uyl-de Groot & Ken Redekop, 2021) Additionally in a RCT by Zeevi et al. (2015), researchers found that personalising diets according to a self-devised machine learning algorithm which integrates various health aspects such as blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiota from 800 individuals resulted in significantly lower postprandial glycaemic responses and consistent alterations to the gut microbiota configuration. Likewise, positive findings were also reported in a recent large-scale Food4me RCT (n=1604) that examined whether dietary advice provided at different levels of personalisation (L0: non-personalised dietary advice based on population guidelines; L1-3: personalised dietary advice according to- L1: self-reported diets; L2: L1 + phenotypic data e.g. blood profile of glucose, total cholesterol, carotenoids and n-3 index; L3: L2 + genotypic data e.g. MTHFR, FTO, APOE4 and FADS1 genes) will produce a greater sustained change in dietary behaviour than standard healthy eating advice. (Celis-Morales et al., 2017) Over a 6-month period, participants in the intervention groups (L1-3) were found to consume significantly lower

amount of red meat, salt and saturated fat, whilst achieving a higher Healthy Eating score and folate intake, as compared to controls (L0). However, there was no evidence that the addition of phenotypic (L2) or phenotypic and genotypic (L3) information as part of the personalisation process could further enhance the effectiveness of PN. (Celis-Morales et al., 2017)

This raises a question of whether using multiple sophisticated technologies to provide increasingly detailed personalised dietary advice is in fact 'worthy', given the additional resources needed to collect and process all the data; or perhaps there may be inherent flaws in the methodology of implementing PN that is contributing to the lack of added value. Evidently, a major limitation uniform across all PN intervention arms is the use of subjective method for dietary assessment (in this instance, using food frequency questionnaires (FFQ)). As there is currently no gold standard method of dietary assessment, subjective dietary assessment tools such as FFQ, 24-hr dietary recalls, weighed food records(WFR) and food diaries are commonly used in nutrition research and many large-scale epidemiological studies. (Shim, Oh & Kim, 2014) However, these methods are archaic and have been criticized for being flawed with systematic and random errors. (Amoutzopoulos et al., 2018; Burrows et al., 2019; Walker, Ardouin & Burrows, 2018) Misreporting, in particular underreporting of energy intake, is a common problem among individuals regardless of age or sex (Mckenzie et al., 2021), and this phenomenon increases with body mass index. (Ravelli & Schoeller, 2020) With an estimated prevalence of misreporting to be between 30 to 88% (Rennie, Coward & Jebb, 2007), researchers face the challenge of accurately capturing and assessing what individuals are consuming, which in turn cast doubt as to whether the subsequent lack of effect of a dietary change at an individual or population level in PN is a result of poor compliance, high inter-individual variability or a true absence of physiological impact. In this regard, the use of objective dietary intake measures is warranted to help researchers better evaluate one's dietary adherence and its corresponding effects on health outcomes.

Metabolomics and its application in nutrition research

Metabolomics has become an emerging area of interest in nutrition research in recent years. In a scoping review that discussed the application of metabolomics to nutrition, Shibutami & Takebayashi (2021) highlighted three primary uses: (1) providing an objective dietary assessment to overcome the limitations caused by conscious or unconscious distortion of self-reported data; (2) metabolic profiling (MP) to explore the

potential health benefits of diet and understand inter-individual variability in metabolising the same foods in health and disease states; (3) predict health risk by characterising individual's susceptibility to diet-induced diseases based on one's physiological or health status biomarkers. As a high-throughput 'omics' technology, metabolomics allows for the identification and quantification of metabolites (small molecules of molecular weight <1kDa) present in cells, tissues and body fluids of an organism and their changes in relation to genes, environment, drug or diet. (Holmes, Wilson & Nicholson, 2008) The theory underlying metabolomics is that an individual's metabolic state provides a close representation of his/her overall health status. (Beger et al., 2016) As metabolites are intermediates or end products of multiple enzymatic reactions in vivo, they are therefore considered the most informative proxies of the biochemical activity occurring in an organism. (Alonso, Marsal & Julià, 2015) Analytical tools such as nuclear magnetic resonance spectroscopy using a hydrogen spectrum (^{1}H-NMR) or liquid/gas-chromatography coupled mass spectrometry (LC/GS-MS) have commonly been employed for metabolomics, through targeted or untargeted analysis. The former enables defined groups of chemically characterized and biochemically annotated metabolites to be measured, whereas the latter involves a comprehensive analysis of all the measurable analytes in a sample, including chemical unknowns, which provides the opportunity for novel metabolite species, pathway and target discovery. (Roberts et al., 2012)

To exemplify its utility in objective assessment of dietary patterns, Garcia-Perez et al. (2017) conducted a randomised controlled crossover trial investigating the effect of different diets on urinary metabolic profiles. The researchers developed four dietary interventions that varies stepwise in agreement with the World Health Organisation (WHO)'s healthy eating guidelines (Diet 1 being the most concordant, Diet 4 being the least concordant, and Diet 2 and 3 of intermediate concordance) and administered them on 19 healthy UK participants in a highly controlled environment. From the third 24-hour urine sample collected for ^{1}H-NMR spectroscopy analysis after test meals consumption, significant stepwise differences were noted in the concentrations of metabolites excreted from Diet 1 and 4, including those with well-known dietary associations such as hippurate, carnitine and tartrate (markers of fruit and vegetable, red meat and grape intake respectively). Although interindividual variability in dietary response was seen from the individual metabolites excreted by each participant, their metabolic profiles were still distinctively separated into two dietary groups, which enabled the researchers to build a mathematical model that can classify participants into consumers of diets associated with low or high risk of non-communicable

diseases. Internal validation of the model was conducted with Diet 2 and 3, in which the model was able to predict the dietary profiles of individuals consuming these intermediate diets based on clear clustering separation across all four metabolite profiles, while external validation was done using the UK INTERMAP cohort (n=225) and a healthy Danish cohort (n=66). (Garcia-Perez et al., 2017) Given the interlink between food intake and metabolite production, the identification of nutrition-specific biomarkers as such can provide actionable information to fill the gaps of inaccuracies arising from self-reported dietary intake. (Tebani and Bekri, 2019) These current evidences put forth the potential of metabolomics as a key enabler of PN, by offering a stepping stone towards mitigating the traditional nutritional challenges of dietary misreporting, compliance and inter-individual response variability. With the highly controlled dietary intervention providing initial proof for the feasibility of using urinary metabolomics as an objective dietary assessment tool, there is a need to further develop the model and determine an optimal approach for translating it into the clinical setting to assist NHS dietitians with their medical nutrition therapy management of CVD, given the concerning rising CVD statistics in the UK.

Patient and Public Involvement in nutrition research

Noting that the successful implementation and translation of clinical nutrition trials hinges on an effective partnership and collaboration between nutrition researchers, dietitians and patients (Jamie Zoellner et al., 2015), it will be helpful to include these stakeholders into the process of shaping clinically translatable nutrition strategies, in order to increase the relevance and acceptability to its end service users. Patient and public involvement (PPI) - defined as research performed ‘with’ or ‘by’ patients and members of the public, instead of ‘to’, ‘about’, or ‘for’ them (NIHR, 2015), has become increasingly popular and recommended in clinical research. Funders such as the National Institute for Health Research (NIHR) raise the importance of establishing an active partnership between patients and/or members of the public and researchers rather than treating PPI as mere tokenism, as it has been shown to facilitate the provision of alternative views based on the lived experiences of diverse individuals, which can lead to improvements in trial design, participant recruitment and outcome measures. (Brett et al., 2014; Crocker et al., 2018; Ennis & Wykes, 2013; Skovlund et al., 2020) Among the rising number of publications on PPI are few nutrition-related researches that have also incorporated PPI and touted the aforementioned benefits in the context of weight management during pregnancy and renal dietetic research (Abayomi et al., 2020; Bridger Staatz et al., 2018; Morris et al., 2017) However, no

study to date have investigated its value in PN or CVD management. PPI may be the key to the development of a clinically translatable, metabolically-personalised nutrition counselling model that NHS dietitians can use to objectively assess dietary intake and enhance dietary advice for free-living UK individuals at risk of CVD.

Therefore to fill the aforementioned literature gaps, a three-study research was established.

Hypothesis

It is hypothesised that a dietary counselling framework is needed to be established to assure the effectiveness of using metabolically-tailored dietary advice for the clinical nutritional management of individuals at risk of CVD.

Aims

This research aims to develop a personalised nutrition toolkit comprising of a urinary metabolic profiling dietary assessment model and metabolically-personalised nutrition counselling model to guide the implementation of personalised dietary advice for the clinical nutritional management of free-living individuals with CVD risk.

To achieve the research aims, three studies will be conducted.

Study 1: Develop a novel metabolic profiling dietary assessment model under controlled conditions for the nutritional management of individuals with CVD risk

Objectives:

1) To investigate changes in the urinary metabolite profiles as a result of following two extreme diets in concordance with NICE guidelines
2) To test the model's capability in predicting dietary habits of individuals at CVD risk

Study 2: Develop a metabolically-personalised nutrition counselling model based on the British Dietetic Association's Model and Process framework for individuals at risk of CVD

Objectives:

1) To combine 24-hour self-reported dietary intake with metabolic profiling dietary assessment model to obtain a comprehensive and accurate dietary assessment
2) To conduct a PPI Plan with clinical dietitians and patients at CVD risk that will inform on the dietary personalisation process and tools involved.

Study 3: Apply the personalised nutrition toolkit for the clinical nutritional management of free-living individuals at risk of CVD.

Objective:

1) To conduct a randomised parallel clinical trial for individuals at risk of CVD to compare traditional vs metabolically personalised dietary advice.

NOTE

1) *I was not involved in conducting the clinical trial for Study 1*
2) *Due to delays in receiving the Enhanced DBS check with Adults' Barred list and delays in starting the clinical trial for Study 3 due to a lack of available consumables and participant recruitment, I will not be presenting results for Study 3 as participants will have to complete at least four weeks in order to have statistically significant (if any) data. The trial is currently ongoing and I have been involved in recruitment, screening and conducting the personalised nutrition counselling.*

Therefore, my MRes thesis includes results from study 1 and 2 (Figure 1).

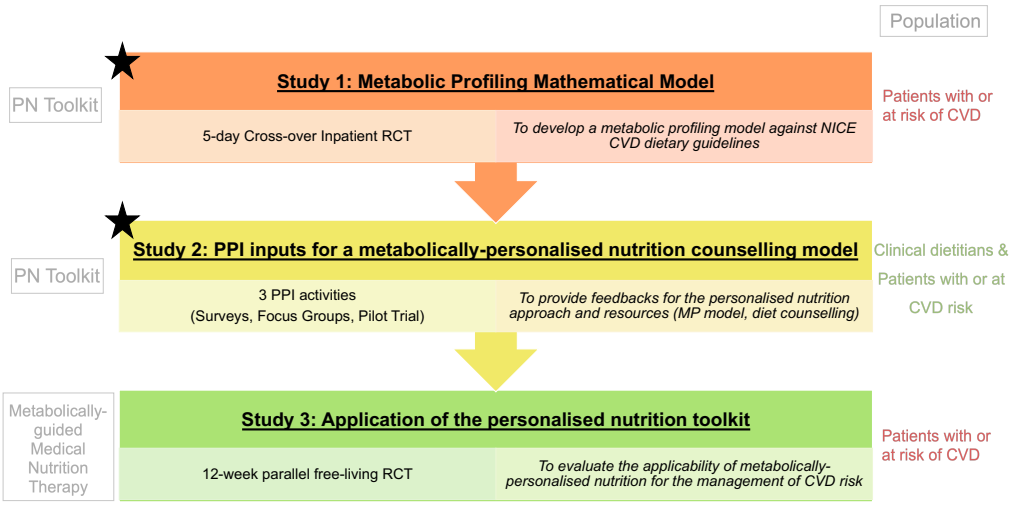


Figure 1: Schematic of the research (Starred sections indicate MRes thesis inputs)

Methods

Ethics

Ethical approval was obtained from the Research Ethics Committee and Health Research Authority (Reference: 18/LO/2042). The trials were run in accordance with the Declaration of Helsinki and Good Clinical Practice.

Study 1: Development of a metabolic profiling dietary assessment model as part of a personalised nutrition toolkit

Clinical trial design

A randomised controlled, crossover study was conducted in participants at risk of CVD over two 5-day inpatient periods. Study participants included males and females aged 30-65 years with body mass index (BMI) of ≥25 and <35kg/m^2, systolic blood pressure (BP) ≥140 or diastolic BP≥90mmHg or those under antihypertensive medication, LDL-cholesterol ≥4.14 mmol/l and HDL-cholesterol ≤1.03mmol/l (men) or ≤1.29 mmol/l (women), waist circumference (WC) >102cm in men or >88cm in women, those with a family history of premature coronary heart disease or are currently smoking. Excluded individuals were pregnant females, those with >3kg weight change in the preceding 3 months, excess alcohol consumers or substance abusers, those with medical conditions such as diabetes, cancer, pancreatitis, HIV, gastrointestinal diseases, kidney or liver disease, or are on medications that can interfere with energy metabolism, appetite regulation and hormonal balance.

Screening visit

Volunteers were examined by a research doctor at the NIHR/Wellcome Trust Imperial CRF at Hammersmith Hospital. BP measurements, an electrocardiogram (ECG) and blood samples for full blood count, HbA1c, urea and electrolytes, liver function test and lipid profile were taken. Anthropometric measurements including height, weight and waist circumference were recorded. All women of child bearing age underwent a urinary pregnancy test. Volunteers had the opportunity to raise any study enquiry, while their medical history, family history of medical illness and drug history were discussed to confirm their eligibility. All eligible volunteers provided written informed consent for study participation.

Pre-study visit

Recruited participants attended the CRF 4 weeks before study commencement. Apart from completing a list of food dislikes, resting energy expenditure was assessed using Bioelectrical Impedance Analysis (BIA) (Tanita BC-418MA) to facilitate the planning of eucaloric diets.

Inpatient Periods

During each five-day inpatient period, each participant followed in a random order, two extreme diets over 72hours (Table 1).

Table 1: Eucaloric diets with portions varying according to participant's estimated energy requirements

Meal type (time)	**Diet 1** (Most concomitant with NICE guidelines)	**Diet 2** (Least concomitant with NICE guidelines)	
	Day 2 to 4	**Day 2**	**Day 3 and 4**
Mixed meal test (08:00)		Ensure plus vanilla 200ml	
Breakfast (09:00)	Tea with milk Swiss style muesli Skimmed Milk Banana		Tea with milk Salted butter White medium bread Whole milk Breakfast cereals (Cornflakes)
Morning Snack (11:30)	Orange Coffee with milk	Chocolate Mousse Tea with milk Salted butter White medium bread Whole milk	Chocolate Mousse Coffee with milk
Lunch (13:00)	Salmon and Dill Potato Bake Mixed vegetables (carrot, peas, cauliflower, cut green beans, sweetcorn) Egg noodles Olive oil Tea with milk	Pork sausages in onion gravy Mashed potato	Pork sausages in onion gravy Mashed potato
Afternoon Snack (15:00)	Coffee with milk Grapes (red)	Chocolate bounty Coffee with milk	Chocolate bounty Coffee with milk
Dinner (18:00)	Chicken Breast in Gravy Mixed vegetables (carrot, peas, cauliflower, cut green beans, sweetcorn) Jacket Potatoes Baked Beans in Tomato sauce Olive oil	Quarter Pound Beef burger with chargrilled onion (C/F 3201) Chips Burger Buns Tea with milk	Quarter Pound Beef burger with chargrilled onion (C/F 3201) Chips Burger Buns
Evening Snack (21:00)	Apple Mixed Nuts	Chocolate milk	Chocolate milk

A researcher not directly involved in the study conducted the randomisation, using opaque, sealed, sequentially numbered envelopes that contained one of the two dietary interventions. The envelopes were stored securely and opened in sequence by an investigator as each participant was enrolled. Although participants and investigators were unable to be masked from the intervention due to the study's nature, investigators analysing the data were masked from the randomisation order.

Intervention duration was chosen based on previous studies indicating urinary elimination of most food-derived metabolites occurs within 48hours. (Favari et al., 2020; Cheung et al., 2017; Cuparencu et al., 2019) The workflow of Study 1 is depicted in Figure 2.

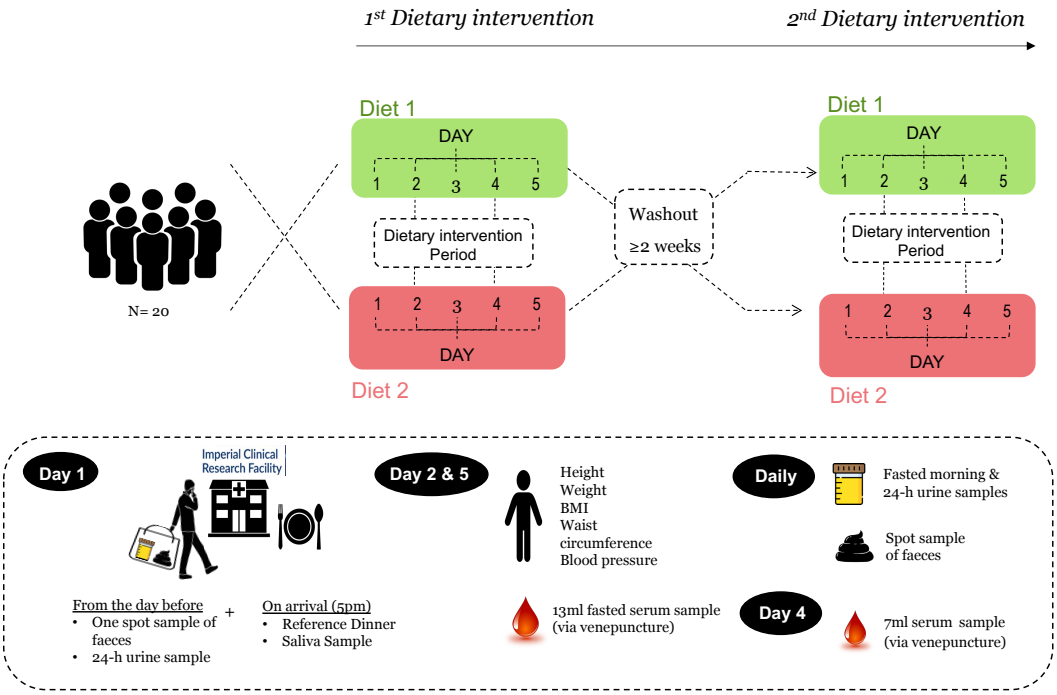


Figure 2: Workflow of Study 1, crossover RCT.

Participants were randomly assigned to Diet 1 - most concomitant to NICE guidelines or Diet 2 - least concomitant to NICE guidelines. Between diets, participants had a 2-week washout period. Participants attended the CRF at 5pm on day 1 and received a standardised dinner. Inpatient sample collection started on day 2. Fasting urine, 24-hours urine and stool samples were collected daily. BP, anthropometric measurements and fasting blood samples were collected at the beginning and end of each study period.

During the intervention period, participants were instructed to consume their allocated diets entirely and were allowed to drink water freely. Such expectation was fully explained prior to trial enrolment. Dietary adherence was strictly monitored by weighing all foods immediately before consumption and all uneaten foods thereafter. Physical activity was also controlled - participants could only engage in very light physical activity (no more strenuous than walking from their hospital bed to the toilet). A minimum washout duration of 2 weeks was planned in between, to minimise any possible carryover effects from the first intervention. Stools, fasting and 24-hour urine samples were collected daily. In addition, fasting blood was collected before and after each diet and 3-hours after lunch on day 4.

Urinary Sample Preparation and 1H NMR spectroscopic analysis

1ml of participants' urine was decanted in 10 1.5ml pre-labelled micro tubes once received. All decanted samples were stored frozen at -80°C until needed. During sample processing, 540 µL of urine was mixed with 60 µL of phosphate buffer (pH 7.4) containing trimethylsilyl-[2,2,3,3,-$^{2}H_{4}$]-propionate as an internal chemical shift reference. ^{1}H-NMR spectroscopy was performed on the urine samples at 300K on a 600 MHz spectrometer (Bruker BioSpin, Karlsruhe, Germany), using a standard one-dimensional pulse sequence with water-presaturation previously exemplified by Garcia-Perez et al. (2017).

Power calculation

Power was calculated based on Garcia-Perez et al. (2017). Effect size was determined using the excretion of hippurate, a urine biomarker derived from consuming fruits and vegetables. Increasing fruit and vegetable intake from 100g to 300g in a highly controlled environment resulted in a rise in urinary hippurate concentration of 3.48 ±4.52 mmol/24-h. With a resulting effect size of 0.772, an alpha of 0.05 and power of 0.90, the study required at least 16 participants (based on a one-tailed difference between two dependent (paired) means). Allowing for a 20% drop-out, 20 participants were deemed appropriate for recruitment.

Statistical analysis

Statistical methods used by Garcia-Perez et al. (2017) was employed. In essence, ^{1}H-NMR spectra (16000 spectral variables) were manually phased, corrected for baseline distortions and digitised over the range δ0·5–9·5 using an in-house MATLAB (version R2021a, The Math-Works, Inc.; Natwick, MA) script. Probabilistic Quotient Normalisation was conducted on the median spectrum of diet 1 and 2 to normalise the spectra to the same virtual overall concentration. As a method that accounts for variations in the overall concentrations of samples caused by different dilutions (Dieterle et al., 2006), such spectra scaling is important to ensure that metabolite concentrations are corrected for differences in urine osmolality which may arise from different hydration status between participants and/or different amount of foods ingested (e.g. caloric density). Following the spectroscopic techniques, multivariate data from Day 4 samples was modelled with Partial Least Squares Discriminant Analysis (PLS-DA) in a repeated-measures Monte Carlo Cross-Validation (RM-MCCV) framework. Day 4 were chosen as it was timed to be 72h after commencing the dietary interventions and ensured metabolic profile stability. (Heinzmann et al., 2012; Hughes et al., 2019) Data centering and scaling were done to account for the repeated-

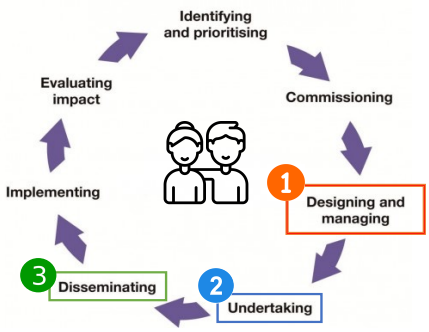
measures design while 1000 iterations were used to assess model robustness. For each iteration, samples left out of the training set were used as the test set. The mean predicted score (T_{pred}) of each participant's urine sample was derived from all 1000 iterations and the variance of T_{pred} was estimated from 25000 bootstrap models, obtained by running an additional 25 bootstrap resampling from the training set of each of the 1000 iterations. As T_{pred} relates to the healthiness of one's diet, a positive T_{pred} indicates that one's urinary metabolite profile mirrors more closely to the metabolite profile generated from a study-controlled diet that is of 100% concordance with NICE dietary guidelines i.e. resembling Diet 1 more than Diet 2, and vice versa for a negative T_{pred}. Kernel density estimate (KDE) was calculated by summing the resulting Gaussian distributions of all samples within each group. The fit and predictability of the model were obtained and expressed as R^2_Y (explained variance) and Q^2_Y (capability of prediction), where higher values indicates a better model. To detect the most robust contributors to the model, p-values were acquired for each spectra variable and further adjusted for multiple testing to obtain a q-value (false discovery rate adjusted p-value). Variable importance was assessed and a q-value of ≤0.01 indicates significance. Thereafter, internal validation was done by using the model to predict the scores of the participants' urine samples obtained from both diets on day 2 and 3, based on the T_{pred} of samples from Day 4. Metabolites' identification were subsequently ascertained from an in-house database and by applying Statistical total correlation spectroscopy (STOCSY).

