# Assessing inflammatory markers and developing dietary counseling guidance to enhance the provision of metabolically personalised dietary advice in patients at CVD-risk and in diabetic patients with NAFLD

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**This research project is submitted in partial fulfilment of the requirements for the degree of MRes in Clinical Research (Diabetes and Obesity)**

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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* | 20 | 80 |
| Determination of Methodology* | 0 | 100 |
| Collection of specimens/material/patient recruitment | 80 | 20 |
| Conducting experiments/ collation of questionnaires etc | 80 | 20 |
| Data analysis | 100 | |
| Write up | 100 | |
| Production of submission | 100 | |
| Problems encountered if any | Anastasia has faced several difficulties: i) ) it has been very challenging to receive Anastasia's DBS which was essential for her to be able to work with the patients. ii) The essential consumables for the clinical trial had a three months delayed; iii) there was also an issue with ethical approval for the first project that Anastasia was assigned and therefore she was moved to a different project, which due to consumables delay there was no data collected and therefore; she was given a different data set to analyse. | |

Any other comments: Anastasia is a very hard worker and bright students with excellent critical thinking. Moroever, her fenomenal team working ability has become very valuable member of my team. She has acquired experience in conducting clinical trial, PPI activities and has attended to the Imperial Great Exhibition. Finally, she has been involved in metabolomic analysis of urine samples by 1H-NMR.

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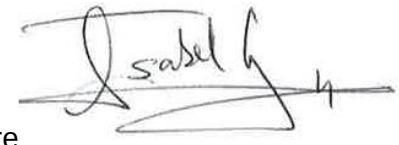
Supervisor Signature  .

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## ABBREVATION LIST

| | |
|---|---|
| CVD | Cardiovascular Diseases |
| BMI | Body Mass Index |
| CRP | C-reactive protein |
| DALYs | Disability-Adjusted Life Year |
| DASH | Dietary Approachers to stop hypertention |
| EASD | European Association for the Study of Diabetes |
| EASL | European Association for the Study of the Liver |
| EASO | European Association for the Study of Obesity |
| ESPEN | European Society for Clinical Nutrition and Metabolism |
| HCC | Hepatocellular carcinoma |
| IL | interuleukin |
| LCD | Low carbohydrate diet |
| LFD | Low fat diet |
| MedD | Mediterranean Diet |
| MetP | Metabolic phenotyping |
| MetPD | Metabolically personalised dietary advice |
| NAFL | Non-alcoholic fatty liver |
| NAFLD | Non-alcoholic fatty Liver disease |
| NCDs | Non-communicablle diseases |
| NHS | National Healthcare Services |
| NICE | National Institute of Health and Care excelence |
| PPI | Patient adn Public Involvement |
| UK | United Kingdom |
| WHO | World Health Organisation |

**AKNOWLEDGEMENTS:**

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**ABSTRACT:**

**Background:** Non-communicable diseases (NCDs) present a major public health concern, with cardiovascular disease and non-alcoholic fatty-liver disease (NAFLD) been two of the key NCDs. NCDs could be prevented and/or managed via dietary interventions. There is a shift from one-size-fits-all nutritional care towards personalised nutrition; due to current limitations of the nutritional assessment. Inflammation has been associated with diet; with literature demonstrating a negative correlation. The project hypothesises that the development of a dietary counselling guidance will enhance the provision of metabolically personalised dietary advice on people at risk of CVD (study 3) but also in diabetic individuals with NAFLD.

**Methods: Study 1:** Patient and Public Involvement (PPI) was conducted which consisted of two focus groups, one including dietitians and one with CVD-risk and NAFLD participants. Each of the two focus groups completed three activities, with the third activity been a shared activity between dietitians and participants. The activities aimed to enhance the dietary counseling guidance. **Study 2:** Healthy participants' data was obtained from a previous study. The participants were randomly assigned to different diets, for 72-hours, following World Health Organisation Dietary Guidelines with diet 1 been the most cohesive to the guidelines and diet 4 the least, in highly controlled environment. Participants at CVD-risk were also invited to follow 2 different dietary interventions with different adherence to the NICE guidelines in highly controlled setting for a 72-hour period. Wilcox sum-rank test was performed to assess the difference between the diet 1 and 4, and p-value<0.05 was set as a cut-off value. **Study 3:** Study 3 aimed to address the use of metabolic phenotyping (MetP) in dietary advice.

**Results: Study 1:** Dietary counselling guidance developed by the research team was amended according to the PPI feedback and tailored to be applied in clinical trial. **Study 2:** A 72-hour of fully adherence to WHO guidelines, significantly decreased urinary IL-6 (P=0.036) and serum TNF-alpha (P<0.001), and statistically increased Glyc/SPC ration, however, the effect was lost after multivariable adjustment (unadjusted p=0.02, adjusted p=0.21 Q=0.01). Full adherence to NICE guidelines in highly-controlled environment statistically

significantly increased Glyc/SPC ratio (unadjusted p=0.000; adjusted p=0.002, Q=0.001).

**Discussion:** The trial demonstrates the need of MetP in clinical settings through PPI. In addition, adherence to dietary guidelines for 72-hours improved inflammation markers specifically the Glyc/SPC was statistically significantly increased. The evidence appear to be more strongly related with individuals at CVD-risk. The findings are in agreement with previously conducted trials regarding the association of diet, inflammation and lipid profile.

**Conclusion:** The trial suggests the benefit of MetP in clinical settings. The enhancement of the dietary counselling guidance with inflammation markers, could be beneficial in the future trials, due to the proven statistical association between inflammation and NICE most cohesive diet.

**Keywords: metabolomics; metabolic phenotyping; CVD-risk, NAFLD, diabetes, personalised nutrition; adherence**

# 1. INTRODUCTION

## 1.1 Non-communicable diseases (NCDs)

NCDs are classified as chronic diseases, with the four major ones been cardiovascular diseases (CVD), cancer, chronic respiratory diseases and diabetes (WHO,2021). In Europe, it has been estimated that approximately 90% of the deaths and 80% of disability-adjusted life year (DALYs) are accounted to NCDs (Charalampous et al., 2022), therefore, accounting for the leading causes of mortality. Taking into consideration that NCDs are a major public health problem, within the current agenda of Heads and State of Government; it has been agreed to achieve a third of reduction in NCDs deaths in individuals younger than 75 years (WHO, 2021).

NCDs are linked to four major modifiable risk factors, which include use of tobacco, lack of physical activity, excessive intake of alcohol as well as poor nutrition (Palmer et al., 2018; Toebes er al., 2020). Elevated blood pressure, obesity, hyperglycemia and hyperlipidemia also represent risk factors for the development of NCDs (Alamnia et al., 2021), however, they could be classified as semi-modifiable as they are also influenced by diet (NICE, 2016). It has been proposed by the Global Burden of Disease, that in 2017 around 11 million deaths and 255 million DALYs globally were due to diet-related risk factors (Afshin et al., 2019). Published guidelines therefore, suggest the need of altering diet to optimize the individuals' nutritional status for both prevention and management of NCDs (Arends et al., 2017; FAO, 2016; NICE, 2015; NICE, 2016; NICE, 2017; Lichtenstein et al., 2021; Plauth et al., 2019).

## 1.2 Non-alcoholic Fatty Liver Disease (NAFLD) and diet interventions

NAFLD is one of the most frequently recognised chronic liver diseases; with approximately 20 in 10,000 incidents per year (Byrne and Targher, 2015). It is classified as the second leading cause of hepatic transplantation and hepatocellular carcinoma (HCC) (Perdomo, Frühbeck and Escalada, 2019) and has been grown to be one of the most common liver-related causes of mortality and morbidity (Byrne and Targher, 2015; Liu et al., 2019). The European Association for the Study of the Liver (EASL) accompanied with the European

Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO) define NAFLD as accumulation of excess lipids presented in more than 5% of hepatic cells (EASL - EASD - EASO, 2016). A number of liver diseases fall under the umbrella of NAFLD, extending to different severity forms of liver disease ranging from hepatic steatosis, also known as non-alcoholic fatty liver (NAFL) to cirrhosis (Rikhi, Singh and Modaresi Esfeh, 2020). The progression of different clinical stages is subjected to the individual and any clinical conditions present (Friedman et al., 2018). Within the United Kingdom (UK), it is estimated that rate of premature deaths as a result of NAFLD has significantly been raised between 2019 to 2020 (GOV.UK, 2021).

Weight reduction is positively correlated with improvement in NAFLD steatosis, with different dietary intervention appearing to positively influence the disease (Aboubakr et al., 2021). The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommend weight loss and lifestyle interventions for the management of NAFLD in overweight or obese (Plauth et al., 2019). The National Institute of Healthcare Excellence (NICE) guidelines suggest obesity management regardless of the individuals' body mass index (BMI) (NICE, 2014; NICE, 2015; NICE, 2016). The conclusions for the guidelines were drawn from a number of studies suggesting that weight reduction through lifestyle interventions, including dietary modifications and/or exercise, in overweight and obese individuals promotes better measurements of hepatic steatosis, inflammation and fibrosis indicators. Weight reduction is also related to disease progression, decrease in fat accumulation around the liver, and improved glucose control (Abdel Moneim et al., 2018; Aboubakr et al., 2021; Hannah and Harrison, 2016; Thoma, Day and Trenell, 2012). It is suggested that an achieved weight reduction of 5% or more of total body weight, results in a decline in NAFLD progression (Aboubakr et al., 2021); with a systematic review of 23 studies suggesting that the strongest relationship between weight loss and decline in disease markers when >7% weight loss is achieved (Thoma, Day and Trenell, 2012). Different dietary interventions, including hypocaloric diet, low-carbohydrate diet (LCD), low-fat diet (LFD), Mediterranean diet (MedD), have been promising in improving disease markers and progression (Abdel Moneim et al., 2018; Aboubakr et al., 2021; Worm, 2020). Clinical trials comparing MedD to

LFD or LFD to LCD; suggest that no diet in superior to the other with respect to weight loss, liver fat, lipid and glucose biomarkers (Properzi et al., 2018; Rodrŕguez-Hernández et a., 2011), therefore, concluding that weight loss regardless of the dietary intervention would be beneficial.

1.3 Cardiovascular Diseases (CVD), risk of CVD and dietary interventions

CVD represent the leading cause of mortality worldwide and has been associated with approximately a third of worldwide fatalities (WHO,2021). Disorders of the heart and circulatory system, which include coronary heart disease, stroke and peripheral vascular disease, caused by atherosclerosis fall under the terminology of CVD (Francula-Zaninovic and Nola, 2018; WHO, 2021). A decline has been observed between 1990 to 2013 in CVD incidence in the UK, however, the numbers of individuals being hospitalised due to CVD remains high; being has increased between 2010/11 to 2013/14 (Bhatnagar et al., 2016). Males of older age, with family history of CVD and from specific ethnic groups, such as South Asians (Chaturvedi, 2003), are at higher risk of CVD incidences (Bachmann et al., 2012; North and Sinclair, 2012; WHO, 2021; Wild et al., 2007). Evidence also demonstrates that in a number of lifestyle behaviors, including unhealthy dietary patterns, tobacco usage, low physical activity levels and insomnia, partially influence the development of hypertension, hyperglycemia and hyperlipidemia; and ultimately an individual's CVD risk (Khera et al., 2016; Livingstone et al., 2021; Masana et al., 2017).

It has been estimated that lifestyle interventions could eliminate 8 in 10 major CVD-related incidents (Celis-Morales et al., 2017). The NICE, American Heart Association and the European Heart Journal Guidelines suggest weight decline in obese or overweight individuals is crucial for CVD risk population (Lichtenstein et al., 2021; NICE, 2016; Visseran et al., 2021), to reduce hypertension, high circulating lipids and glucose levels, hence reducing CVD-risk. NICE guidelines for individuals at CVD risk are presented on **Table 1**.

MedD and Dietary Approaches to stop Hypertension (DASH) diet, are also recommended for CVD risk reduction in terms of weight loss or weight maintenance in to reduce cardiometabolic risk factors (Chiavaroli et al., 2019;

Estruch et al., 2013). Both of these diets are rich in vegetables, fruits, legumes and whole gains, while incorporating moderate amounts of dairy and restricting the amount of red and processed meat. The difference between the two dietary interventions is that MedD is high in olive oil; while DASH diet suggests 3 tablespoons of oil/fat per day (Patel et al., 2021). The Prevención con Dieta Mediterránea (PREDIMED) trial was a large multi-centred trial, which assessed two MeD interventions (one arm added an extra olive oil while the other supplemented extra nuts) compared low fat diet (Estruch et al., 2013). It was observed that MedD had a significant reduction in blood pressure and reduced incidence of major CVD. DASH diet also reducing cardiometabolic incidence (Chiavaroli et al., 2019), with a recently published meta-analysis of 67 trials demonstrating that among different diets including MedD, DASH diet had the most influence on reducing both systolic and diastolic blood pressure, however, the evidence are low to moderate-quality (Schwingshackl et al., 2018).

***Table 1:Dietary Guidelines for the management of CVD risk population (NICE, 2016)***

| |
|---|
| Total fat intake contributing less than 30% of total energy per day |
| Saturated fat been less than 7% of energy per day |
| Less than 300 mg of cholesterol per day |
| Consumption of less than 2% of total energy been trans fatty acids per day |
| Sugar intake been less than 5% of total energy per day |
| Encouragement of the use of olive oil, rapeseed oil or spreads based on these oils in food preparation |
| Consumption of at least 2 portions of fish per week, including a portion of oily fish. |
| Intake of at least 4 to 5 portions of unsalted nuts, seeds and legumes per week |
| Less than 70g/day of red meat intake |
| Increase fiber intake to 30g-45g per day |
| Consumption of at least 5 servings (400 g) per day of fruits and vegetables |
| Encouragement of the wholegrain varieties of starchy food choices in food intake |
| Alcohol:<br>Men: Less than 3-4 units/d<br>Women: Less than 2-3 units/d |

| Less than 2.4g of sodium intake per day |
|---|

## 1.4 Personalised Nutrition

Traditionally, individuals have been offered dietary interventions based on general guidelines. The guidelines published provide the cornerstone of dietary advice, however, they are based on the general population (Pérez-Beltrán et al., 2022). Using nutritional guidelines accompanied by different biochemical, clinical and anthropometric markers, evidenced-based practice for prevention and management of different conditions; dietitians offer recommendations. The traditional one-size-fits-all approach has been long argued and the necessity for personalised nutritional advice has risen. Currently, there is a lack of a universal definition for stratified nutrition, with inconsistency in different studies. Ordovas and his collogues, defined personalised nutrition as tailored nutritional advice given based on individuals' characteristics, such as genetics and metabolomics (Ordovas et al., 2018). Researchers have also argued that personalised nutrition definition should be based on different levels of a personalization-based model. Within the level-based personalization, level 1 personalised advice is based on the utilisation of dietary information given and general guidelines for age and gender, level 2 incorporates both dietary information and individual's phenotype, including anthropometric, clinical and biochemical biomarkers, while the third level involves dietary advice based on eating habits, phenotype and genetics (Ferguson et al., 2016; O'Donovan et al., 2015; Palmnäs et al., 2020). Stratified nutritional advice is pivotal as every individual is unique and some dietary modification suggested by published guidelines might not be applicable for optimal results in specific individuals, due to inter-individual variability (Gibney, 2019).

Concrete evidence suggests that tailoring dietary advice to the individual, according to specific participants' information is beneficial compared to standard nutritional care (Kaliora et al., 2017; Zeevi et al., 2021). The Food4Me trials, multi-centered randomised controlled trials (RCT), provide strong evidence that individualised advice had greater benefit than traditional advice on motivating participants within the study, to implement and sustain healthy dietary alterations to their habitual intake (Celis-Morales et al., 2017; O'Donovan et al., 2017). Within the Food4Me trial, individuals were randomised to four diets, with the four

diets being standard advise, personalised nutritional advice-based baseline diet, baseline diet and anthropometric and biochemical data, and baseline dietary advice based on anthropometry, biochemistry and genotype (Celis-Morales et al., 2017). The study suggests that participants in the personsalised nutrition group had a diet closer towards the World Health Organisation (WHO) recommended, by reporting significantly less meat, salt, saturated fat and energy consumption. The study, however, did not suggest any advanced effect when dietary advice was enhanced by phenotypic or genotypic information. A secondary analysis of the Food4Me trial, utilising the same information demonstrates that personalised nutrition was more effective in reducing energy-dense and poor in nutrients food and beverages, compared to standard dietary advice with respect to energy, salt, total fat and saturated fatty-acid contribution (Livingstone et al., 2021). Given that the trial suggests that stratifying nutritional advice does provide a greater benefit to the individual, compared to one-size-fits all approach, it could be suggested that the use of a different tool other than genetics might promote higher dietary adherence.