Study 2: PPI in the development of a metabolically-personalised nutrition counselling model as part of a PN toolkit

PPI design

Although PPI can take place at all research cycle stages illustrated by INVOLVE (Figure 3, left), a study-focused framework that helps make trials more relevant and appealing (Greenhalgh et al., 2019), we deemed PPI incorporation as the most appropriate once the metabolic profiling and nutrition counselling models have been developed. Hence, we specifically focused on engaging PPI during the designing and managing phase of study 3 in order to strengthen the 'voices' of key stakeholders right from the start. Future plans are also being made for PPI in undertaking and analysing results, and dissemination and implementation as study 3 progresses but these are outside the thesis scope.

Clinical dietitians and patients with CVD risk were chosen as PPI contributors as we hope to incorporate the perspectives of key stakeholders to whom our research aims to benefit. We strived to avoid any power differentials by viewing all stakeholders as 'equal partners' within the group and their respective roles are detailed in Figure 3 (right). PPI activities were planned in accordance with guidance from INVOLVE (NIHR Research Design Service, 2018) and all PPI participants were reimbursed for their time according to rates recommended by the NIHR's Payment guidance for Researchers and Professionals.



Stage of the Research Cycle	Dietitians	Patients
1	- Identify barriers and facilitators to integrating metabolic profiling in clinical practice - Provide feedbacks (positive and negative) on the experience of integrating the metabolic profiling tool in clinical practice & the research materials used - Provide expertise on the dietary counselling process	- Provide an understanding of the drivers of food choice and dietary experiences among patients - Identify potential concerns regarding biological samples collection
2	- Provide feedback on the study results to ensure the interpreted data encompass diverse perspectives (patient, clinician, researchers), improve the quality of the discussion, correct misinterpretations and examine the validity of the conclusions	
3	- Produce lay summaries/materials of findings for dissemination (media, community and CVD groups)	

Figure 3: INVOLVE's study-focused framework: stages of the research cycle (left), roles of each PPI stakeholder group within stages of the research cycle (right)

An online survey (Appendix A) was first used to gain insight into the current state of dietetics practice, which was disseminated through social media platforms such as Twitter and LinkedIn, intending to reach out to dietitians worldwide. Dietitians' views on personalised nutrition (PN) and their nutrition care approach were sought. With the global perspectives identified, local stakeholder groups were engaged for the PPI activities - a clinical group (CG) comprising dietitians with NHS experience recruited through the British Dietetic Association, and a reference group (RG) comprising patients with or at risk of CVD sourced from the CRF database.

Three activities were planned and the PPI workflow is exemplified in Figure 4. Activity 1 and 2 focused on seeking stakeholders' perspectives for integrating the novel tool into nutrition counselling. Activity 3 was created to gather their experiences from pilot testing the integration.

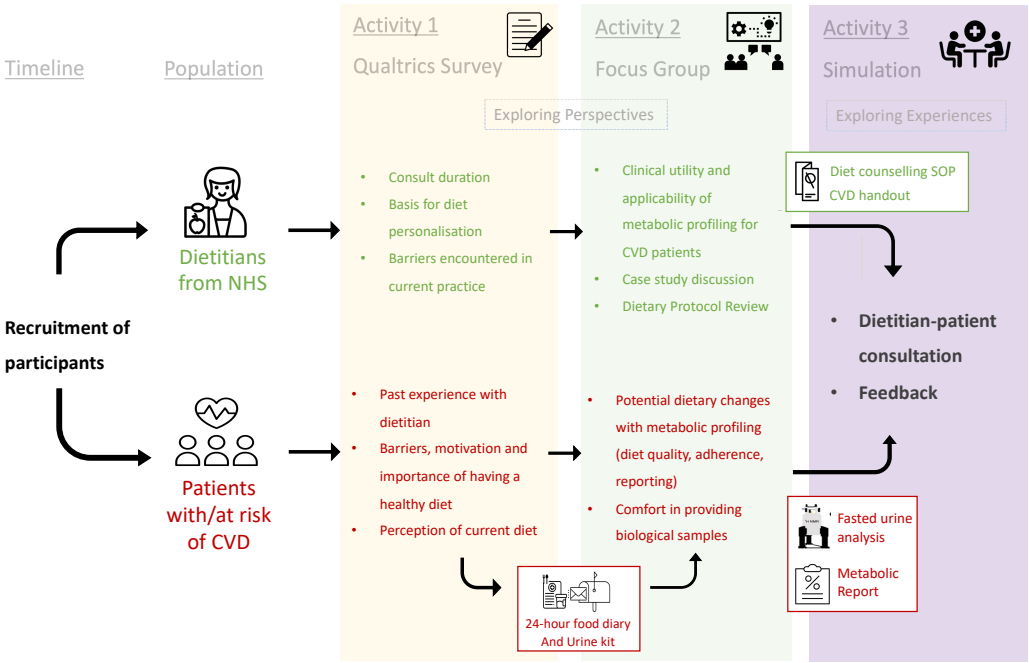


Figure 4: Workflow of the content of each PPI activity conducted.

Activities 1 and 2 were conducted independently for dietitians and patients through surveys and focus groups, while Activity 3 was the result of the interaction between dietitians and patients, through piloting the metabolically-personalised nutrition counselling.

Activity 1 (Appendix B)

Recruited RG and CG respectively received a diet-related questionnaire. Questions for UK dietitians were regarding considerations and barriers faced in personalising diets, while patients were asked about their barriers to healthy eating, experiences with dietitians, their perceived importance of a healthy diet and perceived healthiness of their current diet.

Activity 2 (Appendix C)

An hour-long virtual focus group was held separately for each group to freely express their opinions around metabolically-personalised nutrition counselling. Discussions with dietitians included gathering views regarding the clinical utility of the tool, its applicability to CVD and hypothetical case scenarios of two metabolic profiling reports - one where the dietary recall matches the urinary metabolic profile and another of discordance, to understand how dietitians will approach each situation. Each dietitian was subsequently asked to review the nutrition counselling model protocol that researchers will utilise for personalising diets in Study 3. Discussions with patients included their comfort level in providing biological samples, views regarding potential dietary changes with objective assessment and preferences for diet personalisation.

Upon session conclusion, they were instructed to complete a 24-hour dietary recall using Intake-24 and collect fasted urine sample the next morning using a mailed urine kit. A metabolic profiling report was generated for each patient upon sample return in preparation for Activity 3, using Study 1's model.

Activity 3 (Appendix D)

Each patient was randomly matched to a PPI dietitian for the pilot intervention. Dietitians were instructed to utilise the nutrition counselling protocol, revised based on feedbacks from previous activities and the metabolomics report for information gathering and provision of dietary advice. Semi-structured interviews were held separately for both parties post-consultation, to explore their experience in greater depth and provide feedback about the research materials.

Data Analysis

Focus group discussions from Activity 2 were digitally recorded, transcribed verbatim and each transcript was anonymised. Manifest content analysis was conducted using a thematic approach for the responses from the global survey, activity 1 and 2. This involves a reflexive and iterative process with initial open coding followed by selective and more detailed coding. Codes were thereafter connected based on the questions posed, which led to emergent subthemes and themes.

Study 3: Evaluate the applicability of providing metabolically-guided medical nutrition therapy using the PN toolkit for CVD management

Clinical trial design

A 12-week randomised control parallel-group study is currently being conducted in 134 free-living participants at CVD risk and the study workflow is depicted in Figure 5.

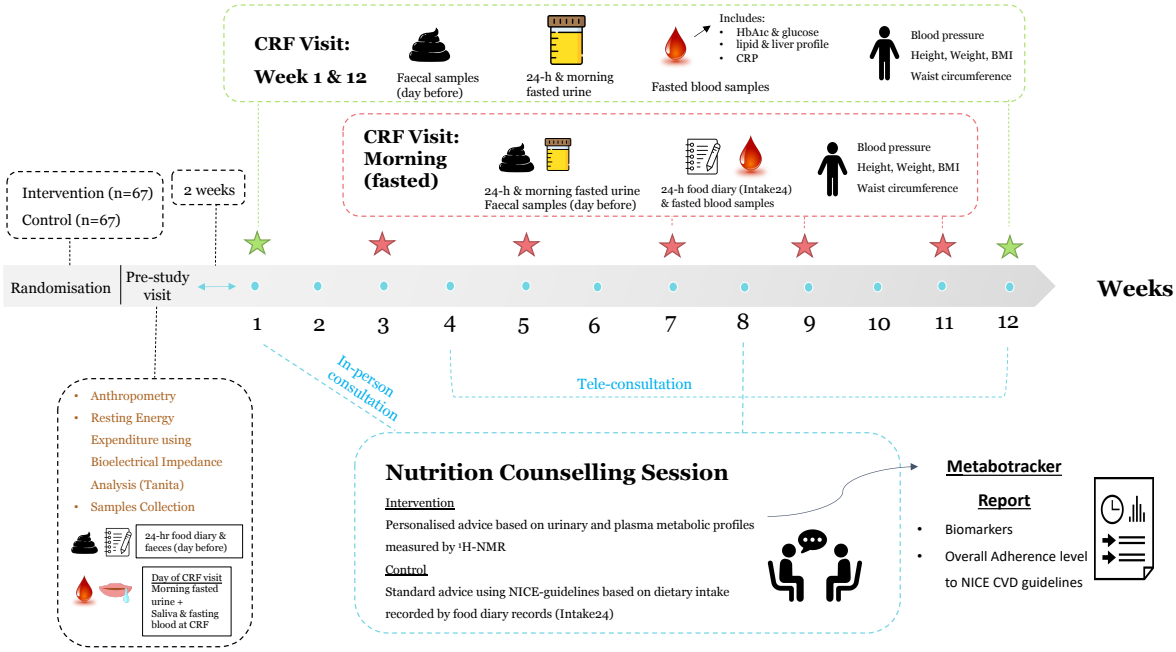


Figure 5: Workflow of Study 3, parallel group RCT.

Participants will receive either a metabolically-personalised nutrition counselling based on the metabolic profiling report (intervention) or standard nutrition counselling based on 24-hr dietary recall (control) over 12 weeks. 4 nutrition counselling sessions with be provided and samples of urine, stool and blood will be collected once fortnightly.

Screening visit

Inclusion and exclusion criteria are identical to Study 1 and screening will be conducted as above. Recruited participants will be randomised to either the control or intervention group using a computer-generated allocation sequence and all will attend the CRF once fortnightly upon study commencement.

CRF study visits

Participants' baseline information will be obtained during their pre-study visit, two weeks before study commencement. Anthropometric measurements will be collected, alongside BP and biological samples such as faeces from the day before, fasted urine in the morning before CRF visit, and saliva and fasting blood samples upon arrival. Participants will be instructed to record their food intake from the day before on

Intake24, an open-source self-completed computerised dietary recall system based on multiple-pass 24-hour recall and their resting energy expenditure will be measured using BIA. 24-hour recall was chosen to mirror clinical dietetics practice. During each fortnightly visit, participants will provide faecal sample and 24-hour urine sample from the day before, fasted urine collected in the morning of their CRF visit and corresponding 24-hour food diaries from Intake24. Anthropometry, BP and fasting blood samples will be obtained upon arrival.

Dietary intervention

All participants will receive tailored dietary advice in accordance with NICE guidelines, through in-person and tele-consultation. Four regular sessions are established for dietitians to set and review dietary progress with participants, to support good dietary compliance. To reduce fidelity between dietitians, they will be trained to execute the protocol of the metabolically-personalised nutrition counselling model. Participants will be encouraged to stay active but no specific physical activity advice will be given as adjunct to diet.

Dietary intake of controls (n=67) will be assessed from Intake24. The breakdown of dietary components will be compared against NICE guidelines to determine overall adherence level, and thereafter scored according to the number of recommendations met (Table 2).

Table 2: NICE Dietary guidelines checklist, used to determine whether participant met each CVD dietary recommendation and their overall adherence to NICE guidelines

Dietary Components	NICE Dietary Recommendation	Did the participant meet the recommendation? (YES/NO)
Energy	Based on the participants requirement, 600 kcal will be deducted for those who need to lose weight	
Total Fat	Less than 30% of energy	
Saturated Fat	Less than 7% of energy	
Dietary cholesterol	Less than 300 mg	
Trans fat	Less than 2% of energy	
Unsaturated fat (MUFA & PUFA)	Using olive oil or rapeseed oil or spreads based on these oils, and to use them in food preparation.	
Fish	At least 2 portions of fish per week, including a portion of oily fish.	
Unsalted nuts, seeds and legumes	At least 4 to 5 portions of unsalted nuts, seeds and legumes per week	
Red meat	Less than 70g/d	
Dietary fibre	30g-45g/d	
Fruits and Vegetables	At least 5 servings /d	

Wholegrain starch	Choose wholegrain varieties of starchy food	
Free sugar	Less than 5% of energy	
Alcohol	Men: Less than 3-4 units/d Women: Less than 2-3 units/d	
Salt	Less than 2.4g of sodium /d	
	Total number of recommendations met: Adherence score (%):	/15 /100

In contrast, diets of the intervention group (n=67) will be assessed from a 'metabotracker' report predicting participants' dietary metabolic response, generated from Study 1's mathematical modelling of their 1H-NMR urinary analysis. Participants' dietary intake will be monitored according to their overall adherence level and individual urinary biomarkers changes (Figure 6), while the accuracy of self-reporting will be determined by examining whether metabolites associated with the reported foods are found in the urine.

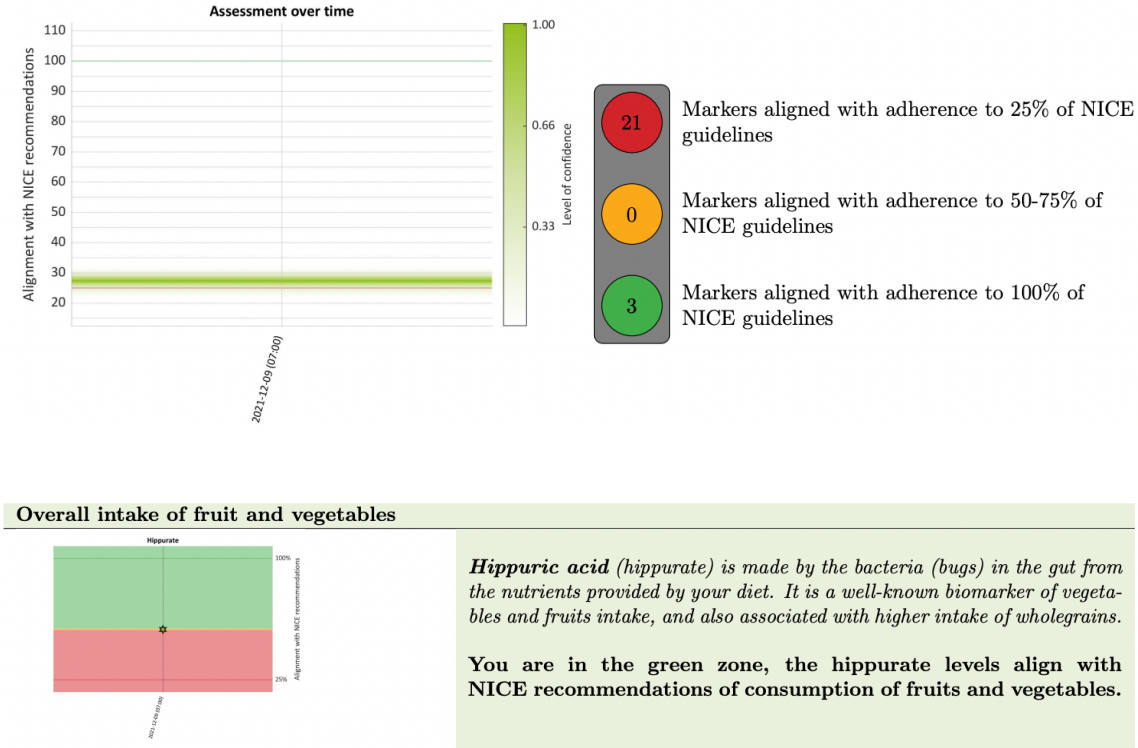


Figure 6: Overall dietary adherence level based on participant's overall metabolic profile (%) (top) and alignment of individual biomarkers (bottom) to NICE's CVD guidelines

Dietitians will utilise these information to engage and educate participants regarding their metabolically-personalised nutritional plan. For example, if participant's metabolic

profile indicates low hippurate levels, which is related to low fruits and vegetables consumption, then increasing these intake will become a key dietary target**.**

Power calculation

Sample size was calculated from Garcia-Perez et al. (2017). With urinary hippurate concentrations rising by 1.05±3.48mmol/24-h and 3.48±4.52mmol/24-h when fruits and vegetables intake increased respectively from 100g to 180g and 300g, an effect size of 0.603 was attained. Assuming 90% power and α=0.05, 118 participants are required (two-tailed difference). Therefore, adjusting for a 13% drop-out according to Celis-Morales et al. (2016) study, 134 participants are needed.

Statistical analysis

An inhouse MATLAB script will be used for multivariate and univariate analyses. Intra-individual change in dietary adherence level from baseline to week 12 will be compared using Paired t-test or Wilcoxon signed-rank, while between-group differences will be compared using the two-sample t-test or Wilcoxon rank-sum test as appropriate, with P-values of <0.05 considered statistically significant.

Results

Study 1: Development of a metabolomics-based dietary assessment model

20 eligible participants were enrolled, 18 successfully completed both inpatient periods and were included for analysis. Participants' baseline characteristics are shown in Table 3.

Table 3: Baseline characteristics of participants who completed the inpatient study

Participants' demographics (n=19)	
Sex	
Male	9 (50%)
Female	9 (50%)
Age (years)	51 (9.9; 30-62)
Anthropometry	
Weight (kg)	86.7 (11.6, 60.2-104.8)
BMI (kg/m^2)	29.9 (2.8, 25.1-34.9)
Waist Circumference (cm)	103.7 (6.5, 92.5-112)
Fat (%)	31.3 (8.5, 18.4-43.9)
Muscle (%)	65.2 (53.5-77.5)
Energy expenditure	
Basal Metabolic Rate (kcal/day)	1780.8 (345.3, 1194-2281)
Estimated Energy Requirements (kcal/day)^	2137 (414, 1433-2737)
Blood sugar	
Glucose (mmol/L)	4.7 (0.6, 3.0-5.6)
HbA1c (mmol/mol)*	35.9 (4.4, 26-46)
Lipid profile (mmol/L)	
Total cholesterol	5.1 (1.3, 3.7-8.0)
LDL-cholesterol	3.3 (1.1, 1.71-5.8)
HDL-cholesterol	1.3 (0.4, 0.8-2.6)
Triglycerides	1.0 (0.3, 0.58-1.4)
Liver function test (IU/L)	
Alanine transaminase (ALT)	25.3 (8.9, 11-44)
Alkaline phosphatase (ALP)	79.8 (24.7, 53-140)

Data are n (%) or mean (SD, range). *Missing data (three for glucose and one for HbA1c) are not included in calculation. ^Estimated with a physical activity correction of 1.2 for all participants

The RM-MCCV-PLS-DA model built using ^{1}H-NMR spectra data of the third 24-hour urine sample demonstrated good predictive capability, given an R^2_Y of 1.0 and Q^2_Y of 0.96. (Figure 7) Cleared separations between diets of least and most concordance to NICE guidelines were observed on the score plots, with the associated T_{pred} ranging roughly from −1.0 to 1.0. KDE of the T_{pred} scores showed inter-individual variability within each diet group even though identical diets were provided to all participants.

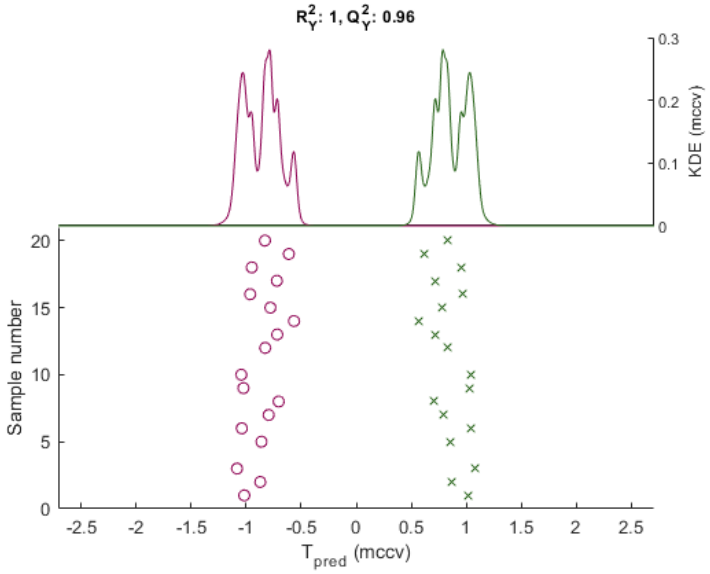


Figure 7: Scores plot from RM-MCCV-PLSDA of Day 4's urine samples.

Tpred relates to the healthiness of a metabolic profile to diets of most of least concordance to dietary guidelines. Positive Tpred indicates a healthier dietary profile and vice versa. Each circle dot represents a participant urine sample.

Twenty-seven urinary metabolites identified as being significantly associated with the difference between Diet 1 and 2 are summarised in Table 4. Sixteen significantly elevated metabolites from Diet 1 include 3-aminoisobutyrate, Rhamnitol, Dimethylamine, N-acetyl-S-methyl-cysteinesulfoxide, S-methyl-cysteine-sulfoxide, 1-methylhistidine, 3-methylhistidine, Trimethylamine-N-oxide, N-methyl-2-pyridine-5-carboxamide, Glycolate, 4-hydroxyhippurate, Hippurate, Tartrate, N-methylnicotinate, N-methylnicotinamide and Urea. In contrast, eleven metabolites significantly elevated after Diet 2 include Fatty acids (C5-C10), Alanine, lysine, N-acetyl-S-(1Z)-propenylcysteine-sulfoxide, N–acetyl neuraminate, Phenylacetylglutamine, O-acetylcarnitine, Carnitine, Creatine, Glucose and Glycine.

Table 4: List of metabolites whose urinary excretion is significantly associated with the difference between Diet 1 and 2

Number	Metabolite name	Chemical Shift (multiplicity) *	Association^	Dietary sources'	P-value"	Q-value"
1.	Fatty acids (C5-C10)	0.88 (m), 1.31 (m), 2.19 (m)	↓	Fats	1.99×10^{-50}	1.07×10^{-47}
2.	3-aminoisobutyrate	1.19 (d), 2.6 (m), 3.02 (t), 3.09 (d)	↑		5.55×10^{-39}	2.08×10^{-37}
3.	Rhamnitol	1.28 (d)	↑	Fruits	8.73×10^{-37}	2.44×10^{-35}
4.	Alanine	1.48 (d)	↓		2.24×10^{-27}	2.10×10^{-26}
5.	Lysine	1.73 (m), 1.91 (m), 3.02 (t)	↓		2.65×10^{-7}	4.88×10^{-7}

6.	N-acetyl-S-(1Z)-propenylcysteine-sulfoxide	1·96 (dd), 2·03 (s), 6·49 (dq), 6·65 (dq)	↓	Onion	1.44×10^{-30}	1.83×10^{-29}
7.	N–acetyl neuraminate	2.06 (s)	↓		3.76×10^{-47}	6.99×10^{-45}
8.	Phenylacetylglutamine	2·11 (m), 2·27 (m), 3·67 (m), 4·19 (m), 7·36 (t), 7·43 (t)	↓		3.52×10^{-29}	3.90×10^{-28}
9.	O-acetylcarnitine	2·15 (s), 3·19 (s)	↓	(Red) meats	2.86×10^{-18}	1.27×10^{-17}
10.	Carnitine	2·44 (dd), 3·23 (s), 3·43 (m)	↓	(Red) meats	1.98×10^{-14}	6.56×10^{-14}
11.	Dimethylamine	2.72 (s)	↑	Fish	1.26×10^{-24}	9.19×10^{-24}
12.	N-acetyl-S-methyl-cysteinesulfoxide	2.78 (s)	↑	Cruciferous vegetables	3.45×10^{-48}	8.92×10^{-46}
13.	S-methyl-cysteine-sulfoxide	2.84 (s)	↑		5.11×10^{-24}	3.55×10^{-23}
14.	Creatine	3·04 (s), 3·93 (s)	↓	(Red) meats	4.31×10^{-20}	2.19×10^{-19}
15.	1-methylhistidine	3·17 (2d), 3·22 (2d), 3·78 (s), 3·99 (dd), 7·17 (s), 8·12 (s)	↑	Lean (white) meats	1.17×10^{-36}	3.20×10^{-35}
16.	3-methylhistidine	3·25 (2d), 3·30 (2d), 3·78 (s), 3·99 (dd), 7·23 (s), 8·27 (s)	↑ (shift)	Lean (white) meats	2.29×10^{-40}	1.03×10^{-38}
17.	Trimethylamine-N-oxide	3.27 (s)	↑	Fish, meats	2.35×10^{-39}	9.13×10^{-38}
18.	Glucose	3·42 (m), 3·49 (m), 3·54 (dd), 3·74 (m), 3·84 (m), 3·91 (dd)	↓	Sugars	2.36×10^{-30}	2.92×10^{-29}
19.	Glycine	3.57 (s)	↓		9.22×10^{-8}	1.76×10^{-7}
20.	N-methyl-2-pyridine-5-carboxamide	3·65 (d), 6·67 (d), 7·83 (dd), 8·34 (d)	↑	Niacin (vitamin B3)	1.36×10^{-10}	3.32×10^{-10}
21.	Glycolate	3·95 (s)	↑		3.62×10^{-3}	3.86×10^{-3}
22.	4-hydroxyhippurate	3·95 (s), 6·97 (d), 7·76 (d)	↑	Fruits	9.60×10^{-23}	6.05×10^{-22}
23.	Hippurate	3·98 (d), 7·55 (t), 7·64 (t), 7·84 (d)	↑	Fruits, vegetables	2.17×10^{-7}	4.03×10^{-7}
24.	Tartrate	4·34(s)	↑	Grapes	2.23×10^{-21}	1.25×10^{-20}
25.	N-methylnicotinate	4·44 (s), 8·10 (t), 8·84 (d), 9·11 (s)	↑	Niacin (vitamin B3)	2.54×10^{-47}	5.04×10^{-45}
26.	N-methylnicotinamide	4·48 (s), 8·19 (t), 8·90 (d), 8·96 (d), 9·29 (s)	↑	Niacin (vitamin B3)	6.29×10^{-36}	1.54×10^{-34}
27.	Urea	5·80 (broad s)	↑	Protein	1.57×10^{-2}	1.43×10^{-2}

*Metabolites are ordered based on chemical shift. Peaks are listed only if they are in the range of the processed data. Multiplicity key is abbreviated as follows: s – singlet, d – doublet, t – triplet, q – quartet, dd – doublet of doublets, dq – doublet of quartets, 2D – two doublets, m – mulitplets
^Sign of association (↑ indicates higher excretion after Diet 1, ↓ indicates higher excretion after Diet 2)
'Only known dietary sources are listed
"P-values are unadjusted while Q-values are adjusted for False Discovery Rate

Internal validation of the model with data from Day 2 and 3 further justified its predictive ability. From the model's predictions of the 24-h urine samples collected on day 2 and 3, good reproducibility was observed, given that the T_{pred} scores of each participant clustered next to Day 4, albeit with greater variability on Day 2 compared to Day 3. (Figure 8)

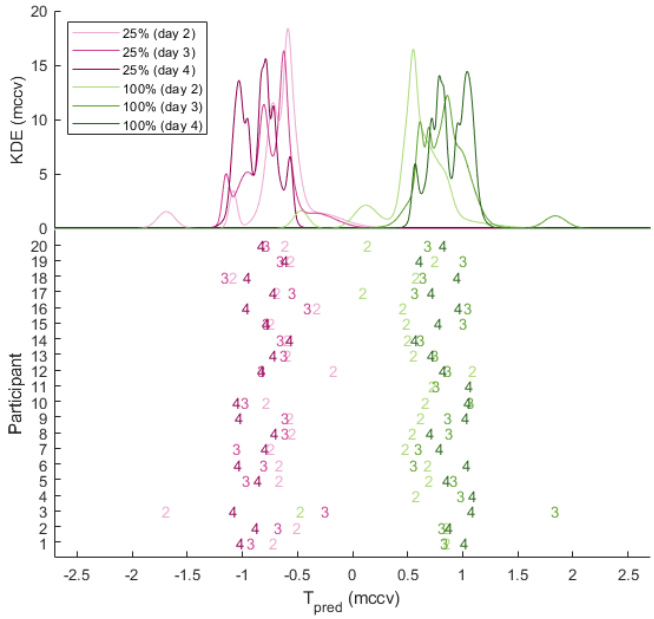


Figure 8: The model's predicted scores for each individual's 24-h urine samples obtained on day 2 and 3, based on MCCV of day 4's samples presented in Figure 7

Study 2: PPI in the development of a metabolically-personalised nutrition counselling model

Thirty-three dietitians completed the global survey, four dietitians and seven patients participated in activity 1 and 2, but only three dietitians and four patients attended activity 3 due to reasons of unavailability, logistical constraints in collecting urine samples or patients deemed ineligible due to engagement in special diets that can alter their metabolism. Table 5 presents the characteristics of participants involved at the start.

Table 5: Characteristics of PPI respondents

Online survey respondents: *Clinical dietitians* (n=33)

Location of Practice		**Years of dietetics experience**	
United Kingdom (total)	22	Less than 1 year	3
England	15	1-3 years	5
Wales	1	5-10 years	5
Scotland	3	10-15 years	7
Northern Ireland	2	15-20 years	4
Yorkshire	1	More than 20 years	5
Iraq	1	Missing	4
Singapore	6		
Missing	4		
Clinical background		**Number of patients seen in a week**	
Diabetes and/or Obesity	7	None	2
Renal	4	1-3 patients	1
Gastroenterology	3	4-10 patients	5
Eating Disorders	3	11-25 patients	10
Nil specialty	3	More than 25 patients	12
Nutrition support	2	Missing	3
Oncology	2		
Critical care	2	**Time spent for preparing consult**	
Ketogenic	1	0-15 minutes	13
Paediatrics	1	16-30 minutes	9
Primary Care Network	1	31-45 minutes	5
Missing	4	46-60 minutes	1
		Missing	5
PPI Clinical Group: Dietitians with NHS experience (n=4)			
Clinical background		**Years of dietetics experience**	
Renal	1	1-3 years	1
Community	1	10-15 years	2
Clinical Research	2	More than 20 years	1
		Time spent preparing consult	
		0-15 minutes	2
		16-30 minutes	1
		Missing (depends)	1
PPI Reference Group: Patients with or at risk of CVD (n=7)			
Gender		**BMI**	
Male	3	18.5-24.99kg/m^2	1
Female	4	More than 25kg/m^2	4
		Missing	2
Ethnic Group		**Family History of Chronic Disease**	
White and Asian	1	Obesity	5
Any other white background	1	Diabetes	5
Indian	1	High Blood Pressure	5
Pakistani	2	Heart Condition	5
Other ethnic groups	2	Missing	1

BP and/or Cholesterol Levels		
	Normal	2
	Elevated	4
	Missing	1

We first affirm the importance and direction of our research by exploring from the global survey, dietitians' understanding of PN and their views regarding dietary assessments. Subsequently we identified from the PPI activities, outcomes and impacts of stakeholders' feedbacks on our research.

Dietitians' understanding of PN and views of dietary assessment tools

Dietitians' overall interpretation of PN were similar across the board – providing nutrition that is specific to individuals' needs. However, dietitians reported different ways in which nutrition can be tailored, as shown in Figure 9.

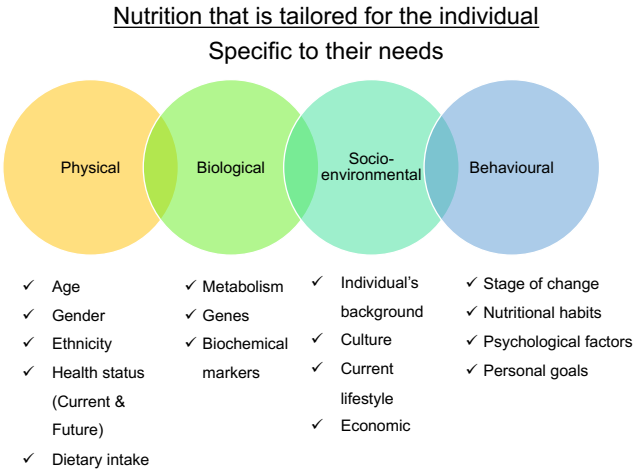


Figure 9: Dietitians' understanding of personalised nutrition

Responses are summarised into four categories: physical, biological, socio-environmental and behavioural

From their opinions about dietary assessments, more than 30% felt that dietary recalls and 24hr food diary are inaccurate to some extent, and at least 80% reported an accurate assessment will be useful and beneficial for them. (Figure 10)

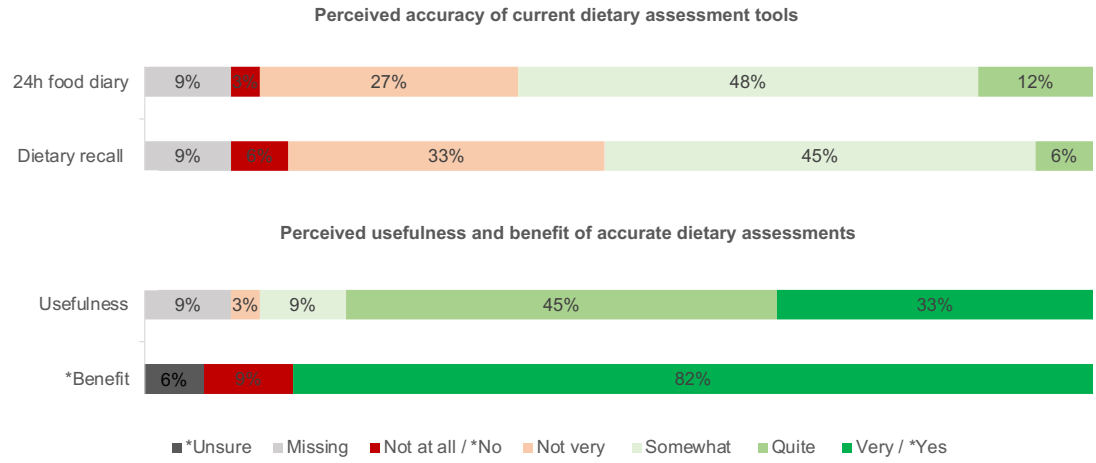


Figure 10: Dietitians' perceived accuracy, usefulness and benefit of assessment tools

Outcomes of PPI on the metabolically-personalised nutrition counselling model

PPI outcomes were summarised into three themes: perspectives relating to nutrition counselling, metabolic profiling in clinic, and their pilot intervention experiences, and classified into positives/facilitators to be leveraged or negatives/barriers to be addressed. (Figure 11)

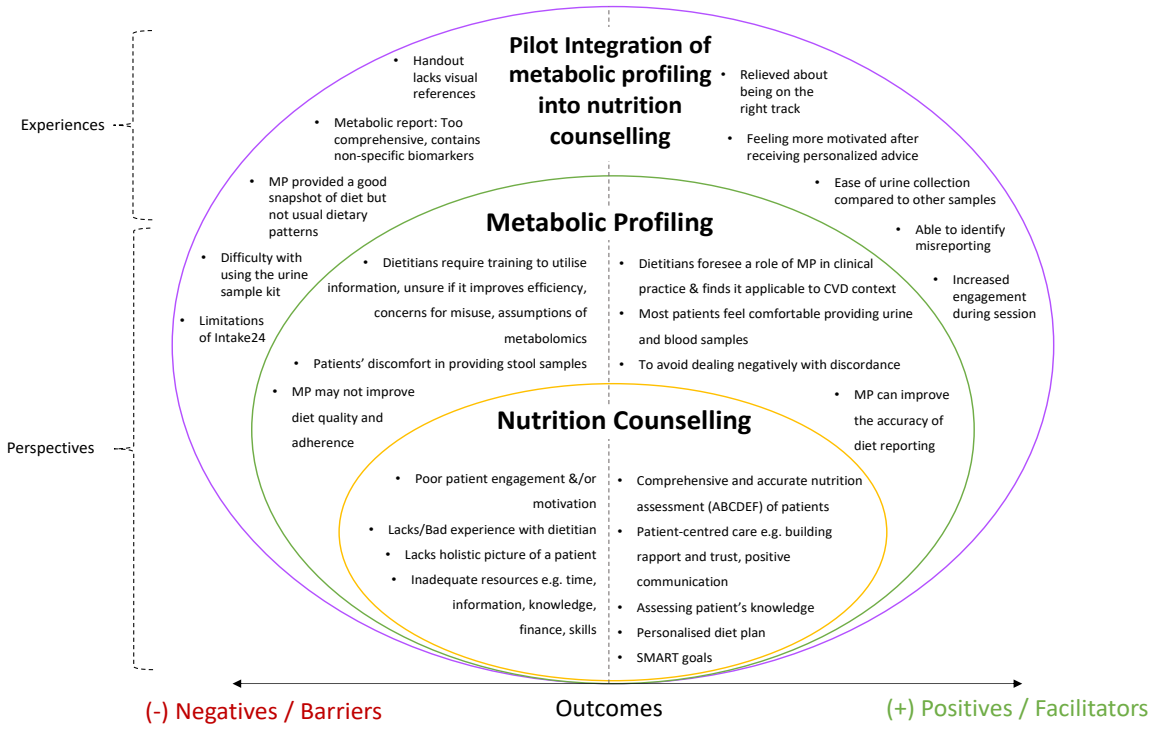


Figure 11: Outcomes from each PPI activity.

The diagram is broken down into three components – 1) facilitators (right) and barriers (left) of nutrition counselling, 2) positive and negative perspectives of incorporating metabolic profiling into clinical dietetics practice, and 3) positives and negative experiences from piloting the intervention

Theme 1: Nutrition Counselling Process

Facilitators from dietitians' perspectives

When conducting nutrition assessments, the ABCDEF framework (Appendix A) allows dietitians to comprehensively gather subjective and objective information and assess patients holistically, which facilitates the provision of evidence-based personalised dietary advice. Alongside comprehensiveness, accuracy of nutrition assessment was also deemed important and a PPI dietitian with research experience suggested to include procedures for conducting anthropometric and clinical measurements in Study 3, so that fluid balances and BP readings are stabilised before measuring, and to reduce fidelity between researchers.

When implementing nutrition interventions, dietitians felt it will be helpful to assess patient's existing knowledge and perception, and thereafter correct or build on it.

> "*what counts as a fruit and vegetables, how is she recording her food diary, does she record snacks, even drinks like juices and smoothies….Having a look at the food diary to see if it is a general overall theme of all foods being underreported, or is it just the socially desirable or undesirable foods being over or underreported".*
> *(D4, response from Activity 2)*

> *"…and also exploring what someone's understanding is before going into advice giving as well. So you're establishing what they already know and then helping them to myth bust or go over things"*
> *(D3, response from Activity 2)*

One dietitian further recommended setting SMART intervention goals within patient's means.

> *"..means nothing if it doesn't fit into their day-to-day lifestyle. And so yes, setting realistic goals that, that person feels able to achieve and checking with them, you know, do you feel this is achievable? Do you feel this is realistic?"*
> *(D3, response from Activity 2)*

Apart from hard skills, soft skills are equally essential for nutritional care. Building rapport and trust with patients, and fostering positive communication were the

foundations raised by dietitians in establishing a positive dietitian-patient relationship, an important factor for successfully implementing nutrition interventions.

> *"A lot of people find it very hard to sit down and write it all down, and then show it to someone else. You need an incredible amount of trust in order to do that with someone" (D2, response from Activity 2)*

> "*Kind of positively reinforcing things as well. So if someone has made changes and they've done really well, trying to keep the motivation with positive reinforcement…. Generally a lot of it boils down to communication. So trying to get on someone's level and trying to speak to someone in a friendly way, but still maintaining professionalism*" *(D3, response from Activity 2)*

<u>Barriers from both stakeholders' perspective</u>

Barriers to nutrition counselling were sought from dietitians and detailed in Appendix A. Time was the most common resource constraint reported by global dietitians in conducting nutrition assessments, given how comprehensive nutrition assessments would ideally be. Additionally, PPI dietitians identified patient's engagement and/or motivation as another barrier, however this appear to vary depending on patient's life circumstances.

> *"to know the person that is coming into your room……are they coming because they have been told that they have diabetes, are they socially or economically deprived, have all sorts of things going on, too many children, unhealthy eating habits, too much pressure on them compared to someone coming into the clinic and paying for your time……a completely different set of skills that will be used with these individuals" (considerations raised by D2, an NHS dietitian)*

> "*my current clients want to be told what to do, the more extreme the better… otherwise they will leave the room disappointed, not following what I say". (D1, dietitian engaging in private practice with clientele of high socio-economic status)*

Consistent with dietitians' views, lack of motivation was also commonly reported by PPI patients. (Figure 12)

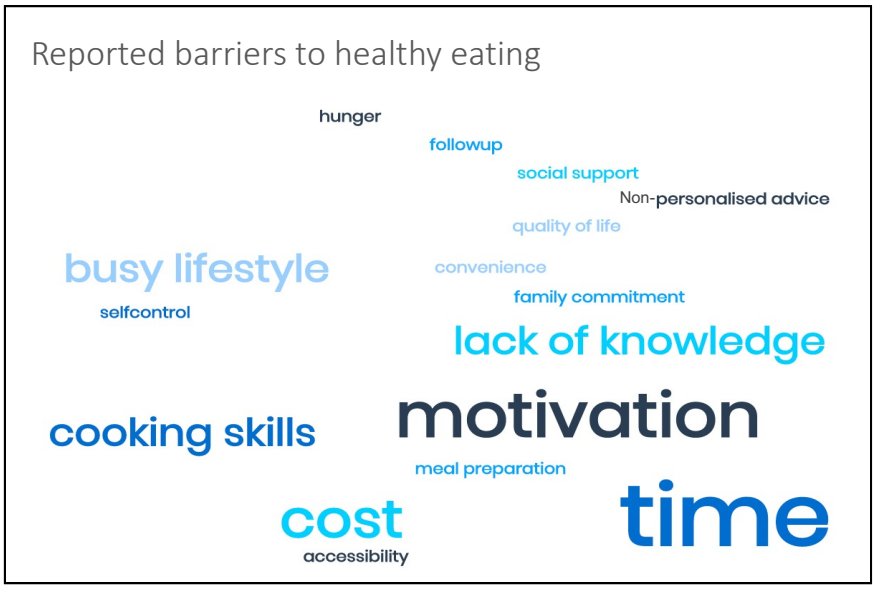


Figure 12: Word cloud of the barriers to healthy eating from Activity 1, reported by PPI individuals at CVD risk

As dietitians are equipped with the expertise to alleviate healthy eating barriers, PPI patients were asked if they had prior experiences with dietitian services. While most patients have never seen a dietitian, two patients who have had prior exposure did not find the given advice helpful and raised desire for effective personalised advice. (Appendix B)

One patient felt that nutrition care from dietitians should be realistic and not overstretching, raising further concerns about deteriorating quality of life and dietary lapses with eating healthily.

> *"if you said eat better, I said yes because it is going to be better thing for my health, that's a matter of fact. But will I eat better to my taste? Am I happier? That's the question..... when I'd been through this program for 3 months…I was deprived of all food almost. It's just vegetables that are steamed… it was very tough, at the time when there was no quality of life at all..finished the 3 months I lost about 8kg, and the target was 15 but I couldn't make it, so I was like, depressed, badly depressed and then I gave up. I just ate everything, I just went back, actually worse than before…. you can't be very strict with the instructions from the dietitians, especially if it deprives you or disbalance you from your normal style of life or style of eating". (P6, male, response from Activity 2)*

Another patient appeared to have reduced his faith in future dietary advice following a negative dietetics experience.

> *"2008 when I became diabetic, I was given awful advice by the dietary people who send me down a spiralling road of more and more carbs until the point where I have to go on insulin…I'm happy to follow what any dietitian says but I will be doing finger-prick test constantly to see its effect on my day-to-day blood sugar levels"* (P3, male)

Theme 2: Perspectives on using metabolic profiling for nutrition counselling

Facilitators to metabolically-personalised nutrition counselling

Positive attitudes about the applicability of MP to patients at CVD risk were expressed by all PPI dietitians and two foresee its role in their clinical practice (Appendix C). They felt that patients' metabolic profiles can be used to highlight areas where patients have done well, with one dietitian reported feeling "*comfortable to use it as a positive reinforcement*".