### 1.5 The problem of dietary assessment for personalised nutrition

Both in clinical and research setting, dietitians and/or researchers assess an individual's intake using food frequency questionnaires (FFQ), 24-hour recall and food diaries (Garcia-Perez et al., 2020); which are subjective to bias due to the numerous limitations. Evidence suggests that under-reporting is significant in nutrition studies which use the current methods for nutritional assessment. Under-reporting ranges approximately between 30% to 90% (Rennie, Coward and Jebb, 2007), and is even more predominant in obese individuals (Mendez, Wynter, Wilks and Forrester, 2004). Recall inaccuracies and difficulty in objectively evaluating the portion sizes are other issues of recall-related nutritional assessments, which may result in both under- or over-estimation of food intake (Brennan et al., 2021; LeVatte et al., 2021). A key drawback exclusively for FFQ, is that food grouping challenges the recording of the food diversity that an individual's consumes, even when then FFQ is large and extensive (Manach, Brennan and Dragsted, 2015). Research has also established that food recording assessments might alter individuals' habitual consumption, therefore, changes in eating habits might be present (Primrose et

al., 2011). It could, therefore, be concluded that the current methods used to assess dietary intake and compliance to dietary interventions, are subjective to inaccuracies and bias, which make provision of personalised dietary advice challenging.

1.6 The use of metabolic phenotyping (MetP) to accurately assess diet

MetP is defined as the science of identification of low-molecular weight substances (metabolites) that could either been intermediates, products or substrates of chemical reactions by enzymes in an organism (Andraos et al., 2022; Trimigno et al., 2020). Metabolites are products of genetic and exogenous factor interaction including dietary intake (Garcia-Perez et al., 2020; Guasch-Ferré, Bhupathiraju and Hu, 2018), and may act as a tool for both delivery of dietary intervention and assessment of intake. It is proposed that nutritional intake could influence the metabolome via host biological metabolome interactions, as well through the food obtained metabolites (Guasch-Ferré, Bhupathiraju and Hu, 2018). Metabolites could represent a variable group of substances ranging from specific amino acids to polyphenols (Zhang et al., 2012), ultimately giving details around the nutritional intake of an individual. There are two gold standard analytical techniques for determining the MetP of an individuals, Proton Nuclear Magnetic Resonance ($^{1}$H NMR) spectroscopy and mass spectrometry (Guasch-Ferré, Bhupathiraju and Hu, 2018). By using those methods, urine and blood samples are analysed to examine the metabolites present in the sample, hence, assess the dietary intake of an individual.

Hundreds of metabolites are present in a urinary metabolic phenotype and some of them can be utilised as an objective biomarker of food intake to enhance accurate dietary intake. Nutritional biomarkers are defined as measurable biological metabolites, which could act as an objective measure of assessment of nutritional status (Potischman and Freudenheim, 2003), for a specific period of time after food intake. MetP has recently emerged as an exceptional tool for dietary assessment (Garcia-Perez et al., 2017; Garcia-Perez et al., 2020; Hedrick et al., 2012) that could be used to identify dietary biomarkers and ultimately assess dietary patterns (Hedrick et al., 2012 ; Heinzmann et al., 2010; Lloyd et al., 2011; Rago et al., 2013). Dietary biomarkers identified included

trimethylamine-N-0xide, S-methyl-L-cysteine-sulfoxide (SMCS) and alkyl resorcinols, which reflect the intake of oily fish (Lloyd et al., 2011), cruciferous vegetables (Edmands et al., 2011) and wheat (Marklund et al., 2010), respectively, are identified through MetP.

Studies have also showed that MetP could also act as a useful indicator of dietary pattern. Garcia-Perez *et al.* assessed the variability of metabolomic profile and the intra-person variability in 19 individuals following four pre-standardised diets in four visit of 72-hours in a highly controlled environment (Garcia-Perez et al., 2017). Using $^{1}$H NMR analysis, the study analysed urinary metabolic phenotype and concluded that they could accurately assess adherence to dietary guidelines. Within the same study, validation was also performed using data from INTERMAP UK cohort and a healthy-eating Danish cohort. Another study published in 2019, aimed to investigate the effect of MedD adherence, based on MedD score (MDS), on plasma metabolites on almost 11,000 healthy individuals of the general population in Britain (Tong et al., 2019). The results of the study illustrate that 66 plasma metabolites are associated with MDS, hence, those metabolites could be essentially used to evaluate the adherence to MedD.

### 1.7 Inflammation and NCDs

Inflammation is defined as a response of an organism triggered by stimuli or conditions; and could be generally presumed as a beneficial response of the host organism against an infection (Medzhitov, 2008). Multiple factors could stimulate an inflammatory response, including an immune system disorder, cancer, exposure to chemicals, smoking etc. (Roe, 2020), and a microbial or viral infection (Medzhitov, 2008). Due to inflammation, numerous cells, pro-inflammatory cytokines and chemokines are secreted, with C-reactive protein (CRP) being the most predominately used biomarkers of inflammation (Galland, 2010). CRP is a hepatic-secreted acute phase inflammatory protein, which increases as a result of injury, infection and any stimuli, especially interleukin- 6 (IL-6) (Zakynthinos and Pappa, 2009). Concentrations could increase in the blood by 100- to 200-fold or even more in systemic inflammation (Yeh, 2004). Other biomarkers of inflammation include cytokines, such as interleukin-1 (IL-1), IL-6, interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) (Hart et al.,

2021; Zakynthinos and Pappa, 2009). Cytokines are described as small molecules which are associated with the response of an organisms to a disease or an infection, and could either be pro-inflammatory, meaning promoting inflammation, and anti-inflammatory, reducing inflammation, acting as a homeostatic balance to inflammation promotion and reduction (Dinarello, 2000).

Glycoprotein signals (Glyc), including GlycA (δ 2.03) and GlycB (δ 2.07), are acutely synthesised by the hepatic cells as a result of the secretion of inflammatory cytokines, including IL-1, IL-6 and TNF-α (Kettunen et al., 2018; Lodge et al., 2021; Moreno-Vedia et al., 2022). GlycA, a low grade systemic inflammatory marker (Mokkala et al., 2020), is related with the N- acetyl groups of the N-acetylglucosamine and N-acetylgalactosamine. Thus, $^{1}$H NMR detection of these N-acetyl groups correlates with assessment of inflammation (Moreno-Vedia et al., 2022), and is associated with inflammatory state diseases including obesity, diabetes and CVD (Lodge et al., 2021). The $^{1}$H NMR GlycB signal is due to glycoproteins containing the N-acetylneuraminidino groups (Lodge et al., 2021). Both GlycA and GlycB are strongly associated with circulating CRP levels (Lorenzo et al., 2017; Malo et al., 2020) and adiposity, however, GlycB has a weaker association to those measures and insulin resistance biomarkers compared to GlycA (Lorenzo et al., 2017). Therefore, glycoprotein $^{1}$H NMR signals have been used as biomarkers of inflammation, with GlycA signals shown to increase by 2 to 5-fold in acute inflammation (Ritchie et al., 2015). Another signal detected by $^{1}$H NMR, the supramolecular phospholipid composite (SPC), a component of HLD-cholesterol, has also been associated with inflammation.

Inflammation could be categorised to acute- and chronic-level; with acute-level inflammation being predominately associated with an infection (Medzhitov, 2008). Chronic inflammatory response, which is associated with NCDs (Galland, 2010; Medzhitov, 2008), is due to continual exposure to stimuli of inflammation, or inappropriate immune reaction to self-molecules (Germolec et al., 2018). **Figure 1** illustrates the association between inflammation and NCDs.

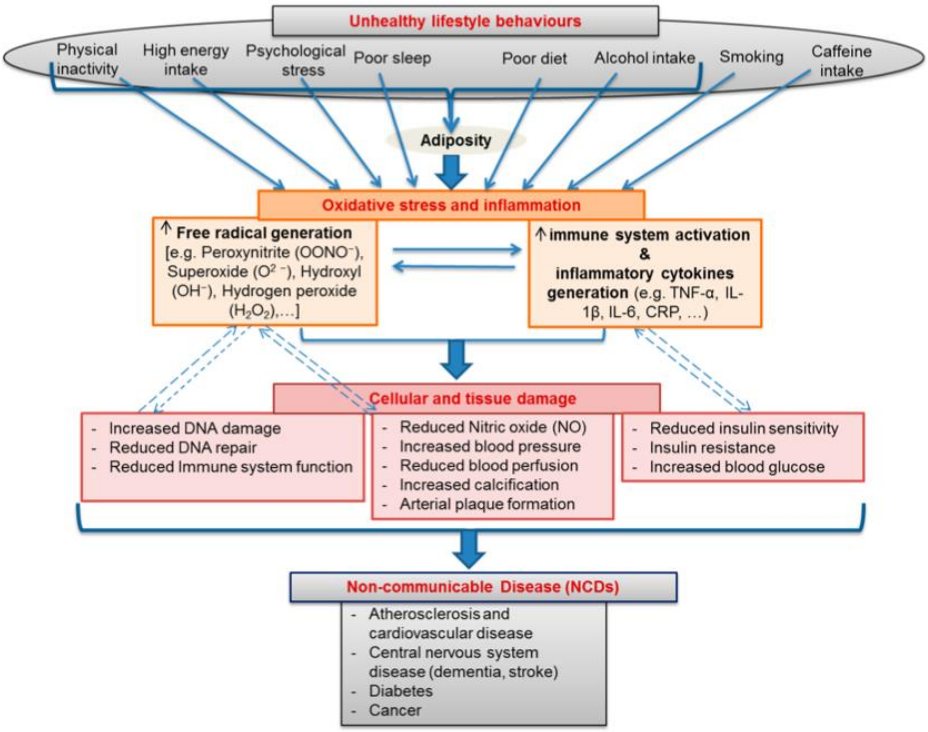



*Figure 1:* ***Representation of the association between lifestyle factors (including intake of unhealthy diet, use of harmful substances etc.) and the response of the body through raise of inflammation. Consequently, the rise of inflammation may result in the incidence or the progression of NCDs. (Diagram by: Seyedsadjadi, N. and Grant, 2020).***

## 1.8 The link between inflammation and dietary intake

The link between diet and inflammation is currently widely established (Salas-Salvadó et al., 2008). Observational and interventional trials suggest that inflammation is not only associated with adherence to dietary patters, but also with specific nutrients and/or food-group(s) (Phillis et al., 2019). Increased intake of fruits and vegetables (Calder et al., 2011), mono- and poly-unsaturated fatty-acids, including ω-3 fatty-acids (He et al., 2009; Kalogeropoulos et al., 2010) and high intake of fiber with reduced intake of high glycemic index foods (Du et al., 2008; North, Venter and Jerling, 2009); decline in consumption saturated and tras-fatty acids (Galland, 2010) have been associated with decrease in pro-inflammatory cytokines and CRP. The western dietary pattern (i.e. processed carbohydrates, high saturated fats etc.) has been correlated with increase of inflammation (Christ, Lauterbach and Latz, 2019). Contradicting, adherence to MedD has been demonstrated to reduce inflammatory markers (Galland, 2010). A Spanish trial did in fact demonstrate that adherence to MedD was inversely

correlated with IL-6. The trial did in fact suggested that CRP and IL-6 concentration were the lowest to individuals with the highest olive oil and nut intake (Salas-Salvadó et al., 2008).

1.9 Scope of the project

The project consists of three distinct studies (the summary of the project plan is demonstrated in **Figure 2**). The intended outcomes of the first two studies, will enhance the provision of metabolically personalised dietary advice (MetPD) on people at risk of CVD (study 3) an also in diabetic individuals with NAFLD (this research will be conducted as part of my future PhD).

***Study 1:***

*Hypothesis:* Establishing dietary counseling guidelines will allow the effective provision of MetPD in individuals at CVD-risk and in diabetic patients with NAFLD.

*Aim:* To develop a dietary counselling guidance to metabolically personalise dietary advice that will be translated in clinical setting for people at CVD-risk and NAFLD

*Objectives:*

- To utilise MetP in combination with traditional 24-hour recall to accurately assess dietary intake in individuals within Patient and public involvement (PPI) groups.
- To conduct PPI focus groups with dietitians working in practice and patients at CVD risk and NAFLD which will feedback on the dietary counselling guidance and urinary metabolic phenotyping tool for personalised dietary advice.

*Outcome:* Dietary counselling guidance developed by the research team was amended according to feedback and been tailored to be applied in clinical trial (**Supplementary Figure 7**)

***Study 2:***

*Hypothesis:* The measurement of glycoprotein, lipid $^{1}$H NMR signals and serum lipid and inflammatory markers can be used to monitor the impact of personalised dietary advice on inflammation in healthy volunteers and participants at CVD-risk.

*Aim:* To explore the impact of 100% adherence to dietary guidelines (WHO and NICE) on specific inflammatory and lipid markers in a healthy and CVD-risk cohort.

*Objectives:*

- To compare differences in inflammatory and lipid markers in healthy participants after following 4 diets with different levels of adherence to the WHO healthy eating guidelines.
- To compare differences in inflammatory and lipid markers in CVD-risk participants after following the most and the least cohesive dietary interventions to NICE CVD-management guidelines.

*Outcome:* The addition of quantitative inflammatory markers in the urinary metabolically personalised dietary report, which would be used in the provision of MetPD.

***Study 3:***

*Hypothesis:* MetPD will enhance adherence to personalised dietary advice and decrease misreporting to intake in free-living individuals at risk of CVD.

Aim: To apply the dietary counselling guidelines developed and inflammatory markers on the provision of MetPD for patients at CVD-risk. (methodology presents on **Supplementary Figure 1**).

*****NOTE:** Due to ethical delays, I was moved from personalised nutritional advice provision to NAFLD population to CVD-risk cohort. Followed by delays in the DBS clearance and consumables' arrival, it was challenging to recruit participants for the completion of study 3, regarding the personalisation of dietary advice in

CVD-risk cohort through MetP. Due to the above stated reasons, data collected through PPI, and data pre-collected by two different previously conducted trials, was given to perform statistical analysis and improve dietary counselling guidance and enhance the urinary metabolically personalised report for study 3. I will pursue a PhD program on metabolically personalised dietary advice on diabetic patients with NAFLD, therefore, the dietary counselling guidance and the amendments to the urinary metabolic personalised dietary report.

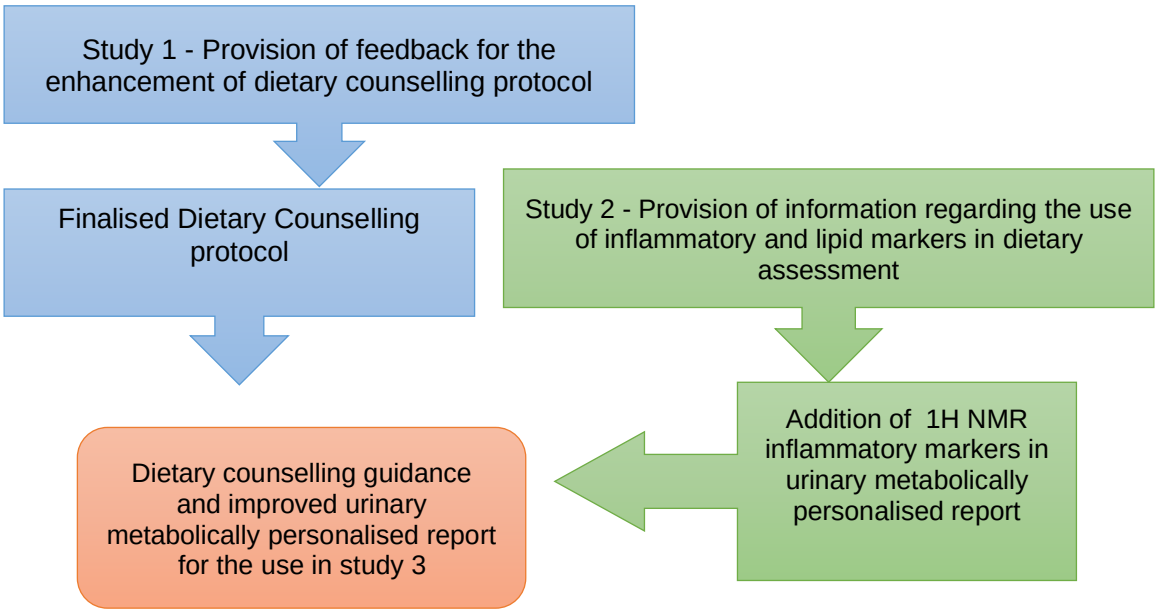



***Figure 2: Project Plan. The plan of the project was to enhance the dietary counselling report utlising PPI sessions (study 1). Though study 2; the urinary metabolically personalised report was enhanced by the addition of inflammatory markers for the assessment of inflammation. Both the dietary counselling guidance and the urinary metabolically personalised report will be used in study 3 to enhance MetP personalised dietary intervention in free-living participants at risk of CVD.***

## 2.0 Study 1

### 2.1 Materials and Methods:

#### 2.1.1 Ethical Approval

The study was approved by Research Ethics Committee (REC) and Health Regulatory Authority (HRA) in accordance with the Declaration of Helsinki (13/LO/0078). All the participants were asked consent to proceed with the PPI. Each participant was reimbursed for their time with a with £25 per focus group session.