Discussion with PPI patients revealed that most feel comfortable with providing blood and urine samples for MP. (Figure 13)

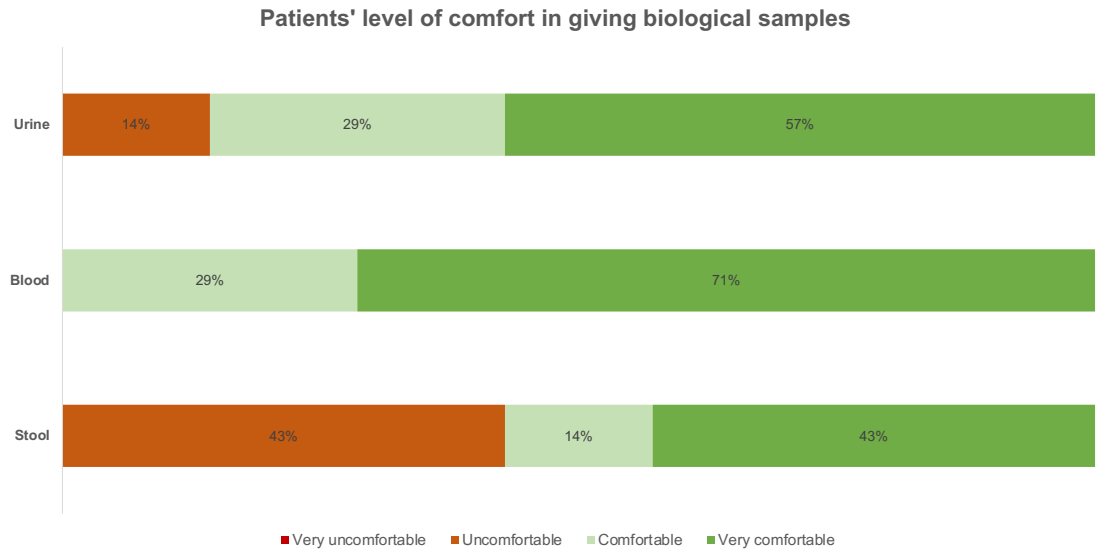


Figure 13: Patient's level of comfort in providing biological samples of urine, blood and stool

One patient attributed feeling comfortable to his positive past experience with the process.

"*for a small inconvenience it was a great service…the purpose of testing is to see how well the body is able to sustain itself…for a good cause*". (P4, male)

All patients also agreed that using metabolic profiling will help them to improve their accuracy of dietary reporting. (Figure 14)

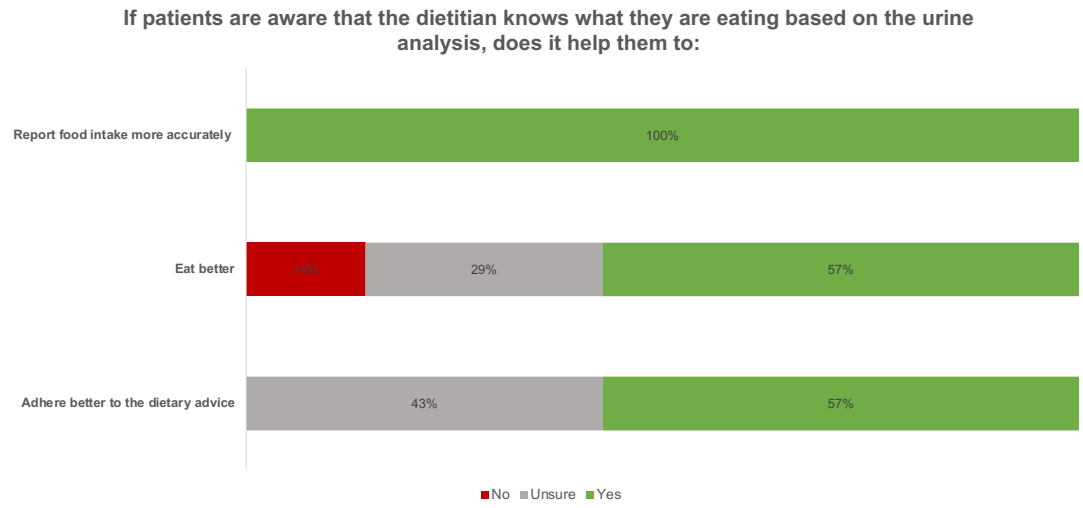


Figure 14: Patients' Activity 2 responses.

Participants were asked whether they will adhere better to the dietary advice, eat better and report food intake more accurately if they are aware that the dietitian will know what they are eating based on their urine samples.

<u>Barriers to metabolically-personalised nutrition counselling</u>

From dietitians' perspective, as metabolomics is a relatively novel concept within the clinical setting, some dietitians (especially those with more clinical experience) may not feel confident in applying the science of metabolomics to nutrition.

"*making the assumption that the dietary biomarker information that we get has already accounted for the variability, and we can trust that it means the patient is having a high intake of fruits and vegetables".*
(D2, 23 years' experience)

PPI dietitians felt that training is needed to utilise information from MP and were unsure if it can improve the efficiency of their pre-consult preparation. When given the scenario of discordance between the self-reported diet and metabolic report, experienced dietitians responded rather apprehensively about the need to delve into reasons

behind dietary misreporting as they were concerned that raising discordance may strain the dietitian-patient relationship.

> *"It could have been the patient was working really hard that week, it was just a difficult week for her to sit down and record things, or it was because of recording on paper and pen, or anything that is completely unrelated to dietary, so I will just be focusing potentially on that, if I even thought it was necessary…...You need to be sensitive as to whether you push the point on this or not…we shouldn't be so dogmatic about it. People are allowed to go and have a good time. So the point that the food diary of the past 24 hours doesn't match it, is that a problem, even?....we have to enjoy things as well, otherwise it becomes a very stick approach…if you focus on what the patient has done wrong, you will be on a highway to nowhere...worry that it can get misused a lot as the negative…and in that context I am incredibly uncomfortable with that approach (D2, 23 years' experience)*

> *"In my head I always have some sort like a negative motivation tool, which I'm really uncomfortable with… It's really dodgy or really awkward to walk that line between. You are not accusing somebody of lying, it's more I want to understand how this discrepancy came up…won't be comfortable having that conversation". (D4, 13 years' experience)*

In contrast, one dietitian with lesser experience felt otherwise.

> *"being honest in a non-judgemental way…needs to be addressed to get the most accurate result that you can…having conversation about why there is disparities and if it is a real problem, it might be a case of trying to develop a tool to help that person".* (D3, <10 years' experience)

Few patients were unsure or certain they will not improve their diet quality and dietary adherence if MP was used for nutrition counselling (Figure 14), with reasons linked to intrinsic and extrinsic motivation and being on the contemplation stage of change.

> "*I think I will eat what I will eat and knowing that somebody will know doesn't really make a difference. if I want to eat better and know what I should do, it will only be sustainable if I decide that I want to do it*." (P2, female)

"*I attempt to eat healthily already… if you change your diet because you are having a urine analysis, then the urine analysis isn't going to be as valid as if you stick to your normal diet and then have the urine analysis...I wouldn't change what I normally do just because I have a urine sample*." (P3, male)

"*I'm currently on a diet but I eat fruits sometimes and drink stuff that I shouldn't eat and drink, so I don't know if I would follow the advice to the maximum...in the past I have been told that I shouldn't eat or drink certain foods but I have done it afterwards…contemplating if I should follow*" (P1, male)

Those who felt metabolic profiling will help similarly responded that it depends on one's motivation.

"*I think it depends on a combination of factors. I think having somebody who you know, look at your urine sample and you know, record what you are eating actually is a motivator for you. well it's like a support to you really. um but again you have to have some motivation yourself to change your eating behaviour. And um I suppose one of the things that is linked to this is um illness as well*." (P5, female)

Theme 3: Experiences from piloting the metabolically-guided medical nutrition therapy

Positive experiences

All PPI dietitians mentioned that the metabolic report generated from the mathematical model was useful to varying degree, and ascribed usefulness to the report's ability to increase patient's engagement, detect misreporting and aspects in line with patient's self-reporting, in which a dietitian felt was encouraging for the patient. He also found the inclusion of Intake24's dietary analysis against NICE guidelines in the nutrition counselling model protocol helpful for conducting dietary assessment.

From PPI patients' feedbacks, they were satisfied with the ease of urine sample collection and dietary advice received. Apart from commenting that the sessions were personalised and informative, two patients reported feeling relieved and more motivated post-consultation as the metabolic report provided a good snapshot of their diet, which enabled them to see that they are on the right track in terms of dietary adequacy. When reviewing the CVD educational handout, most PPI patients felt that

the healthy plate concept and exchange list are understandable, aids in demonstrating healthy portion/serving sizes and further suggested for different combinations of exchanges to be provided to enhance the flexibility of dietary options.

Negative experiences

All dietitians felt that 24-hour recall limits the representativeness of one's dietary habits and recommended to gather additional information about patient's usual diet. They also reported difficulties navigating, interpreting and extracting relevant information within the metabolic report during the counselling session, given the vast amount of food intake biomarkers available and non-specific nature of some biomarkers e.g. high urinary carnitine levels being observed in a South Asian vegetarian participant.

Negative experiences from PPI patients were related to the research materials. Firstly, one patient reported experiencing difficulties in understanding the pictorial instructions on the urinary sample kit and suggested an instructional video may help. Secondly regarding the use of Intake24, one patient raised the need to consider the digital literacy of future research participants and to provide a hardcopy 24-hour recall form for those who may experience difficulty with the online tool. Another patient felt that the database is not sufficiently comprehensive as she was unable to source for food items that is relatively new to the market e.g. lentil pasta, which she considered important in showing dietitians that she has chosen a healthier option. Thirdly for the CVD handout, PPI patients felt that pictorial references should be included to aid visualisation about foods to consume or avoid.

Impacts of PPI

Based on the outcomes above, their associated impacts were identified for study improvements to be made as appropriate. A table summarising each outcome and impact is detailed in Appendix E.

Theme 1: Nutrition Counselling Process

Changes were made to improve the nutrition counselling process. We recognised that we had not previously appreciated the value dietitians placed on taking time to conduct thorough nutrition assessments and build rapport, which prompted us to extend the counselling sessions for Study 3. In conducting nutrition assessments, the research team agreed to standardise information collection according to 'ABCDEF', and to include instructions for collecting anthropometric and clinical measurements. Subjective measures such as self-rated motivation, readiness to change, confidence, knowledge, hunger (timing, levels, triggers) and reported barriers were also included to better understand the patient. The feedbacks also prompted us to place greater emphasis on establishing positive patient-dietitian relationships. We initially assumed that communication is self-explanatory and occurs naturally during counselling. However reflecting on stakeholders' strong emphasis about building rapport and trust, sections on 'effective communication' and 'monitoring and review' were explicitly included in the protocol. Instructions will include taking patient's circumstances into consideration when tailoring dietary advices, such as providing flexible dietary options within patient's budget and maintaining nutrition-related quality of life. Dietitians will encourage patients to celebrate wins and nudge them towards taking actions for improvement in a non-judgemental way. This will be done by setting SMART goals and action plans alongside behaviour change techniques to minimise the risk of dietary lapses.

Theme 2: Perspectives on using metabolic profiling for nutrition counselling

We were glad to learn that dietitians see the relevance of MP within the CVD context while patients will report dietary intake more accurately with the use of MP. These positive findings provided encouragement for the continuation of the research. Taking into consideration PPI dietitians' feedback and concerns, training will be implemented for future users of the metabolic profiling report, in aspects such as the concept of metabolomics, biomarkers interpretation and its use. Ensuring that all dietitians have the same level of understanding may help increase their confidence and knowledge on how to best integrate MP with existing dietary assessment tools, and communicate the results to patients as practical intervention strategies. It was also heartening to note that most patients feel comfortable with providing urine and blood samples – which are

key specimens for MP, hence we will continue to implement samples collection fortnightly in Study 3. We considered patients' discomfort towards stool collection but eventually decided to maintain its frequency as it is important for further research purposes i.e. gut microbiota analysis. As stool collection was not involved in Activity 3, we aim to resolve any concerns that potential study participants may flag up during the study, and improve the stool collection process according to their inputs.
Understanding from PPI patients that MP may not improve their adherence to dietary advice and guide them to eat better was also constructive as it reinforced that no matter how effective a tool may be, patients' attitudes remain a major determinant in the success of a dietary intervention and the need to assess the aforementioned motivation levels of patients.

Theme 3: Pilot intervention of metabolic profiling for nutrition counselling
It was comforting to receive positive comments from stakeholders regarding their experience e.g. increased engagement and patients' satisfaction with the ease of collection, which reaffirmed the need for PN and the usability of urinary MP. The pilot intervention provided us an opportunity to observe different dietetics practice and learn how discordance can be positively dealt with. Based on the observations and dietitians' feedbacks about nutrition counselling, questions regarding usual eating patterns and how it differs from Intake24 reporting were retained in the nutrition counselling model protocol, in addition to the comparison template for nutrient intakes against dietary recommendations. We also ensured the negative experiences of stakeholders were addressed as we foresee them as potential issues that may arise during Study 3. Firstly as suggested for the urine sample collection, an instructional video link was included in its instruction handout to improve clarity. Secondly, based on PPI patients' experience of using Intake24, we prepared a hardcopy 24-hr recall that can be offered to future participants who have difficulties utilising Intake24, and noted the need to review patient's search terms when analysing dietary results from Intake24. Thirdly, to ease the process of gathering relevant data from the metabolic report for dietary counselling, we created a summary table that includes food (groups), associated biomarkers and participants' urinary levels (Appendix E). Lastly, serving size pictorials were retrieved from the British Heart Foundation's website and included into the CVD handout based on PPI patients' feedback.

Discussion

Garcia-Perez et al. (2017) developed and validated a mathematical model to accurately classify dietary patterns in relation to WHO dietary guidelines. To develop the model, a randomised controlled inpatient clinical trial was conducted on healthy participants and validated externally on free-living cohorts. While this well-validated model have shown potential in assessing dietary adherence among free-living populations without requiring subjective dietary data collection, there is a need to determine how to best translate it into the clinical context within the UK setting. This is crucial as the UK government have emphasised the importance of having diets in line with recommended NICE dietary guidelines in light of rising CVD prevalence but there is a lack of quantitative metrics that allow UK individuals at CVD risk understand where they are against recommendations. This makes it challenging for individuals to implement specific dietary changes that are necessary for improving CVD-related health outcomes. Given the mechanisms driving metabolic and nutritional pathways for CVD are often complex and multi-factorial particularly in free-living conditions (Iliou et al., 2021), mathematical modelling may be useful in such respect due to its ability to incorporate individualised data into nutrition care plans, optimise decision-making for dietitians and monitor one's dietary progress. (Vanagas, Krilavičius & Man, 2019) Furthermore, as the validity and reliability of patients' self-reported dietary intake are often challenged by the under-reporting of foods deemed 'unhealthy' e.g. red and processed meats or sugary products, and over-reporting of perceived 'healthy' foods e.g fruits and vegetables, wholegrains and legumes (Subar et al., 2015), the establishment of well-validated models may be key to resolving the long-standing impediment to accurate dietary assessments that dietitians face with patients in clinic. Therefore, Study 1 was conducted as a follow-up to Garcia-Perez et al. (2017), specifically aimed at targeting CVD among UK individuals at risk. Through validating the current model in a CVD population, we demonstrated that a metabolic profiling approach is capable of identifying overall dietary intake patterns e.g. variety and diversity within and across food groups and the adequacy of nutrients and food groups in relation to requirements, as well as individual dietary elements e.g. food or nutrients, as the associated metabolites are reflected accordingly in the urinary metabolomics readouts of individuals at CVD risk. It was shown that three days of adherence to diet 1 and 2 significantly changed one's urinary metabolic profile and T_{pred} scores, with a total of 22 diet-discriminatory metabolites identified. Consistent with urinary excretions from two diets of concomitant extremes to WHO guidelines by Garcia-Perez et al. (2017), our results highlighted that Diet 1 exhibited significantly elevated concentrations of

metabolites associated with healthy foods contributing to positive T_{pred} scores, such as hippurate and 4-hydroxyhippurate (fruit and vegetables), N-acetyl-S-(1Z)-propenylcysteine-sulfoxide (vegetables), N-acetyl-S-methyl-cysteinesulfoxide (cruciferous vegetables), rhamnitol (apples), tartrate (grapes), Dimethylamine and trimethylamine-N-oxide (TMAO) (fish/oily fish), 1-methylhistidine and 3-methylhistidine (lean meat), N-Methylnicotinamide, N-methylnicotinate and N-methyl-2-pyridine-5-carboxamide (niacin). On the contrary for Diet 2, its overall negative T_{pred} scores were contributed by urinary biomarkers of unhealthy foods, few of those known are o-acetylcarnitine and carnitine (red meat), phenylacetylglutamine (animal protein from meat and dairy) and C5-10 fatty acids (fats). Hippurate and 4-hydroxyhippurate are known to be associated with the mammalian-microbial co-metabolism of polyphenol-rich dietary components such as fruits and vegetables. (Lees et al., 2013) In addition to ours, previous work have identified higher presence of these metabolites in recommended diets such as the Mediterranean (González-Guardia et al., 2015) and DASH (Chan et al., 2022) diets, as well as within healthy dietary clusters of metabolomics-based models. (Gibbons et al., 2017; Prendiville et al., 2021) While these metabolites may be indicative of one's diet quality and metabolic health (Brial et al., 2021), it should be noted that their urinary concentrations can also be modulated by gut microbiome diversity (Pallister et al., 2017), hence results interpreted alongside gut microbiota composition may be more helpful. Among protein foods, their urinary metabolites excretion and associated CVD risk varied depending on the quality of proteins consumed, as observed from Diet 1 and 2. As the catabolism of both plant and animal-based proteins produces niacin derivatives, N-methyl-2-pyridine-5-carboxamide, N-methylnicotinate and N-Methylnicotinamide, higher urinary excretion of these metabolites from Diet 1 may be attributed to the wider variety of niacin-rich foods present, derived from plant-based proteins e.g. wholegrains, nuts and legumes, as well as animal proteins. Being able to differentiate the type of dietary protein may be important as Naghshi et al. (2020) previously shown from meta-analysing prospective cohort studies that plant-based protein sources are associated with improved cardiovascular outcomes and lower all-cause mortality risk, whereas total and animal protein intakes did not show significant associations. Therefore further examination into other dietary protein metabolites are warranted. Fish consumption (including oily fish), as recommended by NICE guidelines for CVD management, strongly affects urinary TMAO levels (Loo et al., 2022) and this can be seen from its higher excretion following Diet 1. However, Yin et al. (2020) noted that urinary TMAO alone is inadequate to determine fish intake in free-living population, as TMAO can also be derived from the breakdown of dietary carnitine by the gut microbiota. L-carnitine-rich foods e.g. red

meat contribute to microbiota-dependent TMAO excretion (Koeth et al., 2013), which promotes atherosclerosis and increases CVD risk. (Sun et al., 2021, Miller et al., 2022) Therefore, given these observed synergistic effects or complex interactions among foods within the body, there is a need to examine the totality of diets from the global pattern of urinary metabolites instead of focusing on individual dietary biomarkers. Furthermore, inter-individual variability in dietary responses as seen from our inpatient, tightly controlled study adds to the complexity of dietary assessment. Although identical diets were provided to all participants and the T_{pred} scores clustered in the same direction according to the diets consumed, we noted variability within each positive and negative T_{pred} group scores for all participants, throughout the dietary intervention period (Day 2 to 4). Such observations were likewise substantially identified by Garcia-Perez et al. (2017) for concentrations of hippurate and carnitine, which reinforces the need to tailor diets that matches with individual's metabolism, instead of providing one-size-fits-all recommendations. Our results of high R^2_y and Q^2_y values, coupled with the ability of Day 4's model to predict Day 2 and 3's 24-hour urine samples based on similar clustering patterns, suggest that the model is robust and reproducible, and can therefore be used to predict the percentage of adherence and report intake of specific foods and food groups in a subsequent free-living personalised nutrition study (Study 3). This also provided a proof-of-concept for the feasibility of using mathematical models for advancing nutrition care, a useful and much-needed, yet lacking area of dietetics practice. (Sak & Suchodolska, 2021)

Nevertheless, dietary assessment only constitutes one part of the equation to PN. In order to facilitate the delivery of metabolically-guided medical nutrition therapy for UK individuals at CVD risk and ensure ongoing effectiveness of dietetic consultations in lowering their risk and lipid levels (Ross et al., 2019), a nutrition care process model (Swan et al., 2017) that encompasses nutrition assessment, diagnosis, intervention, monitoring and evaluation is needed for the PN trial. Therefore, we utilised the 'Model and Process' framework (British Dietetic Association, 2020) that clinical NHS dietitians employ as a standardised foundation for our dietary counselling protocol. While such a systematic approach helps create structure for dietary counselling, we recognised that further inputs are needed from its end users to support direct translation of the novel MP tool into clinical dietetics practice, hence PPI was integrated to strengthen the quality of Study 3. Consultations with stakeholders enabled the research team to identify barriers and facilitators to nutrition care and the implementation of metabolic profiling. For example, although dietitians recognise the importance of providing personalised nutritional plans, which is in line with patients' desire for tailored advice as

reported by PPI patients and in the literature (Endevelt & Gesser-Edelsburg, 2014; Tosunlar et al., 2021), dietitian-patient consultations are often less thorough than intended due to time and resource limitations. This may inevitably result in poorer adherence and loss to follow-up from patients (Endevelt & Gesser-Edelsburg, 2014; Harper et al., 2022), hence tools that are neither time-intensive nor cost-prohibitive and permits accurate and comprehensive nutrition assessments, will likely be welcomed based on majority of survey responses. This encouraged the continuation of our research as novel tools like ours may help to address the issues dietitians faced when personalising diets in clinic. However their insights also highlighted a different set of challenges that such novel tools can bring about. Utilising objective, non-invasive MP complementary to traditional approaches will require overcoming potential hesitancy from experienced dietitians, as PPI dietitians with more clinical experience were concerned that highlighting discordance misappropriately can come across to patients as confrontational and become a possible hindrance to patient's engagement. Similar barriers were previously discussed by Abrahams (2019), who qualitatively investigated factors influencing dietitians' integration of nutritional genomics into clinical practice. She detailed the degree of nutrigenetics application as being associated with dietitians' knowledge and confidence in the science, their job environment (acute, private practice or research) and attitudes towards such emerging fields. Hence, in order to alleviate dietitians' concerns, the provision of user training prior to implementation should be considered to help familiarize dietitians with the concept of metabolic profiling and educate them on it can be used to enhance their practice. Additionally, the barriers to healthy eating reported by PPI patients also raised our awareness about the complex system underpinning determinants of diet and health, and highlights the importance of harmonising other subjective and objective information when comprehensively evaluating individual's nutrition status, in order to make patients partners in their own health and decision making. We inferred from PPI responses that the success of metabolically-guided advice largely depends on patient's acceptance of MP and their motivation to make lasting dietary changes, which prompted us to include several subjective measures for our nutritional assessment component. This aspect was similarly raised by Palmnäs et al. (2020) during their discussion about the practical considerations for implementing metabolically-personalised nutrition in clinic, such as the need to determine the acceptance and attitudes towards metabotyping, and factors that will impact PN outcomes.

Strengths and limitations

As a follow-up study to Garcia-Perez et al. (2017), Study 1 was refined by incorporating a reference diet before commencing the interventions to ensure dietary standardisation, and increasing the variety of foods provided in the controlled diets for biomarker discovery and classification. Findings from Study 1 enabled enhancement of the previously validated model, by catering it specifically to the UK context with reference to NICE guidelines and test its performance on individuals with CVD risk. Additionally, we acknowledged that an optimal approach to translate nutrition research into clinical dietetics practice was to incorporate the views of service users into the development of a metabolically-guided medical nutrition therapy. With PPI being increasingly recognised as a vital component of the research process (Brett et al., 2014), we aimed to ensure that the feedbacks obtained from all activities were fully considered instead of perceiving PPI as a 'box ticking' exercise, which helped to shape multiple aspects of Study 3. Current model's robustness, coupled with urinary metabolomics being less costly (£20 per sample, reported by Garcia-Perez et al., 2017) and non-invasive compared to other biological tests, can be well-suited for patients and healthcare providers in future if proven effective in Study 3. Nonetheless, several limitations exist in the current research. Firstly, the non-specific nature of certain biomarkers e.g. TMAO can confound dietary interpretation and make it challenging for users to decipher the results from MP. However as aforementioned, users will benefit most from being trained on examining one's overall diet based on their global urinary metabolites patterns rather than individual metabolite trends. Additionally, instead of interpreting urinary results as a standalone, a complementary systems-wide approach that combines lipid profiles from blood and gut microbiome data from stool samples may confer more enriching findings for PN in a CVD context. Secondly for the PPI activities, while the research team recognised the importance of involving PPI stakeholders at an early stage of research, greater consideration should have been taken in its planning, such as PPI participants dropping out, conducting activities online or in-person given that activity 2 had to be repeated for both stakeholder groups due to individuals' unavailability, and providing stakeholders various research materials (e.g. SOP and handouts) to review in advance of the sessions as the brief stint during the activities felt rushed.

Study implications and directions for future research

Our research outcomes suggested that the research 'toolkit' comprising of the metabolic profiling tool and dietary counselling strategy enhanced with PPI inputs is ready to be applied for testing the effectiveness of metabolically-personalised nutrition in Study 3. As the PPI process has enabled the team to gain a deeper understanding of

the value and importance of PPI in research, we hope to continue collaborating with our PPI stakeholders in subsequent phases of the research, such as analysing study results to ensure the interpreted data encompass diverse perspectives, improving the quality of the discussion, correcting misinterpretations and examining the validity of the conclusions, as well as producing lay summaries of our study findings for dissemination (media, community and CVD groups). Such frequent engagement with end users has also been recommended by Wilson et al. (2021), who raised the importance of treating user engagement as a process rather than isolated events when discussing practical tips for successful incorporating artificial intelligence in healthcare. If the MP approach is able to improve dietary adherence to NICE CVD guidelines in Study 3, it is hopeful that consistent adherence among individuals may assist in improving CVD-related clinical outcomes in the long run. Furthermore, with mathematical modelling increasingly gaining recognition in healthcare given its ability to aid personalised treatments, such objective models may also be useful for other nutritional diseases e.g. chronic kidney disease and diabetes, therefore its value should be adapted and tested accordingly.

Conclusion

The development of a novel, validated mathematical model for individuals at CVD risk and metabolically-personalised nutrition counselling approach that have been refined with PPI inputs, have facilitated the conception of a well-rounded toolkit which will be used to deliver personalised nutrition for patients with or at risk of CVD in study 3. If this strategy proves successful, it can offer a new route for clinical dietitians to assess diets objectively and provide enhanced personalised advice to patients' benefit, thereby improving nutrition care practice within the NHS.

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Appendix A: Global Qualtrics survey disseminated to dietitians via social media

Questions

1. Please confirm that you are a dietitian in practice?
    a. Yes, please state your specialty (i.e. oncology, obesity etc.)
    b. No
2. How many years of experience do you have in dietetics practice?
    a. None
    b. Less than a year
    c. Between 1 and 3 years
    d. Between 3 and 5 years
    e. Between 5 and 10 years
    f. Between 10 and 15 years
    g. Between 15 and 20
    h. More than 20 years
3. In which of these UK regions are you located?
    a. East
    b. East Midlands
    c. London
    d. North East
    e. North West
    f. Northern Ireland
    g. Scotland
    h. South East
    i. South West
    j. Wales
    k. West Midlands
    l. Yorkshire
    m. Other UK region, please specify
    n. A country other than UK, please specify
4. What do you understand by the term 'personalised nutrition'?
5. How many patients do you see for dietary advice in a typical week?

a. None
b. Between 1 and 3
c. Between 4 and 10
d. Between 11 and 25
e. More than 25

6. Which sources of tools do you use to base your personalised dietary advice on?
    a. Anthropometry (for example, weight change, BMI, waist circumference)
    b. Biochemistry (for example, HbA1c, full blood count, etc)
    c. Clinical factors
    d. Self-reported food intake (dietary recalls, food frequency questionnaires, Food diaries, etc)
    e. Lifestyle
    f. Others, please specify

7. In your opinion, how accurate are these sources or tools to personalise dietary advice?

	Not accurate at all	Not very accurate	Somewhat accurate	Quite accurate	Very accurate
24-hour Food diary	○	○	○	○	○
Dietary recall	○	○	○	○	○

8. In your opinion, how useful will be having accurate dietary intake in your personalised dietary advice?

Not useful at all	Not very useful	somewhat useful	Quite useful	Very useful
○	○	○	○	○

9. How much time (in minutes) do you typically spend in preparing a patient's consultation to personalise their diet?

10. Which difficulties or barriers do you encounter when personalising a diet plan?

11. Which difficulties or barriers do you think the patient encounter to follow a personalised diet plan?

12. In your daily practice, would you benefit from having an accurate dietary assessment from your patients?

Responses to question 6 of the global survey

Supplementary Table 1: Nutrition assessment framework used by dietitians for information gathering from patients, with examples provided by dietitians for each component

Components of the nutrition assessment framework	**Examples of each component**
Anthropometry	Weight change, BMI, waist circumference, body composition analysis via bioimpedance
Biochemistry	HbA1c, full blood count, genetic testing, biomarkers of nutrition status
Clinical/physical	Current symptoms that the patient needs help to cope with Treatment outcomes i.e. weight maintenance or weight restoration organ support eg ventilator, dialysis, LVAD, ECMO Comorbidities or medications that influence diet Dentition/chewing/swallowing issues GI issues Presence of any factors that might increase nutrition requirements
Dietary	Dietary recalls, Food frequency questionnaires, Food diaries, etc
Environmental/ Behavioural /Social	Psychological aspects e.g. current views on body image and diet Personal responsibilities or work commitments Health literacy Patient's preferences
Functional	Eye ball assessment - gait, muscle strength, ability to do ADL, get up from seated position, sit to squat etc Self-care abilities of the patient Physical activity level

Responses to question 10 and 11 of the global survey

Supplementary Table 2: Barriers to nutrition care in the current dietetic practice, according to dietitians

Subthemes to barriers	**Challenges that dietitians face in personalising diets**	**Difficulties that dietitians think patients encounter in adhering to dietary advice**
Patient's engagement and/or motivation	- Lack of influence on what the dietitian said - Difficulty negotiating changes - Providing repetitive advice - Patient's ability to follow advice	- Intrinsic and extrinsic motivators e.g. weight concerns and social desirability, patient's stage of change – attitudes, behaviours and perception - Lack of options and/or flexibility in the prescribed diet e.g. day-to-day food intake variability and diversity - Lack of encouragement - Insufficient desire and readiness for change - Conflicting health priorities - Receiving repetitive advice - Inability to break old habits - Lack of self-control - External influences e.g. environment, convenience, social, dependents
Lacking a holistic picture of the patient	- Symptoms reported e.g. GI discomfort - Multiple therapeutic needs - Limited physical ability - Patient's preferences - Cultural and religious differences	- Symptoms experienced e.g. GI function, tiredness, treatment side effects - Past medical and social history
Limited resource availability	- Financial considerations - Insufficient time to conduct nutrition assessment thoroughly and build rapport - Limited tools - Limited availability and accuracy of information e.g. dietary recalls, quantity and frequency of intake - Experience in translating scientific evidence into actionable recommendations	- Financial concerns - Insufficient time - Lack of social support - Inability to recall given advice - Exposure to conflicting online information - Limited cooking skills and knowledge

Appendix B: Activity 1 - Qualtrics survey

Activity 1 - Questions to UK dietitians

Supplementary Table 3: Questions to and responses from PPI dietitians in the UK

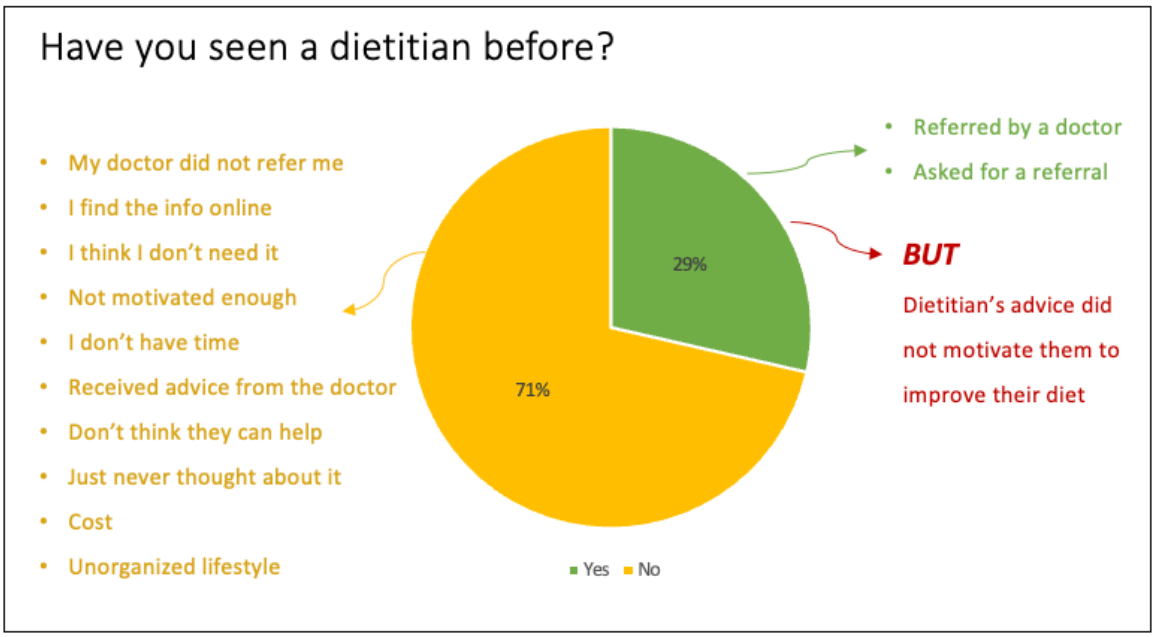
1) What do you base your personalised dietary advice on?	
D1	Appropriate guidelines and research (evidence base), Biochemistry and genetic testing
D2	Evidence on the dietary principles that are suitable for the condition(s) of the patient; existing guidelines and the existing knowledge and experiences of the patient and their educational level
D3	Scientific research, clinical outcomes e.g. anthropometry, patient reported outcomes, self-reported diet histories/diaries, symptoms, clinical conditions/diagnosis, Taste/food preferences and products available on local formulary (medications/nutritional supplements available for prescription in the local area)
D4	This varies in different clinical conditions but ultimately I base it on a holistic assessment of the patient which will always include aspects of the areas I've listed below. The assessment is also tailored based on what's relevant for the problem the patient is presenting with. I've tried to give examples below of how this information might be used, but this varies so it is not exhaustive for every condition/patient-type. - anthropometry with reference to suitable standards (e.g. weight change, BMI, waist circumference, MUAC, body composition etc). This might influence dietary advice in several ways e.g. if a patient is severely underweight with significant weight loss and poor dietary intake I may advise supplements as a first line treatment, whereas if normal BMI but moderate weight loss (5%) then I might advise a food first approach to manage the risk of malnutrition. - biochemistry (if available/relevant). E.g. if diabetic, what is the patient's HbA1c to monitor adherence/medication type and dose suitability and if they keep a blood glucose self-monitoring record. Or if they are relevant, are biomarkers of nutritional status/micronutrient status available? E.g. if anaemic then ideally I would like to have full blood count/vit B12/folate/iron status to determine the specific nutritional cause, or rule out a nutritional cause. This will indicate where the diet could be improved to address the problem, or may prompt further investigations by the GP if not dietary related. - clinical factors such as comorbidities that influence diet (e.g. chronic kidney disease, coeliac disease), any medications that might impact diet, dentition/chewing/swallowing issues, nausea/vomiting, constipation/diarrhea. Presence of any factors that might increase requirements (e.g. infection, pyrexia, wounds, COPD etc.). - the patient's current reported diet including energy, protein, fibre and fluid deficit or excess compared to estimated requirements/broad macro/micronutrient trends/dietary preferences/eating patterns/allergy/cultural and religious aspects etc. As part of assessing current diet, I compare this to relevant standards for the condition to identify areas where changes might be most beneficial. E.g. percentage of energy from fat/CHO/protein/EtOH, how many portions of fruit/veg are consumed per day vs. 5-a-day recommendation etc.

	- environmental factors such as physical activity level/occupation/living situation/dependents/time available. This is so that any advice fits within the patient's lifestyle. - functional capacity including nutrition knowledge/ability to cook/self-feed. - The patient's reported goals
2) How many minutes do you spend on preparing your patient consultation?	
D1	Depending on the patient/ complexity 5-30mins
D2	Preparing for my patient consultation: 5-10mins
D3	Variable, depends on complexity of patient/information included documented referral form and notes.
D4	5-10mins prior to a 45-min initial appointment if taking a diet history during the session – during this time I'll review the referral, any medical history/biochemistry etc included. If I've asked the patient to collect a diet diary and send beforehand I might spend 15-20minutes additional time assessing this and preparing additional questions to clarify anything.
3) Which barriers do you encounter on your current practice while personalising your diet?	
D1	Food preferences, health conditions, religious/cultural preferences, socio-economic factors (family, time, finance, knowledge, skills etc.), motivation/stage of change
D2	Knowledge of the patient's foods if they are from a cultural background I am unfamiliar with, Having a full understanding/ appreciation of what the patient eats day to day and their cooking methods, Time to adequately build trust with the patient so that I can gain an understanding of their diet, their diet-related beliefs and their dietary habits and cooking styles/habits.
D3	Patient engagement/motivation, barriers related to mental health and wellbeing, symptomatic e.g. bowel symptoms, nausea, vomiting, behavioural e.g. food declining.
D4	Conducting a thorough holistic assessment takes time (about 45mins face-to-face time), which is not always available. The alternative of sending (and analysing) lots of questionnaires before meeting patients is burdensome. Often a lot of the information I would like to know is not available or would cost too much or take too much time to fully investigate. An example is biochemistry availability as an objective marker of nutritional status – it is frequently incomplete, out of date, or just not available at all. It also costs a significant amount of money to the NHS (or private patient). If encountering a patient from a culture/religion that I might not be very familiar with, it can be harder to tailor advice for some specific conditions

Activity 1 - Questions to PPI individuals at risk of CVD

1. Do you think it is important to have a healthy diet?
2. From 1 to 10, how healthy do you think your diet is?
3. What barriers do you face when following dietary advice?
4. Have you visited or seen a dietitian before?
    a. If no, what are the reasons?
    b. If yes, what are the reasons?
5. Did the given dietary advice motivate you to improve your diet? and what barriers do you face in following the given advice?

Responses to Question 4

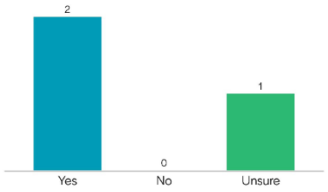


Supplementary Figure 1: Survey responses from patients on whether they have seen a dietitian before

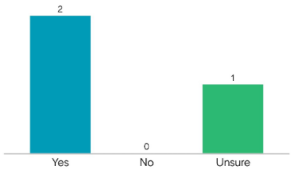
Appendix C: Activity 2 - Mentimeter questions and responses from each focus group

Focus group with PPI dietitians

Can you forsee a role of dietary metabolic profiling in your clinical practice?

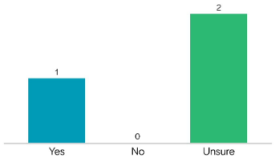


Do you think you will require training to utilise the dietary report?

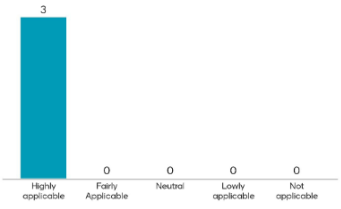


In which aspects do you think you will require training?

With the information provided by the dietary report, do you think it can help you to be more efficient in preparing your dietary advice for patients?



How applicable do you think metabolic profiling will be for patients at risk of and with cardiovascular disease?



Supplementary Table 4: Activity 2 - Dietitians' Focus Group Transcript

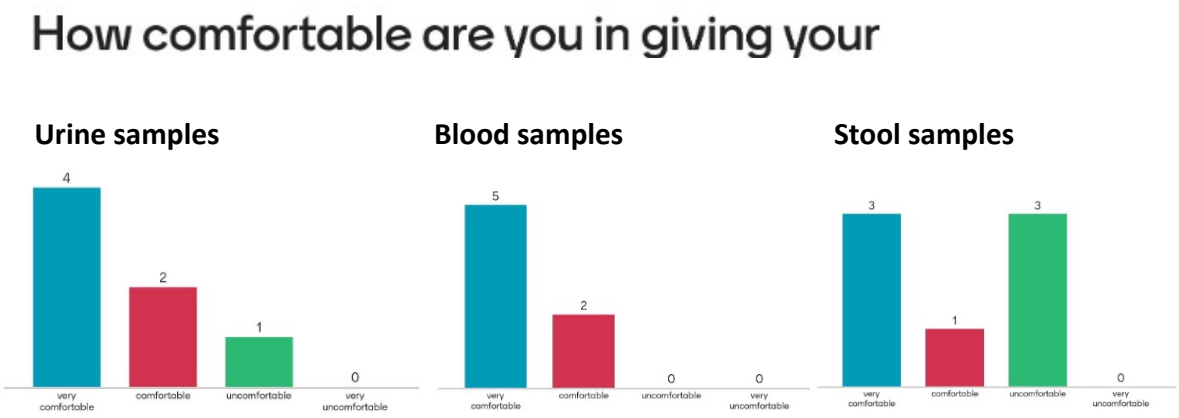
PPI Dietitian Number	Verbatim	Codes
Scenario 1 (overreporting)		
1	- Clients frequently misreport what they wants to remember or doesn't want to remember - That's why using a 24 hour recall + FFQ (14 questions – adherence to the Mediterranean diet, asking on a weekly to monthly basis, weekday and weekend) is a more holistic approach	Acknowledge that misreporting is a Common occurrence in dietetic practice Combination approach may work better
4	- Probably wants to start figuring out what the patient's understanding was, what counts as a F/V, how is she recording her food diary, does she record snacks, even drinks like juices and smoothies, does she consider them as things that she should be recording? Which if they can explain away the discrepancy - Don't know in that circumstance if I will end up using it massively to personalise the advice because it looks like the advice was being followed - If I am able to uncover the issue – whether it was just " I didn't report fruits because I didn't count that particularly as fruit or the pt just does not record snacks for some reason – then that will be the issue - But if the dietary recall that the patient has provided is accurate and the patient may just be a high excreter for some reason, I will need to learn more about how I will communicate that information to the patient – e.g. that the patient Is a high excreter of certain things but that still does not mean that the patient is having enough f/v	Assessment of patient's baseline knowledge and attitudes – identifying contributing factors to discrepancy Questioning the utility of metabolic profiling in certain circumstances The relevance of problem identification in clinical practice Further training in effective communication (conveying information)
2	- My view is that this is an issue of recording it in a food diary, not an issue of understanding the diary, making the assumption that the dietary biomarker information that we get has already accounted for the variability and we can trust that it means the patient is having a high intake of fruit and veg - To me, it's just that the communication or understanding why they haven't wanted to record it, what the issues were – it could have been that the patient was working really hard that week / a difficult week for her to sit down and record things or anything that is completely unrelated to dietary - I will just be focusing on that, if I even thought that it is necessary – is it necessary? I do not think so	Using the tool will fall back on the objectivity and accuracy of metabolomics The relevance / necessity of problem identification in clinical practice Underlying reasons for underreporting (no purpose as it does not provide ++ benefit)
4	- Perhaps having a look at the food diary to see if it is a general overall theme of all foods being underreported, or is it just the socially desirable / desirable foods being over / underreported. If it is an overall observation, it may be just due to a relationship with food and if she is having concerns about her weight even though her BMI is slightly above normal --- the relationship with food that potentially made the patient feels uncomfortable reporting	Assessment of patient's behaviour and perception – the importance of social desirability and potential weight concerns
2	- This is where the recording is a real problem b/c putting focus on something that is deeply personal thing for a lot of people and a lot of people find it very hard to sit down and write it all down, and then show it to someone else - You need an incredible amount of trust in order to do that with someone, and if you don't have that, then why would the patient do that if it is something that is deeply sensitive to them - So I am not sure whether I will be comfortable to do so. You need to be sensitive as to whether you push the point on this or not. If someone has an issue with food, which can happen at any BMI, not necessarily linked to BMI - We shouldn't be so dogmatic about it – people are allowed to go and have a good time - So the point that the food diary of the past 24 hours doesn't match it, is that a problem, even? - b/c we are human, we have to enjoy things as well, otherwise it becomes a very stick approach	Consider patient's views and emotions, establishing trust, respect and equality instead of enforcing a top-down approach Patient-centredness and recognising patient's vulnerability helps enhance the provision of higher quality of care Dietetics profession – image as a "food police" – singling out people's indulgent behaviour