#### 2.1.2 PPI Participation

The PPI focused on two groups, one which involved dietitians, and one which consisted of individuals with NAFLD patients at CVD-risk. Recruitment methods were chosen attempting to minimize any potential bias. Both free-lance and NHS working dietitians were invited to participate, of whom five registered dietitians were selected to attend the PPI focus group. The patients were screened to assess their eligibility to participate in the PPI using the selection criteria for the studies. Forty-nine members of the public expressed interest on participating in the CVD-risk PPI session via advertisement, of whom 6 participants were selected ensuring a representative sample. Five NAFLD public responders, expressed interest through the Imperial College London NHS clinics, of whom 3 were selected. All data gathered from the PPI was collected either through MediMeter, online survey and by the investigators (AC, DL, LA, and IG-P). The diagram for the PPI is demonstrated on **Figure 3**.

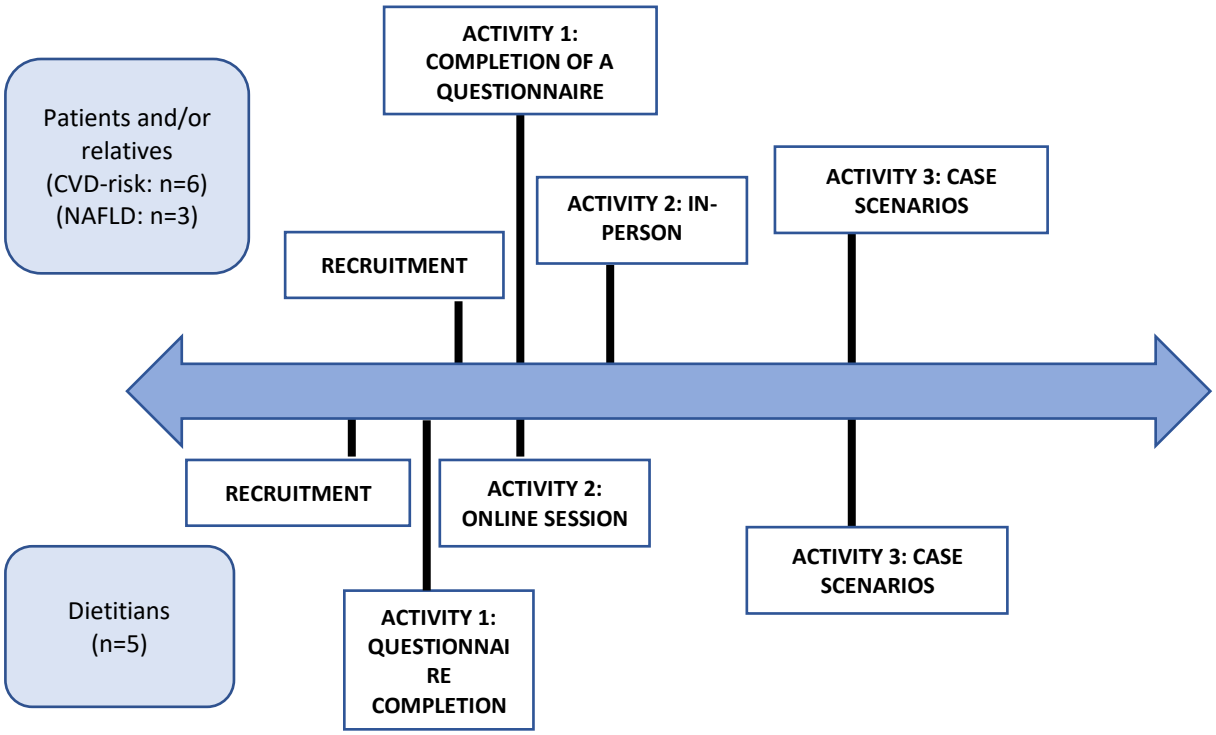



***Figure 3: Figure demonstrates the PPI plan. The activity 2 for the dietitians was conducted on was delivered via teams. Activity 2 for patients was conducted on twice once at South Kensington Campus of Imperial College London and one online. The third shared activity was conducted online and a patient was allocated to a dietitian and a member of the team observed the consultation.***

### 2.1.3 PPI Dietitians

#### 2.1.3.1 Activity 1 - Dietetic Questionnaire

The first activity consisted of an online questionnaire (**Supplementary Figure 2**) aiming to assess information regarding current practice, to examine preparation time and barriers to personalisation of dietary advice. A similar questionnaire was also promoted through different social media accounts of the researchers, and through the British Dietetic Association.

#### 2.1.3.2 Activity 2 – Dietetic workshop

The dietitians recruited were invited to an online workshop, which introduced the MetP methodology accompanied with MetP-related questions and related case studies. The aim of the workshop was to educate the dietitians around the methodology and explore any of their presumed barriers to the use of MetP within the current practice. The case studies focused on understanding the issues and difficulties of under-reporting and misreporting in dietary practice when a urine

metabolically personalised report is available, therefore, aiming to explore how dietitians would overrule the discrepancies. The case studies were developed based on information collected from a previous trial (**Supplementary Figure 3**).

2.1.3.3 Dietary Counselling Guidance Review

Dietitians were invited to assess the dietary counselling guidance designed by the researchers (LA, AC and DT). The aim was to evaluate the reflectiveness of the dietary guidance with the current practice.

2.1.3.4 Activity 3 – Counselling Sessions

During activity 3 participants were offered a dietary consultation by PPI dietitians in on-to-one virtual meetings. Based on Intake24, an online 24h recall (Simpson et al., 2017), research dietitians calculated patients' energy intake, and assessed adherence to the NICE guidelines based on a developed NICE-checklist (**Supplementary Figure 4**). The research dietitians estimated patients' basal metabolic rate (BMR) using Henry's equation (Henry, 2005), and reported physical activity via International Physical Activity Questionnaire (IPAQ) (CRAIG et al., 2003). PPI dietitians were given all the information in advance to performing the dietetic consultation. The activity aimed to collect dietitians' perspectives on the effectiveness and applicability of the urinary metabolically personalised report.

2.1.2 PPI – Patients

2.1.2.1 Activity 1 - Questionnaire

The selected public members were asked to complete a survey which aimed to understand the background of the selected cohort in terms dietary knowledge and readiness to follow a dietetic advice.

2.1.2.2 Activity 2 – Focus Group

The focus groups aimed to introduced MetP to participants and assess barriers to dietary intake adherance, their thoughts on dietitian knowing their accurate intake, and more practical questions required for data collection.

2.1.2.3 Activity 3 – Counselling Sessions

Participants were asked to record their dietary intake via Intake24 and to collect a fasting urine sample the day after the 24h recall completion. Participants were also asked to complete IPAQ. The counselling sessions aimed to feedback on practicability of the use of Intake24, urinary collection, views on MetP use during the consultation and research-developed dietary booklet.

2.2 PPI Results

2.2.1 PPI dietitians

Three of the five dietitians were involved in all the PPI activities, while one dietitian was only involved in the questionnaire and dietary counseling guidance feedback; while one dietitian was involved only in the questionnaire. Three dietitians were involved in dietetic consultation activity.

2.2.1.1 Dietetic Practice questionnaire

Thirty-three dietitians completed the online questionnaire. All dietitians reported that personalised nutrition refers to a nutritional advice “based on the individual”. Almost 80% of the dietitians reported that they feel that personalised nutrition would benefit individuals. It was also reported that that they use dietary assessment following ABCDE (Anthropometry, Biochemistry, Clinical, Diet and Everything Else) format, with body composition analysis, social and lifestyle assessment, phycological aspects and patients’ goals been taken into consideration as enhanced aspects of personalisation. The dietitians also reported that the current food assessment tools could be inaccurate. Time was one of the major barriers for the dietitians to personalize the nutrition, with 8 dietitians reporting, followed by lack of information and motivation. The major barrier to change for personalised nutrition in patients as per dietitians is motivation followed by finances.

2.2.1.2 Activity 1

The majority of the dietitians reported that personalisation to dietary practice was based on existing guidelines on the condition and on assessment methods (ABCDE) and one reporting personalisation of diet also based on genetic testing.

Preparation time varied between dietitians ranging from 5 minutes to 30 minutes. With respect to barriers, patient-centered barriers were more predominant. including patient's knowledge, understanding of cooking methods, understanding influence of cultural/religion, routine, and time required to build patient-clinician trust to gain some more insight information on their eating and cooking behaviours. A single dietitian suggested that misreporting could act as a barrier to personalised dietary advice.

2.2.1.3 Activity 2

Two of the three dietitians felt that there is need of MetP in clinical practice with all feeling that training will be required for interpretation and communicating the information. After viewing the urinary metabolically personalised report, one dietitian reported that the information given by the report would enhance his dietary advice giving. Finally, 3/3 of the dietitians felt that the urinary metabolically personalised report will be applicable in both patients was CVD risk or NAFLD. With respect to case studies, the dietitians believed that the report should be used in a positive way, to reinforce a healthy eating behaviour, however, they felt that it might be used negative manner and suggest that the participant is lying in presance of discrepancies. Dietitians also reported that trust building and "working with the patient" to formulate personalised SMART goal are important on dietary counseling.

2.2.1.3 PPI dietary counselling guidance assessment

All dietitians (3/3) stated that a personalised approach taking into consideration people's perceptions, understanding individuals' lifestyle, and barriers to follow dietary recommendation is crucial. Education and dietary knowledge level of an individual, as well as the motivation was emphasised in the meetings. The dietitians also strongly enforced trust-building even before the initial consultation should be considered, with 1/3 dietitians suggesting that a single dietitian within the research team should review a participant over the 12-week period.

2.2.1.4 Activity 3

From the consultations, it was concluded that dietitians utilised the urinary metabolically personalised report when required; and mostly used to reinforce

individuals' current intake and to make suggestions. Based on dietitians' feedback 1/3 dietitians found the urinary metabolically personalised report extremely useful while the other two found it slightly useful. The dietitian who did two consolations, even though on the real-life scenario reported that the use of the report was slightly useful, on the case-study real-life setting consultation described the report very useful; as he was able to reinforce the participant. The challenges faced by the dietitians were that the report might not be representable to participants' diet and might not be always in agreement with the 24h recall. Two dietitians also reported that it is difficult to collect some of the key information from the report, with the dietitian doing two consultations also explained that some markers, such as carnitine, might not be applicable to be assessed in specific populations. For two of the consultations, it was stated that the report should be more concise and contain some information which is more general and not specific for some fruits and vegetables. A dietitian also reported that additional days or a usual diet might be more applicable in the utlisation of the tool as well as having multiple day urine samples. Ultimately, it was believed that the report helped in patient engagement, however, a tailored version for the participants might have been more applicable.

### 2.2.1 PPI Public

#### 2.2.1.1 Activity 1

Eleven public members completed the online questionnaire to assess their dietary intake. All participants stated that they thought they were following a healthy diet. Six people reported that time was a barrier to follow a healthy diet, while 3/11 members reported that money was a barrier. Cooking skills were reported by a single member of the public, while other issues reported by individuals were motivation (reported by 2/11), health, following diet, food availability, high intake of unhealthy food and difficulty in healthy eating adherence. With respect to assessing dietary review, 3/11 patients have visited a dietitian, with two being referred by their doctor and 1/3 asking for a referral. Individuals who did not have had a dietary consultation stated they found the information online or they were unable to attend due to due to the lack time or motivation. Some also reported that they believed that they did not require a dietary advice from a dietitian or they believed that it was too expensive.

2.2.1.2 Activity 2

Three participants with NASH and six patients at CVD-risk were invited. **Table 2** represents the information obtained from the public regarding giving samples. All the participants reported that they would report more accurately if they knew that the dietitian knew what they have been consuming, however; 5 reported that they would adhere to the recommendation given while 4 stated that they were unsure whether they would follow the diet. Six of them reported that they would also eat better in the specific scenario and1 reported that he wouldn't. Finally, the public reported on how they would want to receive dietary recommendation in the trial, with the majority of the PPI (6/9) preferring weekly menus in accompanied with cooking instructions. Post-discussion, it was suggested that weekly menus are preferred with cooking instructions in order to understand the type of oil (fat) to use as well as the cooking methods. It was also recorded, that online completion of a 24h recall (Intake24) might not be applicable for individuals of older age due to barriers including technological incompetence and language barriers and a healthcare professional-driven 24h recall might be a suitable alternative for the specific cohort.

***Table 2 : Results of PPI analysis on how comfortable individuals will feel giving the different samples included in the trial***

| | Urine Samples | Stool Samples | Blood Samples |
|---|---|---|---|
| **Very Comfortable** | 5 | 3 | 5 |
| **Comfort** | 3 | 2 | 4 |
| **Uncomfortable** | 1 | 3 | 0 |
| **Very uncomfortable** | 0 | 1 | 0 |

2.2.1.3 Activity 3

Three public members were selected to participate in the PPI consultations, based on their urinary metabolite report, while a case-study was presented by a member, not directly linked with the trial, helping with the PPI for the clinical trial. All members of the public found the use of the Intake24 easy, however, it was reported that it might be difficult to use for individuals with low accessibility to technology, while urine collection was easy for 2/3 individuals with 1/3 individuals

suggesting a difficultly in collection. All the participants felt that they would improve their diet post-consultation and 1/3 reported that would be able to report their diet more accurate, however 2/3 participants were unsure. The handout was considered useful, however, it was concluded that it could have been more interactive, with two participants suggesting the use pictures.

2.3 Outcome

This activity reinforced the use of ABCDE format for the nutritional assessment as a part of the dietary counselling guidance developed. From both dietetic perceptions and patients' views, key barriers to change were identified (lack of motivation, finances, education etc.) and ways to overcome those challenges were discussed within the research team. Addition of more assessment information (i.e. Hunger status and satiety); as well as considerations to amend the MetP report and leaflet were made based on feedback.

## 3.0 Study 2

### 3.1 Methodology

#### 3.1.2 Ethical Approval

Both of the trials were approved by the London-Brent Research Ethics Committee (REC) and Health Regulatory Authority (HRA) (References: 13/LO/0078 and 18/LO/2042); in accordance to the declaration of Helsinki. Informed consent forms were obtained prior to the initiation of the trial and after capacity and capability confirmation for each participant was assessed.

#### 3.1.3 Study Design and Participants

*Healthy Cohort:*

Data from a previously published open-label randomised controlled trial, where healthy participants were recruited from a database of healthy volunteers at the UK National Institute for Health Research (NIHR)/ Wellcome Trust Imperial Clinical Research Facility (CRF) (Garcia-Perez2017) were obtained. The healthy volunteers invited, have previously been pre-screed at the CRF. Volunteers were eligible to participate if: (1) aged between 21-65 years and (2) had a BMI of 25-30 kg/$m^2$. Volunteers who expressed interest for the trial were invited for a pre-screening visit at the CRF. Potential participants were excluded in the presence of: (1) clinically significant illness, (2) experienced weight loss or gain of 3 kg or more in the last 2 months, (3) were prescribed medication, (4) were current smokers or substance abuser, (5) presented any abnormalities during physical examination, electrocardiography or pre-screed biochemical analysis, (6) were pregnant of breastfeeding.

*CVD-risk Cohort:*

Data from an open-label randomised controlled trial, recruiting participants at CVD-risk were collected for the analysis (n=20). Participants were eligible to participate if they had at least 3 of the following criteria: (1) aged 30 – 65years, (2) have a BMI ≥25 kg/m2 and <35 kg/m2, (3) have a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or taking hypertensive medication, (4) have LDL-cholesterol ≥4.14 mmol/L and HDL-cholesterol ≤1.03 mmol/L (males) or ≤1.29 mmol/L (females), (5) been currently a smoker, (6) have

a history of premature coronary heart disease (CHD) and (7) have a waist coreference of >103 cm in males or >88 cm in females. Individuals were excluded if: (1) have been recruited in other studies during the 12 week time frame, (2) excess alcohol consumption was observed (> 21 units/week for males and 14 units /week for females), (3) weight gain or loss of ≥5 kg was reported in the last 12 weeks, (4) are substance abusers, (5) have taken or currently taking any dietary supplements in the last 6 months, (6) were diagnosed with malignancy, (7) have diabetes, (8) pregnant (9) have any gastrointestinal (i.e. inflammatory bowel disease or irritable bowel syndrome) or renal or hepatic diseases, (10) have pancreatitis, (11) are suffering from chronic illnesses or are HIV positive and (12) use any medications which may interfere with energy metabolism, appetite and hormonal homeostasis (i.e. anti-inflammatory medications, antibiotics, androgen, erythromycin or thyroid hormones).

3.1.4 Randomisation and masking

A researcher not directly linked with the trial was involved in the randomisation of the participants. Randomisation was achieved by sealed envelopes via the use of opaque sealed folders. The sealed envelopes were securely stored away from the trial site and opened by the researchers on a participant to participant basis.

*Healthy Cohort:*

Nineteen participants from the healthy cohort (power calculation provided in **supplementary figure 6**) followed, in a random order, four different diets with different levels of WHO healthy eating dietary adherence. Participants were randomised to which of the four diets they would have followed during the course of the trial. The order and which of the dietary interventions were followed by the participants were randomly assigned.

*CVD-risk Cohort:*

Twenty participants (power calculation provided in **supplementary figure** 6) were followed ,in random order two diets.

3.1.5 Procedures:

*Healthy Cohort:*

Healthy participants were invited to attend four 72-hours inpatient stays, with a wash-out period of at least. During each of the 72-hour period, participants received different levels of adherence to WHO guidelines. Through the 72-hour period, 24-hour urine samples were obtained. Blood samples were obtained at the baseline and at the end of the 72-hour period (Garcia-Perez et al., 2017). Figure 4 shows the study plan.

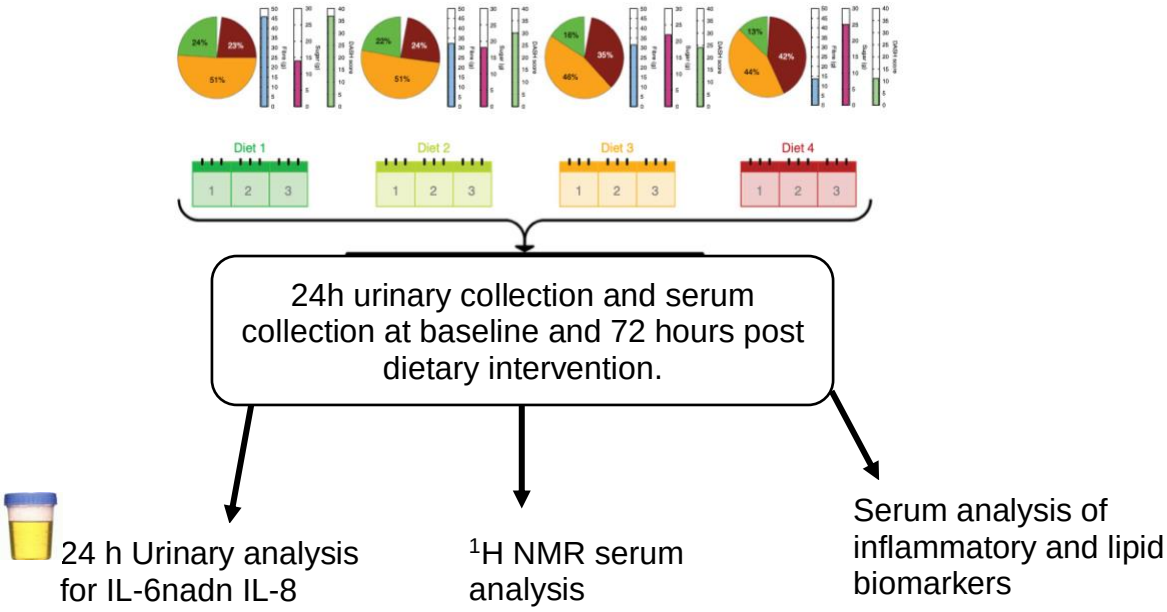



***Figure 4: Study Design of Healthy Cohort following 4 distinct 72-hour dietary intervention with a wash-out period of at least 5 days between dietary interventions.***

*CVD Cohort:*

The selected participants, were invited to attend the NIHR/Wellcome Trust Imperial CRF at Hammersmith hospital for two 5-day (and 4 nights) inpatient periods. There was a minimum of two-weeks wash out period between each 5-day period. During each period participants received different levels of adherence to the CVD risk management NICE guidelines diet, Diet 1, being the most concomitant with the guidelines and Diet 2 being the least concomitant.

Information of dietary interventions is given in the following section. Prior attending the clinic for each of the 5-day periods, participants were asked to bring a spot faecal sample and a 24-hour urine sample of the previous day. Anthropometric (BMI, waist circumference), biochemical, clinical (blood pressure and saliva samples) measures were taken prior and post-dietary intervention period. Spots of urine samples, 24-hour urine collection and faecal samples were collected on each day of both study periods. Research dietitians, calculated participants' energy expenditure based on Schofield equation (Schofield, Schofield and James, 1985). **Figure 5** presents the flow diagram of the trial.

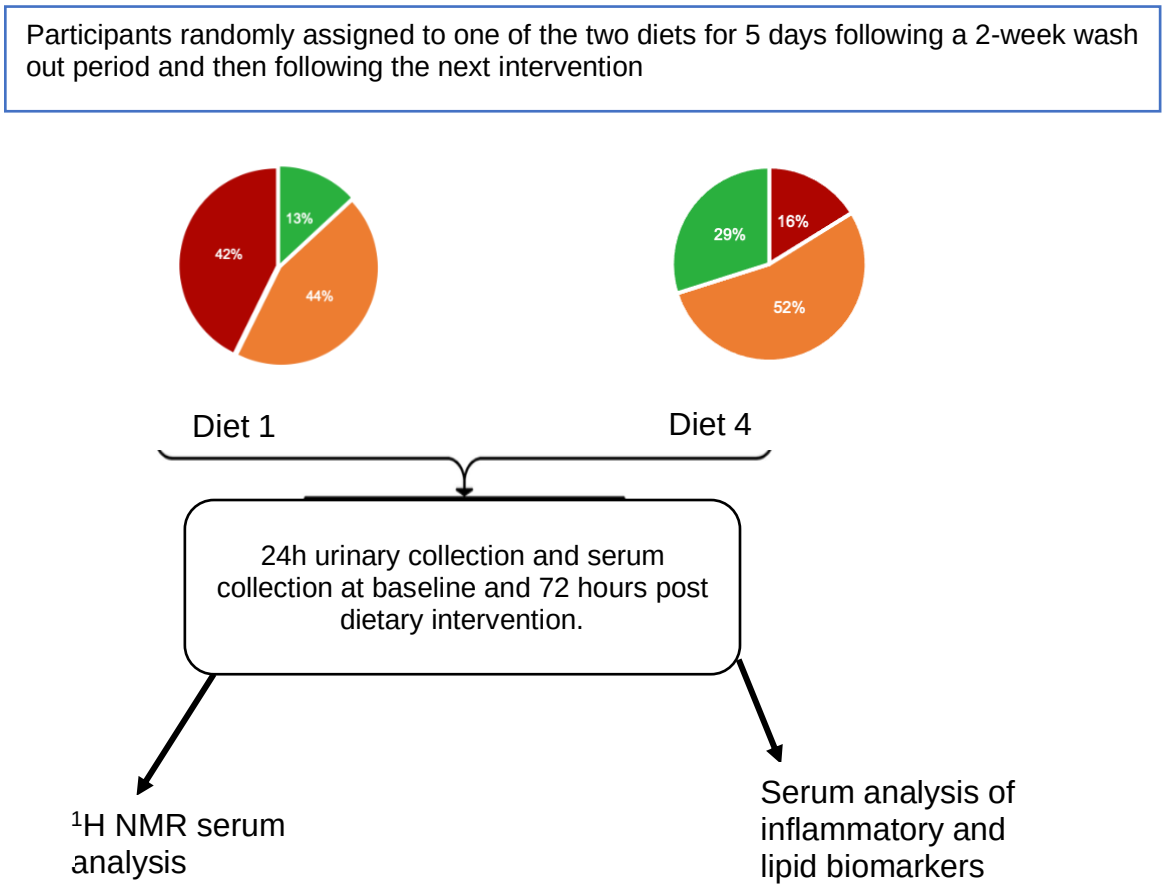



***Figure 5: Study Design of CVD-risk cohort following 2 5-day period of 2 different dietary interventions in controlled-settings with samples for analysis obtained on day 2 and day 5.***

### 3.1.6 Dietary Counseling

The Nutrition and Dietetic Research Group of Imperial College London, led by GF helped in the development of the dietary intervention for the two cohort. Food menus were developed and given to participants through their in-patient stay at

CRF. Participants were asked to consume all the food given by the research team. The only drink allowed during their in-patient stay was water as they wished. Participants were closely monitored by the members of the research team and it was expected for them to consume all the food provided. All food was weighted prior and post being given to the participants, and any uneaten food was weighted to control adherence. Participants were not encouraged to do strenuous exercise, however they were allowed to engage in light activity.

*Healthy Cohort:*

Based on the WHO guidelines on healthy eating, the research group, developed four different dietary interventions in a stepwise variance. Diet 1 was the one closer to WHO guidelines; whilst diet 4 was the one with the list adherence to the NICE guidelines (Garcia-Perez et al., 2017). Nutritional analysis of the food is presented on **Figure 6** and supplementary report provides the food menu given.

*CVD-risk cohort:*

Based on the NICE guidelines on healthy eating for people at CVD risk (NICE, 2016), the nutrition and dietetics research group developed two extreme diets. Diet 1 represented the diet with the highest adherence to the NICE guidelines, whilst diet 4 was the one with the list adherence to the NICE guidelines. Diet 1 consisted of 52% of the diet being carbohydrate, 24% of the total energy was given from protein and the rest (16%)was from fat. Protein, carbohydrate and fat provided 13%, 44%, and 42% of the total energy in diet 4, respectively. Supplementary Table represents the difference between the two different diets. The same monitoring protocol as per study 2 was followed for the CVD risk population.

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| Energy (kcal) | 2260 | 2259 | 2427 | 2490 |
| Energy density (kcal/g) | 1·2 | 1·5 | 1·6 | 1·9 |
| Proportion of protein | 24% | 22% | 16% | 13% |
| Proportion of carbohydrate | 51% | 51% | 46% | 44% |
| Total sugar (g) | 14 | 18 | 22 | 25 |
| Proportion of fat | 23% | 24% | 35% | 42% |
| Saturated fatty acids (g) | 5 | 7 | 19 | 20 |
| Monounsaturated fatty acids (g) | 8 | 6 | 14 | 12 |
| Polyunsaturated fatty acids (g) | 8 | 5 | 4 | 2 |
| Total trans fatty acids (g) | 0·5 | 0·5 | 1 | 1 |
| Fibre (g) | 45·9 | 32·1 | 31·5 | 13·6 |
| Sodium (mg) | 2367 | 2261 | 3812 | 3066 |
| Fruit and vegetables (g) | 600 | 300 | 180 | 100 |

***Figure 6: Figure taken from Garcia et al., (2017). Macronutrient content and characteristics of dietary interventions.***

3.1.7 Sample analysis and storage

I was not involved in the sample analysis, and date acquired was received from Bruker (Bruker.com, 2022). Fasting serum and 24-hour urine samples were examined to identify the interleukin content. The inflammatory markers, Glyc, GlycA, GlycB and SPW were quantified in the fasting serum samples of both cohorts by Bruke using the Bruker IVDr 600 MHz spectrometer.

3.1.8 Statistical analysis:

Data is presented as mean ± standard deviation unless stated otherwise. Statistical analysis of the data MATLAB (2014a, MathWorks). Changes between baseline and end-point of inflammatory and lipid beermakers' concentrations (Δchange) were calculated to assess the impact of change of the two extreme diets. Non-parametric Wilcoxon signed-rank test was performed to compare the significant differences between baseline and end-point of different dietary intervention and to assess the difference between diet 1 and diet 4 results utilising the data gathered. The same statistical test was applied to explore the difference

between Δchange of extreme diets (diet 1 and diet 4). Statistical significance was set at $p<0.05$ p-value was further adjusted for age, gender and BMI. Storey-Tibshirani method was also applied to calculate a specific False Discovery Rate (FDR) and a q-value were calculated and significance was also set at $q<0.05$.

## 3.2 Results

### 3.2.1 Participants characteristics

Participants characteristics of both cohorts are presented in **Supplementary Tables 2**.

### 3.2.2 Relationship of pro-inflammatory cytokines within the serum and 24h urine collection and the extreme WHO diets in healthy volunteers

The project investigated the changes in inflammatory markers in healthy volunteers following four different levels of adherence to WHO healthy eating guidelines. **Supplementary Figure 4** provide results of TNF-α. Following statistical analysis, a statistically significant association between IL-6 within the 24h urine collection and Delta changes in serum IL-12 were observed (**Figure 7**). Urinary IL-8, serum IL-10 and TNF-α did not present statistically significant difference between the two extreme diets. Figure 3 represent the changes IL-6 and IL-8 within the 24h recall. IL-12 is difference is demonstrated on **Figure 8**.

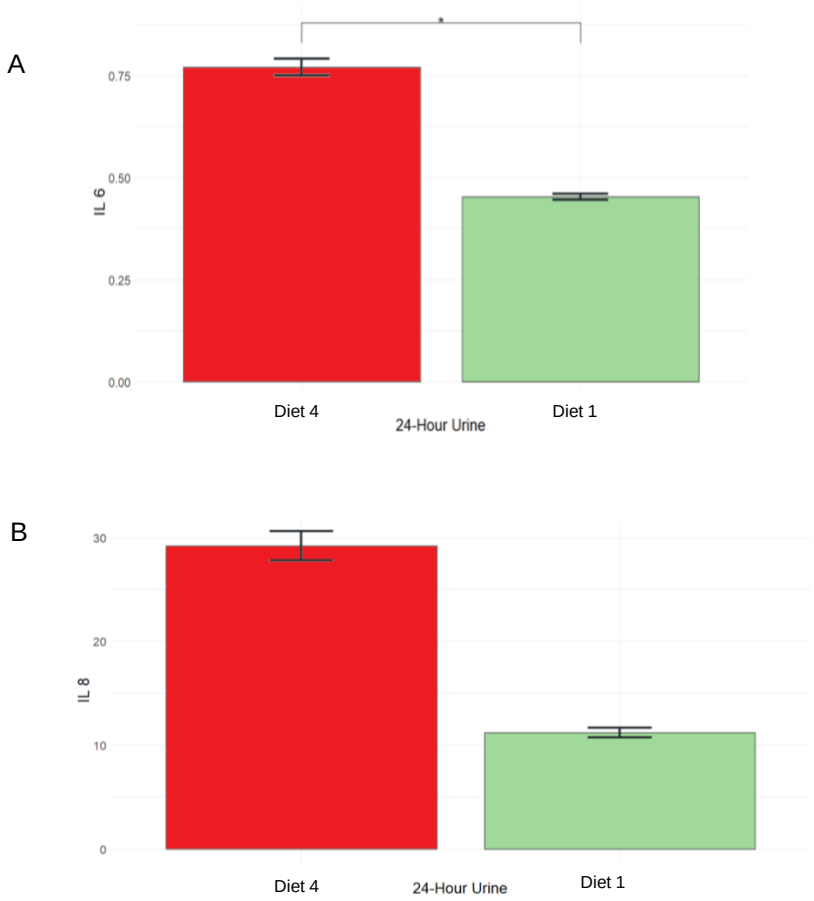



***Figure 7: Comparison between interleukin (IL) changes. Figure A demonstrates the IL--6 concentrations within the 24h urine collection between diet 4 (mean: 0.77 ± 0.13 (standard error)) and diet 1 (0.45 ± 0.04). It suggests a significant difference between diet 1 and diet 4 (p=0.03; n=19) in favor of diet 1 which reduced IL-6. Figure B shows the 24h urinary concentration of IL-8 between diet 4 (29.15 ± 8.65) and diet 1 (11.22 ± 2.75). The difference between the IL-8 concentration is not significant diet 1 and diet 4 diet 1 had lower urinary levels of IL-8.***

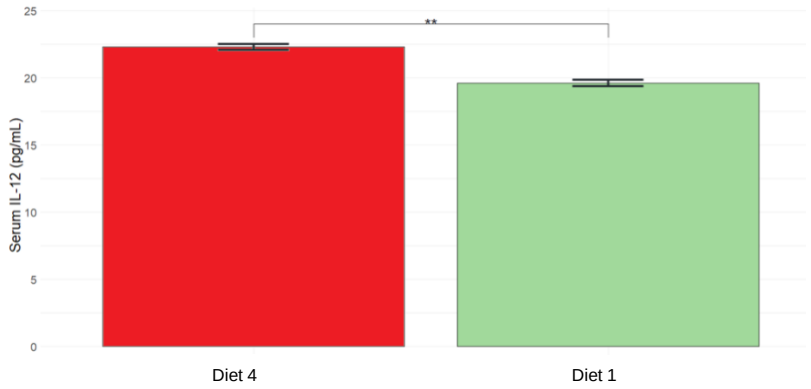



***Figure 8: Figure 8 shows the association between serum interleukin (IL)-12, diet 1 and 4. It is shown that individuals following Diet 4 had statistically significant more serum IL-12 concentrations compared to individuals following diet 1; essentially representing higher inflammatory status following diet 1 (p<0.05; n=19).***

3.2.3 Relationship between $^{1}$H NMR signals and WHO dietary interventions

**Table 3** represents the mean and standard deviation values of GlycA, GlycB and Glyc/SPC for the different diets and days. Comparing the different diets, it could be concluded that creatinine levels remained similar between baseline (day 1) and end-of-intervention (day 4). The highest GlycA and GlycB value is observed at baseline following diet 4, however, both $^{1}$H NMR N-acetyl signals decline in all groups after the intervention. Glyc/SPC ratio is the highest after following diet 1, while the lowest value is observed post-diet 3 end of intervention-period.