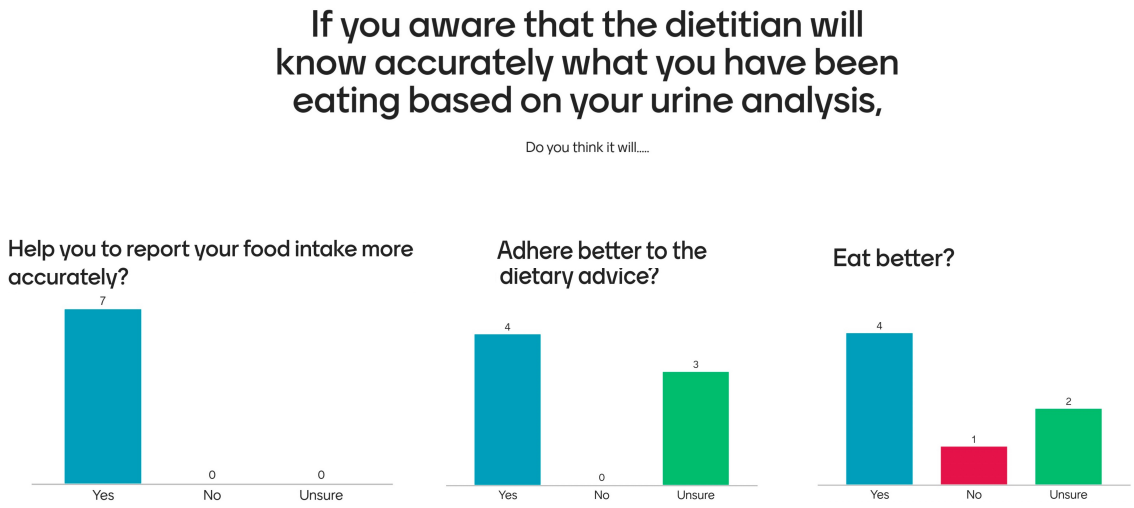
		Understanding that food also has a social component embedded. Embracing the idea of moderation is key instead of instilling fear & apprehension (putting one's life under a microscope)
4	- Yes, not being fixated about the Specific 24 hour window, w/o doing it on a repeated basis, just the day to day variation of what people eat, you might overeat on one day and undereat on another – it's just weird to eat the exact same thing	Repetitive dietary consumption is simply illogical – consider variability and diversity
1	- That's why they need to combine the measurement tool with something else e.g. FFQ, to reflect what they do on a weekly basis, or monthly basis	Combination approach may work better
Scenario 2 (underreporting)		
4	- Would that be overreporting of perceived-to-be desirable food? - It will be awkward in a sense, because you are not really wanting to accuse someone of lying but rather to understand how this discrepancy came up - if there are certain foods that the patient think is healthier, or why the patient think they should report consuming that food more - I won't be comfortable having that conversation	Identifying contributing factors to discrepancy Forsee the experience to be an uncomfortable one – potential hinderance to patient engagement
2	- I won't be comfortable having that conversation as well - I would park that food diary entirely and just have a conversation about something else - I feel that if I focus on what the patient has done wrong, the dietitian will be on a highway to nowhere	Potential hinderance to patient engagement Defeats the purpose of the dietary counselling The issue of trust
1	- from what D2 said it's a lot about behavioural communication and these things, where instead what I see with my current clients is that they want to be told what to do - the more extreme the better - for me personally, I will normally need to tell my clients directly, otherwise they will leave the room disappointed, not following what I say - It's the shift from NHS to private	Differing opinion Dependent on the context and patient demographic (e.g. socio-economic status, motivation stage of change) Aspects relating to behavioural change techniques / communication
2	- The description D1 made hits the nail in showing how important context is in this and how important it is to know the person that is coming into your room / the consultation, what are they, are they coming because they have been told that they have diabetes, they are socially / economically deprived, all sorts of things going on, too many children, unhealthy eating habits – too much pressure on them compared to someone coming into the clinic, paying for your time – wants more extreme measures, the honest the better - a completely different set of skills that will be used with these individuals - An example: when I was doing a diet history with someone who was quite overweight, and similar to the case study, the reality did not match their reporting and then I said: I think you need to be kind to yourself – and the person just said all the truth, all the emotions, b/c that was a shift in the element of trust, and that worked - You won't necessarily be able to get the same by pushing a food diary in front of someone and instructing them to fill it out e.g. come back and report to me what you have done - I'm not sure how I would use this tool - Because I avoid using a food diary, or even at times avoid using a 24 hour recall because it's quite an invasive approach to understanding how someone eats. You don't talk about food, you let them talk about what they want to bring to you	Importance of understanding patient's background in the provision of tailored dietary advice Element of trust (dietitian patient relationship reinforced, instead of a top-down approach Reluctance for usage - Invasive approach to understanding how someone eats
1	- Perhaps the girls can continue the trial with a continuous glucose monitor. It helps to show people the spikes with what they have had	Combination approach may work better for measuring dietary intake quantitatively
4	- where I see a use for or where I think might be using food diaries is less of me using it and monitoring, It's more of people being at	Confirmatory / reaffirmation tool that an individual is on the right path – positive

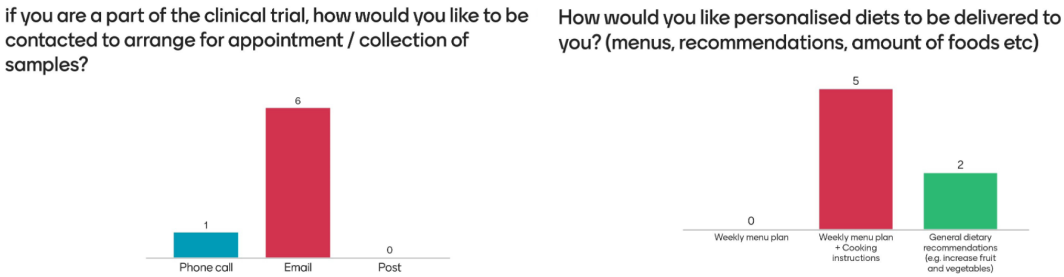
	the early stage of making dietary change and helping themselves in terms of self-efficacy and self-monitoring for longer term - I wonder if this information could almost bypass the dietitian, more of it as in, the patient feels that he is doing really well, but would like an external check on his progress and see if he can confirm he is doing it right e.g. similar to wearable tech where patients can continue to monitor their actions - because they have already done the steps, they know how much they have done. they already know how well they are doing in a broad sense o maybe even for goal setting in an abstract way	reinforcement rather than a negative connotation Reinforce and encourage appropriate behaviours
2	- I'm interested to see how to use it with the people that needed it the most. You already have the committed people, you almost don't need it with them. They are on the health picture - how do you maximise the usage for where it is really needed o for people with chronic conditions o that part is still a bit unclear - I do see that self-monitoring is a very educational process, teaches people about how they are eating, because they have never look at that aspect before - There's a place for this tool but not for everyone	Maximising tool's utility – for those who needs it the most (helpful for specific cohorts) - strengthen the idea behind its utility in chronic disease mx
1	- Perhaps use as a screening tool by the GP, whatever that flags up can be sent for further advice (low / high profile)	Suggestion (Screening tool for GP)
2	- It's a good idea as a screening tool, because if you have someone with confirmatory result, so in a screening way I can see that working	
4	- In my head I always have some sort like a negative motivation tool, which I'm really uncomfortable with - Looking it in the way, like, oh no you are doing much better than you actually think - I feel a lot more comfortable to use it as a positive reinforcement	Suggestion as a positive reinforcement to motivate continued change in dietary behaviour (helpful to boost confidence of those who thinks they are not adhering)
2	- I worry that it can get misused as the negative, a stick approach - I feel extremely uncomfortable to use the tool to flag out people who does not follow the advice and the dietitian following that up - Maybe, for people who have been screened, similar to the screening of people with protein or glucose in their urine and then the people can get sent on for further support	Barriers/Concerns for use – misused as a negative criticism/connotation
2	- So yes, dietitians will not be doing it, maybe the doctors, maybe the GP in primary care	Suggestion (Screening tool for GP)
1	- GP uses it, those who gets flagged up with a bad score gets referred to the dietitian - Something they can work on with the dietitian	Suggestion (Screening tool for GP)
4	- Might not be for everyone but if there is a subset / certain type of people - This should be investigated and refined further - Perhaps for people who have not seen effectiveness of dietary changes as a tool? o Offering it as an additional measure to prevent drop-out, because it allows for further personalisation - Rather than it being used as a blanket referral - People who is not going to respond positively to it - Not sure to stratify the patient group - Behavioural and personality type thing o Personality type screening?	Positive reinforcement for those who have low confidence Stratification using measures of individual's personality to identify appropriate weight loss/mx strategies for individuals
2	- When I see someone, I hope that I get a good feel of how healthy or unhealthy, or suitable or unsuitable their diet is - Have you look at the dietitians perception of someone's diet compared to the diet score? Rather than the patient recording it, have you compared it to the dietitian's assessment - because I am not sure how it will enhance my practice, because when I see someone, I usually have a fairly good feel of how someone rank on a score	Experience of a dietitian in identifying how well the patient's diet is Suggestion: look at the dietitian perception of someone's diet compared to DMS (dietitian's assessment) Apprehension regarding applicability (experience has helped her to have a good gauge of how ideal an individual's diet is
4	- Instead of getting into a negative relationship with the patient, maybe you can ask them: from your food diaries it seem like things are going well -- explore any challenges or barriers they might have encountered or couldn't report on? - Something like an exploratory tool	Prevent negative connotations and break the rapport built Explore other challenges or barriers

		Exploratory tool
2	- Conclusion: Currently we are unsure how to use in a clinical setting, but as a screening in the GP, it may potentially be feasible for people with CVD. They need to know how much dietary input they need, so if someone comes up as 25%, you will clearly know that they will need dietary advice - I will be interested to know how does I personally compare to the tool. How does my intuition / experience / judgement compare to the tool.	Barriers for implementation in the clinical setting but potential screening tool Comparing dietitian's aassessment to the tool

Focus group with PPI patients







Supplementary Table 5: Activity 2 - Patients' Focus Group Transcript

PPI Patient Number	Verbatim
Comfort level in providing urine sample	
1	- should be a private thing for me when giving your urine samples, just don't like it from the bottom - I've done it before - happy to do it but not too keen on it, wouldn't rather not do it if given a choice
Comfort level in providing stool sample	
1	- happy to do it but not too keen on it, wouldn't rather not do it if given a choice..not too pleasant
2	- never had to do it and but I'd rather not have to do it, rather than any specific issues
3	- not pleasant to do.. although its par for the course
4	- did one recently and felt very grateful because the hospital immediately within 2 days contacted me and inform me that you have to do a colonoscopy in which they found polyps that they removed. pre-cancerous. grateful that these things are discovered… felt that for a small inconvenience it was a great service - the purpose of the testing is to see how well the body is able to sustain itself.. for a good cause
If you are aware that that the dietitian will know accurately what you have been eating based on your urine analysis, will it help you to adhere better to the advice?	
3	- Unsure. 2008 when I became diabetic, i was given awful advice by the dietary people who send me down a spiralling road of more and more carbs until the point where I have to go on insulin. and I got off insulin a year by excluding carbohydrate basically from my diet… felt that it has been good at stabilising my weight and blood sugars. I'm happy to follow what any dietitian says but I will be doing fingerpick test constantly to see its effect on my day to day BSL, e.g. tomatoes peak my BSL. I'm trying to preserve my BSL - I've learnt to eat much more, I was very lazy, I was just listening and saying yes, but I dont want to be challenging anything that you are saying, that's why i said unsure because in that sense, we all have our personal responsibility to understand why and how our bodies work and so forth. and I see what you are doing is a fantastic tool, to help individuals tailor their diet, particularly if there is something missing in their diet
1	- Unsure. I dont know if I would follow the advice, I'm currently on a diet but I eat fruits sometimes and drink stuff that I shouldn't eat and drink, so I dont know if I would follow the advice to the maximum. I'm not sure how much I will follow the advice because in the past I have been told that I shouldn't eat or drink certain foods but I have done it afterwards…contemplating if I should follow
If you are aware that that the dietitian will know accurately what you have been eating based on your urine analysis, will it help you to eat better?	
2	- No. I think I will eat what I will eat and knowing that somebody will know doesn't really make a difference. if I want to eat better and know what I should do, it will only be sustainable if I decide that I want to do it. I don't think that somebody knowing what I eat will make me eat better
3	Unsure - similar to what I've just said, another reason is that I attempt to eat healthily already. at the same time, I'll be very honest with you about what I eat or drink, because if we change it, the feedback that we are going to get from you will be more tailored. it will be tailored to.. if you change your diet because you are having a urine analysis, then the urine analysis isn't going to be as valid as if you stick to your normal diet and then have the urine analysis. if you usually have one of the sweet but you stop having it because you know a urine analysis is coming up, then I think it invalidates no doubt the urine sample. any analysis will make you change your habit, mi absolutely convinced of that. in the past that blood test, you intervene that… no I wouldn't ignore it, I'm just unsure if… what I'm trying to say is i wouldn't change what i normally do just because i have a urine sample
5	- I ticked yes. I think depends on a combination of factors. I think having somebody who you know, look at your urine sample and you know, record what you are eating actually is a motivator for you. well it's like a support to you really. um but again you have to have some motivation yourself to change your eating behaviour. um but then I suppose one of the things that is linked to this is um illness as well. if somebody has got different chronic condition, you know they may be impacting so much that they can't really adapt this behaviour or adhere to that dietary advice. so it's a combination of things it not always just yes/no
6	- I totally agree with P2. She said no I said yes. but I totally agree with her that at the end you are instructed to follow specific diet but depends on the circumstances, how you behave.. I mean that circumstances will control how you behave.. and if you said eat better, if said yes because it is going to be better thing for my health, that's a matter of fact. but will I eat better to my taste? am I happier? that's the question. because when I been through this program for 3 months, i was deprived of all food almost. it's just vegetables that are steamed and if i want to and specific vegetables. it was very tough, at the time when there was not quality of Life at all. we were in lockdown, at home for 3 months, can't step out, and there is nothing for you left as a QOL except food. you are not going out, you are not travelling you are not doing anything. that limitation and that pressure went on and on. finished the 3 months i lost about 8kg, and the target was 15 but I couldn't make it, so I was like depressed, badly depressed and then I gave up. I just ate everything, I just went back, actually worse than before. now I'm catching up quite a bit, not eaten everything, not abusing everything, honestly speaking, I'm trying to try everything as well, regardless, regardless because that's the main point. it's good to know, it's good thot you have a dash board after this, sampling of you know… of urine.. giving us a better idea of where one is standing and where we are going, which direction, then you can moderate, but you can't be very strict with the instructions from the dietitians, especially if it deprives you or disbalance you from your normal style of life or style of eating

Appendix D: Activity 3 - Post-pilot intervention semi-structured interviews

Interview questions and responses from dietitians

** Dietitian 4 participated in Activity 3 twice, hence his sessions will be labelled as 4a and 4b

1. How useful is it for you to utilize the report generated from the urine analysis? (Extremely useful, moderately useful, slightly useful, not useful at all, unsure)

PPI dietitian	Responses
1	Extremely useful
2	Slightly useful
4a	Slightly useful
4b	Very useful

2. What challenges do you face when utilising the information?

PPI dietitian	Responses
1	- She did not eat high red intake but had a burger that day (intake24 doesn't capture dietary habits just snapshot) - Recommendation: Ask more information regarding diet
2	- Dietitian describes that the report itself is difficult to use and pull out information required (Dietitian required some time to read and extract information required and ultimately created a new document) - Dietitian reports conflict between different metabolomics (i.e. carnitine and other protein, fruits and vegetables – from dietary and metabolomics mismatch from patient)
4a	- Some markers seem non-specific (e.g. those relating to red meat intake were high, but Reena is a vegetarian - this happened during the documentary filming also for another vegetarian). - Other markers require significant investigation/interrogation to interpret such as glucose - in this patient it was unlikely related to sugar, but more likely carbohydrate intake, and possibly insulin resistance.
4b	- Dietitian thinks the participant misreported the diet - Participant did not mention HbA1c, insulin resistance (even if not in diagnostic level). It is known that sugar from test was high – Consider that the tool is validated in younger healthy population, where no insulin resistance is present (?Limitation).

3. What improvements can we make on the applicability of this method?

PPI dietitian	Responses
1	- Need to use more than 24h recall - Consider the need to have a weekday and a weekend (different days) dietary recall
2	- Some aspects of the report could ultimately be removed (i.e. less information regarding specific fruits and more information around general fruit and vegetable intake). - Dietitian is unsure when disagreements are observed how much of the truth lies within the actual report.
4a	- Including additional days recall/urine tests so it is more representative of the 'usual' diet. In patients who do not have regular meal patterns and high inter-day variation, it might not be as useful.
4b	- Dietitian reports that he would have had the report or a tailored version of the report on the desk.

	- It would be beneficial to have a discussion on some aspects that might have been more useful to give to the patient.

4. How do you think we can improve the dietary protocol for the clinical trial dietary counselling sessions?

PPI dietitian	Responses
1	- Metabolomic report is an add on to the consultation - Recommend a Stricter advice compare to the recommendation - Leaflet Advice: include more pictures
2	- Dietitian practices differently than SOP (believes it is more important in creating a report with the patient rather than using the SOP). - Dietitian mentions that before talking about food understand what goes around food (could potentially be included in the SOP). - Dietitian suggests that the consultation should Not a number approach but human approach (building a report with the patient and siding with their truth).
4a	- A single day recall was only of limited use, and this was not a representative day (esp evening meal) for the patient. Some probing revealed significant snacking behaviours related to hunger/stress/boredom, that ended up being the most significant aspects to address through satiety methods. Having diet and urine for several days to capture variation/usual patterns might be more meaningful. - The dietary documentation is very large, and possibly too prescriptive. For some patients discussing energy deficits/allowances is not suitable/effective. This may be suitable for a study, but is probably not for day-to-day clinical use. In a way it felt more like automating some of the process. - Having all of the diet history details, comparison to NICE, energy/macronutrient calculations was very useful, even though not all was used in this first appointment. I expect more of this will be discussed in future appointments.
4b	- To be tailored for different populations (i.e. If an individual is vegetarian, meat intake information could be removed from the report). - It would be good for them to know things do well and things to improve –considering a smaller report for the patient (i.e. provide markers specific to NICE).

5. In your opinion, do you think the tool has helped you to engage the patient? (made the session more interesting? Caught the patient's attention?)

PPI dietitian	Responses
1	- Dietitians view that patients in general find metabolomic report, genetic testing etc. more interesting
2	- The report was used for alcohol intake, processed meat, and sugar in different ways (i.e. dietitian reports that she might not have asked for alcohol if it was not observed in the report). The dietitian hasn't brought the report up to the patient (belief that it could be utilised in a bad manner). - With respect to fibre intake, the report helps.
4a	- Yes in some respects - being able to show areas that supported the dietary analysis (e.g. fruit/veg intake being adequate/high) was reinforcing/encouraging. - Other areas did highlight an avenue for discussing/investigating portions size (high urine glucose not related to free sugar intake but carbohydrate). - In others it was more distracting - like the indication of red meat intake in a vegetarian, although some of this might be explained by fish intake the previous night. The core of the consultation still revolved around behaviours/relationships with food and managing these practically, which might not have been any different without the tool in this example.
4b	- Good for reinforcing (as seen patient was taking huge force of reinforcement) - Dietitian didn't use it in aspects where the report and patient's report did not line up

	- Phrase used to show discrepancies “We could see from your urine that you are consuming high amounts of apples but there might be lack in variety of fruits”

Interview questions and responses from PPI individuals at CVD risk

1. How did you find the process of:
    a. using Intake24 to record your diet? (Very difficult / Difficult / Neutral / Easy / Very easy)
    b. collecting urine using the urine kit? (Very difficult / Difficult / Neutral / Easy / Very easy)

PPI Patient	Responses
1	a. Fine, did not experience any difficulties as she had a phone and is digitally literate (Understands why we used Intake24 because "that's the future".). Suggestion: if researchers were to scale up to the general population: consider accessibility -- for those who are not digitally competent, to be done via email instead of a website b. Very easy and the collection was prompt, finds it a useful thing compared to blood
2	a. no difficulties using, pretty self-explanatory. one issue is that she is unable to key in lentil pasta as an option (not available in intake24) and she doesn't want to choose pasta (as she wants to show that she is consuming a healthier alternative rather than a regular pasta) b. very easy, no difficulties at all.
3	a. Able to follow the instructions from the video. Not too difficult, pretty straight forward and self-explanatory b. Quite confused and instructions are hard to follow (unsure what to do with the sticky label on the container. Needed to ask his dad for help to fill the urine container. Would be good if there is a instruction video to follow

2. Now that you have received the advice,
    a. In general, does the personalised dietary advice given by the dietitian motivate you to improve your:
        - diet (Yes/No/Unsure)
        - accuracy of dietary reporting (Yes/No/Unsure)
    b. Do you think the addition of the urine test results has further motivated you? (Yes/No/Unsure)

PPI Patient	Responses
1	- Diet: Yes (finds it important to make the dietary changes as well) - Accuracy : Yes (feels that it is good for the diet to be reported ahead of the consultation as she cannot hide anything during the consultation session itself) - Urine results further motivated her to improve on her diet in her daily life, because of the urine can be collected in real time. She sees how it can be applied into the NHS setting in future.
2	- Diet: yes, the 1 on 1 consultation is useful, gives her a better understanding of her diet as a whole. Knows that the dietitian is looking at her diet individually and providing specific advice that is tailored for her (rather than one that is for the general population. likes that she has been given solutions and tips on how to improve her diet - accuracy - neutral (reports that she is already reporting accurate and knows that she needs to. further comment re: diet reporting: gathering the dietary information (e.g. details about meal patterns, social aspects etc) on a once-off basis will help but not on a regular basis as she does not see how it will impact the consultation - Further motivated her, gives a good snapshot of her diet BUT there is a few limitations (she feels that the urine report tells you what you had on the particular day and not on a usual basis, as she usually don't eat much protein (perhaps much less than the general population / does not eat meat at all (mainly fish, so she is unsure why the report indicates high carnitine). feels that it is not as useful for informing usual dietary patterns

3	- Diet: finds the advice quite helpful, will try to utliise the strategies to improve his diet - Accuracy: Unsure because he has experience with recording his dietary intake in food diaries. (currently doing another research study which requires him to complete a food and drink diary) - Further motivatation: Unsure. feels that he is already reporting accurately at baseline and that the dietitian did not mention much about the urine test

3. The dietitian has noticed that the information you have provided is (not) in agreement.
    a. How does it make you feel?
    b. Does it motivate you to do better? (Yes/No/Unsure)

PPI Patient	Responses
1	- Satisfied / Relieved, knows that she can and need to be honest because the results are there
2	- Feels relieved knowing that her vegetable intake is not a problem
3	- feels comfortable conversing with the dietitian, does not feel antagonized by her. does not feel judged because he is open to sharing what he does with his diet. Feels that the dietitian is there to help him at the end of the day and in the long run + he is doing the activities for the money
4	- the session was informative, comfortable and engaging - the rapport and trust that the dietitian built during the session allowed her to feel safe to have an open and honest conversation with him - Having a general run through of her diet (diet history) with the dietitian allowed her to mentally 'visualise' what she has eaten on a daily basis and the personalised dietary advice helped her to identify areas to work on as a start

4. Do you have any feedbacks that you would like to provide regarding the collection process and the dietary counselling?

PPI Patient	Responses
1	Found the session great and helpful. It gives her an overview of her dietary pattern and provides confirmation that she is on the right track in some way. Finds that she couldn't hide anything from the dietitian. Participant mentioned she cannot actually remember the diet that she has eaten even though she previously reported it. Feels that the session increases her dietary knowledge in general Feedback for the handout: Can be more interactive for the general public e.g. more pictures of the foods rather than just tables. will be easier for participants to remember portion sizes, commented that what the dietitian show is more memorable, handout is slightly too long, participants may prefer a more concise version Provision of urinary dietary report to participants: As the actual research are volunteers, she feels that it will be good (in terms of ethical standards) to offer the report as an option to the participants
2	handout: easy to follow, colour coded, explains about portion size (which is something she has struggled with), the "healthy" plate example is helpful, knows how much carbohydrates she should consume
3	Feedback for the handout: understandable and straightforward but may be too a bit too lengthy
4	Feeback regarding the handout: - felt that the exchange list table was good in the sense that it brings people away from having to count calories and directly informs the readers the number of servings - Suggested to have different combinations of food group exchanges (tailor according to patient's usual diet e.g. if pts usually consume 3-4 servings of CHO, felt that it might not practical to increase to 8-9 portions esp for those who have a small intake at baseline) - might be useful to include graphics - felt that everyone interprets portion size differently and it will be good to have a standard to make reference to (e.g. a small banana might be viewed as medium size for another individual)

	- Suggest to do it for snacks as well - reason being it may help individuals be more aware of the amount of snacks that are consuming relative to the recommended portion

Appendix E: Summary of the outcomes and impacts from PPI activities

Supplementary Table 6: Feedbacks received (Outcomes above) are listed in detail according to the sub-themes and the action taken (impacts) by the research team are indicated on the right.