***Table 3: Comparison between $^{1}$H NMR signals and different WHO dietary adherence interventions***

| | | GLYCA (P.D. U) | GLYCB (P.D.U) | GLYC/SPC (P.D.U) |
|---|---|---|---|---|
| **DIET 1** | *Day 1* | 0.80 ± 0.09 | 0.31 ± 0.03 | 0.59 ±0.18 |
| | *Day 4* | 0.77 ± 0.13 | 0.29 ± 0.05 | 0.64 ± 0.16 |
| **DIET 2** | *Day 1* | 0.80 ± 0.07 | 0.31 ±0.03 | 0.55 ± 0.11 |
| | *Day 4* | 0.78 ± 0.09 | 0.30 ±0.04 | 0.59 ±0.13 |
| **DIET 3** | *Day 1* | 0.81 ± 0.14 | 0.31 ± 0.05 | 0.54 ± 0.13 |
| | *Day 4* | 0.75 ± 0.10 | 0.29 ± 0.04 | 0.56 ± 0.13 |
| **DIET 4** | *Day 1* | 0.83 ± 0.12 | 0.32 ± 0.05 | 0.55 ± 0.14 |
| | *Day 4* | 0.76 ± 0.12 | 0.30 ± 0.05 | 0.57 ±0.14 |

Wilcox sign-rank statistical analysis test was applied to examine the differences of diet 1 and diet 4 between day 1 and 4. It was demonstrated that since day 1, Glyc/SPC ratio were statistically higher following the Diet 1 (p=0.004). GlycA, GlycB and SPC were significantly lower on day 1 compared to day 4 for diet 1. Following the same statistical analysis for diet 4, individuals showed to also have higher levels of Glyc/SPC ration on day 4, however the results are not significant (p=0.434). **Table 4** presents the relationship between $^{1}$H NMR N-acetyl signals and diet 1 and 4 at baseline and end-of-study-period.

*Table 4: Comparison between $^{1}H$ NMR N-acetyl signals between different WHO dietary adherence interventions (n=16)*

| **DIET 1** | | | | | | |
|---|---|---|---|---|---|---|
| | **Day 1** | | **Day 4** | | **p-value** | **Adjusted p-value** | **Q-Value** |
| GLYCA (P.D.U) | 0.798 0.086 | ± | 0.766 0.126 | ± | 0.396 | 0.986 | 0.164 |
| GLYCB (P.D.U) | 0.311 0.035 | ± | 0.293 0.052 | ± | 0.162 | 0.705 | 0.098 |
| SPC (P.D.U) | 2.031 0.563 | ± | 1.707± 0.393 | | 0.001 | 0.013 | 0.002 |
| **DIET 4** | | | | | | | |
| | **Day 1** | | **Day 4** | | **p-value** | **Adjusted p-value** | **Q-Value** |
| GLYCA (P.D.U) | 0.829 0.116 | ± | 0.761 0.124 | ± | 0.63 | 0.77 | 0.23 |
| GLYCB (P.D.U) | 0.318 0.051 | ± | 0.300 0.056 | ± | 0.77 | 0.77 | 0.24 |
| SPC (P.D.U) | 2.189 0.507 | ± | 0.571 0.138 | ± | 0.01 | 0.14 | 0.02 |

A statistical analysis was not performed to examine the mean difference between intermediate diets, however, the statistical analysis was performed to compare the difference between Δchange in diet 1 and diet 4. A statistically significant difference was observed between Δchange of Glyc/SPC (p= 0.02, n=16) before adjusting the p-value in favor of diet 1. The association between diet 1 and inflammatory markers are demonstrated on **Table 5**.

*Table 5: Δchange statistical difference between GlycA and GlyB with WHO extreme diets in healthy individuals post-72h intervention (n=16)*

| **BIOMARKER** | **DIET 1** | **DIET 4** | **P-VALUE** | **ADJUSTED P-VALUE** | **Q-VALUE** |
|---|---|---|---|---|---|
| **GLYCA (P.D.U)** | -0.025 ± 0.115 | -0.074 ± 0.102 | 0.192 | 0.717 | 0.067 |
| **GLYCB (P.D.U)** | -0.016 ± 0.048 | -0.019 ± 0.038 | 0.538 | 0.850 | 0.096 |

**Figure 9** demonstrates the changes in Glyc/SPC ratio following different diets from baseline (day 1) until day 4, including delta (Δ)change. When investigating on Δchange of Glyc/SPC, it was concluded that there was a significant difference between diet 1 and diet 4, with individuals in diet 1 having a statistically significant

higher Glyc/SPC ration with the effect been lost after multi-variable adjustments (p=0.02; adjusted p-value=0.21; Q-value=0.01).

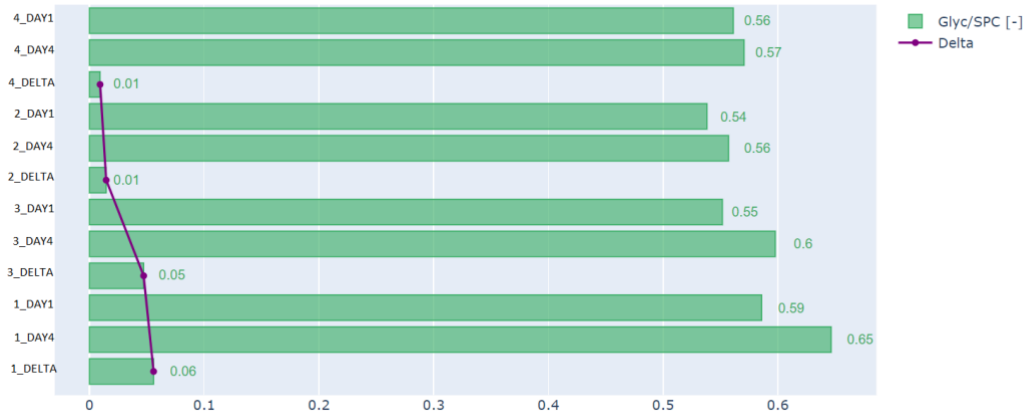



***Figure 9: : The figure demonstrates correlation between Glyc/SPC ratio values (x-axis) and different adherence of WHO diets (diet 1 to diet 4) at baseline (day 1) and on day 4 on the y-axis. The figure also shows the delta (Δ)change between baseline and day 4 for each of the dietary interventions. Diet 4 shows the lowest levels of Gly/SPC on day 4 (Glyc/SPC= 0.57); while diet 1 demonstrates the highest levels of Glyc/SPC post-intervention (Glyc/SPC=0.65). When looking on changes between baseline (day 1) and end-of-intervention (day 4), the highest Glyc/SPC was achieved following diet 1. The highest Δchange between day 1 and day 4 was achieved via diet 1.***

### 4.2.2 Changes in inflammatory markers in people at CVD risk after 72hours of two extreme diets

When investigating changes in inflammatory markers on diet 1 between baseline and end of intervention, it was concluded that creatinine and $^{1}$H NMR N-acetyl signals Glyc/SPC ratio demonstrated an association. Creatinine demonstrated a statistically significant negative correlation with Diet 1 (p=0.008), however, after adjusting for confounding factors, the effect was lost (p=0.115). Glyc/SPC ratio represented a statistically significant positive correlation with diet 1 even after adjustment for confounding factors (p=0.001). When investigating for confounding factors, the relationship of creatinine, GlycA, GlycB, SPC and consequently Glyc/SPC ration in individuals following diet 4, it was concluded everything was statistically significantly increased 72hours post-intervention, whereas the Glyc/SPC ratio even though it was higher post-intervention, the difference between baseline and end-of-intervention was not statistically significant. The association between inflammatory markers and the two extreme diets on day 1 and day 4 are shown on **Table 6**.

*Table 6: Comparison between inflammatory markers and two extreme diets on day 1 and 4 (n=20)*

**DIET 1**

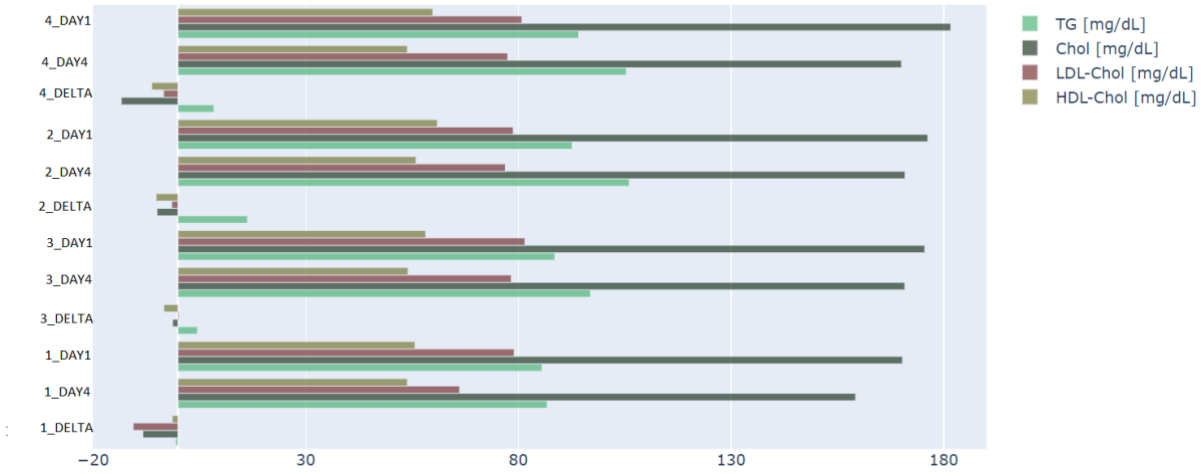
| | **Baseline** | **End-of-trial** | **p-value** | **Adjusted p-value** | **Q-Value** |
|---|---|---|---|---|---|
| GLYCA (P.D.U) | 0.800 ± 0.087 | 0.845 ± 0.086 | 0.021 | 0.277 | 0.061 |
| GLYCB (P.D.U) | 0.305 ± 0.035 | 0.321 ± 0.036 | 0.075 | 0.752 | 0.125 |
| SPC | 1.795 ± 0.502 | 1.703 ± 0.414 | 0.289 | 0.907 | 0.306 |
| GLYC/SPC | 0.658 ± 0.177 | 0.718 ± 0.168 | 0.000 | 0.001 | 0.001 |
| **DIET 4** | | | | | |
| | **Baseline** | **End-of-trial** | **p-value** | **Adjusted p-value** | **Q-Value** |
| GLYCA (P.D.U) | 0.784 ± 0.073 | 0.824 ± 0.081 | 0.0164 | 0.115 | 0.010 |
| GLYCB (P.D.U) | 0.295 ± 0.031 | 0.312 ± 0.038 | 0.0212 | 0.149 | 0.010 |
| SPC (P.D.U) | 1.659 ± 0.424 | 1.720 ± 0.398 | 0.0435 | 0.261 | 0.018 |
| GLYC/SPC | 0.684 ± 0.164 | 0.687 ± 0.151 | 0.8060 | 1.000 | 0.262 |

The project consequently investigated the Δchange between the two extreme diets (diet 1 and diet 4). The results suggested that Glyc/SPC ratio following diet 1 (0.058 ± 0.054) was statistically significantly higher when compared to diet 4 (0.003 ± 0.059) (unadjusted p=0.000; adjusted p-value: 0.002; Q-value: 0.001); whilst GlycA (diet 1: 0.041± 0.071; diet 4: 0.041 ± 0.062) and GlycB (diet 1: 0.013 ± 0.027; diet 4: 0.017 ± 0.027) were higher in diet 1 compared, however, the difference observed was not statistically significant.

3.2.4 Changes in lipid markers in controlled settings following the 72h of Diet 1 and Diet 4 in healthy individuals in controlled settings:

Mean values and standard deviations for Δchange for the intermediate diets are presented on supplementary data. Individuals consuming diet 1 have shown to lower Cholesterol, Triglycerides (TG), LDL-Cholesterol, LDL-Phospholipids and HDL-Cholesterol, with cholesterol not been statistically significant different. **Figure 10** shows the relationship between the lipid profile and the different diet adherence levels.

A




B

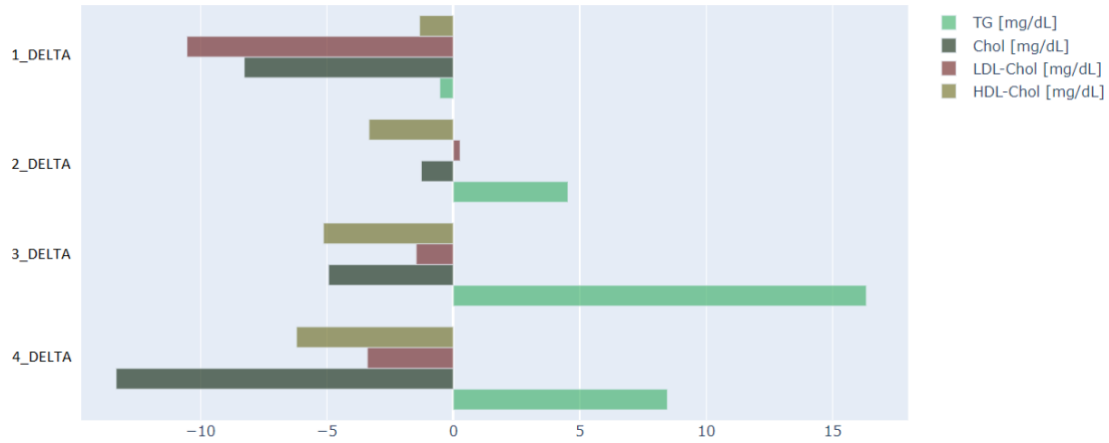



***Figure 10: Figure 10 shows the lipid profile observed at baseline and end-of-intervention in different levels of WHO adherence diet. It also demonstrates the delta difference between each diet. The x-axis of figure A represent the mean values for triglycerides (TF), cholesterol (Chol), Low-density-lipoprotein (LDL)-chol, and High-density-lipoprotein (HDL)-Chol. When comparing the diets, it could be viewed that the lower values for each of the dietary levels are presented on Diet 1_day 1. Figure B represents a part of the 5.1 graph, showing only the Δchange. From the graph, it is viewed that TG levels are increased to the highest extend on diet 2. The biggest reduction on LDL-chol is observed following diet 1, while the highest HDL-cholesterol and total cholesterol decrease between baseline and day 4 is observed following diet 1.***

4.2.4 Changes in lipid markers in controlled settings following the Diet 1 and Diet 4 in participants at CVD-risk in controlled settings:

Following statistical analysis to find if there are intra-diet changes between baseline and end-point, it was found that 5-day intake of diet 4 intake statistically significantly increased total cholesterol (p=0.002; adjusted p= 0.023; Q-value=0.003). Exposure to diet 4 also statistically increased LDL-cholesterol. The effect was lost following adjustment for age, gender and BMI (p=0.019; adjusted p= 0.131; Q=0.010). HDL-cholesterol was higher at the end of the diet 4 period, but there was no statistically significant difference between baseline and end-point (**Supplementary Table 6**). Diet 1 results similarly did not show a statistically significant difference from baseline to endpoint (**Supplementary Table 6**).

When investigating the difference between Δchange (end-point – baseline), LDL-cholesterol LDL-Phospholipids and total cholesterol were significantly reduced in diet 1 arm compared to diet 4 arm. HDL-Cholesterol, HDL-Phospholipids and triglycerides had a higher difference between baseline and end-point when diet 4 was followed, with the difference between diet 1 and diet 4 Δchange was not significant. **Table 7** shows the results of the analysis.