PPI inputs for the nutrition counselling model	Themes	Feedbacks received (outcomes)	Action taken (impacts)
Perspectives regarding Nutrition Counselling	Theme 1a Facilitators of nutrition counselling from dietitians' perspective	▪ Comprehensive information gathered for nutrition assessment – most dietitians utilise the ABCDEF framework	▪ Standardise the nutrition assessment framework that will be used according to ABCDEF, focusing on factors important to CVD management
		▪ Having accurate information for nutrition assessment o Dietitian suggested to: include procedures for conducting Bioelectrical Impedance Analysis, waist circumference and blood pressure measurements, and to gather information about usual diet for capturing dietary habits	▪ Provided clear instructions for conducting procedures (Appendix A, B and D of SOP) ▪ Confirmed inclusion of the section on 'usual eating habits'
		▪ Patient-centred care being provided e.g. building rapport, trust and positive communication for successful nutrition advice uptake	▪ Included a section on effective communication: Emphasise rapport building and establishing trust, behaviour change techniques
		▪ SMART goals that are achievable for patients, checking in with the person to find out whether they feel the goal is achievable or realistic	▪ Reaffirm the importance of setting SMART goals with patients
		▪ Understand patient's knowledge and help them to debunk misconceptions	▪ Included measures to assess patient's dietary knowledge & specify the type of intervention done during consult (intervention category)
	Theme 1b Barriers to nutrition counselling from dietitians and	Dietitians ▪ Insufficient time for rapport building and conducting a thorough nutrition assessment to obtain a holistic picture of the patient	▪ Extended the dietary counselling session (Initial consult from 30 to 45 minutes, follow-up consult from 15 to 30 minutes)

	patients' perspectives	▪ Patient's engagement and motivation and as a barrier to dietary uptake <u>Patients</u> ▪ Lacking/having bad experience with dietitians ▪ Lack of resources to effect positive dietary change ▪ Concerns of deteriorating quality of life and dietary lapses	▪ Included subjective measures of readiness to change, motivation and confidence level, barriers (if any) ▪ Emphasise on effective communication and being realistic within patient's means in the dietary protocol ▪ Included measures of hunger triggers, level and usual timing of occurrence to assess environmental, behavioral and social influence on dietary change ▪ Included a section on monitoring and review of dietary progress ▪ SMART goals as above
Perspectives on using metabolic profiling for nutrition counselling	<u>Theme 2a</u> Facilitators to using metabolic profiling for nutrition counselling	▪ Dietitians' positive perception of using metabolic profiling in the CVD context	▪ Nil impact on research, motivation for researchers
		▪ Highlighting to patients the positives in their metabolic profile	▪ Using the report for positive reinforcement rather than flagging the negatives (can be taught during user training)
		▪ Most patients feel comfortable with providing urine and blood samples	▪ Continue with fortnightly samples collection
		▪ Metabolic profiling helps patients to report food intake more accurately	▪ Reaffirm the applicability of the tool in clinical dietetic practice
	<u>Theme 2b</u> Barriers to using metabolic profiling for nutrition counselling	▪ Lacks familiarity and confidence in the utility of metabolomics	▪ To consider conducting user training for metabolomics e.g. metabolic report interpretation
		▪ Concerns for misuse (e.g. highlighting discordance between metabolic report and self-reported diet history in a negative manner), which can break the rapport with patients	▪ Avoid flagging our the negatives and to use the report as a positive reinforcement tool)
		▪ Patients' discomfort in providing stool samples	▪ Maintain frequency of stool sample collection – only for research purpose, not for the clinical setting

		▪ Metabolic profiling may not improve patient's adherence to dietary advice and guide patient to eat better	▪ To first assess where patient sits on the 'stages of change' model (included in protocol as above)
Integration of metabolic profiling into nutrition counselling	Theme 3a Positive experiences with pilot intervention	Dietitians ▪ Increase motivation and engagement of patients	▪ Reinforced to researchers the need for personalised nutrition
		▪ Improved ability to identify misreporting	▪ Observation of the pilot intervention provided researchers an understanding on how to deal with discordance if it occurs in Study 3
		▪ NICE and energy/macronutrient calculations comparisons in the nutrition counselling protocol are helpful	▪ To continue with the comparison of nutrient intake to recommendations ▪ Created a NICE guideline checklist for the control group to determine patient's degree of adherence to NICE guidelines
		Patients ▪ Felt urine was easy to collect compared to other biological samples	▪ Reaffirm the use of urine samples for metabolic profiling
		▪ Participants felt exchange list is understandable and found the healthy plate concept helpful	▪ Retained healthy plate and exchange list in the CVD handout as a guide for food groups portions
	Theme 3b Negative experiences with pilot intervention	Dietitians ▪ Experienced challenges in using the metabolic report i.e. too comprehensive, hard to pull out information relevant to patient	▪ Created a metabolomics report summary table for easy reference during consult
		▪ Non-specific biomarkers e.g. carnitine for vegetarians	▪ To be aware of these limitations (can be flagged out during user training)
		Patients ▪ Unclear instructions for using the urine sample kit	▪ Included instructional video link into sample collection instruction handout
		▪ Digital literacy of participants in using Intake24 ▪ Comprehensiveness of Intake24 database ▪ Intake24 is a snapshot of diet, not usual dietary patterns	▪ Consider a hard copy version of 24-hr recall for digitally illiterate patients ▪ To review patients' search terms when analysing diets from Intake24 ▪ To ask patient about their usual diet (as mentioned above in Theme 1)

	▪ Pictorial references in the educational handout will be better for visualising serving sizes	▪ Included serving size pictorials from British Heart Foundation into handout

Appendix F: Protocol for the metabolically-personalised nutrition counselling model

METABOLICALLY-PERSONALISED NUTRITION COUNSELLING MODEL

Protocol

1.0 Background

Cardiovascular diseases (CVD) account for approximately a third of the total deaths worldwide and cost the NHS approximately £7 billion per year. Although genetic predisposition plays a role in CVD, lifestyle, particularly diet, is known to modify disease risk. Healthy diets such as the Mediterranean diet have been shown to improve CVD risk factors (blood pressure, obesity, cholesterol) and are critical to the UK government's policies to reduce CVD-risk. However, it is known that people respond differently to dietary changes and in order to find the best strategy for an individual it is necessary to identify objective measures of dietary intake, dietary adherence and dietary effect. It has been estimated that 50% of the self-reported food diaries within the 2000 cohort of the National Diet and Nutrition Survey significantly under-reported, which makes these national data impossible to interpret.

The premise of this clinical trial is that metabolic profiling can be used to improve the accuracy of monitoring dietary intake, behaviour and adherence to diet guidelines for people at risk of CVD and can be a useful tool for establishing inter-individual variation in response to diet. This project aims to evaluate the applicability of providing a metabolically-informed personalised dietary advice to help people at risk of CVD to change their dietary habits within their own environment. A model for predicting adherence and response to diet has been built from the blood and urine metabolic profiles of participants in the first part of the study.

In the second phase of the project, the model will be tested on a larger number of individuals at risk of CVD, in their home environment and the viability of using these metabolic profiles as an adjunct to nutritional management in a clinical setting will be evaluated. The intervention group will receive advice based on measurements of their urinary metabolic profiles and the effect of metabolically-informed personalised dietary advice on reducing CVD risk factors will be compared with a control group receiving standard dietary advice provided by the dietician.

In order to facilitate the dietary counselling process and reduce intra-individual variability in the advice provided during the session, this standard operating procedure (SOP) has created for research dietitians undertaking the diet counselling to standardize the practice.

2.0 Purpose

To facilitate dietary counselling for both the intervention and control group of the clinical trial study in order to ensure standardisation of the consultation process and completeness of documentation done by all research dietitians.

3.0 Scope

This SOP applies to all dietitians involved in the clinical trials for the nutritional management of cardiovascular disease or non-alcoholic fatty liver disease risk, using metabolic profiling strategies.

4.0 Responsibilities of the Dietitian

- Undertake a nutritional assessment of patients who are enrolled into the clinical trial.
- Provide a nutrition diagnosis
- Implement a nutritional care plan that is personalized for the participant, using the report derived from participant's urinary metabolomics analysis (intervention group) or self-reported dietary intake from Intake-24.
- Monitor and review participant's progress, providing changes as appropriate.

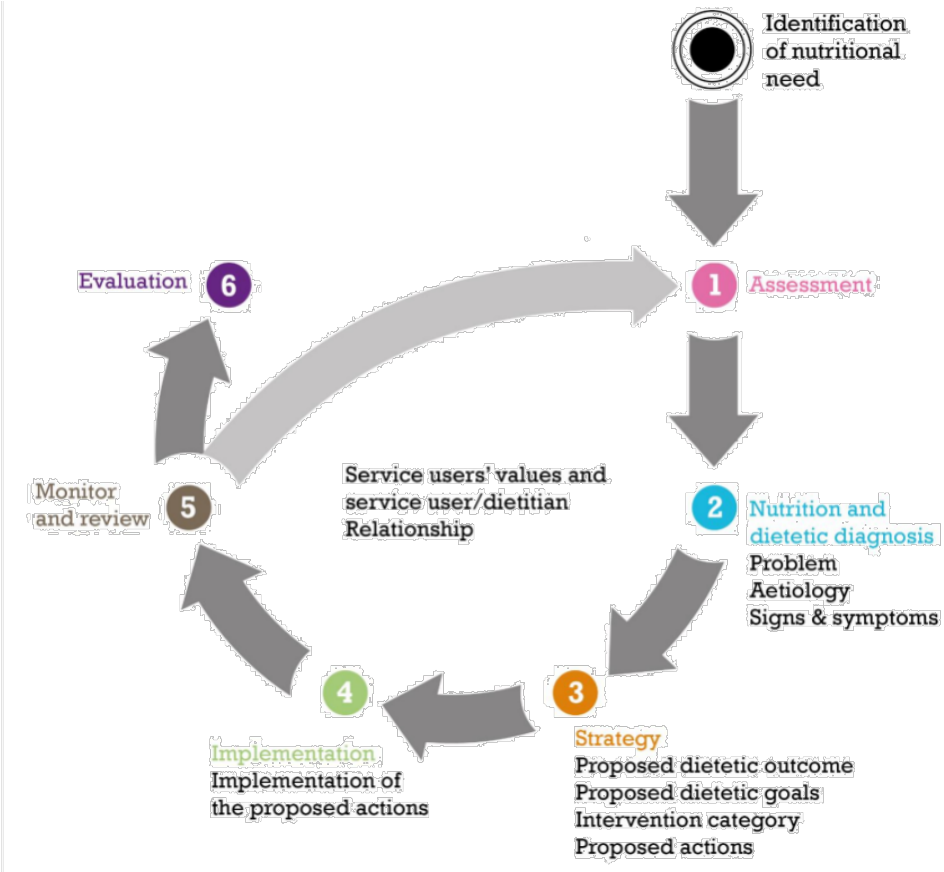
5.0 Glossary

SOP	Standard Operating Procedure
NCP	Nutritional Care Plan

6.0 Overview

6.0.1 This SOP has been established using the revised British Dietetic Association Model and Process for Nutrition and Dietetic Practice, abbreviated to 'Model and Process' (BDA, 2020). The purpose of the Model and Process is to describe, through the six steps highlighted in *Figure 1*, the consistent process dietitians follow in any dietary intervention with individuals in the clinical setting.

6.0.2 Each step of The Model and Process has been adapted to be in aligned with our personalised dietary intervention. The systematic application of the six steps will demonstrate the unique skills of the dietitian and provide consistently high standards of dietetic practice. In addition, it will support an agreed structure for dietetic records.



Protocol Figure 1: Model and Process for Nutrition and Dietetic Practice (BDA, 2020)

This SOP will describe in detail the method of each step: 1. Assessment, 2. Nutrition and Dietetic diagnosis, 3. Strategy, 4. Implementation, 5. Monitor and review and 6. Evaluation, to provide a personalised dietary advice for people at risk of cardiovascular disease in the clinical setting.

6.1 Procedure

6.2 Assessment

6.2.1 Assessment is a systematic process of collecting, grouping, analysing and interpreting relevant information to make decisions about nutritional status and the nature and cause of nutrition-related problems that affect a participant.

6.2.2 The assessment demonstrates the critical reasoning that informs decisions made around the nutrition and dietetic diagnosis as well as the development and monitoring of the intervention.

6.2.3 The data collection prompt acronym (ADCDEF) may be used as a helpful tool to ensure that all appropriate data has been collected from relevant areas to help inform the assessment: **A**nthropometry, **B**iochemistry, **C**linical/physical, **D**ietary, **E**nvironmental/ behavioural/social, and **F**unctional. (Table 1). The data collection and the collection method of each assessment component is exemplified in Table 1.

Protocol Table 1: Nutrition assessment table (the data collected and collection method for each assessment type)

Assessment type	**Data collection**	**Collection method**
Anthropometry	Body Mass Index (BMI), Body weight, Body fat, Body water, Lean body mass, and Basel Metabolic Rate (BMR)	Bioelectrical Impedance Analysis (BIA)/ Tanita. Instructions in Appendix A will be followed.
	Waist circumference	Meter. Instructions in Appendix B will be followed.
	Weight history, Usual body weight and their perception on the current weight	Participant will be asked about this information in the dietary care record (Appendix C).
	Weight change	Weight change will be calculated as follow: Current weight – Usual weight
Biochemistry	C-reactive protein (CRP), HbA1c, urea, Electrolytes, liver function, lipid profile, full blood count and fasting glucose	Blood tests will be done by medical doctor at the beginning and the end of the intervention (after 12 weeks)
Clinical/physical	Blood pressure	Blood pressure monitor will be used to measure

		blood pressure. Instructions in Appendix D will be followed.
	Health/disease status Medication & dietary supplements Bowel movements Food allergies Any relevant symptoms or signs	Participant will be asked about this information in the dietary care record.
Dietary	24 dietary recall	Online tool (Intake24) will be used by participant to record their dietary intake. Instruction about using the tool will be given (Appendix E)
	General eating habit: meal frequency/timing, skipping of meals, eating outs, food cooking and shopping, appetite/Hunger, and alcohol intake.	Participant will be asked about this information in the dietary care record.
	Current adherence to NICE Dietary guidelines	NICE Dietary guidelines checklist (Appendix F) will be used to assess dietary adherence to NICE guidelines.
	Metabolic report (if participant was in the intervention group), the report will provide objective information about the dietary intake. Information provided by the report: adherence level to NICE dietary guidelines and some specific dietary biomarkers for some foods	Urine and serum samples will be collected and analysed using NMR to produce metabolic report
Environmental/ behavioural/ social	Readiness to change, motivation level, confidence level, dietary knowledge level, smoking, occupation, educational level, type of work, specific food culture, and any environmental barriers.	Participant will be asked about this information in the dietary care record.
Functional	Physical activity level	International Physical Activity Questionnaire (IPAQ) will be used. Participant will be asked about this information in the dietary care record.

6.2.4 Participant will be fully assessed before the initial visit, and all collected data will be documented in the Dietary care record.

6.3 Nutrition and dietetic diagnosis

6.3.1 The NDD is the identification of nutritional problem(s) to be addressed that may impact on the physical, mental and/or social well-being of an individual, and where the dietitian is responsible for

action. Firstly, a PASS statement is created, which is then formulated into the NDD. Each nutritional problem is formulated into the NDD using the following three separate components (known as the 'PASS statement'):

6.3.2 **Problem** – identification of the key nutrition related problem(s) that the dietetic intervention will aim to address.

6.3.3 **Aetiology** – cause of the nutrition related problem(s)

6.3.4 **Signs and Symptoms** – a cluster of signs and symptoms that evidence the problem

6.3.5 All NDD will be documented in the Dietary care record (Appendix C). The NDD is written as: (problem) related to (aetiology) as evidenced by (signs and symptoms). *Table 2* includes (but not limited to) some nutritional problems, aetiologies, signs and symptoms related to people at risk of cardiovascular disease

Protocol Table 2: Some nutritional problems, aetiologies, signs and symptoms related to people at risk of cardiovascular disease

Problems	**Aetiology**	**Signs and Symptoms**
Weight (BMI >25kg/m2) **Waist circumference** more than 88 cm for women or more than 102 cm for men (abdominal/central obesity) **Dietary intake**: (e.g high fat, low fibre etc..) **Abnormal lipid profile** **Uncontrolled blood pressure**	Poor dietary behaviours Lack of dietary knowledge Lack of motivation Others	Anthropometry measurement Blood test clinical parameters Dietary recall Others

6.4 Strategy

6.4.1 The strategy outlines what the dietitian and participant want to achieve, the indicators that will be used to measure this, and how they will achieve this. These provide evidence of improvement, or not, in nutritional or health status.

6.4.2 **Proposed dietetic outcome**: The outcome is what the dietitian and participant aim to achieve by the end of the intervention. The outcome must relate directly to the nutritional 'Problem' section of the dietetic diagnosis. In our clinical trial we aim to improve participant's dietary behaviours to reduce their risk of cardiovascular disease.

6.4.3 **Outcome indicators:** a parameter or tool that measures a change in status relating to the proposed outcome. In our trial we will use dietary recall and metabolic report as indicators of improving dietary behaviours. In addition, we will use BMI, waist circumference, lipid profile and blood pressure as indicators of reducing risk of cardiovascular disease.

6.4.4 **Dietetic goals** – SMART goals will be set to be achieved by the next consultation. The goals enable monitoring of progress towards achieving the outcome, therefore they should relate directly to the

proposed outcome and must also relate directly to the nutritional 'Problem' section of the dietetic diagnosis. In our intervention, the dietetic goals will be focused on improving the participant adherence level to NICE dietary guidelines.

- Total fat intake is 30% or less of total energy intake.
- Saturated fats are 7% or less of total energy intake.
- Dietary cholesterol is less than 300 mg/day.
- Eat at least 5 portions of fruit and vegetables per day.
- Eat at least 2 portions of fish per week, including a portion of oily fish.
- Eat at least 4 to 5 portions of unsalted nuts, seeds and legumes per week.
- Increase mono-unsaturated fat intake with olive oil, rapeseed oil or spreads based on these oils and to use them in food preparation.
- Dietary fibre intake is at least 30g/day.
- Free sugar intake is 5% or less of total energy intake.
- Choose wholegrain varieties of starchy food reduce their intake of sugar and food products containing refined sugars including fructose.
- Limit red meat intake to less than 70g/day
- Limit salt intake to 6g/day (Sodium=2400mg).
- Limit alcohol intake to 14 units per week

6.4.5 **Goal indicators:** a parameter or tool that measures a change in relation to the goal. In our trial, we will use the participant dietary recall as a goal indicator of improving the participant adherence level to NICE dietary guidelines in which we will compare the reported dietary intakes with NICE dietary guidelines.

6.4.6 **Intervention category** – an intervention category should meet the proposed outcome and goals. Our intervention categories include knowledge building, specialised diet, behaviour change, and counselling.

6.4.7 **Proposed Action Plans –** these are the proposed activities that should be carried out to meet the dietetic goals that have been identified. Similarly, to goals, actions should be SMART. The actions, together with the dietetic goals, will be documented, reviewed, and changed (as required) at each visit. Our action plans will be as per the dietetic goals stated above. In addition, if participant needs to lose weight, energy restricted diet will be offered with 600 kcal deficit (that is, they contain 600 kcal less than the participant needs to stay the same weight).

6.5 Implementation

6.5.1 This step requires the implementation of the proposed actions and the communication, coordination, management and leadership required by the dietitian to effectively deliver the strategy. The intent of this stage is to change nutrition related behaviours, risk factors, environmental factors or aspect of physical or psychological health or nutritional status of the individual.

6.5.2 Energy requirements will be calculated based on the participant BMR measured by BIA/Tanita and multiply it by the physical activity

level (PAL) assessed by IPAQ (BMR X PAL). PAL values will be 1.4 for inactive category, 1.6 for minimally active category and 1.8 for HEPA active category. 600 kcal will be deducted from the total energy intake if the participant needs to lose weight.

6.5.3 Nutrients calculations will be done in alignment with NICE guidelines in which total fat intake is 30% or less of total energy intake, saturated fats are 7% or less of total energy intake, intake of dietary cholesterol is less than 300 mg/day and where possible saturated fats are replaced by mono-unsaturated and polyunsaturated fats. In addition, dietary fibre intake is 30g/day, and the free sugar intake is 5% or less of total energy intake.

6.5.4 To minimise variations in the dietary intervention, macronutrients will be calculated as a percentage of the total energy in which: Fat: 25-29%, Protein: 15-20%, and Carbohydrates: 45-55%. Calculations will be translated to food servings using *[food exchange list]*, Table 1 shows the nutritional values per one food serving.

Protocol Table 3: Nutritional values per one food serving

Food group	Energy (kcal)	Carbohydrates (g)	Protein (g)	Fat (g)
Starch/ Bread	80	15	3	--
Meat/ Meat substitute				
Lean	55	--	7	3
Med Fat	75	---	7	7
High Fat	100	---	7	7
Vegetables	25	5	2	7
Fruits	60	15	--	2
Milk				
Skim	90	12	8	----
Low fat	120	12	8	5
Whole fat	150	12	8	8
Fat	45	---	---	5

6.5.5 Participant will be advised to do all the following: choose wholegrain varieties of starchy food reduce their intake of sugar and food products containing refined sugars including fructose, eat at least 5 portions of fruit and vegetables per day, eat at least 2 portions of fish per week, including a portion of oily fish, and eat at least 4 to 5 portions of unsalted nuts, seeds and legumes per week. Advice on salt intake will be given for people with high blood pressure. Educational handouts will be given to the participants.

In the first counselling visit a detailed information about the personalised diet and the general NICE dietary advice will be given to the participant by the dietitian considering the participant's dietary knowledge, lifestyle, and socioeconomic status. This will include education about measuring food portion size, reading food label, explaining the quantities of the food servings needed per day, using the food exchange list, explaining the types of foods need to be reduced (such as fat or sugar) or increased (such as fibre), explaining healthy food sources and cooking options. According to the participant's circumstances, dietary advice will be tailored, and food alternatives will be offered. CVD

booklet handout will be given (Appendix G), the booklet will be tailored to the participant's dietary needs. Dietitian will use simple and clear words to ensure that the participant understands the information. All dietary plans and interventions will be documented in the Dietary care record. Participant will have chance to ask at any point. Standard behaviour change techniques will be used; these include:

- Goal setting.
- Action planning.
- Providing information on health consequences and benefits of the behaviour.
- Environmental restructuring: this involves altering the environment to make healthy living easier (e.g. not having unhealthy food in the house, and putting fruit/healthy snacks).
- Time management: this involves working with the participants to help them identify how they can best manage their time in order to prioritise a healthy behaviour (e.g. physical activity as a family).

6.5.6 **Effective communication:** In order to achieve effective communication, the patient must feel that he/she is in a safe and comfortable environment. During the initial consultation, in order to establish report, post greeting; formal introductions and explanation of our role in the study and practice will be done. Throughout the consultation, the dietitian will use a combination of open and close questions, focusing on open questions in order to obtain higher level of information. Within the initial consultation, a shared setting agenda will be set by both participants and dietitians to establish boundaries (i.e. time and confidentiality) as well as to define the purpose of the visit and aims. Dietitians will also demonstrate active listening skills throughout the consultation via verbal and non-verbal behaviours, paraphrasing, reflecting and summarising. Finally, before closing the consultation in order to establish that there was an effective communication throughout the consultation, dietitians will summarise and confirm the aims and action plans agreed.

6.6 Monitor and Review

6.6.1 Monitoring refers to the review and measurement of the participant's nutritional status and/or dietary intake in the follow up visits at planned intervals which will take place in the week 4, 8 and 12.

6.6.2 This will be done by measuring progress towards outcomes and goals using goal indicators and evaluating any barriers and facilitators to progress. New nutritional issues or a lack of progress will lead to reassessment and possibly a new NDD, strategy and/or implementation. This stage involves assessment of the following, modified accordingly to enable progress to be made:

- Participant understanding, and adherence to, strategy and implementation
- Whether the current NDD is still appropriate, or a new NDD is now a higher priority
- Whether the current outcome, dietetic goals and actions are still appropriate

- Progress towards the dietetic goals through measuring change in goal indicators
- Whether actions are or are not improving or resolving the nutrition and dietetic problem, its aetiology and/or signs and symptoms
- Whether actions are being implemented as prescribed
- Barriers and facilitators to progress

6.6.3 In our intervention, changes in the anthropometric parameters will be assessed, including body weight, body fat, body water, lean body mass, BMI, BMR and waist circumference. Relevant biochemical results will be checked in week 12. Blood pressure reading, changes in medication or dietary supplements will be reviewed. Participant will be asked if there are any problems in the bowel movements, or any relevant symptoms and signs. Dietary intake will be reassessed using Intake24 considering energy and macronutrients intakes. NICE dietary guidelines checklist will be used to monitor the dietary adherence (Appendix F). In the intervention group, an additional report of the participant's metabolic profile will be used to monitor dietary intake. Physical activity will be reassessed using International Physical Activity Questionnaire.

6.6.4 Significant changes in the anthropometric parameters and physical activity level will require reassessment of the energy and macronutrients requirements using the same method described in the initial visit. Goal outcomes, actions plan will be reviewed.

6.6.5 Dietitian will find out what is working well, and what participant are finding challenging. If the participant is struggling to achieve an action plan, dietitian will amend the action plan as appropriate.

6.6.6 Encourage participant not to worry if they don't always stick to their plan and explain to them it is normal that life will get in the way sometimes. Participant will be encouraged to celebrate achievements and discuss challenges.

6.6.7 This will be documented in Dietary care record.

6.7 Evaluation

6.7.1 Evaluation is the systematic comparison of current findings against previous status at the end of the dietetic intervention (after 12 weeks). Outcome indicators will be used to measure changes, to establish whether the proposed outcome has been met and whether this has resolved (corrected) the NDD. This will either be a 'yes' or a 'no'. If not met, the reason for this will be evaluated. Any other positive/negative outcomes will also be documented.

6.7.2 This stage will identify what went well and not so well. Further action to be taken, research gaps and learning will be identified and communicated as necessary. Comments and compliments will also be documented.

6.7.3 In our trial we will use dietary recall and metabolic report as indicators of improving dietary behaviours. In addition, we will use BMI, waist circumference, lipid profile and blood pressure as indicators of reducing risk of cardiovascular disease.

7.0 Appendices

Appendix A: Instructions of using the Bioelectrical Impedance Analysis (BIA)/ Tanita

TANITA is a body Composition scale, that is used to assess individuals body composition, and gives information regarding Basal Metabolic Rate (BMR).

Before TANITA measurements – Please ask participants to:

1. Avoid exercise prior to measurement.
2. Ask participant to not consume any alcohol or caffeine the day before.

Steps on how to use TANITA:

1. Ask the participant to empty his/her bladder before doing any measurements.
2. Ask the participant to remove his/her shoes and socks as well as any heavy objects (keys, belts etc.).
3. Ask the participant to stand on the scale with bare feet.
4. Switch on the bioelectrical impedance and enter the following:
    a. Press enter when asked about the patient's NHS number and press enter.
    b. Physical activity status (standard/athletic) and press enter.
    c. Enter the participant's gender and press enter.
    d. Enter participant's age and press enter.
    e. Ener participant's height and press enter.
5. Ask the participant to stand complete still during the measurements, with his arms not touching his inner thighs.
6. When TANITA demonstrates ask the participant to pull the grips and hold them next his body.
7. Ask the participant to step out of the TANITA.
8. Note down:
    a. Body fat in kg and %
    b. Muscle mass in kg and %
    c. Body water in kg and %
    d. Basal Metabolic Rate (BMR) in kcals
    e. Body mass index (BMI) in kg/m^2
9. Switch off TANITA and clean it using antiseptic wipes.

Appendix B: Waist circumference measurement:

1. Place the tape measure directly on the participant skin, or over no more than 1 layer of light clothing.
2. The correct place to measure the waist is halfway between your lowest rib and the top of your hipbone. This is roughly in line with the participant belly button.
3. Ask participant to breathe out normally and measure.
4. Make sure the tape is snug, without squeezing the skin.

Appendix C: Dietary record form

Patient ID: Date: Group: CONTROL/INTERVENTION Patient consented to being seen by a Lina/Delyse/Anastasia Communication method: face-to-face/online

1st VISIT DIETETIC FORM

SECTION A:
ANTROPOMETRY (dd/mm/yyy @hh:mm):
Height (cm):
Weight (kg):
Fat (kg):
Fat (%):
Water (kg):
Water (%):
Muscles (kg):
Muscles (%):
BMI (kg/m2):
BMR (Kcal):
Waist circumference (cm):
Weight history (kg):
dd/mm/yyyy:

Usual weight (kg):
Weight change (kg):
Perception on current weight:

BIOCHEMISTRY:
(dd/mm/yyyy):
CRP:
HbA1c:
Na:
K:
Liver Function Tests:
LDL-C:
HDL-C:
FBC:
Fasting glucose:

CLINICAL:
Blood pressure (mmHg):
Reading 1:
Reading 2:
Reading 3:
Mean BP reading (mmHg):

Past medical history:

Family history: CVD/diabetes/blood pressure/cholesterol/emotional issues

Allergies (food/drug etc.):

Relevant medications:

Vitamins or supplements:

Bowels: open/not open (indicate frequency/changes in stool and BM)

Signs/symptoms (i.e. frequent urination, tiredness, pain, numbness)

DIETARY:
24h Recall (Intake24):

Estimated intake / requirements based on Intake24:
Estimated energy intake:
Estimated CHO intake:
Estimated protein intake:
Estimated fat intake:
Estimated SFA intake:
Estimated free sugar intake:
Estimated fibre intake:

Difference between usual day and Intake24 (usual day/unusual day):

General Eating habits:
Food Diary:
Breakfast
MS:
Lunch:
MS:
Dinner:
Pre-sleep:

Meal frequency (3 constructive meals/2 meals/day, 1 meal/day, non-pattern):
Skipping of meals: yes/no (reason)
Take outs/delivery/eating at restaurants: times per week
Alcohol use (never/daily/occasionally/weekly):
Alcohol consumption: drinks per day/week (indicate if binge drinking)

Food preparation (self/spouse/other):
Oil used regularly:

Dietary Questions:
Usual Hunger Timing:
Usual Hunger Levels (0-10):
Triggers of Hunger (i.e. job/environment):

Metabolic Report:
Total Score on Metabolomic Report (%):

Dietary intake	Biomarkers	High/Medium/Low
Fruits and vegetables	Hippuric acid 4-Hydroxyhippuric acid	
Cruciferous vegetables	N-acetyl-S-methylcysteine sulfoxide	
Onions	N-acetyl-S-(1Z)-propenyl-cysteine sulfoxide	
Apples	Rhamnitol	
Citrus foods	Proline betaine	
Grapes	Tartaric acid	
Fibre (AOAC)	Acetate	
Free sugar	Glucose	
Alcohol	Ethanol	
Fish and oily fish	Dimethylamine Trimethylamine-N-oxide	
Red meat	O-Acetylcarnitine Carnitine	
Animal protein from meat and dairy	Phenylacetylglutamine	
Lean meat	1-Methylhistidine and 3-Methylhistidine	
Plant based protein	Trigonelline 1-Methylnicotinamide N-methyl-2-pyridine-5-carboxamide	

Comments on Metabolomic Report:

<u>ENVIROEMNTAL/BEHAVIOURAL/SOCIAL:</u>
Race/Ethnicity:
Employment: employed/unemployed/retired

Type of work: sedentary/physical
Shifts: 9am – 5pm/ 7pm – 7am/mixed shift
Occupation:
Household occupation:
Education level (high school/ diplomas/college/technical school/ university/ graduate of school):
Dietary knowledge level:
Smoking: Smoker/non-smoker
Readiness to change (0-10):
Motivation Level (0-10):
Confidence level (0-10):
Barriers to change (if any):

FUNCTIONAL:

Physical Activity:
Limitations to Physical Activity (if any):

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe

somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐

Don’t know/Not sure

PAL Calculations:

SECTION B:

NUTRITIONAL DIAGNOSIS:
Problem:
Aetiology:
Evidence (signs/symptoms):

STRATEGY:

-
-
-

DIETETIC OUTCOME:

-
-

OUTCOME INDICATORS:

-
-
-

GOALS:

- Total fat intake is 30% or less of total energy intake.
- Saturated fats are 7% or less of total energy intake.
- Dietary cholesterol is less than 300 mg/day.
- Eat at least 5 portions of fruit and vegetables per day.
- Eat at least 2 portions of fish per week, including a portion of oily fish.
- Eat at least 4 to 5 portions of unsalted nuts, seeds and legumes per week.
- Increase mono-unsaturated fat intake with olive oil, rapeseed oil or spreads based on these oils and to use them in food preparation.
- Dietary fibre intake is at least 30g/day.
- Free sugar intake is 5% or less of total energy intake.
- Choose wholegrain varieties of starchy food reduce their intake of sugar and food products containing refined sugars including fructose.
- Limit red meat intake to less than 70g/day
- Limit salt intake to 6g/day (Sodium=2400mg).
- Limit alcohol intake to 14 units per week

GOALS INDICATOR:

Dietary intake	NICE Dietary Recommendation	Did the participant meet the recommendation? (YES/NO)
Energy	Based on the participants requirement, 600 kcal will be deducted for those who need to lose weight	
Total Fat	Less than 30% of energy	
Saturated Fat	Less than 7% of energy	
Dietary cholesterol	Less than 300 mg	
Trans fat	Less than 2% of energy	
Unsaturated fat (MUFA & PUFA)	Using olive oil or rapeseed oil or spreads based on these oils, and to use them in food preparation.	
Fish	At least 2 portions of fish per week, including a portion of oily fish.	
Unsalted nuts, seeds and legumes	At least 4 to 5 portions of unsalted nuts, seeds and legumes per week	
Red meat	Less than 70g/d	
Dietary fibre	30g-45g/d	
Fruits and Vegetables	At least 5 servings /d	
Wholegrain starch	Choose wholegrain varieties of starchy food	
Free sugar	Less than 5% of energy	
Alcohol	Men: Less than 3-4 units/d Women: Less than 2-3 units/d	
Salt	Less than 2.4g of sodium /d	

INTERVENTION CATEGORY (knowledge. Building/specialised diet/behavioural change/counselling):

ACTION PLANS:

-
-

IMPELEMETATION:
Estimated energy requirements Based on TANITA:

Estimated energy requirements (BMR x PAL from IPAQ):
Estimated energy requirements (-600 kcal):
Estimated CHO intake (45-55%):
Estimated protein intake (15-20%):
Estimated fat intake (25-29%):
Estimated SFA (>7%):
Dietary Cholesterol: >300 mg/day
Dietary Fiber: 30 g/day
Fress Sugar intake (>5%)
Estimated Fluid Intake: mL/day

SECTION C:
Progress Notes Completed by Dietitian:
Discussion during the consultation (i.e. patient concerns, patients perception of current diet, patient's personal aims)

Barriers to change identified via the consultation:

FOLLOW UP DIETETIC FORM (WEEK 4, 8, 12_D1)

Date:
Patient consented to being seen by a Lina/Delyse/Anastasia

DIETARY ASSESSMENT FORM (F/U VISIT)

SECTION A:
ANTROPOMETRY (dd/mm/yyyy @hh:mm):
Height (cm):
Weight (kg):
Fat (kg):
Fat (%):
Water (kg):
Water (%):
Muscles (kg):
Muscles (%):
BMI (kg/m2):
BMR (Kcal):
Waist circumference (cm):

CLINICAL:
Blood pressure (mmHg):
Reading 1:
Reading 2:
Reading 3:

Changes to medication:

Bowels: open/not open (indicate frequency/changes in stool and BM)

Signs/symptoms (i.e. frequent urination, tiredness, pain, numbness)

DIETARY:

24h Recall (Intake24):

Estimated intake / requirements based on Intake24:
Estimated energy intake:
Estimated CHO intake:
Estimated protein intake:
Estimated fat intake:
Estimated SFA intake:
Estimated free sugar intake:
Estimated fibre intake:
Estimated free sugar intake: %/day
Estimated fibre intake: g/day

Metabolic Report:
Total Score on Metabolomic Report (%):

Dietary intake	Biomarkers	High/Medium/Low
Fruits and vegetables	Hippuric acid 4-Hydroxyhippuric acid	
Cruciferous vegetables	N-acetyl-S-methylcysteine sulfoxide	
Onions	N-acetyl-S-(1Z)-propenyl-cysteine sulfoxide	
Apples	Rhamnitol	
Citrus foods	Proline betaine	
Grapes	Tartaric acid	
Fibre (AOAC)	Acetate	
Free sugar	Glucose	
Alcohol	Ethanol	
Fish and oily fish	Dimethylamine Trimethylamine-N-oxide	

Red meat	O-Acetylcarnitine Carnitine	
Animal protein from meat and dairy	Phenylacetylglutamine	
Lean meat	1-Methylhistidine and 3-Methylhistidine	
Plant based protein	Trigonelline 1-Methylnicotinamide N-methyl-2-pyridine-5-carboxamide	

Comments on Metabolomic Report:

FUNCTIONAL:

Physical Activity (IPAQ as above)
Changes to Physical Activity:

PAL Calculations:

SECTION B:
STRATEGY:

-
-

DIETETIC OUTCOME:

-
-

OUTCOME INDICATORS:

-
-

GOALS INDICATOR:

Dietary intake	NICE Dietary Recommendation	Did the participant meet the recommendation? (YES/NO)
Energy	Based on the participants requirement, 600 kcal will be deducted for those who need to lose weight	
Total Fat	Less than 30% of energy	
Saturated Fat	Less than 7% of energy	
Dietary cholesterol	Less than 300 mg	
Trans fat	Less than 2% of energy	
Unsaturated fat (MUFA & PUFA)	Using olive oil or rapeseed oil or spreads based on these oils, and to use them in food preparation.	
Fish	At least 2 portions of fish per week, including a portion of oily fish.	
Unsalted nuts, seeds and legumes	At least 4 to 5 portions of unsalted nuts, seeds and legumes per week	
Red meat	Less than 70g/d	
Dietary fibre	30g-45g/d	
Fruits and Vegetables	At least 5 servings /d	
Wholegrain starch	Choose wholegrain varieties of starchy food	
Free sugar	Less than 5% of energy	
Alcohol	Men: Less than 3-4 units/d Women: Less than 2-3 units/d	
Salt	Less than 2.4g of sodium /d	

INTERVENTION CATEGORY (knowledge. Building/specialised diet/behavioural change/counselling):

ACTION PLANS:

IMPELEMETATION:
Estimated energy requirements Based on TANITA:
Estimated energy requirements (BMR x PAL from IPAQ):
Estimated energy requirements (-600 kcal):
Estimated CHO intake (45-55%):
Estimated protein intake (15-20%):
Estimated fat intake (25-29%):
Estimated SFA (>7%):

Dietary Cholesterol: >300 mg/day
Dietary Fiber: 30 g/day
Free Sugar intake (>5%)
Estimated Fluid Intake: mL/day

SECTION C:
Progress Notes Completed by Dietitian:
Discussion during the consultation (i.e. patient concerns, patients perception of current diet, patient's personal aims)

Barriers to change identified via the consultation:

EVALUATION:

Appendix D: Blood pressure measurements

Before measuring blood pressure

- Don’t measure participant blood pressure within half an hour of eating, smoking, drinking caffeinated drinks such as coffee, or exercising. These can all raise the blood pressure temporarily.
- Ask participants if they need to use the toilet before measuring the blood pressure.
- Ask participant to wear loose-fitting clothes, or a short-sleeved t-shirt or something with sleeves you can push up easily, nothing tight. This is so that you can fit the cuff around participant arm.
- Let the participant take a rest for five minutes before taking the reading.
- Make sure that the participant sits down somewhere quiet, ideally at a desk or table. Have participant back supported with arm resting on a firm surface and feet flat on the floor. Participant should stay in this position while taking blood pressure.
- Make sure participant arm is supported and at the same level as heart. Position the participant so that the arm is resting on a surface and is at the same height as heart. Make sure the participant arm and hand relaxed, not tensed.
- Make sure the participant is relaxed and comfortable. If participant is anxious or uncomfortable, wait till they relaxed.

While measuring blood pressure

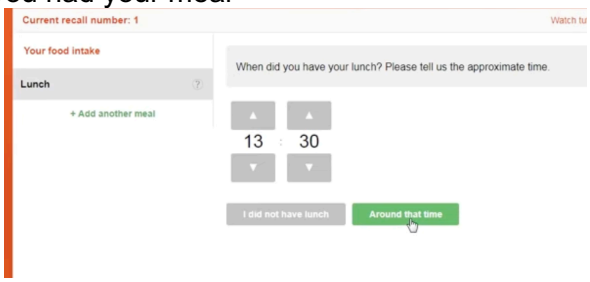
- Follow the instructions that came with monitor. Make sure you place the cuff around participant arm as described above.
- Place the arm cuff just above participant elbow. The cuff should be about 2cm above the elbow to make sure it can detect the artery in arm, just under the skin.
- Make sure the participant is still quiet while you take the reading**.** Moving, chewing, talking and laughing can affect the reading. Make sure participants don't cross their legs, as this will raise reading too.
- Take three readings, each about one to two minutes apart. Once you have three readings, you can work out the average.
- Keep a record of participant measurements. Record all your readings in the dietary record form.

Appendix E: Instructions for participant to fill dietary recall using intake24

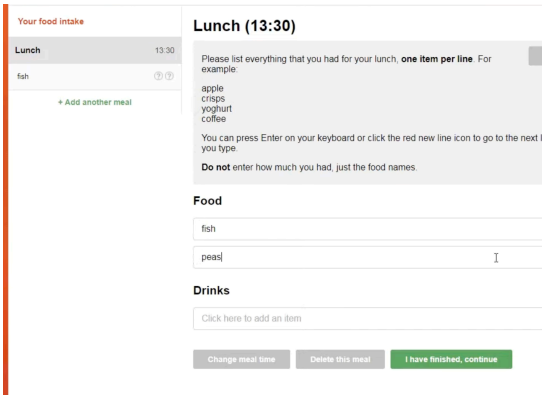
We would like you to record, as accurately as possible, what you eat and drink for 24 hours, on the 8th of June from breakfast till 9th of June before breakfast.
Please record ALL food and drink consumed using Intake24.
You should include all meals and snacks, plus sweets, drinks etc. When recording the foods eaten during meals, please include any sauces, dressing or extras eg: gravy, salad dressing, pickles, as well as the main dish.

Below is an example of how the Intake24 food record will look like:

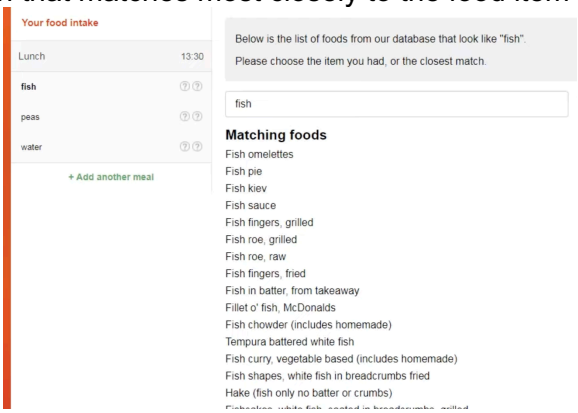
1. Enter the time you had your meal



2. Record what you have consumed for the meal

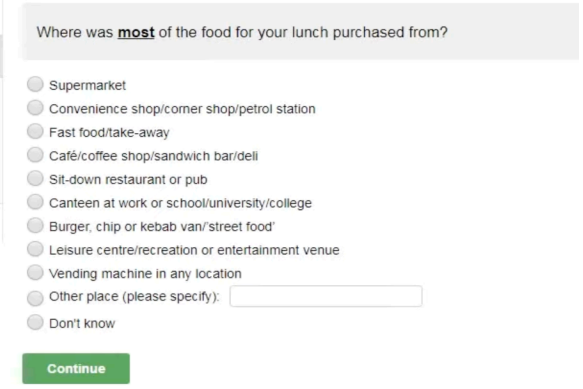


3. Select the option that matches most closely to the food item you have consumed.

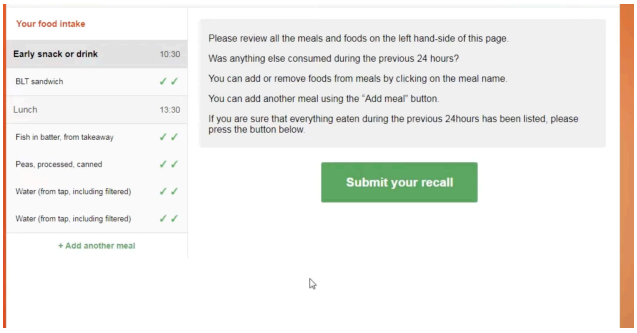


4. Based on these pictorial guide, choose the portion of food that most closely resemble the amount you have consumed.

5. If it is a takeaway food item, select where you have purchased it.



6. At the end of the dietary record, you will be asked to confirm if these are the food items that you have consumed over the past 24 hours. If everything is right, click on submit your recall.



You can refer to the video instructions (https://youtu.be/70Wm_kyxpvg) at any point in time if you are unclear.

Appendix F: NICE Dietary guidelines checklist

	Dietary intake	NICE Dietary Recommendation	Visit 1	Visit 2	Visit 3	Visit 4
1	**Energy**	Based on the participants requirement, 600 kcal will be deducted for those who need to lose weight				
2	**Total Fat**	Less than 30% of energy				
3	**Saturated Fat**	Less than 7% of energy				
4	**Dietary cholesterol**	Less than 300 mg				
5	**Trans fat**	Less than 2% of energy				
6	**Unsaturated fat (MUFA & PUFA)**	Using olive oil or rapeseed oil or spreads based on these oils, and to use them in food preparation.				
7	**Fish**	At least 2 portions of fish per week, including a portion of oily fish.				
8	**Unsalted nuts, seeds and legumes**	At least 4 to 5 portions of unsalted nuts, seeds and legumes per week				
9	**Red meat**	Less than 70g/d				
10	**Dietary fibre**	30g-45g/d				
11	**Fruits and Vegetables**	At least 5 servings /d				
12	**Wholegrain starch**	Choose wholegrain varieties of starchy food				
13	**Free sugar**	Less than 5% of energy				
14	**Alcohol**	Men: Less than 3-4 units/d Women: Less than 2-3 units/d				
15	**Salt**	Less than 2.4g of sodium /d				