***Table 7: Comparison of lipid markers and Δchange between the two extreme diets (n=40)***

| LIPID MARKER | DIET 1 | DIET 4 | P-VALUE | ADJUSTED P-VALUE | Q-VALUE |
|---|---|---|---|---|---|
| **TOTAL CHOL (MG/DL)** | 14.833 ± 15.198 | 1.000 ±17.543 | 0.013 | 0.141 | 0.019 |
| **LDL-CHOL (MG/DL)** | 8.000 ± 18.266 | -6.389 ±16.985 | 0.009 | 0.095 | 0.019 |
| **HDL-CHOL (MG/DL)** | -0.333 ± 6.029 | -1.556 ± 6.336 | 0.479 | 0.940 | 0.323 |
| **LDL-PHOS (MG/DL)** | 4.000 ± 8.388 | -2.889 ± 7.315 | 0.014 | 0.158 | 0.019 |
| **HDL-PHOS (MG/DL)** | 0.611 ± 3.837 | -1.667 ± 9.204 | -0.238 | 0.940 | 0.189 |
| **TG (MG/DL)** | 16.889 ± 33.711 | 14.444 ±29.254 | 0.488 | 0.940 | 0.323 |

## 5.0 Discussion

This is the first project that utilizes PPI focus groups, lipid and inflammatory data for the development of a dietary counselling guidance and enhancement of urinary metabolically personalised report. The current project enhances dietary counseling guidance through PPI by utilizing the information given by both public member and dietitians, as well as through observations made in PPI counseling sessions. The project also utilised data collected from two previous studies, which used healthy participants and participants at CVD-risk who followed WHO and NICE guidelines, respectively. The project utilised individuals' inflammatory and lipid profile to demonstrate that the highest adherence to WHO dietary guidelines (Diet 1) positively influence inflammatory and lipid markers in healthy individuals in a highly-controlled environment. The same trend was observed in the CVD-risk cohort when the most cohesive diet to the NICE guidelines was consumed. A negative relationship was observed between high cohesive WHO diet and cytokines (IL-6, IL-8, IL-12 and TNF-α) within the healthy participant cohort. Data on cytokines was not available for CVD-risk cohort, although, It could argued that similar or better results to healthy volunteers would have been observed if the data were available. With respect to lipid biomarkers, no association was observed in neither healthy or CVD-risk cohort; although a trend of a worst lipid profile been observed following the least cohesive diet to the guidelines. Data on NAFLD cohort was not collected due to delays in ethical approval of the trial. It could be hypothesised that a similar pattern to CVD-risk cohort observations would also have been observed in the NAFLD cohort.

Through study 1, the research team identified crucial issues required to be addressed in the dietary counselling guidance developed. The key areas raised by PPI dietitians were: building report with the patient, trust building and make personalised recommendations by viewing the individual as a whole. The main areas identified by the PPI patients were: understanding main barriers to change, explore on participants' perceptions on the use of the urinary metabolically personalised report, find if they would have engaged more in the consultation and ultimately dietary advice given. Through PPI, the research team was also able to experience the real-life use of Intake24 in accompanied with the urinary metabolically personalised report; to assess the dietary intake of an individual.

To current knowledge, there is no published dietary counselling guidance regarding the use of MetP in clinical and research setting. The document therefore, produced as a result of the PPI would benefit the provision of improved personalised nutrition, especially for individuals at CVD-risk as the document was primarily build for this population.

The results obtained from the serum and $^{1}$H NMR signals were not statistically significantly improved after multivariable adjustments in the healthy cohort following diet 1, even though a trend was observed. Within the CVD-risk population, we have also shown the presence of an association with serum inflammatory markers and $^{1}$H NMR signals of glycoproteins and SPC. The positive effect observed between high adherence to NICE guidelines and Glyc/SPC ratio been one of the predominant findings. Glyc/SPC ratio remains statistically significantly higher in diet 4 in CVD-cohort even after multi-variable adjustment, unlike healthy cohort results. Glyc/SPC ratio is negatively associated with inflammatory status. As diet 4 in the CVD-risk population showed greater Glyc/SPC even after adjustments for age, gender, and BMI, the effect of the NICE guidance on inflammatory status changes could be observed in short-term periods. The differences in serum inflammatory markers between the two populations may be related to potentially higher inflammation status in individuals at CVD-risk, although baseline data cannot be compared between the two cohorts to confirm this. The EURIKA trial, has shown that is high in a substantial proportion of individuals with at least a single CVD risks factor (Halcox et al., 2014). Studies have also correlated low-grade inflammation with increased risk of CVD-event (Ferrucci, and Fabbri, 2018; Shivappa et al., 2018) hence, the reason of statistically significant decrease in inflammatory markers being observed in CVD-risk cohort when following most-cohesive dietary advice could be partially hypothesised due to higher levels in the serum.

Our data adds the evidence to previously published studies suggesting the association between healthy dietary intake and inflammation (Du et al., 2008; Galland, 2010; North, Venter and Jerling, 2009; Kalopgeropoulos et al., 2010; Salas- Salvadó et al., 2008); however, no current research examines the association between high adherence of NICE or WHO dietary intake with

inflammation status. Most of the published trials explore the relationship between inflammation and diet either in a macronutrient level, or with dietary patterns limited to the MedD, or diets following Healthy Eating Index (Hart et al., 2021). WHO and NICE dietary guidelines mention increasing the consumption of fruits and vegetables to 400g per day, increasing intake of legumes, nuts and whole grains and reducing intake of free sugars, saturated and trans-fats reduces inflammatory markers (WHO, 2020; NICE; 2016). The difference between the two guidelines is the strickted trans-fatty acid intake mentioned by WHO guidelines, while additional guidance in fish intake and cholesterol is described in the NICE guidelines. Trials illustrate the intake of saturated fatty acids, trans-fats and high intake of high GI foods, which contain high concentrations of free sugars, increase serum inflammatory markers and lipid markers (Kontogianni, Zampelas and Tsigos, 2006). **Table 8** demonstrates the association of different macronutrients with inflammatory markers and their association with CVD risk and NAFLD. Fat intake and high glucose intake, mostly presented in refined processed high GI products, tend to increase inflammation via enhancement of reactive oxygen species, as well as enhancing the activation of pro-inflammatory transcription factors, resulting in the secretion of pro-inflammatory cytokines (Biobaku et al., 2019). However, WHO and NICE guidelines suggest the restriction of such macronutrients via increasing of whole grains, limiting processed foods, and replacing of saturated and trans-fatty acids with poly-unsaturated fatty acids.

*Table 8: Macronutrient association between inflammatory markers, CVD-risk and NAFLD.(Table modified by: Kontogianni, Zampelas and Tsigos, 2006). ((1)Aboubakr et al., 2021; (2) Galland, 2009; (3) Kechagias, Blomdahl and Ekstedt, 2019; (4)Ikehara et al., 2008; (5)Pérez-Montes de Oca et al., 2020; (6) Stromsnes et al., 2021; (7) Zaloga, 2021).*

| Nutrient | Response of inflammatory marker due to Intake | CVD risk | NAFLD risk |
|---|---|---|---|
| **Saturated FA** | ↑ CRP, ↑ IL-6, | ↑ | ↑ |
| **Trans FA** | ↑ CRP, ↑ IL-6, ↑ VCAM-1, ↑ ICAM-1 | ↑ | ↑ |
| **MUFA** | ↓ CRP, ↓ IL-6 | ↓ | ↓ |
| **PUFA (ω-3)** | ↓ IL-1β, ↓ IL-6, ↓ IL-8, ↓ TNF-α, ↓ CRP, ↓ IL-12 [(6)] | ↓ | ↓ |
| **High GI carbohydrates** | ↑ CRP | ↑ | ↑ |
| **Fiber** | ↓ CRP | ↓ | ↓ |

Studies on intake of GI, refine carbohydrates suggest that a 10-unit GI food could cause a 30% increase of CRP (Du et al., 2008). Fiber, which is a low GI carbohydrate, has been shown to reduce CRP by up to 55% with an increase of ≥ 3.3 g/MJ (North, Venter and Jerling, 2009). Therefore, the trial agrees with the results observed, that increasing the consumption of wholegrains reduces inflammation as per WHO and NICE recommendations. Oily fish was also part of the two dietary intervention cohort, with ω-3 fatty acids associated with reduction of inflammation. The Multi-Ethnic study of atherosclerosis (MESA) conducted in individuals aged 45-84 without CVD, and the ATTICA study conducted in healthy adults, reinforce the findings of the current project as they suggest that ω-3 fatty acids were inversely correlated with decrease in IL-6 (He et al., 2009). The ATTICA study also suggested an inverse association with CRP and TNF-a (Kalogeropoulos et al., 2010). WHO and NICE guidelines promote the intake of such products likely due to their anti-inflammatory properties, could be due to the proposed mechanism of action of reduction.

The project additionally demonstrates a negative association between high cohesive diets and lipid profile. The results demonstrate a negative correlation between LDL-cholesterol, HDL-cholesterol and triglycerides concentrations, but not with total cholesterol, with the most cohesive diet in healthy volunteers. There was no association observed with higher diet adherence to the WHO guidance

intervention. The results do agree with precious studies, however, there is lack of evidence of short-term changes in lipid profile. The results within the CVD-risk cohort followed the same pattern as with the healthy volunteers group. Literature suggests an association between healthy eating and reduction of total cholesterol and LDL-cholesterol, and an increase of HDL-cholesterol (Son et al., 2021; Tsaban et al., 2020). Although the studies do suggest of improvements in lipid profile, the follow up period ranges from 4-weeks to 12-months, with a recently published study demonstrating statistically significant changes within the HDL-cholesterol lipodomics within a 4-days of MedDier intervention when compared to fast food trial (Zhu et al., 2019). Lipodomics were not assessed for the purpose of this project, and therefore, no conclusions were made. As the trial shows a statistically significant improvement in some of the lipid profile markers in 4 days of WHO or NICE guidelines adherence; the 12-week NICE guideline intervention through personalised nutrition is expected to provide significant improvements.

Future studies should use markers of lipids and inflammation for the assessment of dietary adherence, considering the significant changes in inflammatory levels observed within this project, as well as the improvement in lipid levels, despite not all results being statistically significant. Through the PPI, key areas were identified with respect to barriers to dietary adherence, and key areas to consider when personalising nutritional care utilising MetP. MetP was accepted by both dietitians and patients as a tool to give personalised care and assess adherence and mis-reporting, however, as emphasised by the dietetic focus groups it is required to be used as a positive reinforcement as not as a tool to judge individuals. Future research should focus on assessing the changes within the intermediate diets (diet 2 and diet 3) within the healthy volunteers cohort, or compare any of the intermediate diets with one of the extremes. Diet 2 and diet 3 are similar to diet 1 and diet 4, respectively, in terms of food options used. The major difference between intermediate diets and extremes were the cooking methods used as in diet 4, which was the most cohesive to the WHO guidelines, all food prepared was steamed and no oils were added. Taking into consideration the low adherence of the high cohesive dietary guidance in community-based interventions, further statistical analysis exploring the association between

intermediate diets could have been performed, reflection less controlled environment.

The project has several strengths and weaknesses. Firstly, the data collected for inflammatory and lipid analysis was conducted in a highly-controlled environment, and therefore, it ensures that the results obtained are due to the changes in dietary intake. Even though this is not a real-life scenario, the trial provides the foundation of the assessment of the tool within current NHS practice. Secondly, the trial was adjusted for age, gender, and BMI, which all influence inflammation and lipid markers. With respect to the PPI, the trial involved a research team, who contacted MetP in order to have good guidance on the development and reporting of the PPI. Finally, the study uses a number of inflammatory markers as well as $^{1}$H NMR glycoprotein signals to assess inflammation. $^{1}$H NMR glycoprotein signals are more reflective of inflammatory status compared to CRP, they are stable and demonstrating less intra-variability (Connelly et al., 2017; Moreno-Vedia et al., 2022). A major limitation of the project is the uncertainty individuals' consumption prior to start of intervention, and ultimately, whether the current results for inflammatory and lipid markers were influenced by habitual dietary exposure. This could partially explain why there was not a significant difference between specific inflammatory and lipid biomarkers and dietary patterns. Another limitation of the trial is the intra-variability of the WHO dietary adherence diets in the healthy volunteers cohort. The differences within the intermediate and extreme diets are very minor, mostly varying in terms of cooking methods and oils used in the food preparation. This again might explain the non-visible differences between intermediate diets and extremes.

## 6.0 Conclusion

The project suggests that high adherence to WHO or NICE guidelines could improve inflammatory and lipid markers in healthy and CVD-risks, respectively. The utilisation of the available biomarkers in combination with improved dietary counselling guidance could enhance the future clinical trials aiming to personalise nutrition for people at risk of CVD and diabetic patients with NAFLD based on their metabolic phenotyping.

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## 8.0 SUPPLEMENTARY DATA

Supplementary Figure 1: CVD-risk methodology

Ethical Approval:

The study was approved by Research Ethics Committee (REC) and Health Regulatory Authority (HRA) in accordance with the Declaration of Helsinki (13/LO/0078). All the participants were provided a participant information sheet and all additional information required prior giving an informed consent form.

Study Design and participants:

In this randomized controlled trial, participants will recruited post-advertisement of the trial by posters. Participants from a pre-completed study, which assessed the levels of adherence to dietary recommendations based utilizing MP in a controlled environment. The participants from this study had an online review about the current trial. All the participants recruited from advertisement, had a screening visit prior been recruited. Participants were eligible to participate if they were classified as been high risk of CVD assessed by the following criteria: (1) aged 30 – 65years, (2) have a BMI ≥25 kg/m2 and <35 kg/m2, (3) have a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or taking hypertensive medication, (4) have LDL-cholesterol ≥4.14 mmol/L and HDL-cholesterol ≤1.03 mmol/L (males) or ≤1.29 mmol/L (females), (5) been currently a smoker, (6) have a history of premature coronary heart disease (CHD) and (7) have a waist coreference of >103 cm in males or >88 cm fin females. Potential participants were excluded: if (1) have been recruited in other studies during the 12 week time frame, (2) excess alcohol consumption was observed (> 21 units/week for males and 14 units /week for females), (3) weight gain or loss of ≥5 kg was reported in the last 12 weeks, (4) are substance abusers, (5) have taken or currently taking any dietary supplements in the last 6 months, (6) were diagnosed with malignancy, (7) have diabetes, (8) pregnant (9) have any gastrointestinal (i.e. inflammatory bowel disease or irritable bowel syndrome) or renal or hepatic diseases, (10) have pancreatitis, (11)are suffering from chronic illnesses or are HIV positive and (12) use any medications which may interfere

with energy metabolism, appetite and hormonal homeostasis (i.e. anti-inflammatory medications, antibiotics, androgen, erythromycin or thyroid hormones).

2.3 Randomisation:

Participants in both cohorts were randomised to either control group, given standard NHS nutritional advice, or target group, which received a metabolomic enhanced nutritional counselling. A computer based blocked randomization was performed by an investigator (IG-P), who is not linked to data collection. The blocked randomisation was operated using a web-based online random allocation system, named “Sealed Envelope”. The trial’s participants and the main investigators could not be blinded from the dietary intervention during the trial period, due to the nature of the trial. The investigator (AC) analyzing the data obtained from the trial was not masked from the randomisation order.

2.4 Procedures:

During the course of the trial, the participants routinely visited Imperial College London NHS outpatient clinics to complete their blood tests. They also attended an online consultation delivered by a trained dietitian, to receive either standard or metabolomic enhanced dietary intervention. Participants were asked to deliver fasted urine samples, anthropometric, clinical and biochemical measures. Participants were also asked to deliver a 24h recalls on intervals. Information around how to compete the 24h recall were given to the participants, prior the study. In order to standardise the date of food assessment collection across the participants, participants were asked to provide a 24h recall of the date they collected the fasted urine sample. The 24h recall was analyzed Intake24 (Simpson et al., 2017). Fasted urine samples were analysed using Proton Nuclear Magnetic Resonance ($^{1}$H NMR) spectroscopy via untargetetd metabolomics analysis

2.5 Sample Analysis and Storage:

The fasted urine sample were collected through the Trust and were delivered to Imperial College London; where they were stored at –80 °C in a stored and safe

environment in accordance to best practice guidance. The samples of fasted urine were analysed using $^{1}$H NMR spectroscopy (1600 spectra). The urine samples were treated with a phosphate buffer of pH 7.4 for the $^{1}$H NMR spectroscopy. The $^{1}$H NMR spectroscopy as preciously been described analysed the samples at 300 K on a 600 MHz spectrometry (Bruker Biospin). The standard one-dimensional pulse sequency with water resonance saturation, which have been in accordance to Garcia-Perez and collogues report (Garcia-Perez et al. 2017). The parameter used for are presented in appendix (Appendx 1). The information generated from the each participants' fasted urine analysis were imported to a MATLAB (2014a, MathWorks), and a metabolomic report was generated.

2.6 Dietary Counselling:

Within the CVD-risk patient cohort, participants received dietary counselling in at week 1, 2, 4 and 8. A standardised dietary protocol was developed prior to the initiation of the trial. The consultation with the dietitian was online. Each participant in each groups had an initial dietary consultation been approximately 30 minutes and the follow-up consultation was completed within 15 minutes. Information from the 24h recall using the mobile application Intake24 (Simpson et al., 2017), which analysed food intake in macro- and micro-nutrient. Information around dietary habits, environmental factors and any other clinical conditions was collected during the first consultation, in order to help inform the dietary plan. The participants within the control group, received standardized dietary information based on current risk factors and the 24h recall following the National Institute of Health and Care Excellence (NICE) advice. For each participant, personalised goals were achieved by the end of each consultation. Patients in the intervention group (n=67) received dietary counselling based on the 24h recalls and a supplementary stratified nutritional advice, which was based on the metabolic response of their diet via urinary and serum metabolic profiling deriving from faster urine and blood samples given.

2.7 Statistical analysis:

To the best of my belief, this is the first trial that examines the use of metabolomic profile for patients at CVD-risk; and therefore, no universal power calculation

could be performed. In order to perform a sample size calculation; preliminary data was used from a previously conducted study published by Lancet (Garcia-Perez et al., 2017). Garcia-Perez and collogues, suggested that a higher consumption fruit and vegetable from 100g to 180g and from 100g to 300g, would result in the increase of specific urinary biomarkers urine concentration by 1.05 mmol/24h and 3.48 mmol/24h, respectively. the standard deviation (SD) for an increase in intake of fruit and vegetable from 100g to 180g is expected to be 3.48 mmol/24h; while the SD when fruit and vegetable consumption rises from 100g to 300g is calculated to be 4.52 mmol/24h. The effect size therefore; results to have the value of 0.603. Supposing we are aiming for a power of 0.90 and an alpha of 0.05, 59 participants per group (n=118) are required to perform a two-tail difference statistical test. Celis-Morales and collogues, in a previously published review on personalised nutrition accounted for a 13% drop-out rate (Celis-Morales et al., 206). Taking into consideration the noted drop-out rate in Celis-Morales study, the trial aimed to recruit a total of 67 participants per trial - arm (n=134) for each patient group cohort. Due to the nature of the study, and the relative short duration of time the trial analyses x participants who are at CVD-risk.

Supplementary Figure 2: Dietetic PPI questionnaire – activity 1

Imperial College London

**Public and Patient Involvement project for Personalised Nutrition**

Kindly answer the three questions below and send it back by email. We really appreciate your time and help.

**Q1:** What do you base your personalised dietary advice on?

**ANSWER:**

**Q2:** How many minutes do you spend on preparing your patient consultation?

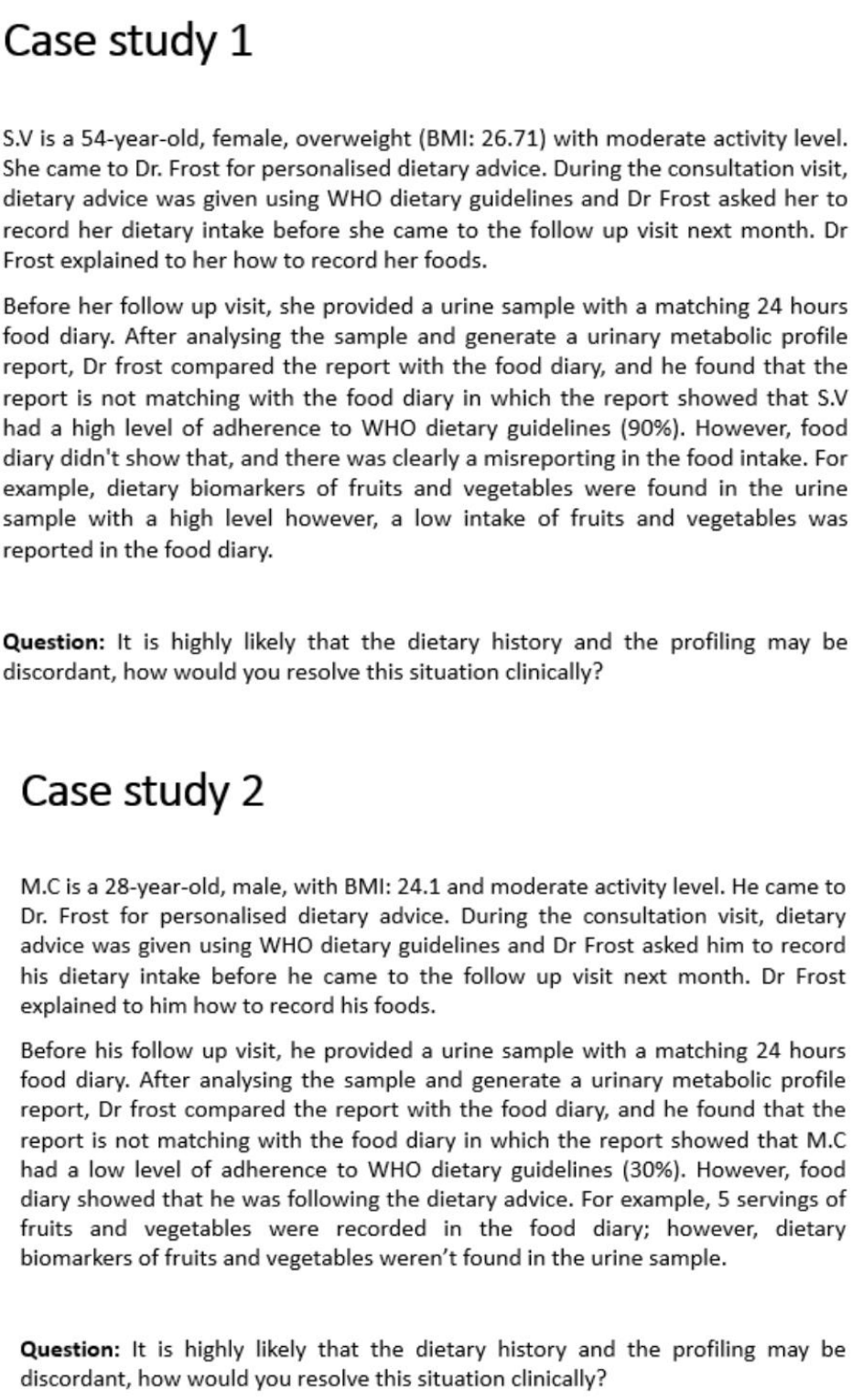
**ANSWER:**

**Q3:** Which barriers do you encounter on your current practise while personalising the diet?

**ANSWER:**

*Supplementary Figure 1: The figure demonstrates the PPI questionnaire used to assess current practice and barriers to personalised nutritional care. The questionnaire was completed by the PPI dietitians with a similar one (asking more information around current dietary statues (i.e. working within the NHS, band level etc.) was given to 33 dietitians to complete.*

Supplementary Figure 3: Case-studies scenario – activity 2



# Case study 1

S.V is a 54-year-old, female, overweight (BMI: 26.71) with moderate activity level. She came to Dr. Frost for personalised dietary advice. During the consultation visit, dietary advice was given using WHO dietary guidelines and Dr Frost asked her to record her dietary intake before she came to the follow up visit next month. Dr Frost explained to her how to record her foods.

Before her follow up visit, she provided a urine sample with a matching 24 hours food diary. After analysing the sample and generate a urinary metabolic profile report, Dr frost compared the report with the food diary, and he found that the report is not matching with the food diary in which the report showed that S.V had a high level of adherence to WHO dietary guidelines (90%). However, food diary didn't show that, and there was clearly a misreporting in the food intake. For example, dietary biomarkers of fruits and vegetables were found in the urine sample with a high level however, a low intake of fruits and vegetables was reported in the food diary.

**Question:** It is highly likely that the dietary history and the profiling may be discordant, how would you resolve this situation clinically?

# Case study 2

M.C is a 28-year-old, male, with BMI: 24.1 and moderate activity level. He came to Dr. Frost for personalised dietary advice. During the consultation visit, dietary advice was given using WHO dietary guidelines and Dr Frost asked him to record his dietary intake before he came to the follow up visit next month. Dr Frost explained to him how to record his foods.

Before his follow up visit, he provided a urine sample with a matching 24 hours food diary. After analysing the sample and generate a urinary metabolic profile report, Dr frost compared the report with the food diary, and he found that the report is not matching with the food diary in which the report showed that M.C had a low level of adherence to WHO dietary guidelines (30%). However, food diary showed that he was following the dietary advice. For example, 5 servings of fruits and vegetables were recorded in the food diary; however, dietary biomarkers of fruits and vegetables weren't found in the urine sample.

**Question:** It is highly likely that the dietary history and the profiling may be discordant, how would you resolve this situation clinically?

*Supplementary Figure4: Case studies provided in the PPI workshop for dietitians. Case Study 1 suggesting that both 24h-recall and metabolic report agree, while case study 2 showing discrepancies between 24h recall and urinary metabolite report.*

Supplementary Table 1: NICE checklist developed

| 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<br>Women: Less than 2-3 units/d | |
| **Salt** | Less than 2.4g of sodium /d | |

Supplementary Table 2: Participant characteristics; A (healthy cohorts) and B (CVD-risk cohort)

A

| | Data (n=19) |
|---|---|
| Sex | |
| Male | 10 (53%) |
| Female | 9 (47%) |
| Age (years) | 55·8 (12·6; 29–65) |
| Ethnic origin | |
| White | 18 (95%) |
| Asian | 1 (5%) |
| Weight (kg) | 74·5 (12·5; 52·8–107·9) |
| BMI (kg/m²) | 25·6 (3·2; 21·1–33·3) |
| Energy expenditure (kcal/day)* | 2099 (351; 1668–2995) |
| Glucose (mmol/L)† | 4·8 (0·4; 4·1–5·4) |
| $HbA_{1c}$ (%)† | 5·5% (0·1, 5·1–5·8) |
| $HbA_{1c}$ (mmol/mol)† | 36·4 (0·9; 32–40) |
| Triglycerides (mmol/L)‡ | 0·9 (0·3; 0·5–1·4) |
| Cholesterol (mmol/L)‡ | |
| Total | 5·1 (0·7; 3·9–6·1) |
| LDL | 3·1 (0·7; 1·7–4·2) |
| HDL | 1·6 (0·4; 0·9–2·6) |
| Liver function tests (IU/L)‡ | |
| Alanine transaminase | 21·2 (7·4; 12·3–40·0) |
| Aspartate transaminase | 19·5 (3·2; 15·0–24·3) |

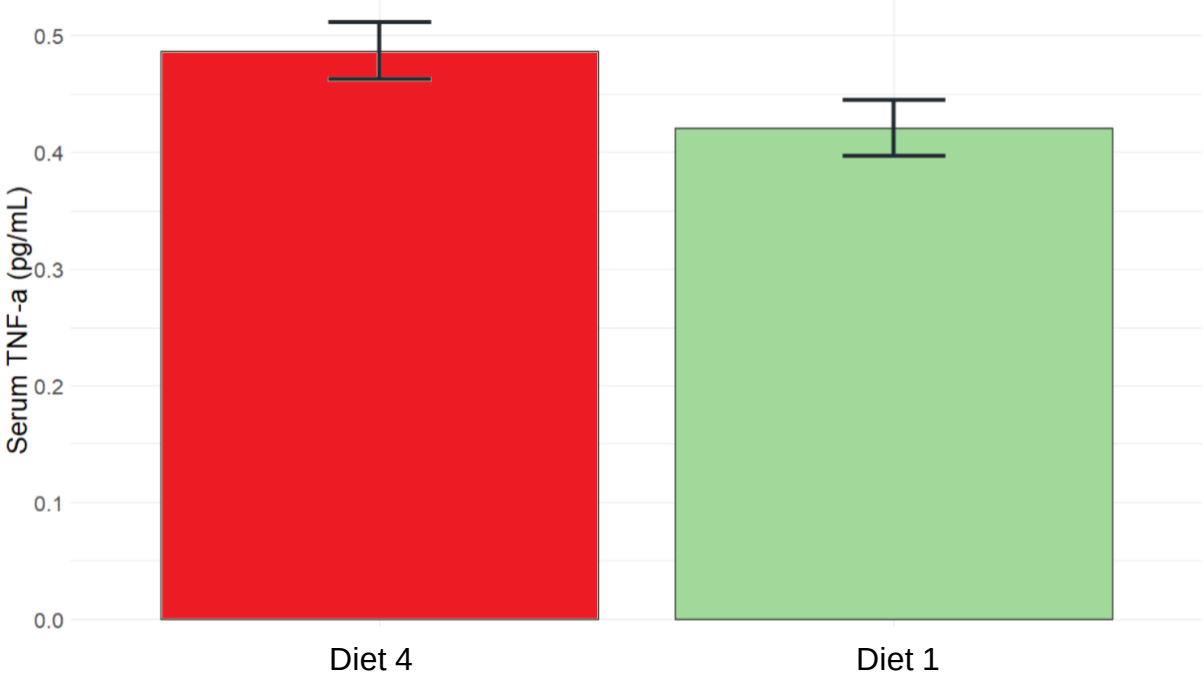
Data are n (%) or mean (SD; range). IU=international units. *Estimated with a physical activity correction of 1·4 in all participants (appendix p 2). †From plasma samples. ‡From serum samples.

***Table 2:*** **Baseline characteristics**

B

| | DATA (N=20 |
|---|---|
| **SEX** | |
| **MALE** | 9 (45%) |
| **FEMALE** | 11 (55%) |
| **AGE (YEARS)** | 51.8 (30-65) |

Supplementary Figure 5: Mean difference between Δchange of TNF-α between diet 1 and diet 4




*Supplementary Figure 2: Demonstration of the difference between baseline and end of intervention and the association between each dietary intervention. There is no statistical difference between diet 4 and diet 1.*

Supplementary Table 4: Example of a dietary Interventions for study 2 – healthy cohort

| | Diet 1 | | Diet 2 | | Diet 3 | | Diet 4 | |
|---|---|---|---|---|---|---|---|---|
| Meal type (time) | **Food** | **Amount (g)** | **Food** | **Amount (g)** | **Food** | **Amount (g)** | **Food** | **Amount (g)** |
| **Breakfast (09:00)** | *Whole wheat cereal* | 60 | *Sugar coated cereal* | 15 | *Sugar coated cereal* | 30 | *Sugar coated cereal* | 60 |
| | *Semi-skimmed milk* | 150 | *Whole milk* | 50 | *Whole milk* | 100 | *Whole milk* | 150 |
| | *Wholemeal bread, toasted* | 60 | *White bread, toasted* | 20 | *White bread, toasted* | 40 | *White bread, toasted* | 60 |
| | *Margarine, polyunsaturated* | 10 | *Butter* | 2·5 | *Butter* | 7·5 | *Butter* | 10 |
| | *Egg, hard boiled* | 60 | *Whole wheat cereal* | 40 | *Whole wheat cereal* | 20 | | |
| | | | *Semi-skimmed milk* | 100 | *Semi-skimmed milk* | 50 | | |
| | | | *Wholemeal bread, toasted* | 40 | *Wholemeal bread, toasted* | 20 | | |
| | | | *Margarine, polyunsaturated* | 7·5 | *Margarine, polyunsaturated* | 2·5 | | |
| | | | *Egg, hard boiled* | 30 | | | | |
| **Morning Snack (11:00)** | *Apple, Granny Smith* | 150 | *Apple, Granny Smith* | 100 | *Low fat yoghurt* | 125 | *Greek yoghurt* | 125 |
| | | | | | *Apple, Granny Smith* | 50 | | |
| **Lunch (13:00)** | *Salmon, steamed* | 150 | *Cod, steamed* | 150 | *Sausage casserole* | 125 | *Pork sausages, fried* | 125 |
| | *Jacket potato* | 200 | *New potato* | 200 | *Oven chips, baked* | 150 | *Potato waffles, grilled* | 120 |
| | *Garden peas, boiled* | 60 | *Garden peas, boiled* | 30 | *Garden peas, boiled* | 15 | *Cola* | 330 |
| | *Carrots, boiled* | 60 | *Carrots, boiled* | 30 | *Carrots, boiled* | 15 | | |
| | *Broccoli, boiled* | 100 | *Broccoli, boiled* | 75 | *Broccoli, boiled* | 50 | | |
| | | | *Diet cola* | 330 | *Cola* | 330 | | |
| **Afternoon Snack (15:00)** | *Grapes* | 150 | *Dark Chocolate* | 50 | *Milk Chocolate* | 22·5 | *Milk Chocolate* | 45 |
| | | | *Grapes* | 100 | *Dark Chocolate* | 25 | | |
| | | | | | *Grapes* | 50 | | |
| **Dinner (18:00)** | *Chicken breast, grilled* | 125 | *Chicken breast, fried* | 125 | *Beef burgers, grilled* | 100 | *Beef burgers, fried* | 100 |
| | *Whole wheat pasta* | 150 | *White pasta* | 150 | *Oven chips, baked* | 150 | *Potato waffles, grilled* | 120 |
| | *Peppers* | 80 | *Peppers* | 40 | *Baked beans in tomato sauce* | 150 | *Processed cheese* | 30 |
| | *Onion* | 40 | *Onion* | 20 | *Cheddar cheese* | 40 | *Tomatoes* | 100 |
| | *Tomato pasta sauce* | 150 | *Tomato pasta sauce* | 150 | *Diet Cola* | 330 | *Cola* | 330 |
| | | | *Diet cola* | 330 | | | | |
| **Evening Snack (21:00)** | *Wholemeal bread, toasted* | 80 | *White bread, toasted* | 40 | *White bread, toasted* | 40 | *White bread, toasted* | 80 |
| | *Margarine, polyunsaturated* | 10 | *Butter* | 2·5 | *Butter* | 7·5 | *Butter* | 10 |
| | | | *Wholemeal bread, toasted* | 40 | *Wholemeal bread, toasted* | 40 | | |
| | | | *Margarine, polyunsaturated* | 7·5 | *Margarine, polyunsaturated* | 2·5 | | |

Supplementary Table 5: Example of a dietary Intervention for study 2 – CVD risk cohort (based on 2500 kcal)

| | Diet 1 | | Diet 4 | |
|---|---|---|---|---|
| **Meal type (Time)** | **Food** | **Amount (g)** | **Food** | **Amount (g)** |
| **Breakfast (09:00)** | | | | |
| | tea with milk | 30 | Ensure plus vanilla | 200 |
| | Swiss Style Muesli | 50 | | |
| | Skimmed Milk | 200 | | |
| | Banana | 150 | | |
| **Snack (11:30)** | | | | |
| | Orange | 143 | tea with milk | 30 |
| | coffee with milk | 30 | Salted Butter | 20 |
| | | | White medium bread | 40 |
| | | | Whole Milk | 150 |
| | | | Chocolate Mousse | 90 |
| **Lunch (13:30)** | | | | |
| | Salmon and Dill Potato Bake | 400 | Pork Sausages in Onion Gravy | 451 |
| | Mixed veggs (carrot, peas, cauliflower, cut green beans, sweetcorn) | 200 | Mashed Potato | 227 |
| | Egg Noodles | 150 | Coffee with milk | 30 |
| | Olive oil | 15 | | |
| | tea with milk | 30 | | |
| **Snack (15:30)** | | | | |
| | coffee with milk | 30 | Chocolate Bounty | 57 |
| | Grapes, red | 250 | tea with milk | 30 |
| | | | | |
| **Dinner (17:30)** | | | | |
| | Chicken Breast in Gravy | 140 | Quarter Pound Beef burger with Chargrilled onion (C/F3201) | 120 |
| | Mixed veggs (carrot, peas, cauliflower, cut green beans, sweetcorn) | 200 | Chips | 57 |
| | Jacket Potatoes | 228 | Burger Buns | 63 |
| | Baked Beans in Tomato sauce | 57 | coffee with milk | 30 |
| | Olive oil | 10 | | |
| **Snack (21:00)** | | | | |
| | Apple | 134 | Chocolate milk | 100 |
| | Mixed nuts | 25 | | |

Supplementary Table 6: Study data on lipid profile

| **DIET 4** | | | | | |
|---|---|---|---|---|---|
| | **Baseline** | **End-of-trial** | **p-value** | **Adjusted p-value** | **Q-Value** |
| LDL-CHOL (MG/DL) | 95.167 ± 33.396 | 103.167 ± 24.474 | 0.150 | 0.907 | 0.179 |
| HDL-CHOL (MG/DL) | 48.278 ± 13.319 | 47.944 ± 11.780 | 0.450 | 0.907 | 0.390 |
| CHOL (MG/DL) | 180.556 ± 46.313 | 195.389 ± 41.157 | 0.601 | 0.907 | 0.477 |
| LDL-PHOS (MG/DL) | 55.278± 16.395 | 59.278 ± 12.271 | 0.092 | 0.824 | 0.125 |
| HDL-PHOS (MG/DL) | 65.944± 16.326 | 66.556 ± 15.538 | 0.755 | 0.907 | 0.514 |
| **DIET 1** | | | | | |
| | **Baseline** | **End-of-trial** | **p-value** | **Adjusted p-value** | **Q-Value** |
| LDL-CHOL (MG/DL) | 93.300 ± 32.068 | 87.400 ± 31.472 | 0.019 | 0.131 | 0.010 |
| HDL-CHOL (MG/DL) | 50.700 ± 13.159 | 48.750 ± 11.433 | 0.937 | 1.000 | 0.284 |
| CHOL (MG/DL) | 182.000 ± 43.030 | 184.150 ± 43.679 | 0.002 | 0.023 | 0.003 |
| LDL-PHOS (MG/DL) | 54.800 ± 15.565 | 52.100 ± 15.580 | 0.021 | 0.145 | 0.010 |
| HDL-PHOS (MG/DL) | 70.400 ± 17.398 | 68.450 ± 14.413 | 0.442 | 1.000 | 0.155 |

Supplementary Figure 6: Power calculations for study 2.

## Healthy cohort power calculation

A formal power calculation is not possible as this will be the first study of its type. However, recent studies have demonstrated significant changes in metabolomics profiles in cohorts of 20 volunteers (1, 2). Allowing for a dropout rate of 33%, we intend to recruit 30 volunteers for this research.

## CVD-risk cohort power calculation

**Study 1**: The power calculation is based on the mathematical model from my previous fellowship (Garcia-Perez *et al.* (2017) Lancet Diabetes and Endocrinology), using the same dietary methodology also proposed here. In order to calculate the effect size, I used the excretion of a previously identified specific urinary biomarker (hippurate) as a result of increasing fruit and vegetable intake. It was shown that a rise in urine concentration of 3.48 mmol/24-h of hippurate is the result of increasing fruit and vegetable intake, in a highly control environment, from 100g to 300g, with an SD 4.52 mmol/24-h. The resulting effect size is 0.772, and with an alpha of 0.05 and power of 0.90 it requires at least 16 volunteers (based on a one tailed difference between two dependent (paired) means). Allowing for a drop-out of 20%, I aim to recruit 20 volunteers.

Supplementary Figure 7: Dietary Counselling Guidance

## 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.

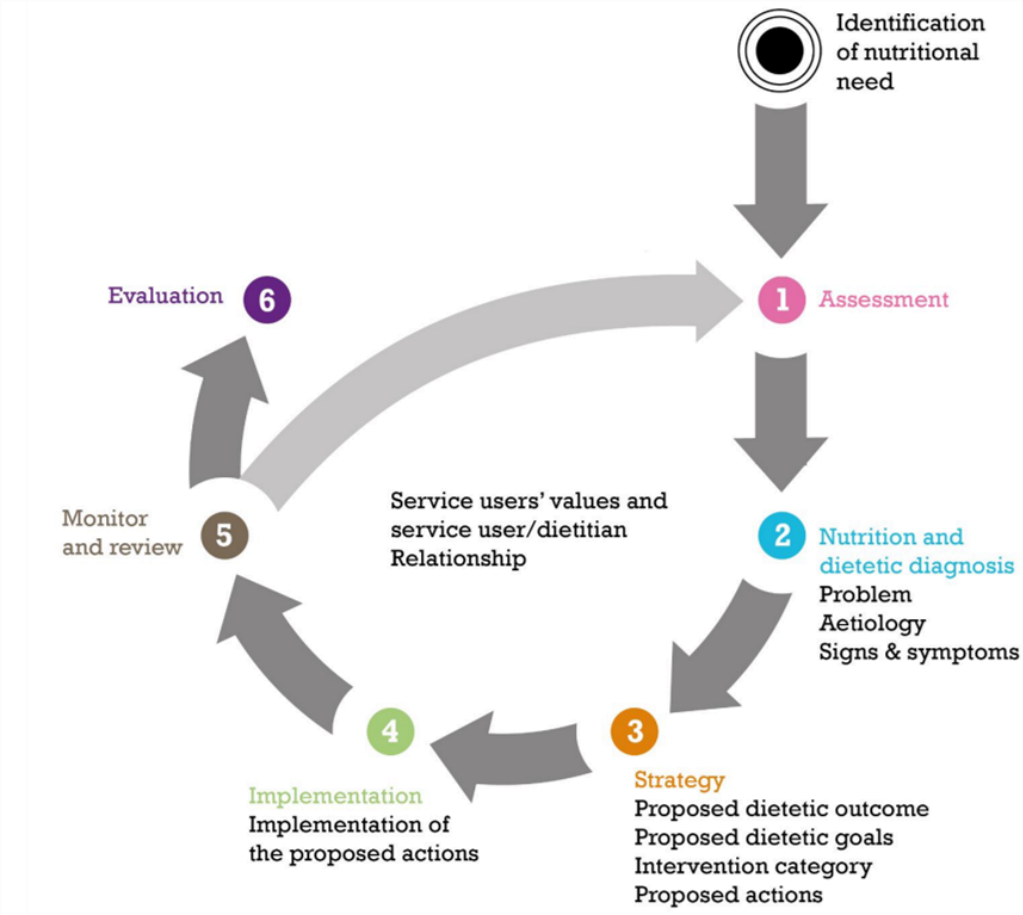



*Figure 3 Model and Process for Nutrition and Dietetic Practice (BDA, 2020)*

**6.0.3** 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.

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

| **Assessment type** | **Data collection** | **Collection method** |
|---|---|---|
| **A**nthropometry | 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 |
| **B**iochemistry | 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) |

| | | |
|---|---|---|
| **C**linical/physical | Blood pressure | Blood pressure monitor will be used to measure blood pressure. Instructions in Appendix D will be followed. |
| | Health/disease status<br>Medication & dietary supplements<br>Bowel movements<br>Food allergies<br>Any relevant symptoms or signs | Participant will be asked about this information in the dietary care record. |
| **D**ietary | 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 |
| **E**nvironmental/ 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. |
| **F**unctional | 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

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

*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

**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<br>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<br>Trimethylamine-N-oxide | |

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

Comments on Metabolomic Report:

ENVIROEMNTAL/BEHAVIOURAL/SOCIAL:
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<br>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_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<br>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<br>Trimethylamine-N-oxide | |
| **Red meat** | O-Acetylcarnitine<br>Carnitine | |
| **Animal protein from meat and dairy** | Phenylacetylglutamine | |
| **Lean meat** | 1-Methylhistidine and 3-Methylhistidine | |
| **Plant based protein** | Trigonelline<br>1-Methylnicotinamide<br>N-methyl-2-pyridine-5-carboxamide | |

Comments on Metabolomic Report:

FUNCTIONAL:

***Physical Activity:***
Changes to Physical Activity:

*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.

5. 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***

6. 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.

7. 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***

8. 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***

8. 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.

9. 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:**

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<br>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 8_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<br>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<br>Trimethylamine-N-oxide | |
| **Red meat** | O-Acetylcarnitine<br>Carnitine | |
| **Animal protein from meat and dairy** | Phenylacetylglutamine | |
| **Lean meat** | 1-Methylhistidine and 3-Methylhistidine | |
| **Plant based protein** | Trigonelline<br>1-Methylnicotinamide<br>N-methyl-2-pyridine-5-carboxamide | |

Comments on Metabolomic Report:

FUNCTIONAL:

***Physical Activity:***
Changes to Physical Activity:

*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.

9. 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***

10. 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.

11. 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***

12. 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***

10. 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.

11. 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:**

STRATEGY:

- 
- 
- 

DIETETIC OUTCOME:

- 
- 

OUTCOME INDICATORS:

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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<br>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:

- 
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- 
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- 

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 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<br>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<br>Trimethylamine-N-oxide | |
| **Red meat** | O-Acetylcarnitine<br>Carnitine | |
| **Animal protein from meat and dairy** | Phenylacetylglutamine | |
| **Lean meat** | 1-Methylhistidine and 3-Methylhistidine | |
| **Plant based protein** | Trigonelline<br>1-Methylnicotinamide<br>N-methyl-2-pyridine-5-carboxamide | |

Comments on Metabolomic Report:

FUNCTIONAL:

***Physical Activity:***
Changes to Physical Activity:

*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.

13. 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***

14. 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.

15. 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***

16. 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***

12. 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.

13. 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:**

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<br>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:

**EVALUATION:**

| | | Did the participant meet the recommendation? (YES/NO) | | | |
|---|---|---|---|---|---|
| **Dietary intake** | **NICE Dietary Recommendation** | **Week 0** | **Week 4** | **Week 8** | **Week 12** |
| **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<br>Women: Less than 2-3 units/d | | | | |
| **Salt** | Less than 2.4g of sodium /d | | | | |

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 til 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.

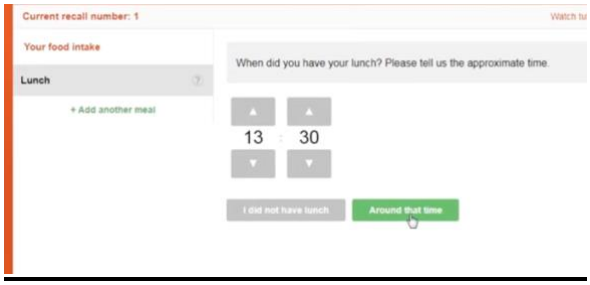
Please record ALL food and drink consumed using Intake24.

You should include all meals and snacks, plus sweets, drinks etc. When recording the foods eaten during meals, please include any sauces,

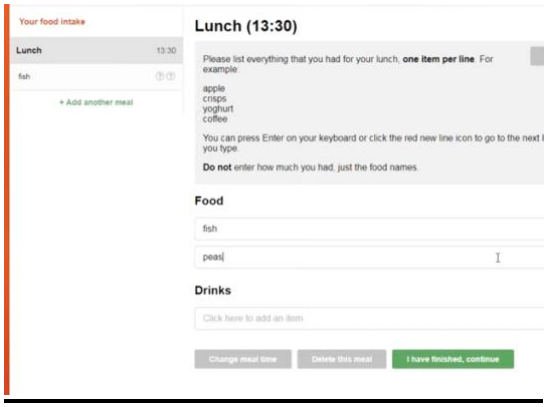
dressing or extras eg: gravy, salad dressing, pickles, as well as the main dish.

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

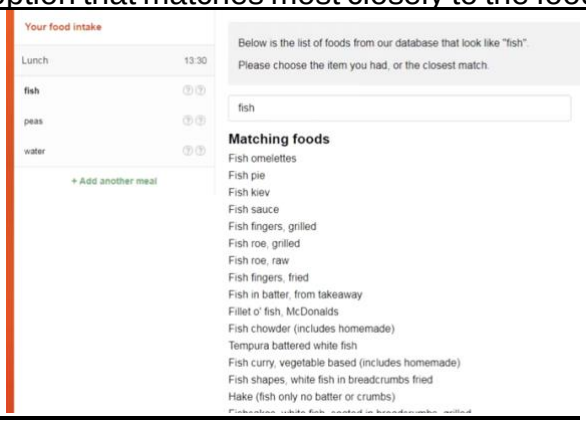
1. Enter the time you had your meal




2. Record what you have consumed for the meal

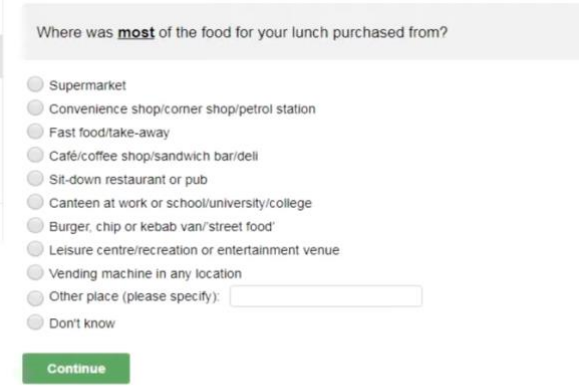



3. Select the option that matches most closely to the food item you have consumed.

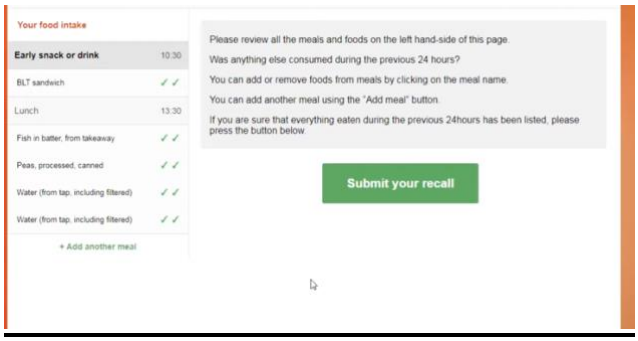



4. Based on these pictorial guide, choose the portion of food that most closely resemble the amount you have consumed.


**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<br>Women: Less than 2-3 units/d | | | | |
| 15 | **Salt** | Less than 2.4g of sodium /d | | | | |

## Appendix G: CVD booklet handout